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U.S. Army Surgical Research Unit

Brooke Army Medical Center

Fort Sam Houston, Texas

Research Report

ANNUAL RESEARCH PROGRESS REPORT

REPORTS CONTROL SYMBOL: RCS-MEDDH-288(R1) 30 June 1966

Project No. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

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FOREWORD

This volume represents a great part of the research effort of the US Army Surgical Research Unit, Fort Sam Houston, Texas. It does not include those studies which are just beginning and much data and many impressions gathered indirectly in pursuit of these projects but not bearing on the specific problem significantly.

It is anticipated that in the immediate future years there will be two major changes in respect to this annual report.

The first relates to distribution of the report which is to be severely restricted. The reason for this primarily is that it is being wasted on individuals and installations who either do not comprehend what is contained therein or, as is most often the case, do not read it. While all of the project reports do not relate directly to nor can the information be applied directly to clinical situations important in military medicine, there are areas in which such actually is the case. In spite of this, the information has yet to be utilized by any other than a few interested individuals. For this reason, only those latter persons are to receive the annual report after this issue and a direct request to this office will be required for its release.

The second anticipated change concerns volume and quality of subsequent reports. It is likely that both will decrease sharply in the next few months and will justify only sporadic reports. The basis for this is the personnel policies which are indiscriminantly decimating the research effort in this installation. While such programs can be readily justified by those responsible for the actions, such explanations do nothing to enhance research activity.

Little can be said that would adequately express my own admiration and gratitude to those whose efforts have made this annual report possible. It is hoped that others will benefit from these efforts.

JOHN A. MONCRIEF
Colonel, MC
Commander and Director

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

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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS Thermal injury, topical therapy, autograft, homograft, heterograft, resuscitation, mortality

24. (U) Tech Objective - To improve care of thermally injured.

(U) Approach - Patients admitted from world-wide areas. Acute care and rehabilitation.

25. (U) Progress (Jan-Dec 65) - This annual report is a summary of the clinical experiences accrued in management of patients with thermal injuries admitted to and discharged from the Burn Study Branch of the Surgical Research Unit in the calendar year 1965. The mission of the Burn Center is described, current modes of therapy outlined, and investigative projects and teaching functions explained. Statistical data are provided as comparison with the experiences of previous years.

As in 1964, the number of admissions for the year again reached a new high, but more important was maturation of an improved burn management program begun in the year 1964 which achieved a marked increase in burned patient survival. This program, 26. continued in 1965, employs topically applied Sulfamylon and special techniques of homografting, the combination of which has effectively controlled lethal burn wound sepsis.

The patient load has markedly increased during this fiscal year with a corresponding increase in operational costs. Patient census for FY 66 is 170% of FY 65.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CLINICAL OPERATION, BURN CENTER

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 January to 31 December 1965

Investigators:

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Carolina F. deLeeuw, Major, AMSC
Ann D. Fritsch, Major, AMSC

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: CLINICAL OPERATION, BURN CENTER

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 January to 31 December 1965

5. Investigators: Walter E. Switzer, Lt. Colonel, MC
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This annual progress report is a summary of the clinical experiences accrued in management of patients with thermal injuries admitted to and discharged from the Burn Study Branch of the Surgical Research Unit in the calendar year 1965. The mission of the Burn Center is described, current modes of therapy outlined, and investigative projects and teaching functions explained. Statistical data are provided for comparison with the experiences of previous years.

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CLINICAL OPERATION, BURN CENTER

The primary responsibilities of the Burn Study Branch of the US Army Surgical Research Unit are to provide specialized care for patients with thermal injuries, to conduct clinical investigative studies pertinent to burn problems, and to teach and train others in this special field of trauma.

The Burn Service, as a section of the Clinical Division of the unit, is physically located in Brooke General Hospital and functions as an Armed Forces specialty center for cases of thermal trauma. Patients authorized care in this facility are active duty or retired military personnel and their dependents, beneficiaries of the Veterans Administration, beneficiaries of the Bureau of Employees' Compensation, and American Indians and Eskimos. Patients are acquired from the local area, from all sections of the United States, and from military overseas facilities. Rapid, long-distance aerial transportation is provided by the Air Force Military Airlift Command, which permits safe evacuation early in the postburn period if resuscitative efforts are adequate and continuous. In urgent situations, a Surgical Research Unit burn team will fly to any location within the continental United States to evaluate and return burned patients to the unit, providing treatment en route. During 1965, burn teams made 50 aerial trips to distant bases, safely evacuating 64 patients.

Principles of Management

Upon admission to the Burn Center, a patient with a major thermal injury is examined to (1) appraise the patient's general physical status, (2) determine the presence or absence of associated injuries, and (3) estimate the extent and depth of the burn.

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

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This annual progress report is a summary of the clinical experiences accrued in management of patients with thermal injuries admitted to and discharged from the Burn Study Branch of the Surgical Research Unit in the calendar year 1965. The mission of the Burn Center is described, current modes of therapy outlined, and investigative projects and teaching functions explained. Statistical data are provided for comparison with the experiences of previous years.

As in 1964, the number of admissions for the year again reached a new high, but more important was maturation of an improved burn management program begun in the year 1964 which achieved a marked increase in burned patient survival. This program, continued in 1965, employs topically applied

sulfamylon and special techniques of homografting, the combination of which has effectively controlled lethal burn wound sepsis.

(1) Thermal Injury
(2) Topical Therapy
(3) Autograft
(4) Homograft
(5) Heterograft
(6) Resuscitation
(7) Mortality

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* Supplied by Sterling-Winthrop Research Institute, Rensslaer, New York.

CLINICAL OPERATION, BURN CENTER

The primary responsibilities of the Burn Study Branch of the US Army Surgical Research Unit are to provide specialized care for patients with thermal injuries, to conduct clinical investigative studies pertinent to burn problems, and to teach and train others in this special field of trauma.

The Burn Service, as a section of the Clinical Division of the unit, is physically located in Brooke General Hospital and functions as an Armed Forces specialty center for cases of thermal trauma. Patients authorized care in this facility are active duty or retired military personnel and their dependents, beneficiaries of the Veterans Administration, beneficiaries of the Bureau of Employees' Compensation, and American Indians and Eskimos. Patients are acquired from the local area, from all sections of the United States, and from military overseas facilities. Rapid, long-distance aerial transportation is provided by the Air Force Military Airlift Command, which permits safe evacuation early in the postburn period if resuscitative efforts are adequate and continuous. In urgent situations, a Surgical Research Unit burn team will fly to any location within the continental United States to evaluate and return burned patients to the unit, providing treatment en route. During 1965, burn teams made 50 aerial trips to distant bases, safely evacuating 64 patients.

Principles of Management

Upon admission to the Burn Center, a patient with a major thermal injury is examined to (1) appraise the patient's general physical status, (2) determine the presence or absence of associated injuries, and (3) estimate the extent and depth of the burn.

A history is obtained, with particular reference to the time and circumstances of the present injury, and the presence of cardiac, respiratory, renal, hepatic or allergic disease. Blood and urine samples are obtained for analysis, and a chest

x-ray and electrocardiograph tracing taken. Initial therapeutic efforts are concentrated on an intensive resuscitative regimen of appropriate intravenous solutions. The effectiveness of the therapy is determined by observation of the vital signs, urinary volume and concentration, central venous pressure, hemoconcentration, status of the peripheral circulation, and the patient's appearance and mental status.

Airway compromise associated with burns of the head and neck is managed by tracheostomy at the level of the second or third tracheal rings; at lower levels, the tracheostomy tube may impinge on the carina, causing bronchospasm, or enter a main stem bronchus, producing obstruction. Complications of the procedure such as pneumothorax, hemorrhage or invasive infection may produce significant morbidity or even mortality.

After resuscitation measures are under way, the entire burn wound is washed with pHisoHex solution and debrided to remove necrotic tissue and to permit more accurate assessment of burn depth. Intravenous medication may be required to allay the discomfort of debridement, but general anesthesia is rarely indicated and may be hazardous at this time.

With patient evaluation completed, it is possible to plan a rational approach to future management of the burn injury. For the purposes of description, the improved burn wound management program initiated in 1964 and continued in 1965 may be divided into four overlapping phases:

(1) Sulfamylon phase.

(2) Homografting (or heterografting) phase.

(3) Autografting phase.

(4) Rehabilitation phase.

The success of the program is indicated by an improvement in the overall mortality rate from an average of 38 per cent in the years 1962-1963 to 20 per cent in the years 1964-1965.

Sulfamylon Phase

In the acute burn period, both full-thickness and partial-thickness burns are treated by complete exposure, early motion of functional areas and daily hydrotherapy. Blood agar plate cultures are taken from representative areas of the surface of the burn wound for bacteriological study, following which the entire burned area is coated with a 10 per cent Sulfamylon hydrochloride* preparation. The use of topical Sulfamylon in 1964 and 1965 represented an important change in the therapeutic regimen for thermal injury patients and was instituted as a method for control of infection in the burn wound. Prior to using Sulfamylon on human burn injuries, a detailed and controlled study had been done utilizing a burned and contaminated animal model. The results of this investigation demonstrated marked inhibition of bacterial growth with nearly 100 per cent survival of treated animals as compared with invasive burn wound infection and death in almost all untreated animals. Clinical trials in December 1963 on patients with serious thermal injuries were so impressive that topical Sulfamylon was adopted as part of the standard regiment for burned patient care in 1964 and continued in 1965.

The Sulfamylon hydrochloride compound is prepared for clinical use by personnel of the laboratory of the Surgical Research Unit. The basic drug is obtained in powder form and blended into a water-soluble pharmaceutical base in a ratio of 10 grams of Sulfamylon hydrochloride powder to 90 grams of base. The cream is usually applied to the entire burn wound by sterile gloved hand or suitable spatula on a twice-a-day schedule until the coagulum of the partial-thickness burn or the eschar of a full-thickness injury separates from the underlying viable tissue. The drug is then discontinued and the wound prepared for initial homografting by cleansing the wound of Sulfamylon and loose debris with saline solution. In addition to the topical therapy, the patient's burn wound is cleansed daily in the Hubbard tank, whirlpool or shower and gentle surgical debridement accomplished along with appropriate physical therapy exercises.

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Sulfamylon hydrochloride in a water-soluble base has several characteristics which make it desirable as a topical agent for control of burn wound sepsis. The finished product is inexpensive, stable at room temperature, white in color, has the consistency of thick cold cream, and spreads easily and smoothly over the burn surface. The 10 per cent concentration is readily absorbed into the burn wound, effectively inhibiting gram-negative bacterial colonization and proliferation. The drug is absorbed into the systemic circulation but is rapidly deaminated, and blood levels of Sulfamylon as such are difficult to identify. Crystalluria is not seen.

Sulfamylon does have several undesirable features. From the standpoint of the patient, there is considerable pain when the drug is applied to second-degree burns within the first several days postinjury. Allergic skin reactions manifested by urticaria and a maculopapular rash occur in approximately 5 per cent of patients. The exact incidence of allergic reactions due to Sulfamylon is difficult to identify precisely, since these patients have usually received a variety of medications which could have produced a drug rash. The pain problem is treated by appropriate analgesics, and the allergic reactions by removal of the Sulfamylon from the burn wound and antihistaminics. In two patients, steroid therapy was required for treatment of an exfoliative dermatitis probably associated with the Sulfamylon therapy.

The most serious complication of Sulfamylon therapy is the development of a hyperchloremic metabolic acidosis in patients with large body surface injuries which is secondary to absorption of hydrogen and chloride ions from the sulfa molecule, the formation of an acid p-carboxysulfonamide degradation product and the inhibition of carbonic anhydrase activity in the renal tubules. While patients with an intact respiratory tree ordinarily compensate for the increased acid load by increased CO_2 expiration, those with significant pulmonic complications resulting from inhalation injury or pneumonia are unable to compensate and become quite acidotic. If this occurs, the drug is removed from the patient's burned areas by washing with a mild detergent, and sodium bicarbonate buffer is given intravenously. It is interesting that children have a lesser tendency toward

the acidotic complication than adult patients; the reason for this tolerance is not clear but may be due to better tracheobronchial function than that of adults who have varying degrees of chronic bronchitis, emphysema or other low-grade pulmonary injury.

Separation of the devitalized burned tissue of Sulfamylon-treated wounds occurs somewhat more slowly than in similar wounds not treated with Sulfamylon in which bacterial activity is unrestricted. Since treated patients are less septic and in generally better physical condition throughout their hospital course, the increased time required for eschar separation has been acceptable.

Homograft (or Heterograft) Phase

As the coagulum of partial-thickness or eschar of full-thickness burn injuries separates, the underlying denuded area is covered with viable homograft, lyophilized homograft or canine heterograft, effecting a gradual closure of the wounds. Fresh, viable cutaneous homograft occasionally is obtained from living donors, but suitable cadaver donors * are equally acceptable and provide the main source of homograft for the burn ward requirements. If special permission to take homograft is obtained from the next-of-kin of the deceased, the body is taken to the Surgical Research Unit operating room where it is shaved, prepared and draped. Skin grafts approximately 0.010-in. thick are cut from all available areas with an electric dermatome, spread on lightly impregnated petrolatum gauze, folded, and placed in large Petri dishes. Penicillin and streptomycin are added, the dishes sealed, labeled and stored in a refrigerator at 4°C. Graft viability decreases rapidly after three days, but satisfactory takes may be obtained for a week or more after removal from the donor.

Late in 1965, a project was undertaken with the Tissue Bank at the Bethesda National Naval Medical Center to determine the usefulness of lyophilized homograft prepared by the Tissue Bank on patients with thermal injuries. These skin

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* Unsuitable donors are those who have skin lesions, a history of hepatitis, or who expire from infection or lymphomatous disease.

grafts are taken from cadaver donors, frozen rapidly in a vacuum, and stored at room temperature in a vacuum bottle. When one desires to use the grafts, they are removed from the vacuum bottle, reconstituted in normal saline solution, and placed on the wound area in precisely the same manner as viable homografts. Freeze-drying, of course, destroys the graft viability but does permit one to store the materials for an indefinite period of time at room temperature. At present, it appears that lyophilized grafts provide a satisfactory cover for burn wound surfaces but do not adhere as readily or stay intact as long as viable homografts.

When homograft is not available in sufficient quantity to meet patient requirements, canine heterograft provides a satisfactory substitute. These split-thickness grafts are taken in the unit laboratory operating room from healthy animals which are clipped, shaved, scrubbed with soap solution, dipped in alcohol, and draped with sterile sheets. The grafts are taken with an electric dermatome, spread on gauze, placed in Petri dishes, labeled and stored in a refrigerator at 4°C. as with homografts. The heterografts are applied in the same way and under the same circumstances as homografts, providing excellent coverage for a period up to four days after application.

Both second- and third-degree burned areas often require one or more applications of homograft before epithelialization from remaining dermal elements has been completed in the partial-thickness burn or before the granulating bed of a third-degree burn is ready for autografting. If purulent material or other debris accumulates under the homograft, the skin is peeled from the site the area debrided, and fresh homograft reapplied. Prolonged take of the homografts or heterografts leading to rejection is avoided by removing the grafts no later than five days after application. If the grafts are permitted to remain in place longer than this period of time, they become so adherent that removal from the recipient bed is technically impossible. If this occurs, one has no alternative but to let the grafts go on to rejection.

Autografting Phase

When a good homograft take is obtained on an area of full-thickness loss, autografting is done. The homograft is removed as described above, exposing an almost sterile, tacky, well-vascularized granulating surface on which autograft will take with minimal or no loss. The exposed wound requires no washing but should be kept moist during the autografting procedure with saline-dampened pads. The grafts need no sutures, and dressings are not applied unless mechanical dislodgement would occur because of the anatomical area grafted. If dressings are placed to prevent graft dislodgement, a dressing change is accomplished on the second or third day postgrafting to inspect the area and accomplish any debridement that may appear necessary. Only a modest amount of care is required when the dressing change is done to avoid graft disruption. If graft take is satisfactory, dressings are reapplied for another two- or three-day period, following which the wounds are exposed and treated without dressings. If the first dressing change is not accomplished within two or three days after the grafting procedure loculated, purulent accumulations beneath the autograft may extend unobserved and destroy a good autograft take in adjacent areas.

When patients have large burn injuries, available autograft is often inadequate to cover all areas of full-thickness loss. This situation is managed in one of two ways; usually, homograft is reapplied at five-day intervals until original donor areas have healed and may be used once again as donor sites. A second method is to permit the homograft to go to rejection; when rejection is completed, the granulating surface is re-prepared with homograft and autografting done be recropping the original donor sites.

Rehabilitation Phase

Completion of wound healing is followed by intensification of the physical therapy and rehabilitation program which has been under way since admission of the patient to the unit. During the acute period of wound care, exercises appropriate to the patient's physical condition are given at the bedside or in the physical therapy section to maintain joint function and muscle tone. Despite diligent efforts, some debilitation does occur and must be corrected prior to

patient discharge. A progressive program of exercise and reconditioning is employed using the facilities of both the Surgical Research Unit and occasionally the Physical Medicine Service of Brooke General Hospital, where more specialized equipment is available. Often the combined efforts of the occupational and physical therapists of the unit are utilized to construct special splints and other devices on an individual patient basis as an aid to rehabilitation.

A discussion of burned patient management would be incomplete without comments relative to the problems of weight loss and nutrition. Metabolic studies at this unit a decade ago demonstrated persistent and irreversible negative nitrogen balance in all patients with severe thermal injuries until healing had occurred.

A significant factor contributing to weight loss during the acute burn period is associated with maintenance of core temperature in the face of large evaporative water losses from the burn wound. Although healing occurs and grafts take despite negative nitrogen balance and weight loss, patients who eat well or whose caloric intake is supplemented by tube feedings become less debilitated and recover more rapidly than those with less adequate nutrition. For these reasons, many patients, both adults and children, are fed a homogenized regular diet through an indwelling nasogastric catheter when spontaneous intake is inadequate. Experience with various tube feedings indicates that the homogenized regular diet is better tolerated than the special high-caloric formulae available from commercial sources which often produce diarrhea The homogenized regular diet gives the patient approximately one calorie per cubic centimeter of fluid, which is fairly adequate from a nutritional standpoint and rarely induces diarrhea. Other important factors in preventing nutritional deficits are a decrease in the number of operative procedures requiring general anesthesia and the use of the anesthetic agent Fluothane * when anesthesia is necessary, from which patients awaken quickly and without side effects of nausea or emesis.

* Ayerst Laboratories, New York, N.Y.

Clinical Research Projects

Investigation of renal plasma flow in the acute burn period was begun on selected patients. A formal project utilizing viable homograft on fresh second-degree burns proved both interesting and promising.

Complicated studies on cardiac output and blood volume determinations were carried out on a few selected burned patients. Although these studies are incomplete, it has been observed that a marked fall in cardiac output may occur in the absence of obvious clinical signs and that the cardiac output may be increased by the use of vasodilator agents if adequate intravenous fluid therapy is maintained.

A continuation of the project on the immunological response in burned patients has demonstrated the ability of severely burned patients to develop a marked increase in antibody titer to an antigen to which the patient has previously been exposed and a failure to respond to an antigen to which the patient has never been exposed.

A cooperative study with the Bethesda Naval Tissue Bank was also undertaken to determine the feasibility and usefulness of lyophilized split-thickness skin homografts on burned patients. This study was followed with appropriate biopsy, bacteriological and photographic efforts.

Education

In 1965, two general surgery residents from the Brooke General Hospital residency program participated in the clinical functions of the Burn Service. These officers served a three-month rotation on the burn ward, assuming direct patient responsibility under the supervision of the permanent staff. A senior medical student from the University of Texas Medical Branch at Galveston was employed for two months as a clinical clerk on Ward 14A.

More than 350 physicians from varying parts of the United States visited the ward, in addition to 24 physicians from 14 foreign countries including Belgium, Brazil, Canada, Germany,

Great Britain, India, Ireland, Israel, Japan, Pakistan, Peru, Portugal, Sweden and Venezuela. Considerable effort is expended by the personnel of the ward to conduct these tours and provide detailed information which the visitors seek.

Staff members participated in numerous local, regional and national medical meetings, presenting material on thermal injury problems to approximately 3,500 physicians, nurses and lay people.

An active in-service training program for nurses, therapists, and enlisted technicians continued to provide practical and current information necessary for good patient care. Instruction was given by experienced physicians, nurses and enlisted specialists of the unit in all aspects of patient management, using appropriate visual aids, demonstrations, and bedside teaching. The Nursing Service Branch of the unit also conducted orientation lectures on burned patient care for numerous nursing personnel of the Air Force flight nurse training program and other groups.

Statistical Resume

During the calendar year 1965, 174 patients were admitted for treatment of burn injuries, more than for any previous year in the history of the unit. There were 159 patient dispositions, including 33 deaths, for an overall mortality rate of 20.7 per cent. Of the disposition group, 115 patients were male and 44 were female, with an age range varying from 42 days to 75 years. Forty-five patients were less than 10 years of age, and 11 patients were 50 years of age or more. The average number of days hospitalized was 51. Patients were received from all Army areas of the continental United States and from Europe, Alaska, the Far East, and the Caribbean. Forty patients were active duty personnel, including 16 Army, 14 Air Force, and 10 Navy. Sixty-two patients were military dependents, 20 were beneficiaries of the Veterans Administration, and 37 others were in various civilian categories (Table 1).

Causes of injuries, shown in Table 2, were flash or flame, or a combination thereof, in 125 patients; 17 patients were

Table 1. Source of Admission, 1965

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	2	0	0	0	0	0	0	1	3
2nd Army	0	2	0	1	0	0	0	4	7
3rd Army	6	10	1	3	4	1	1	2	28
4th Army	3	10	4	16	1	3	13	18	68
5th Army	0	1	5	2	0	0	0	3	11
6th Army	0	1	3	3	0	2	3	3	15
Europe	2	4	0	1	0	0	0	4	11
Far East	2	1	1	0	4	0	0	1	9
Alaska	0	0	0	0	0	0	2	0	2
Caribbean	1	0	0	0	1	0	0	0	2
Congo	0	0	0	0	0	0	0	1	1
Hawaii	0	0	0	1	0	0	1	0	2
TOTAL	16	29	14	27	10	6	20	37	159

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

Others: Bureau of Employees' Compensation Beneficiary (8)
US Public Health Service Beneficiary (2)
Designee of Secretary of Army (17)
Civilian Emergency (9)
Vietnamese Air Force Officer (1)

Table 2. Burn Etiology, 1965

Cause of Burn																						Average % of Burn	
Military						Dependents						VAB			Other								Patients-159
Flash	Flame	Flash & Flame	Chemical	Contact	Electrical & Flame	Flash	Flame	Flash & Flame	Hot Liquid	Contact	Electrical & Flame	Flash	Flame	Flash & Flame	Flash	Flame	Flash & Flame	Hot Liquids	Electrical	Electrical & Flame	Chemical	Contact	Steam	159 Total Body Surface	125 Third-Degree Burn
5	13	15	2	2	3	1	34	7	16	3	1	2	8	10	2	11	17	1	1	1	2	1	1	33.2*	19.6†

* Total body surface involvement in the 159 patients averaged 33.2%.
† One hundred twenty-five of the 159 patients had some third-degree burn; in this group of 125 patients, the third-degree burn averaged 19.6%.

burned by hot liquids, 6 by electricity, one by steam, 4 by chemicals, and 6 by contact with a hot object. Total body surface involvement in the 159 patients averaged 33.2 per cent, slightly higher than the average burn in 1964. One hundred twenty-five of the patients had some full-thickness burn, and in this group, the third-degree loss averaged 19.6 per cent, substantially the same as in 1964.

The relationship of body surface involvement to mortality is shown in Table 3. There were no mortalities in 79 patients with burns totaling less than 30 per cent. This is a slight improvement over the 2 per cent mortality rate in the same burn category for 1964. In the 30 to 40 per cent burn category in 1965, there were 2 deaths in a group of 25 patients, for a mortality rate of 8 per cent. There were no expirations in this category in 1964. Of the 2 patients who expired, one was a 75-year-old man with a 39 per cent total burn who developed a myocardial infarction, and the other a 49-year-old female with a 32 per cent burn who developed acute hemorrhagic pancreatitis complicating her burn injury. Two of 9 patients with total burns between 40 and 50 per cent expired, for a mortality rate of 22.2 per cent, approximately the same rate as for 1964. Five of 18 patients in the 50 to 60 per cent burn group expired, for a mortality rate of 27.8 per cent, which represented a considerable improvement over the 70 per cent mortality rate in this category for 1964. There were 28 patients with burns greater than 60 per cent, and 24 of these expired, for a mortality rate of 85.7 per cent compared with a rate of 88.8 per cent for 27 patients in this category in 1964. Table 4 presents these comparisons in tabular form.

Mortality rates for 1965 in various age groups are depicted in Table 5. In 45 patients below the age of 10 years, the mortality rate was 13.3 per cent as compared with 11.9 per cent in 42 patients for 1964. In the age group between 10 and 50 years, mortality rates were again very similar, with 24.2 per cent in 1965 compared with 20.7 per cent in 1964. The older age group of patients between 50 and 90 years showed a disparity in mortality rate for the two years, with 18.1 per cent in 1965 compared with 41.6 per cent in 1964. This reduction may not be significant, since the total numbers of patients involved were only 11 and 12 respectively.

Table 3. Per Cent Body Surface Involvement and Mortality, 1965

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
No. Burned	35	22	22	25	9	18	13	9	4	2	159
Deaths	0	0	0	2	2	5	11	7	4	2	33
% Mortality	0	0	0	8	22.2	27.8	84.6	77.8	100	100	20.7

Table 4. Comparison of Per Cent Body Surface Involvement and Mortality, 1964-1965

% Burn	1964			1965		
	No. of Patients	No. of Deaths	% Mortality	No. of Patients	No. of Deaths	% Mortality
0-30	109	2	1.8	79	0	0.0
30-40	21	0	0.0	25	2	8.0
40-50	17	4	23.5	9	2	22.2
50-60	10	7	70.0	18	5	27.8
60-100	27	24	88.8	28	24	85.7

able 5. Age Distribution and Mortality, 1965

Age (Yrs.)	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20	20-30	30-40	40-50	50-60	60-70	70-80	Total
No. Burned	5	13	4	3	4	16	11	14	34	28	16	6	3	2	159
Deaths	2	1	0	1	1	1	1	5	7	8	4	0	1	1	33
% Mortality	40	7.7	0	33.3	25	6.3	9.1	35.7	20.6	28.6	25	0	33.3	50	20.7

Table 6 relates mortality to both age and body surface involvement, and is a more informative compilation than that of Table 5. The reduction in mortality rates in nearly all age brackets and burn categories seen in 1964, in comparison with previous years, was again maintained in 1965 and was particularly gratifying in the pediatric age group. In 1964-1965, 24 children less than 10 years old with injuries varying between 30 and 60 per cent had an overall mortality rate of 8.3 per cent compared with a previous mortality rate of 70 per cent for this age group and magnitude of injury.

Data on survivors of major burns (in excess of 30 per cent of body surface) and nonsurvivors since 1955 are recorded in Table 7. There were 47 survivals for 1965, a sizeable increase as compared with any prior years, although the average size of the burn injuries and the third-degree component were almost identical with previous years when there were fewer survivals. The 33 patients who expired had burn injuries averaging 66 per cent of total body surface, approximately the same as for previous years.

Of the 47 survivors in 1965, 10 were children 5 years of age and under (Table 8). All of these children were treated by the Sulfamylon-homograft-autograft program. Two patients 51 years of age survived, and 2 patients 2 years of age survived. Seventeen patients with burns of 50 per cent or over survived, a number which far exceeds any previous year.

Complications in the survivor group were numerous. Contractures of severity were seen in 24 patients, septic chondritis in 17, significant gastrointestinal bleeding in 10 (3 requiring gastric resection), septic phlebitis in 6, serious pulmonary problems of various types in 5, periarticular calcification in 5, ectropion in 5, sepsis in 4 (2 Staphylococcus, and 2 Staphylococcus and Pseudomonas). One or more minor amputations were performed upon 5 patients and major amputations upon 3 patients. Significant decubitus ulcers were seen in 3 patients and infected donor sites in 3 others. There was one complication each of the following categories: acute renal failure, peroneal nerve palsy, generalized seizures, cardiac arrest, glomerular nephritis,

Table 6. Age, Body Surface Involvement & Mortality, 1965

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			
[illegible]	1	[illegible]	1	0	1 (1)	0	1 (1)	0	0	0	5	2	40.0
[illegible]	[illegible]	4	2	1	0	0	1 (1)	0	0	0	13	1	7.7
[illegible]	[illegible]	[illegible]	0	1	0	0	0	0	0	0	4	0	0
[illegible]	[illegible]	0	1	1	0	0	1 (1)	0	0	0	3	1	33.3
4-5		0	0	0	2	0	0	0	1 (1)	0	4	1	25.0
[illegible]		0	5	6	0	2	2 (1)	0	0	0	16	1	6.3
10-15	[illegible]	1	1	1	0	1	2 (1)	0	0	0	11	1	9.1
15-[illegible]	1	2	3	1	0	2	0	4 (4)	1 (1)	0	14	5	35.7
20-30	2	7	5	4	4 (1)	7 (2)	3 (3)	1	1 (1)	0	34	7	20.6
30-40	7	3	1	5	1	3 (1)	2 (2)	3 (2)	1 (1)	2 (2)	28	8	28.6
40-50	6	2	1	2 (1)	1	3 (2)	1 (1)	0	0	0	16	4	25.0
50-60	1	1	2	2	0	0	0	0	0	0	6	0	0
60-70	2	0	0	0	0	0	0	1 (1)	0	0	3	1	33.3
70-80	1	0	0	1 (1)	0	0	0	0	0	0	2	1	50.0
Total	35	22	22	25	9	18	13	9	4	2	159		
Deaths	0	0	0	2	2	5	11	7	4	2		33	
% Mortality	0	0	0	8	22.2	27.8	84.6	77.8	100	100			20.7

Note: Deaths shown in parentheses.

Table 7. Per Cent Burn Versus Survival, 1955-1965

Year	Survivors (burns over 30%)			Deaths		
	No. Cases	Average % Burn		No. Cases	Average % Burn	
		Total	3°		Total	3°
1955	20	39.5	20.3	21	55.6	38.1
1956	22	41.0	17.3	20	57.8	37.8
1957	19	38.4	24.1	17	57.1	38.8
1958	15	42.3	21.6	23	56.5	35.3
1959	29	43.1	20.6	24	63.1	38.1
1960	17	44.2	20.1	30	57.8	37.3
1961	18	44.2	25.0	31	58.0	39.7
1962	18	42.7	21.4	54	59.1	46.2
1963	28	45.8	19.6	57	69.0	41.0
1964	40	41.8	14.8	37	65.0	42.4
1965	47	43.8	21.0	33	66.0	33.4

Table 8. Survivors of Major Burns, 1965

Patient	Age	Sex	% Burn Total	% Burn 3°	Complications and Remarks
1	35	M	71	51	GI bleeding (Curling's ulcer); calcification, elbows; amputation, 5th finger, bilateral; heterograft x 5, homograft x 23
2	28	M	70	40	GI bleeding (Curling's ulcer), sub-total gastrectomy; heterograft x 1, homograft x 9
3	14	M	61	48½	GI bleeding (Curling's ulcer); fractures, femur, bilateral; acute tubular necrosis, hemodialysis x 1; amputation, right hand; chondritis, bilateral, loss of right ear; heterograft x 2, homograft x 13
4	5	F	60	50	GI bleeding (Curling's ulcer); bronchopneumonia, bilateral; multiple flexion contractures; homograft x 13
5	20	M	59	8	GI bleeding (Curling's ulcer); amputation, left mid-forearm; bronchopneumonia; osteomyelitis, left radial stump; septicemia; heterograft x 1, homograft x 4
6	48	M	58	11	Herpes zoster; heterograft x 2, homograft x 3
7	9½	M	57	46	Multiple contractures; homograft x 11
8	6	F	57	43	Multiple contractures; homograft x 11
9	17	M	54½	10	Multiple contractures; thrombophlebitis; heterograft x 1, homograft x 1
10	15	F	54½	9	Immediate homografting; heterograft x 1, homograft x 3
11	20	M	54	21	Homograft x 4
12	39	M	53½	41	GI bleeding (Curling's ulcer); septicemia; pneumonia (inhalation of hydrofluoride gas); pyelonephritis; atelectasis; rupture of patella tendon; I.V. steroids; heterograft x 1, homograft x 21
13	10	M	53	53	GI bleeding (Curling's ulcer); gross hematuria; IVP cystogram, panendoscope; homograft x 2
14	27	M	53	24	Electrical burn; septicemia; periarticular calcification, left elbow; left peroneal nerve palsy; heterograft x 1, homograft x 8

Table [illegible] (Continued)

Patient	Age	Sex	% Burn Total	% Burn 3°	Complications and Remarks
15	22	M	51½	½	Immediate homografting; reaction to Sulfamylon (rash)
16	39	M	51	41	Pneumonia; multiple contractures; steroids; heterograft x 1, homograft x 10
17	29	M	50	8½	Multiple contractures; heterograft x 2, homograft x 1
18	4	F	48	28	Multiple contractures; mediastinal emphysema secondary to tracheostomy; mannitol; homograft x 5
19	44	F	44	13	Heterograft x 1, homograft x 13
20	33	M	43½	25½	White phosphorus burn; GI bleeding (Curling's ulcer), sub-total gastrectomy; partial amputation of index, middle and ring fingers, left, middle, ring and little fingers, right; homograft x 3
21	21	M	43	20	Popliteal contracture, bilateral; heterograft x 1, homograft x 3
22	24	M	4[illegible]	4	Immediate homografting
23	4	M	41	27	Electrical burn; burn of skull with dura exposed; suprapubic cystostomy; multiple contractures; heterograft x 1, homograft x 10
24	20	M	41	7½	GI bleeding (Curling's ulcer), sub-total gastrectomy; postoperative gastrectomy incision wound infection; thrombophlebitis, left greater saphenous, with excision; homograft x 3
25	30	M	39½	25	Multiple fractures, left hand; disarticulation, right wrist; heterograft x 1, homograft x 2
26	9	F	39½	18	Multiple flexion contractures; mannitol; homograft x 6
27	5	F	39½	0	Immediate homografting; homograft x 3
28	51	M	30½	30	Multiple contractures; MP patient; heterograft x 1, homograft x 7
29	7	F	37½	20½	Multiple contractures; heterograft x 1, homograft x 5

Table 8 (continued)

Patient	Age	Sex	% Burn Total	% Burn 3°	Complications and Remarks
30	5	F	37	24½	Immediate homografting; homograft x 5
31	32	M	36	25	Partial amputation, fingers; heterograft x 1, homograft x 7
32	11	M	35½	15	Silver nitrate 0.5% soaks; grand mal seizures, unknown cause; Sulfamylon reaction; multiple contractures; mental deficiency; heterograft x 3, homograft x 2
33	2	M	35	2½	Homograft x 15
34	32	M	34½	15	Multiple contractures; loss of distal digits, both hands; homograft x 6
35	28	M	34½	9½	Bronchopneumonia; homograft x 3
36	43	M	34	2	Multiple contractures; heterograft x 1, homograft x 6
37	5	F	33½	22½	Homograft x 4
38	29	M	32½	24½	Multiple contractures; anterior chamber of eye perforated, bilateral; left eye removed; homograft x 9
39	3	M	32½	15	Bacteremia; homograft x 9
40	2	M	32	20	Multiple contractures; cardiac arrest with external massage; homograft x 4
41	5	M	32	1	Immediate homografting; homograft x 3
42	51	M	31	29	Amputation, left arm; amputation, all toes, left foot; ribs, left side, 6, 7, 8, 9, 10, exposed and excised; heterograft x 1, homograft x 8
43	28	M	31	16½	Silver nitrate 0.5% soaks; acute glomerulonephritis, cause undetermined; axillary contracture; homograft x 10
44	24	M	31	2	Immediate homografting
45	31	M	30½	10½	White phosphorus burn; amputation, right hand
46	15	F	30	28	Silver nitrate 0.5% soaks; pregnant x 3 months
47	22	M	30	0	Immediate homografting; homograft x 3

gross hematuria, herpes zoster, and an infected incisional abdominal wound. An unusual complication occurred in one patient who required removal of one eye and developed a severe corneal ulcer in the other secondary to the wearing of contact lenses which were not removed for several days following his injury.

Table 9 presents a summary of the principal cause or causes of death in the 33 patients who expired, as determined by clinical findings and the 31 autopsies done. Prior to 1964, the principal cause of patient deaths was burn wound sepsis. As this problem was controlled with combined topical therapy and covering of the wounds with homograft prior to autografting, a variety of other conditions have emerged and assumed lethal roles. In 1965, few patients expired because of a single abnormality; in the majority of cases, there were multiple problems, and it was often difficult to determine which abnormality was primary in causing death.

It appears from the records that cardiopulmonary disorders were the most common conditions causing or contributing to demise. Pulmonary congestion and/or edema were seen in 21 cases, pneumonia in 17, and myocardial infarction in one. Although vigorous efforts were made to treat pulmonary problems more effectively, little progress was made. Significant gastrointestinal disease included 7 cases of duodenal ulcer and 9 of gastric ulceration, all presumed to be of the Curling's type. There was one case of acute hemorrhagic pancreatitis which was the principal cause of demise, and a large pelvic abscess in one patient who had been operated upon for a bleeding ulcer problem and later for an acute intestinal obstruction. Renal complications included 2 cases of acute tubular necrosis and 2 of acute pyelonephritis. Burn wound infection which appeared primary or contributory to demise was present in 6 patients, and 4 had serious septic phlebitis. Two patients had no significant findings at autopsy other than the burn wound, and their demise was assumed to be associated with metabolic imbalance.

In 1965, there were 591 operations, the great majority of which were debridement and grafting procedures. Three patients required major amputations, two involving the

Table 9. Causes of Death, 1965

Pt.	Age	Sex	% Burn Total	% Burn 3°	PB Day Death	Clinical Causes of Death	Postmortem Causes of Death
1	39	M	92½	80	4	Burns; gastric ulcer (Curling's)	Burns; bronchopneumonia; esophageal ulcer; multiple gastric ulcers; pulmonary congestion
2	32	F	90	0	18	Burns; septicemia; GI bleeding	Burns; burn wound sepsis; suppurative phlebitis (cutdown site); pulmonary edema
3	21	M	8[illegible]	53	8	Burns; duodenal and gastric ulcers (Curling's); septicemia; pneumonia; cardiopulmonary congestion	Burns; congestive heart failure; burn wound sepsis; duodenal and gastric ulcers; acidosis
4	33	F	84½	46	7	Burns; bronchopneumonia	Burns; pulmonary edema with congestion; focal burn wound sepsis
5	18	M	82	60	2	Burns; inhalation burn, tracheobronchial tree	Burns; smoke inhalation with respiratory tract burn; pulmonary congestion
6	4	M	80	60	2	Burns	Burns; pulmonary congestion; diffuse edema
7	19	M	78½	57½	9	Burns; hyperkalemia due to acute tubular necrosis	Burns; acute tubular necrosis; focal burn wound sepsis; bronchopneumonia
8	33	M	78½	29½	9	Burns; pulmonary edema	Burns; pulmonary edema
9	18	M	78½	28	10	Burns; bronchopneumonia; metabolic acidosis	Burns; bronchopneumonia, with aspiration of foreign material; duodenal ulcer
10	35	M	78	51½	5	Burns (sulfuric acid burn) tabolic acidosis; septicemia	Burns; suppurative phlebitis (cutdown site); pulmonary edema with congestion

Note: Septicemia was diagnosed clinically when a significant infection of some type existed and was associated with one or more positive blood cultures. Infections included pneumonia, peritonitis, suppurative phlebitis, intraperitoneal abscess, and invasive burn wound sepsis singly or in combination.

Table 5 (continued)

Pt.	Age	Sex	% Burn Total	% Burn 3°	PS Day Death	Clinical Causes of Death	Postmortem Causes of Death
11	61	M	76	70	4	Burns; acute tubular necrosis; bronchopneumonia; GI hemorrhage (Curling's ulcer)	No autopsy performed
12	18	M	71½	40½	12	Burns; GI hemorrhage (Curling's ulcer); burn wound sepsis	Burns; burn wound sepsis; focal pulmonary infarcts; laryngeal ulcers; gastric ulcer
13	18	M	70½	24½	10	Burns; bronchopneumonia; spontaneous bilateral pneumothorax	Burns; bronchopneumonia; congenital cysts, lungs; hepatic congestion
14	32	M	69½	46	4	Burns; pulmonary edema; bilateral hydrothorax	Burns; pulmonary edema; bilateral hydrothorax; erosion of anterior wall of trachea
15	20	M	67	37½	7	Burns; bronchopneumonia	Burns; bronchopneumonia; multiple gastric ulcers (Curling's)
16	44	F	65½	26	12	Burns; bronchopneumonia; burn wound sepsis	No autopsy performed
17	28	M	65	29	15	Burns; tracheitis, from smoke inhalation; acute tubular necrosis; septicemia	Burns; pneumonia; pyelonephritis; tracheobronchitis
18	11/12	F	65	23½	4	Burns; bronchopneumonia	Burns; pulmonary congestion
19	5	F	64	46	31	Burns; pneumonia; septicemia	Burns; burn wound sepsis; hematogenous pyelonephritis; pulmonary abscess; multiple gastric ulcers (Curling's)
20	3	F	62	38	3	Burns; acidosis due to cause other than diabetes	Burns; cerebral edema; pulmonary edema
21	4	F	61	29	4	Burns	Burns; hypokalemia
22	11	M	60	35	4	Burns; bronchopneumonia	Burns; bronchopneumonia; pulmonary congestion

Table 9 (continued)

Pt.	Age	Sex	% Burn Total	% Burn 3°	PB Day of Death	Clinical Causes of Death	Postmortem Causes of Death
23	29	M	60	28	5	Burns; bronchopneumonia	Burns; bronchopneumonia; pulmonary edema and congestion; gastric ulcer
24	30	F	60	26	5	Burns; bronchopneumonia	Burns; bronchopneumonia, with hyaline membrane formation; duodenal ulcer
25	72	M	59	3½	1	Burns	Burns; pulmonary edema and congestion, with hyaline membrane formation; duodenal ulcer; bronchopneumonia
26	46	M	53½	19½	14	Burns; cardiopulmonary congestion	Burns; pulmonary congestion
27	35	M	53	0	13	Burns; bronchopneumonia	Burns; pneumonia; gastric ulcer
28	22	F	52	30½	14	Burns; phlebitis; bronchopneumonia; septicemia	Burns; bronchopneumonia; septicemia; pulmonary congestion; septic phlebitis
29	40	M	50	30½	7	Burns; acute tubular necrosis; pulmonary edema; atrial fibrillation; septicemia; multiple fractures, lower extremities	Burns; bilateral pleural effusion; probable congestive heart failure; septicemia
30	23	M	47½	39	49	Burns; bronchopneumonia; encephalopathy; duodenal ulcer (Curling's); gastrectomy; cardiac arrest; subdiaphragmatic abscess; pneumothorax; septicemia	Burns; pelvic abscess (4000 ml); bronchopneumonia
31	8/12	F	42½	15	9	Burns; duodenal ulcer (Curling's); gastrectomy; bronchopneumonia; phlebitis; septicemia	Burns; bronchopneumonia; pulmonary edema; post-gastroduodenostomy
32	75	M	39	0	9	Burns; emphysema; bronchopneumonia; septicemia	Burns; bronchopneumonia; septicemia; myocardial infarction; gastric ulcers with hemorrhage; duodenal ulcers (Curling's) with hemorrhage
33	48	F	32½	24	41	Burns; septicemia; bronchopneumonia; acute hemorrhagic pancreatitis	Burns; acute hemorrhagic pancreatitis; peritonitis; septicemia; focal burn wound sepsis

upper extremities and one a lower extremity. Six patients were operated upon for gastrointestinal hemorrhage, and all underwent gastric resection for control of bleeding from Curling's ulcers of the duodenum or stomach. Four of the 6 patients survived.

Increased homograft requirements were met by using a record number of 87 cadavers as skin donors. Approximately 300 homograftings were done in the operating room, and many others on the ward without anesthesia. Since homograft supply did not always coincide with patient requirements for viable skin, canine heterograft taken from the laboratory animals at the Surgical Research Unit was used 58 times on 32 patients. This type of wound coverage has continued to prove quite satisfactory.

Summary

The burn wound management program which had proved so successful in 1964 was continued in 1965, producing almost identical results. Since this program is applicable to all burns, large or small, simple in application, relatively inexpensive and aesthetically acceptable, it has received considerable attention from clinicians in both the United States and foreign countries. The metabolic acidosis which caused considerable concern during the first year of topical Sulfamylon created less difficulty during the past years. This problem was recognized earlier and managed by removal of the medication and buffering with sodium bicarbonate until compensation occurred. This therapy was effective unless acute or chronic pulmonary disease was severe. In a few patients, positive pressure respirators were of value but not consistently so.

In Table 10, burn mortality rates of four burn categories for the years 1962-1963 are compared with those of 1964-1965. The first two-year period included those patients treated by conventional management prior to the employment of topical therapy and large amounts of homograft. The 1964-1965 period included the two years of the Sulfamylon-homograft program. Mortality rates for 1964-1965 improved in each burn category, particularly in the three groups between 30 and 60 per cent. Comparing these groups would seem to be valid, since the

Table 10. Comparison of Burn Mortality Rates, 1962-63 and 1964-65

Years	Per Cent Burn											
	0-30			30-40			40-50			50-60		
	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
1964-65	188	2	1.1	46	2	4.3	26	6	[illegible]	28	12	42.9

numbers of patients involved are substantial and the only significant change in management in the two periods was that of topical therapy and increased use of homograft. Little or no improvement in mortality rates have been achieved for patients with thermal injuries greater than 60 per cent of body surface; their multiple problems often remained overwhelming.

Although survival rates have improved, many major problems of burned patients remain unsolved. Fluid resuscitative regimens are empirical and require reappraisal. Basic physiology relative to energy requirements, cardiac output and tissue perfusion needs more study. Pulmonary complications secondary to inhalation of combustion products are often fatal, and gastrointestinal ulcerations with bleeding or obstruction contribute to both morbidity and mortality. The immunology of skin homotransplantation remains a mystery, and the dense, unyielding scars of the late burn period continue to produce functional and cosmetic deformities which affect entire life patterns of patients and their families. It is apparent that many problems remain to challenge both the clinician and the research scientist in this special field of trauma.

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2. Zaroff, L.I., Linder, W.R., and Moncrief, J.A. Successful autografting in a burned hemophiliac without systemic or local hemostatic agents. Plast. & Reconstr. Surg., 35:271-275, 1965.

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Exhibits

Mills, W., Jr., Switzer, W.E., Fitts, C.T., and Kaye, M.P.: exhibit entitled, "Electrical Injuries", displayed at the American Medical Association Annual Meeting, New York, N.Y., 20-24 June 1965, and at the Association of the US Army, Washington, DC, 25-29 October 1965.

Presentations

Moncrief, J.A.: The Burn Injury--Pathophysiology as a Basis for Therapy. Staff, St. John's Mercy Hosp., St. Louis, Mo., 11 Jan. 1966.

DeLeeuw, C.F.: Psychiatric Problems of the Burned Patient, with Emphasis on Occupational Therapy and Physical Therapy. Occupational Therapists, San Antonio State Hosp., presentation given at Surgical Research Unit, BAMC, FSHT, 15 Jan. 1965.

Peake, M.C.: Nursing Care of the Burned Patient. Flight Nurses, Brooks Air Force Base, Texas, 29 Jan. 1965.

Switzer, W.E.: Advancements in the Treatment of Burns. San Antonio Assoc.of Oper.Room Nurses, Beach Pav., FSHT, 1 Feb 1965.

Moncrief, J.A. and Alexander, J.W.: Pathophysiology of Thermal Injury. Staff, Robert B. Green Hosp., San Antonio, Texas, 2 Feb. 1965.

Switzer, W.E., and Pruitt, B.A., Jr.: Treatment of Burns. MFSS Management of Mass Casualties Course, BAMC, FSHT, 11 Feb. 1965.

Miller, T.A.: Early Treatment of the Burned Hand. Central District, Texas Chapter, Amer. Physical Therapy Assoc., San Antonio, Texas, 15 Feb. 1965.

DeLeeuw, C.F., and Fritsch, A.D.: Therapy of the Burned Patient. Physical Med. Svc., BGH, BAMC, FSHT, 25 Feb. 1965.

Pruitt, B.A., Jr.: Management of Burns in the Combat Zone. MFSS Off. Orientation Class, BAMC, FSHT, 26 Feb. 1965.

Mills, W., Jr.: Surgical Research in Burns. Class 65, Adv. Aerospace Med. Course, Allied Med. Officers, USAF Sch. of Aerospace Med., Brooks AFB, Texas, 3 Mar. 1965.

Duckett, J.W.,Jr. : Discussion of Causes and Types of Burns. Alamo Heights High Schcol, Sophomore Class, San Antonio, Texas, 4 Mar. 1965.

The following presentations were made at the Seminar on the Surgical and Orthopaedic Aspects of Trauma, BGH, BAMC, FSHT, 8-12 Mar. 1965:
Moncrief, J.A.: Physiological Approaches to Shock Therapy.
Moncrief, J.A.: Moderator, Morning Session: Panel on Burn Injury.
Pruitt,B.A.,Jr.:Initial Care of the Burned Patient.
Switzer, W.E.: Methods of Wound Management.
Mills, W., Jr.: Electrical Burns.
Order, S.E.: Mortality and Complication in Burn Injury.

Switzer, W.E.: Surgical Procedures in Burn Therapy. Annual M.E.N.D. Orientation Tour, USASRU, BAMC, FSHT, 13 Mar. 1965.

Peake, M.C.: Care of Burns. Texas State Nurses Assoc. Ann. Meeting, Austin, Texas, 22 Mar. 1966.

Moncrief, J.A.: (1) Burn Sepsis, (2) Burn Shock. Postgrad. Course in Surgery, Univ. Kansas Med. Ctr., Kansas City, Kans., 22-25 Mar. 1965.

Moncrief, J.A.: Thermal Radiation. DASA Nuclear Med. Oprn. Workshop (NMOW-1-1), Albuquerque, New. Mex., 5-9 Apr 1965.

Switzer, W.E., and Mills, W., Jr.: Management of Burns in Mass Casualties. MFSS Mass Casualty Course, BAMC, FSHT, 8 Apr. 1965.

Moncrief, J.A.: Burns. M.E.N.D. Lecture. Univ. of Nebraska, Omaha, Nebraska, 9 Apr. 1965.

DeLeeuw, C.F.: Workshop on Splinting Materials. Rancho Los Amigos Hosp., Los Angeles, Calif., 12 Apr. 1965.

DeLeeuw, C.F.: Occupational Therapy in Treatment of Burns. Physical Disabilities Study Group, So. Calif. OT Assoc., Los Angeles, Calif., 14 Apr. 1965.

Pruitt, B.A., Jr.: Management of Burns. USAF Sch. of Aerospace Med., Brooks AFB, Tex--Residency in Aerospace Med. Class, Held on Ward 14A, USASRU, BAMC, FSHT, 19 Apr. 65.

Moncrief, J.A.: (1) Management of the Burned Hand; (2) Resuscitation of the Severely Injured Patient. Amer. Coll. Surgeons Trauma Seminar, Las Vegas, Nev., 25-28 Apr. 1965.

Pruitt, B.A., Jr.: Emergency Treatment of Burns (2 presentations). Emergency Med. Treatment Classes, BGH, BAMC, FSHT, 27 Apr. 1965.

Moncrief, J.A., Switzer, W.E.,and Mills, W., Jr.: Panel Discussion on "Burns". 74th Ann. Mtg. Ariz. Med. Assoc., Tucson, Ariz., 29 Apr. 1965.

Miller, T.A.: Emergency Treatment of Burns (2 lectures). Emergency Med. Treatment Classes, BGH, BAMC, FSHT, 30 Apr. 1965.

Pruitt, B.A., Jr.: Standard and Mass Casualty Care of the Burned Patient. Indiana Assoc. of Osteopathic Physicians & Surgeons Ann. Convention, Indianapolis, Ind., 3 May 1965.

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Pruitt, B.A., Jr.: Treatment of the Burned Child. National Osteopathic Child Health Conf., Kansas City, Mo., 5 May 1965.

Peake, M.C.: Nursing Care of Burns. MFSS Adv. Nursing Students, FSHT, 5 May 1965.

Moncrief, J.A.: Energy Expenditures in Patients with Severe Burns and Complications. Univ. Pennsylvania Grad. Sch., Philadelphia, Pa., 6-8 May 1965.

Moncrief, J.A.: Control of Burn Wound Sepsis. M.E.N.D. lecture, Med. College of South Carolina, Charleston, S.C., 17 May 1965.

Switzer, W.E.: Management of Burned Patients. Mass Casualty Course, MFSS, FSHT, 20 May 1965.

Fritsch, A.D. Range of Motion Problems. Amer. Physical Therapy Assoc. State Mtg., San Antonio, Texas, 21 May 1965.

Moncrief, J.A.: Fluid Loss Through the Burn Wound. Postgrad. Seminar--Current Management of Burns, Cook Co. Grad. Sch. of Med., Chicago, Ill., 4-5 June 1965.

Moncrief, J.A.: Treatment of Burns at USASRU. Michael Rees Hosp. & Med. Ctr., Chicago, Ill., 5 June 1965.

Moncrief, J.A.: New Concepts in Treatment of Burns. Mil. Med. & Allied Sciences Course, Walter Reed Army Institute of Research, Washington, DC, 7 June 1965.

Switzer, W.E., Easterling, R.E., and Miller, T.A.: Investigative and Clinical Aspects of Thermal Injury and Renal Failure Problems. Med. Students from Baylor Univ., at Clinical Division, Ward 14A, USASRU, BAMC, FSHT, 28 June 1965.

Peake, M.C.: Nursing Care of Burns. Student Nurses, Sch. of Aerospace Med., Brooks AFB, Texas, 29 June 1965.

Peake, M.C.: Nursing Care of Burns. Student Nurses, Sch. of Aerospace Med., Brooks AFB, Texas, 1 July 1965.

DeLeeuw, C.F.: Occupational Therapy in the Burn Unit. OT Therapy Students, BGH, at USASRU, BAMC, FSHT, 15 July 1965.

Moncrief, J.A.: Practical Aspects of Burn Care. Black Hills Medical Seminar, Rapid City, S. Dakota, 6-7 Aug. 1965.

Pruitt, B.A. Jr.: Recent Advances in Burn Care. Nursing Staff, US Army Hospital, Okinawa, 10 Aug. 1965.

Fritsch, A.D., and DeLeeuw, C.F.: Occupational and Physical Therapy in Treatment of the Burned Patient. PT Students, MFSS, on Clinical Div., Ward 14A, USASRU, BAMC, FSHT, 20 Aug. 1965.

Fritsch, A.D.: Physical Therapy in the Care of the Burned Patient. Basic OT Officers from MFSS, Ward 14A, Clinical Div., USASRU, BAMC, FSHT, 13 Aug. 1965.

DeLeeuw, C.F.: Role of the Occupational Therapist in the Treatment of Burned Patients. OT Students, Veterans Administration, Houston, Texas, 20 Aug. 1965.

Moncrief, J.A.: Initial Treatment of Thermal Injury. 82nd Ann. Mtg., Wyoming State Med. Soc., Jackson Lake Lodge, Wyoming, 24-27 Aug. 1965.

Moncrief, J.A.: Current Therapy of Burns. Amer. College of Surgeons, Montana-Wyoming Chapter, Jackson Lake Lodge, Wyoming, 28 Aug. 1965.

Pruitt, B.A., Jr.: Advances in the Treatment of Burns. Wimberley Chamber of Commerce, Wimberley, Texas, 15 Sep. 1965.

Peake, M.C.: Treatment of Burns. Randolph Registered Nurses Club, Randolph AFB, Texas, 27 Sep. 1965.

Peake, M.C.: Nursing Care of Burns. Brooks AFB Flight Nurses, Brooks AFB, Texas, 30 Sep. 1965.

Peake, M.C.: Treatment of Burns. New Mexico State Nurses Ann. Convention, Albuquerque, New Mex., 12 Oct. 1965.

Moncrief, J.A.: Use of Topical Sulfonamide in Control of Burn Wound Sepsis. Meeting Amer. Assoc. for Surgery of Trauma, Phila., Pa., 14 Oct. 1965.

Switzer, W.E.: Use of Canine Heterografts in the Therapy of Thermal Injury. Meeting Amer. Assoc. for Surgery of Trauma, Phila., Pa., 14 Oct. 1965.

Moncrief, J.A.: Mortality and Morbidity in the Early Postburn Period: A Result of Infection. Panel Discussion on "Burns." Amer. Coll. of Surgeons Surgical Forum, Atlantic City, New Jersey, 20 Oct. 1965.

Moncrief, J.A.: Circulatory Hemodynamics of the Postburn Period. Meeting, Surgical Biology Club II, Atlantic City, N.J., 17 Oct. 1965.

Switzer, W.E.: Dressing the Burned Patient. Sixteenth National Conf. on Disaster Med. Care, Amer. Med. Assoc. Committee on Disaster Med. Care of Council on National Security, Chicago, Ill., 30 Oct. 1965.

Moncrief, J.A.: Practical Aspects of Burn Care. Tri-County Surgical Society, Qtr.y Mtg. and Scientific Program, Palm Desert, Calif., 11 Nov. 1965.

The following presentations were made at the Sixth Annual Burn Seminar, Chicago, Illinois, 12-13 Nov. 1965.
Moncrief, J.A.: Moderator, Session on "Wound Management."
Moncrief, J.A.: Investigation in Burn Centers.
Switzer, W.E.: Use of Homografts

Moncrief, J.A.: The Treatment of Severe Burn Cases. Med. Staff Meeting, San Antonio Community Hosp., Upland, Calif., 16 Nov. 1965.

DeLeeuw, C.F.: Occupational Therapy in the Treatment of Burns. OT Students from Houston Veterans Administration, at Ward 14A, USASRU, BAMC, FSHT, 19 Nov. 1965.

Peake, M.C.: Treatment of Burns. Adv. Nursing Course, MFSS, BAMC, FSHT, 29 Nov. 1965.

Peake, M.C.: Treatment of Burns. Brooks AFB Flight Nurses, Brooks AFB, Texas, 26 Nov. 1965.

Moncrief, J.A.: Treatment of Burns. M.E.N.D. Program, Univ. of So. Dakota Sch. of Medicine, Vermillion, So. Dakota, 1 Dec. 1965.

Peake, M.C.: Function of the Surgical Research Unit. Brooke Gen. Hosp. Nursing In-Service Program, BGH, BAMC, FSHT, 1 Dec. 1965.

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10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 148

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) Vasodilators in the Treatment of Burn Shock (44)

12. SCIENTIFIC OR TECH. AREA	12. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 63	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	c. TYPE NA	PRIOR FY 65	3	4
		CURRENT FY 66	3	3

19. GOV'T LAB/INSTALLATION/ACTIVITY

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NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas

INVESTIGATORS PRINCIPAL ASSOCIATE: Pruitt, B.A., Maj, MC
Moncrief, J.A., Col, MC

TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS:
Vasodilators, burn shock, excess lactate, cardiac output

24. (U) Tech Objective - The effect of vasodilators, specifically hydralazine, in treatment of burn shock is being clinically studied in appropriate patients. The effect of hydralazine on cardiac output, blood volume and pressure, and lactate metabolism is determined. Consistent beneficial effects on circulatory dynamics of such agents in early postburn period would permit resuscitation with lesser volumes of fluid and with improved peripheral circulation preserve viability in marginally injured tissue.

25. (U) Approach - Adult patients admitted within 48 hours postburn are monitored by means of serial cardiac output and blood volume determinations. Blood gas and serum chemistry measurements are made and vital signs closely followed. The influence of hydralazine in a dosage of 0.5 mg/kg on these indices is noted.

26. (U) Progress (1 Jul 65 - 30 Jun 66) - Three patients with greater than 40% burns have been studied. Results indicate that hydralazine was effective in increasing cardiac output in all three patients after 23 hours postburn, the effect persisting for 1-3 hours in two of the three. Improvement occurred in the presence of a decreased blood volume and was accompanied by decrease in systolic pressure within significant pulse rate increase. At 9 hours postburn, neither increased fluid administration nor hydralazine alone would increase cardiac output, but combined, a 22% increase in cardiac output was noted. Changes in excess lactate were inconsistent following hydralazine at these times. Vasodilators appear to improve the circulatory dynamics of the severely burned patient due to the release of vasospasm and subsequent increase in cardiac output secondary to a decrease in peripheral resistance and may well permit utilization of smaller volumes of fluid in the resuscitation of the severely burned patient.

27. COMMUNICATIONS SECURITY	28.	29. DOD CODE	30. BUDGET CODE
[ ] a. COMSEC OR COMSEC RELATED [X] b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) FY	36.

DD FORM 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: VASODILATORS IN THE TREATMENT OF BURN SHOCK

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Basil A. Pruitt, Jr., Major, MC
John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: VASODILATORS IN THE TREATMENT OF BURN SHOCK

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Basil A. Pruitt, Jr., Major, MC
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Three patients, each with burns of greater than 40 per cent of the body surface, have received hydralazine in the early postburn period. Administration of this vasodilator after the twenty-third postburn hour was followed by an increase in cardiac output of from 31 to 67 per cent in all three patients. In two of the three patients, the improvement in cardiac output persisted from one to three hours. One patient also received hydralazine at nine hours postburn with no increase in cardiac output until the rate of fluid administration was increased, with the combined effect increasing cardiac output while neither fluids nor hydralazine alone were effective at that time.

The beneficial effect was achieved in the presence of decreased blood volume and was accompanied by a decrease in systolic blood pressure without significant rise in pulse rate. The response of excess lactate was variable following hydralazine administration.

This agent appears promising as a means to improve the circulatory dynamics without massive fluid loads in the early postburn period.

(1) Vasodilators
(2) Burn Shock
(3) Excess Lactate
(4) Cardiac Output

VASODILATORS IN THE TREATMENT OF BURN SHOCK

The hemodynamic changes noted in the early postburn period appear to represent a summation of the effects of a decreased blood volume and an increased peripheral resistance. The latter results from vasospasm, affecting both arterioles and venules and increased blood viscosity as represented by alteration of serum proteins and by sludging of red blood cells.

The importance of the vasospastic component of the increased peripheral resistance is being investigated by measuring the response of cardiac output to treatment with hydralazine. This vasodilator (a chelating agent) has a direct effect on the small vessel smooth musculature with resultant relaxation and vasodilatation.

All adult patients admitted to this unit within the first 48 hours postburn are carefully monitored with periodic measurements of cardiac output by a dye dilution technique, blood volume utilizing radioiodinated serum albumin, arterial pressure, venous pressure, hematocrit, serum chemistries and blood gases.

Three such patients admitted during the past 15 months have been so studied with hydralazine in dosage of 0.5 mg/kg being administered intravenously at various times during the early postburn period when measured cardiac output was significantly lower than the predicted cardiac output as calculated by the formula:

$$\underset{(L/min)}{C.O.} = \frac{26.5 \times B.W.\ (\text{in grams})^{.854}}{60,000}$$

Results as tabulated in Tables 1, 2 and 3 revealed a prompt increase in cardiac output following adminstration of hydralazine at all times after 23 hours postburn, varying from 30.7 per cent to 67.2 per cent. There appeared to be a persistence of this beneficial effect with further increase in cardiac output of approximately 5 per cent in each of two patients in whom measurements

Table 1. Patient Circulatory Indices

Pt. #1 % Burn 53½ Weight 87.72 kg

Hrs. PB	Observed C.O.* (L/min)	% of Predicted	Hydralazine Given (mg)	% Change	Observed B.V.†
4	2.11	28			5800
6	3.46	47			4525
8½	4.41	60			
9	3.98	54	43	-10	4300
9½	5.41	73		+22§	
12	5.14	69			4100
23	4.95	67			4300
23¼	6.79	92	43	+37	
25	5.74	78			
29	7.45	101			4200

* Predicted cardiac output 7.35 L/min

† Predicted blood volume 6140 cc

§ Patient given 750 cc lactated Ringer's and 600 cc Plasmanate at 18 cc/min for 75 min prior to this measurement

Table 2. Patient Circulatory Indices

Pt. #2 % Burn 43 Weight 86.81 kg

Hrs. PB	Observed C.O.* (L/min)	% of Predicted	Hydralazine Given (mg)	% Change	Observed B.V.†	Lactate (mg%)	Pyruvate (mg%)	Excess Lactate (mMol/L)	Arterial Pressure
4	7.42	102			6400	26.3	1.5	.632	100/50
12	6.82	93.6			5800	16.3	1.0	.326	125/65
15	6.0	82.4			5500				165/75
18	5.57	76.5			5200	20.0	1.2	.398	130/75
24	5.3	72.8			5300	16.2	1.1	.128	130/75
27	7.82	107			5600	12.1	.9		
36	5.68	78			5400				165/65
38	9.5	130	40	+67.2	5300				120/60
39	9.8	135		+72.5	5600				100/65
42	8.28	114			5275	14.8	1.0	.166	130/75
48	9.6	132			5600				180/70
60	11.95	164			5600	16.0	1.0	.296	170/80

* Predicted cardiac output 7.28 L/min

† Predicted blood volume 6077 cc

Table 3. Patient Circulatory Indices

Pt. #3 % Burn 71.5 Weight 63.63 kg

Hrs. PB	Observed C.O.* (L/min)	% of Predicted	Hydralazine Given (mg)	% Change	Observed B.V.†	Lactate (mg%)	Pyruvate (mg%)	Excess Lactate (mMol/L)	Arterial Pressure
19	5.02	90			3900	46.0	3.4	1.332	115/65
23	3.36	60			3850	45.0	3.5	1.112	75/40
28	3.50	63			3600	32.0	2.2	1.120	95/40
37	4.23	76			3200	26.5	1.5	1.281	115/75
39	5.53	98.9	35	+30.7	3500	43.0	3.3	1.120	90/50
42	5.72	102		+35.2					105/60
44	5.57	99.6			4800	30.5	2.0	1.165	75/50
48	6.51	116							100/50
60	7.29	130			4600	8.0	.8		100/50

* Predicted cardiac output 5.59 L/min

† Predicted blood volume 4454 cc

were repeated one to three hours later and a decrease in cardiac output of 14 per cent in the third patient one and three-fourths hours after administration of the hydralazine.

One patient, #1, who received hydralazine within the first nine hours postburn, had no increase and in fact a 10 per cent decrease in cardiac output immediately after administration. With subsequent rapid administration (18 cc/min) of 1350 cc of lactated Ringer's and Plasmanate in 75 minutes, a 22 per cent increase of cardiac output over the prehydralazine level was noted.

That this beneficial effect of hydralazine is due to a decrease in peripheral resistance is suggested by the decrease in arterial systolic pressure with no significant increase in pulse rate noted after administration of hydralazine. In Patient#3, calculated total systemic resistance decreased by 43 per cent and remained at this level or lower for five hours.

In two patients, #2 and #3, arterial lactate and pyruvate were measured at the times of cardiac output determination and excess lactate (XL) calculated using the lowest lactate and pyruvate levels as the "control" levels for the calculations. In Patient #2, the excess lactate four hours posthydralazine was greater than that before hydralazine administration, suggesting a possible "washout" effect secondary to improved peripheral circulation and increased cardiac output. In Patient #3, however, the markedly elevated excess lactate present prior to hydralazine administration showed a slight decrease immediately posthydralazine, with a subsequent slight rise within five hours but to a level lower than that recorded prior to administration of hydralazine.

Summary

Three patients with burns exceeding 40 per cent of the body surface have been given hydralazine in a dosage of 0.5 mg/kg during the early postburn period(within 48 hours postburn). After 23 hours, a prompt increase in cardiac output of 31 to 67 per cent was noted, with persistence of this improvement in two of the three

patients for from one to three hours. There was a simultaneous decrease in systolic blood pressure without any significant increase in pulse rate. In each patient, the improvement in cardiac output occurred in the presence of a decreased blood volume. Prior to this time, it appears that the administration of hydralazine must be accompanied by more rapid fluid administration to obtain improvement in cardiac output.

Excess lactate showed a variable response, increasing in one patient but decreasing in the other patient who showed a markedly elevated excess lactate prior to hydralazine administration.

In the three patients studied thus far, the improvement in cardiac output subsequent to hydralizine administration confirms the value of vasodilators in the treatment of burn shock previously noted in the experimental animal by one of the investigators. This method of treatment offers a means of improving the circulatory dynamics in the burned patient without using massive fluid volumes, a finding of considerable importance in all burned patients but especially in the elderly and the very young.

Publications and/or Presentations

None.

GPO 770884

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6382	CSCRD - 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"D. CHANGE" 04 04 66	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 117

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) Hemodynamics of the Early Postburn Patient (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 62	NA	Other / DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	5	2
		CURRENT FY 66	4	2

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20. PERFORMING ORGANIZATION
NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas
INVESTIGATORS PRINCIPAL ASSOCIATE: Moncrief, J.A., Col, MC
Pruitt, B.A., Jr., Maj, MC
TEL.: 512 - CA1 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Burns, fluid, blood volume, lactate, cardiovascular system

24. (U) Tech Objective - Serial measurements of cardiovascular indices in the early postburn period are being made. The magnitude and mechanism of vascular fluid loss is being quantitated to identify a means of assessing adequacy of resuscitation that may permit modification of fluid therapy.

25. (U) Approach - In appropriate patients admitted within 48 hours postburn, serial measurements of cardiac output, blood volume, arterial pressure, hematocrit, urine output, lactate and pyruvate are performed and extravascular fluid loss rates and excess lactate values calculated. Blood volume and cardiac output determinations are compared with their respective predicted normal levels. Relationship of these indices is examined. Technical difficulty has been encountered in terms of suitable cannulation sites in some burned patients and availability of technical assistance nocturnally. The referral nature of the Unit limits the number of study patients.

26. (U) Progress (1 Jul 65 - 30 Jun 66) - In 4 patients studied as above, an initial decrease in blood volume to approximately 80% of predicted normal occurred within first 24 hrs. postburn. Cardiac output initially paralleled blood volume decrease but later seemed to vary independently. Initially elevated excess lactates decreased only to rise again at the time of a blood volume depression. Extravascular fluid loss paralleled the volume of fluids administered. Loss rate calculations show obligatory losses in the first 24-hr period and suggest resorption during the third 24-hr period with the curve intercepts varying about zero for the intervening 24 hours. The vascular losses appear to represent the summation of a constant plus a fraction of the fluids administered during the immediate postburn period.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC REL./ ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
FY + 1	

DD FORM 1 AUG 66 1498 (Items 1 to 36 identical to NASA Form 1332) REPLACES DD FORM 611 WHICH ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: HEMODYNAMICS OF THE EARLY POSTBURN PATIENT

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

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

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: HEMODYNAMICS OF THE EARLY POSTBURN PATIENT

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: John A. Moncrief, Colonel, MC
Basil A. Pruitt, Jr., Major, MC
Arthur D. Mason, Jr., M.D.

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

In the early postburn period, an initial decrease in blood volume has been documented with the average maximum decrease in four patients to a level of approximately 80 per cent of predicted normal blood volume occurring within the first 24 hours postburn. In two of three patients, the early decrease in blood volume was associated with decreasing cardiac output with subsequent increase in cardiac output whether blood volume was stable or increasing. Maximum depression of cardiac output ranged from a level of 28 to 73 per cent of predicted, with an average of 53.6 per cent.

Neither blood volume nor cardiac output correlated well with urinary output. Urinary output appeared to vary with rate of fluid administration.

Excess lactate, initially, elevated, decreased only to rise secondarily at the time of maximum blood volume depression and bore no constant relationship to cardiac output.

Extravascular fluid loss throughout the study period in each patient paralleled the volume of fluids administered. Loss rate calculations revealed obligatory losses in the first 24-hour period and resorption during

the third 24-hour period, with the intercept of the curves for the intervening 24 hours varying about the origin. A general equation for vascular fluid loss during the first two 24-hour postburn periods has been formulated for these patients as follows: Vl = K + xF, where VL is vascular loss in cc/hr, K a constant varying from - 200 to approximately zero with time, F the volume of fluids given in cc/hr, and x a whole number changing from 0,8 to approximately 1 with time.

(1) Burns
(2) Fluids
(3) Blood Volume
(4) Lactate
(5) Cardiovascular System

HEMODYNAMICS OF THE EARLY POSTBURN PATIENT

The changes in hemodynamic indices consequent to extensive thermal injury and the influence of resuscitative therapy on these disturbances are being documented in burned patients admitted to this unit within the first 48 hours postburn. Blood volume, cardiac output, pulse, arterial pressure, and hematocrit are serially measured during the early postburn period, as well as lactate and pyruvate levels.

Progress

Four acutely burned patients have been studied in the past year utilizing a Cardio-green dye dilution technique with a Sanborn Model 130 cardiac output computer for cardiac output determinations. RISA blood volume measurements have been made with the Volemetron, and standard techniques employed for lactate, pyruvate, blood gas, serum chemistry, and hematocrit determinations.

Predicted blood volume was calculated for each patient on the basis of 7 per cent of the body weight, and predicted cardiac output calculated according to the formula: $\text{C.O. (L/min)} = \frac{26.5 \times \text{weight (in grams)}^{.854}}{60{,}000}$

Vascular loss was calculated as the amount of fluids given plus the decrease in blood volume since the previous measurement minus the blood drawn during the same time period.

Results

Three of the four study patients were admitted and measurements made within six hours postburn. In these patients, the lowest recorded blood volume occurred between 12 and 21 hours, and in general was coincident with an elevated hematocrit. In the other patient, first measured at 19 hours postburn, the most depressed blood volume occurred at 37 hours postburn at a time when the hematocrit was showing a progressive hemodilution effect. The

average lowest blood volume in all four patients was only 79.1 per cent of their predicted blood volumes.

Two of the three patients in whom cardiac output was measured showed a decrease in cardiac output with decreasing blood volume between 4 and 24 and 19 and 23 hours postburn respectively. One of these patients, Patient #3, showed a subsequent increase in cardiac output over the ensuing 36 hours with an essentially stable blood volume varying ± 300 cc. The other patient, #4, showed a similar increase in cardiac output over the ensuing 37 hours, with, in this case, an overall increase in blood volume of approximately 1000 cc. The other patient in whom the initial cardiac output at four hours postburn was 28 per cent of that predicted showed a subsequent increase in cardiac output with a decreasing blood volume from 4 to 12 hours postburn, with a further upward trend and maintenance of cardiac output thereafter with a blood volume which remained relatively stable at 67 to 70 per cent of the predicted blood volume. The lowest recorded cardiac outputs occurred at 4, 23 and 24 hours postburn and were 28, 60 and 72.8 per cent of the predicted cardiac output respectively, averaging 53.6 per cent.

Patients #3 and #4 showed no apparent relation between either blood volume and arterial pressure or cardiac output and arterial pressure. These same two patients both had elevated excess lactate levels on admission which subsequently decreased only to show a secondary rise at the time of greatest depression of the blood volume. There was no apparent relation between cardiac output and excess lactate in either of these patients.

There appears to be no direct relationship between either the cardiac output or blood volume and the urinary output. Such a relationship seems to exist between the rate of fluid administration and urinary output, as could be anticipated from clinical experience.

Calculation of extravascular losses confirms our previous impression that early in the postburn course, the extravascular loss parallels the volume of fluids administered. Loss rate calculation, however, suggests that

in the first 18 to 24 hours postburn, the loss is the summation of a constant obligatory volume and a fraction, approximately 0.8, of the fluids administered. During the second 24-hour period, the loss rate approximates the amount of fluid given per unit time with the intercepts of the loss rate curves varying around the origin. After 48 hours, resorption occurs, as noted by a negative Y intercept and the loss equals a fraction of the fluids given minus the volume resorbed.

A general equation for vascular fluid loss during the first two 24-hour postburn periods has been formulated for these patients as follows: $Vl = K + xF$, where VL is vascular loss in cc/hr, K a constant varying from +200 to approximately zero with time, F the volume of fluids given in cc/hr, and x a whole number changing from 0.8 to approximately 1 with time.

Preliminary examination of the extravascular loss rates expressed in cc/hr/kg of body weight suggests that the increased transcapillary movement of fluids into the extravascular space occurs in unburned as well as burned tissue, with similar variation of loss rate by time period postburn as noted above.

Summary (Tables 1-4)

Four patients, each with burns of greater than 40 per cent of the body surface, have been admitted to this unit within the first 24 hours postburn and serial measurements of cardiac output, blood volume, arterial pressure, lactate, pyruvate and urinary output performed. Excess lactate and extravascular loss rates have been calculated. In three of four patients, the lowest blood volume occurred at or before 24 hours postburn, and average maximum blood volume depression in all four was 79.1 per cent of the predicted blood volume.

Two patients showed a decrease in cardiac output with with an early decrease in blood volume, with subsequent increases in cardiac output whether the blood volume was maintained within relatively narrow limits or increased. The depression in cardiac output had a range of from 28 to

Table 1. Early Postburn Circulatory Indices

Pt. #1 % Burn 53½ Weight 87.72 kg Predicted Blood Volume 6140 Predicted Cardiac Output 7.35 L/min Age 48

Hrs. PB	Observed B.V. (cc)	% of Predicted	Hct	Observed C.O. (L/min)	% of Predicted	B.V.Δ (cc)	Fluids Received Total cc	Fluids Received cc/hr	Blood Drawn	Vascular Loss Total cc	Vascular Loss cc/hr	Urine (cc/hr)
4	5800	94.4	42	2.11	28							
6	4525	73.6		3.46	47	-1275	950	475	75	2150	1075	183
8½				4.41	60							
9	4300	70.0		3.98	54	-225	3450	1150	75	3600	1200	30
9½				5.41	73							
12	4100	66.7		5.14	69	-200	1100	367	100	1200	400	37
23	4300	70.0	47	4.95	67	+200	4200	382	100	3900	355	23
23¼				6.79	92							
25				5.74	78							
29	4200	68.4		7.45	101	-100	5035	839	100	5035	839	62
50	5075	82.6	39			+875	6920	330	100	5945	283	89
100	5360	87.2	33			+285						

Table 2. Early Postburn Circulatory Indices

Pt. #2 % Burn 49 Weight 53.63 kg Predicted Blood Volume 3754 Age 18

Hrs. PB	Observed B.V. (cc)	% of Predicted	Hct	B.V. Δ (cc)	Fluids Received Total cc	Fluids Received cc/hr	Blood Drawn	Vascular Loss Total cc	Vascular Loss cc/hr	Urine (cc/Hr)
6	4300	115	55							
12	4100	109	67	-200	1900	317	70	2030	338	66
17	3900	104	52	-200	2700	540	70	2830	566	28
21	3500	93	54	-400	300	75	70	630	158	199
33	3800	101	49	+300	3200	267	70	2830	236	78
57	4450	119	39	+650	5400 7125*	225 297*	70	4380 6405*	195 267*	117
81	5650	151	35	+1200						
129	4050	108	38	-1600						

* Includes both intravenous and oral fluids

Table 3. Early Postburn Circulatory Indices

Pt. #3 % Burn 43 Weight 86.81 kg Predicted Blood Volume 6077 Predicted Cardiac Output 7.28 L/min Age 54

Hrs. PB	Observed B.V. (cc)	% of Predicted	Hct	Observed C.O. (L/min)	% of Predicted	B.V. Δ (cc)	Fluids Received Total cc	Fluids Received cc/hr	Blood Drawn	Vascular Loss Total cc	Vascular Loss cc/hr	Arterial Pressure	Lactate (mg%)	Pyruvate (mg%)	Ex.Lact. (mg%/L)	Urine (cc/hr)
4	6400	105	49	7.42	102							100/50	26.3	1.5	.632	
12	5850	96	43	6.82	93.6	-550	4700	588	150	5100	638	125/65	16.3	1.0	.326	50
15	5500	91	44	6.0	82.4	-350	1500	500	100	1750	583	165/75				48
18	5150	85	54	5.57	76.5	-350	1750	583	100	2000	667	130/75	20.0	1.2	.398	41
24	5300	87	55	5.3	72.8	+150	1500	250	100	1250	208	130/75	16.2	1.1	.128	23
27	5600	92	49	7.82	107	+300	1800	600	100	1400	467		12.1	9		31
36	5400	89	49	5.68	78	-200	4150	461	100	4250	472	165/65				49
38	5300	87		9.5	130							120/60				
39	5400	89	45	9.8	135	0	800	267	100	700	233	100/65				25
42	5275	87	46	8.28	114	-125	850	283	100	875	292	130/75	14.8	1.0	.166	42
48	5600	92	41	9.6	132	+325	2150	358	100	1725	288	180/70				51
60	5600	92	39	11.95	164	0	2350	196	100	2250	188	170/80	16.0	1.0	.296	53
87	7300	120	31			+1700	2900 6560*	107 243*	100	1100 4760*	41 176*					84

* Includes both intravenous and oral fluids

Table 4. Early Postburn Circulatory Indices

Pt. % Burn 71.5 Weight 63.63 kg Predicted Blood Volume 4454 Predicted Cardiac Output 5.59 L/min Age 55

Hrs. PB	Observed B.V. (cc)	% of Predicted	Hct	Observed C.O. (L/min)	% of Predicted	B.V.Δ (cc)	Fluids Received Total cc	Fluids Received cc/hr	Blood Drawn	Vascular Loss Total cc	Vascular Loss cc/hr	Arterial Pressure	Lactate (mg%)	Pyruvate (mg%)	Ex.Lact. (mMol/L)	Urine (cc/hr)
19	3900	87.5	53	5.02	90							115/65	46.0	3.4	1.332	
23	3850	86.4	50	3.36	60	-50	1100	275	100	1050	263	75/40	45.0	3.5	1.112	
28	3600	80.8	49	3.5	63	-250	1900	380	100	2050	410	95/40	32.0	2.2	1.120	
37	3200	71.8	45	4.23	76	-400	2550	283	100	2850	317	115/75	26.0	1.5	1.281	61
39	3500	78.5	44	5.53	98.9	+300	350	175	100	-50	-25	90/50				32
42				5.72	102							105/60	43.0	3.3	1.120	
44	4800	107.7	46	5.57	99.6	+1300	1000	200	130	-430	-86	75/50	30.5	2.0	1.165	51
48				6.51	116							100/50				
60	4800	103.2	46	7.29	130	+200	3150	197	200	3150	197	100/50	8.0	0.8		63
66	4250	95.4	43			-350	1000	167	100	1250	208					52

73 per cent of predicted, with an average of 53.6 per cent.

Neither blood volume nor cardiac output appears directly related to either arterial pressure or urinary output. Urinary output does appear to vary with the rate of fluid administration.

In two patients in whom excess lactate was calculated, it bore no apparent relationship to cardiac output. Both patients had elevated excess lactate on admission which diminished prior to a secondary rise at the time of greatest blood volume depression.

Throughout the study periods, extravascular loss paralleled the volume of fluid administration. Extravascular loss rates plotted against rate of fluid administration revealed an early obligatory loss during approximately the first 24 hours postburn which approaches zero during the second 24-hour postburn period and becomes negative (reabsorption) during that time period. This is felt to indicate a shift of equilibrium and movement of fluid across the capillary wall. Examination of vascular loss rates in cc/hr/kg of body weight suggests that the transcapillary fluid movement occurs in both burned and unburned tissue. During the first 24 hours postburn, it appears that an average of 80 per cent of administered fluids escapes from the vascular compartment, while during the second 24-hour postburn period the loss of administered fluids approximates 100 per cent.

Publications and/or Presentations

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 0A6955	CSCRD - 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / SRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	D, CHANGE 270865	U / U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 01 140	NONE

11. TITLE:
(U) Renal Function in Burned Patients (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	01 65	NA	Other , DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 65	1	4
		CURRENT FY 66	1	5

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME Headquarters ADDRESS US Army Med Res & Dev Command Wash DC 20315 RESP. INDIV. Kovaric, J.J., Lt Col TEL 8 22 63737	NAME US Army Surgical Research Unit ADDRESS Fort Sam Houston, Texas INVESTIGATORS PRINCIPAL ASSOCIATE O'Neill, J.A., Jr, Capt, MC Pruitt, B.A., Jr., Maj, MC TEL. 512 - CAI 2720 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Burns, renal function, renal blood flow

24 (U) Tech Objective - Since urinary output is utilized to judge the adequacy of fluid resuscitation in burn therapy, it seemed warranted that direct studies of renal function should be performed. If renal function is impaired, criteria for the adequacy of resuscitation should be altered and perhaps changes should be made in the method of fluid administration.

25 (U) Approach - Adult patients with severe burns are being studied in the early post-burn period, preferably within 48 hours, since this is the period when fluid requirements are greatest. Determinations include inulin, PAH, urea, endogenous creatinine, osmolar and free water clearance. Serum and urine electrolytes are studied as well. The results of early studies are compared with repeat studies made at five days. There is still some shortage of acute burns, so progress has been somewhat delayed.

26 (U) Progress (Jul 65-Jun66) - Seven patients have been studied thus far. The most striking finding thus far has been a decrease in renal plasma flow in patients with burns over 25 per cent. These findings correlate with decreases in cardiac output and increases in peripheral resistance noted in other studies of acutely burned patients. If further studies are confirmatory, this will be evidence in favor of further investigation of the use of vasodilator agents during the early postburn period

27. COMMUNICATIONS SECURITY	28.	29. DOD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) FY + 1	36.

DD Form 1 AUG 66 1498 (Items 1 to 36 identical to NAVY Form 1121) REPLACES DD FORM 613 & 6150 WHICH ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: STUDIES OF RENAL FUNCTION IN BURNED PATIENTS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

James A. O'Neill, Jr., Captain, MC
Basil A. Pruitt, Jr., Major, MC
Neil Lempert, Captain, MC
Timothy A. Miller, Captain, MC
Robert M. Vertel, Captain, MC

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: STUDIES OF RENAL FUNCTION IN BURNED PATIENTS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: James A. O'Neill, Jr., Captain, MC
Basil A. Pruitt, Jr., Major, MC
Neil Lempert, Captain, MC
Timothy A. Miller, Captain, MC
Robert M. Vertel, Captain, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Studies of renal function in seven burned patients during the early postburn period have been performed. The most striking finding was a depression of renal plasma flow without proportional decrements in glomerular filtration rates. An antidiuresis was also noted.

The decreases in renal flow are most likely accounted for on the basis of increased peripheral resistance and decreased cardiac output.

Further studies combined with determinations of cardiac output are warranted and are in progress.

(1) Burns
(2) Renal function
(3) Renal blood flow

STUDIES OF RENAL FUNCTION IN BURNED PATIENTS

All who treat severely burned patients utilize urinary output as the main criterion for the adequacy of fluid and colloid replacement. Despite this fact, very little information has been obtained in the form of direct measurements of renal function. In the absence of such data, there is reason to doubt that urinary output is a valid reflection of adequate fluid replacement. It is conceivable that patients with marginal circulatory status might put out what would be considered acceptable volumes of urine.

Studies by Graber and Sevitt indicate that the glomerulus is the principle site of renal pathological changes in that they noted deposition of lipoid material in the endothelium of the glomerular tuft.[4] On the other hand, others have found no alteration of glomerular filtration in small groups of patients studied from 5 to 10 days postburn.[5,6] Taking the above into consideration, it seemed warranted that direct measurements of renal function should be made in the early postburn period when fluid resuscitation is a prime concern.

Methods

Acutely burned adult patients were studied during the early postburn period while fluid resuscitation was in progress. All patients were given fluids either according to the Brooke formula or in greater amounts. These measurements were compared with similar ones made on the fifth postburn day.

The determinations included inulin and PAH clearance studies as indications of glomerular filtration and renal plasma flow respectively. In addition, serum and urine studies were performed in order to determine clearances of urea, endogenous creatinine, osmotically active constituents, free water, and electrolytes. This included determinations of urea, creatinine, osmolality, sodium, potassium and chloride in both serum and urine.

Results (Tables 1,2, Figs. 1-7)

Table 1. Clinical Data

Patient	Per Cent Total Burn	Per Cent Burn Index	Interval Postburn Studied	Hematocrit
1	28	15	48 hours	45
2	34	20.5	48 hours	46
3	78	65	48 hours 5 days	48 37
4	65	47.5	48 hours	38
5	65	47	8 days	40
6	45.5	24	36 hours	45
7	53.5	33.5	5 days	31

Table 2. Representative Serum and Urine Electrolytes

Patient	Time Postburn Studied	Serum				Urine			
		Na	K	Cl	SUN	Na	K	Cl	UUN
1	48 hours	132	4.5	110	11.3	151	94	45	697
2	48 hours	137	6.0	111	16.3	68	219	75	939
3	48 hours	134	4.1	111	27.8	40	128	23	476
	5 days	128	5.1	108	49.5	3	87	51	1215
4	48 hours	140	5.9	114	43.0	19	74	97	271
5	8 days	158	3.1	119	52.0	27	53	30	979
6	36 hours	140	4.7	---	22.7	28	100	144	1286
7	5 days	147	4.0	126	30.0	8	87	80	325

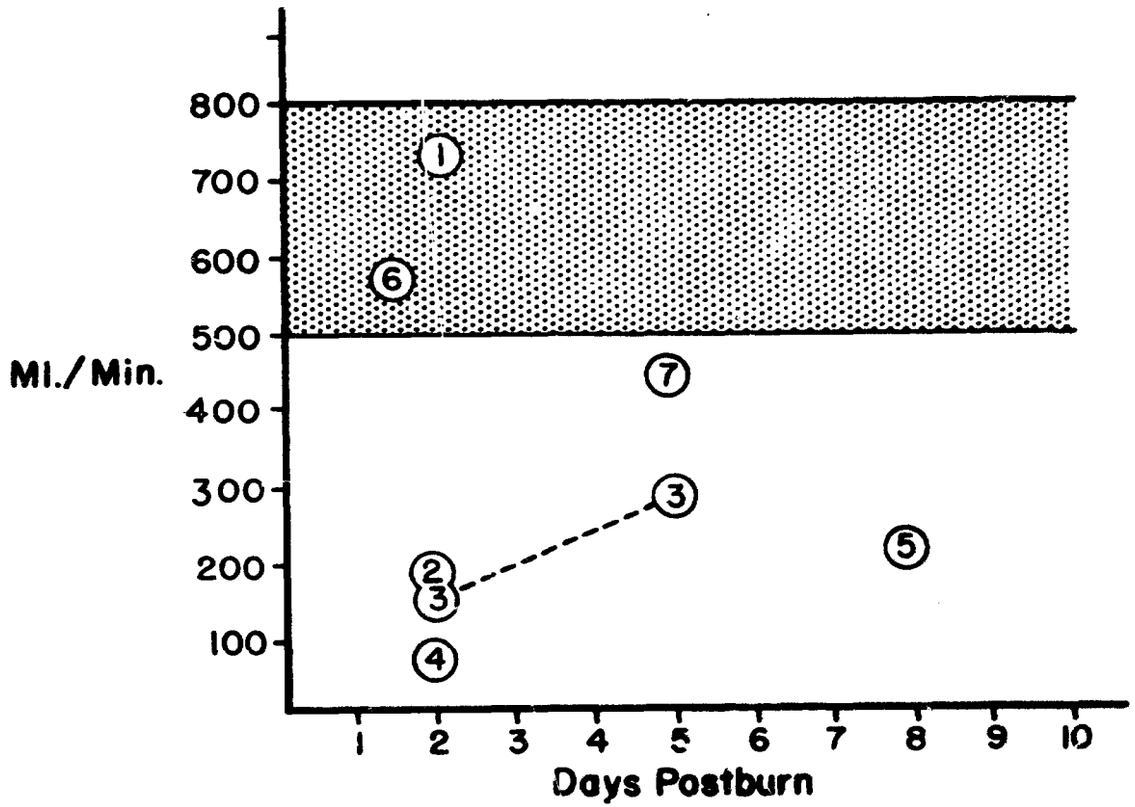

Fig. 1 RENAL PLASMA FLOW

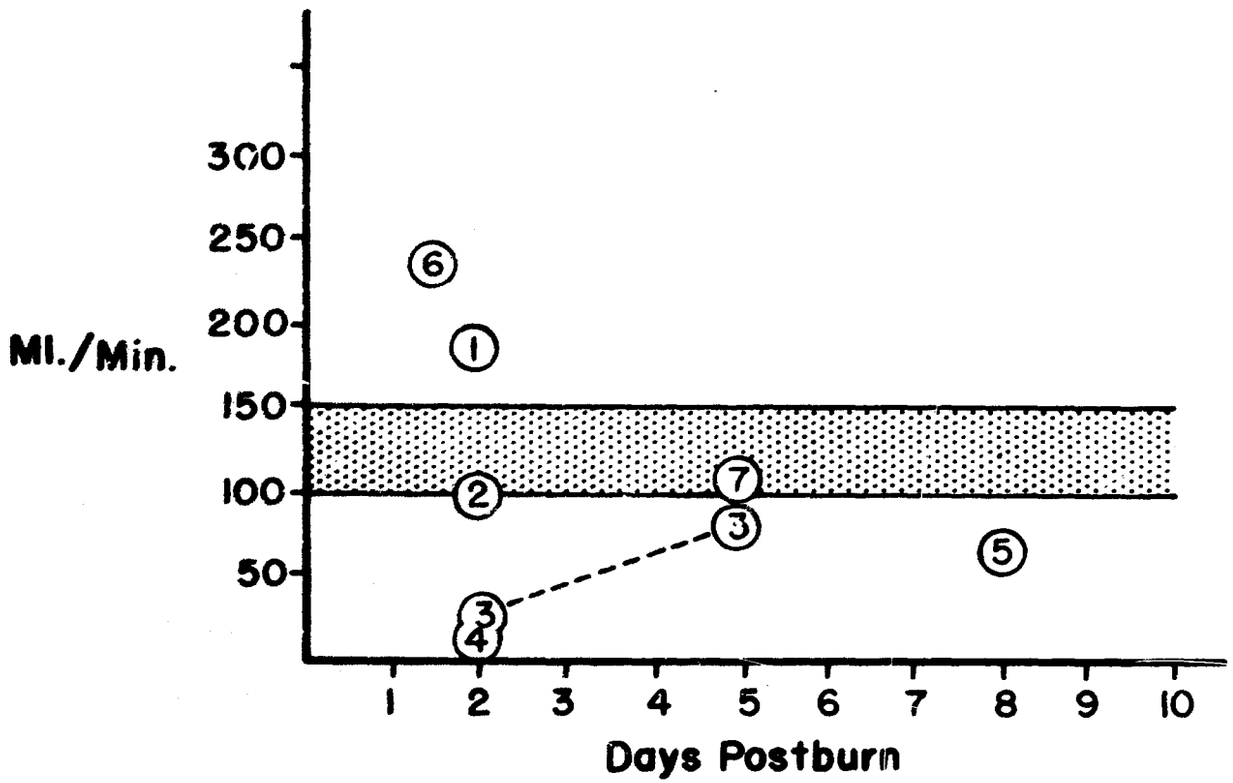

Fig. 2 GLOMERULAR FILTRATION RATE

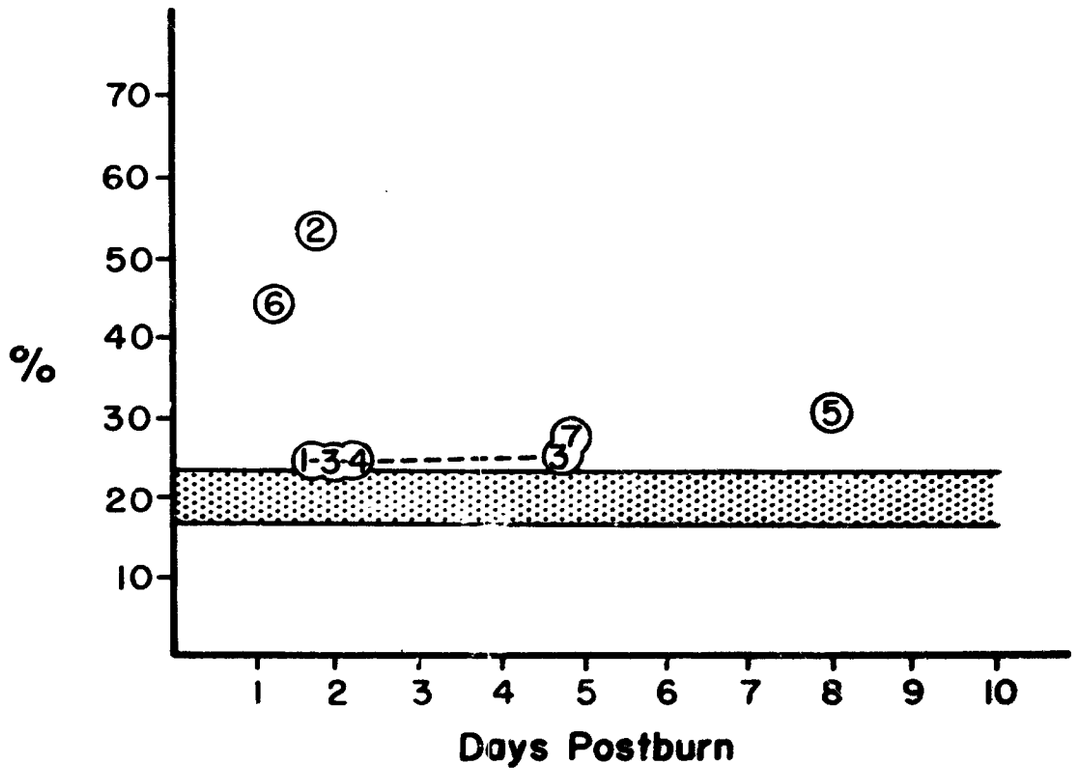

Fig. 3 FILTRATION FRACTION $\left(\frac{GFR}{RPI} \times 100\right)$

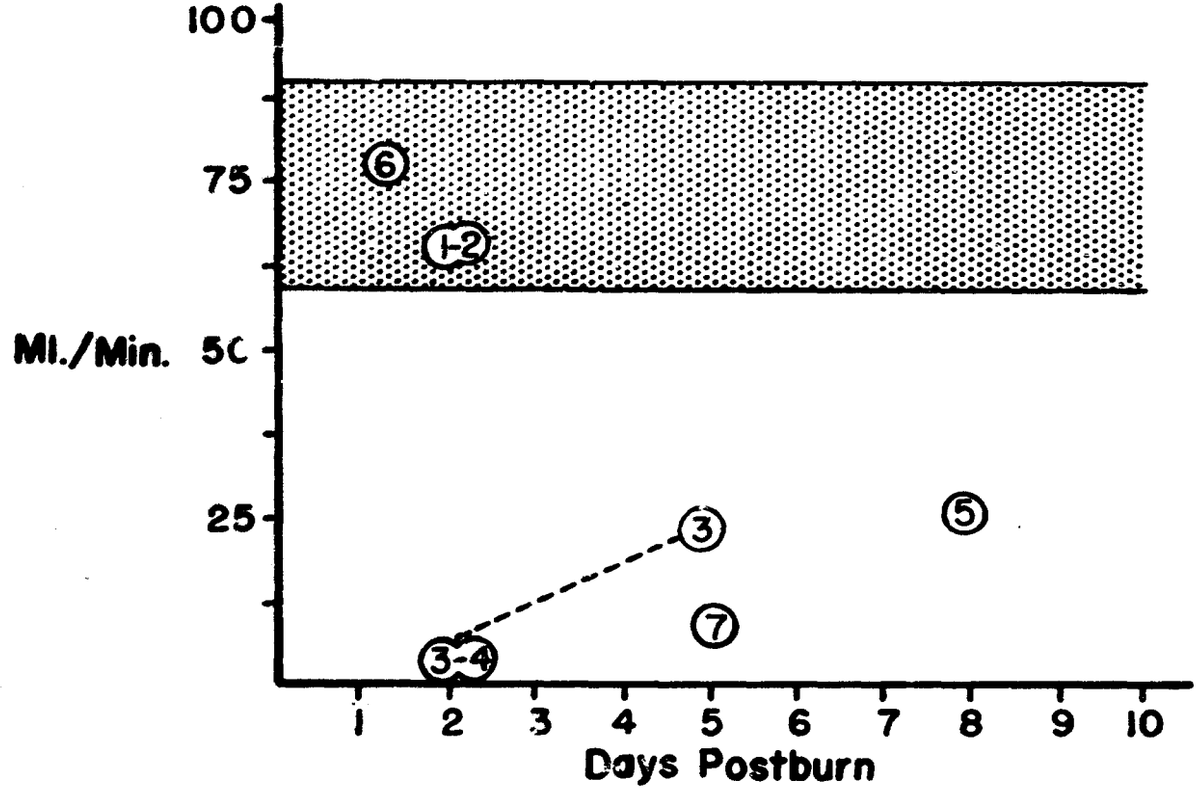

Fig. 4 UREA CLEARANCE

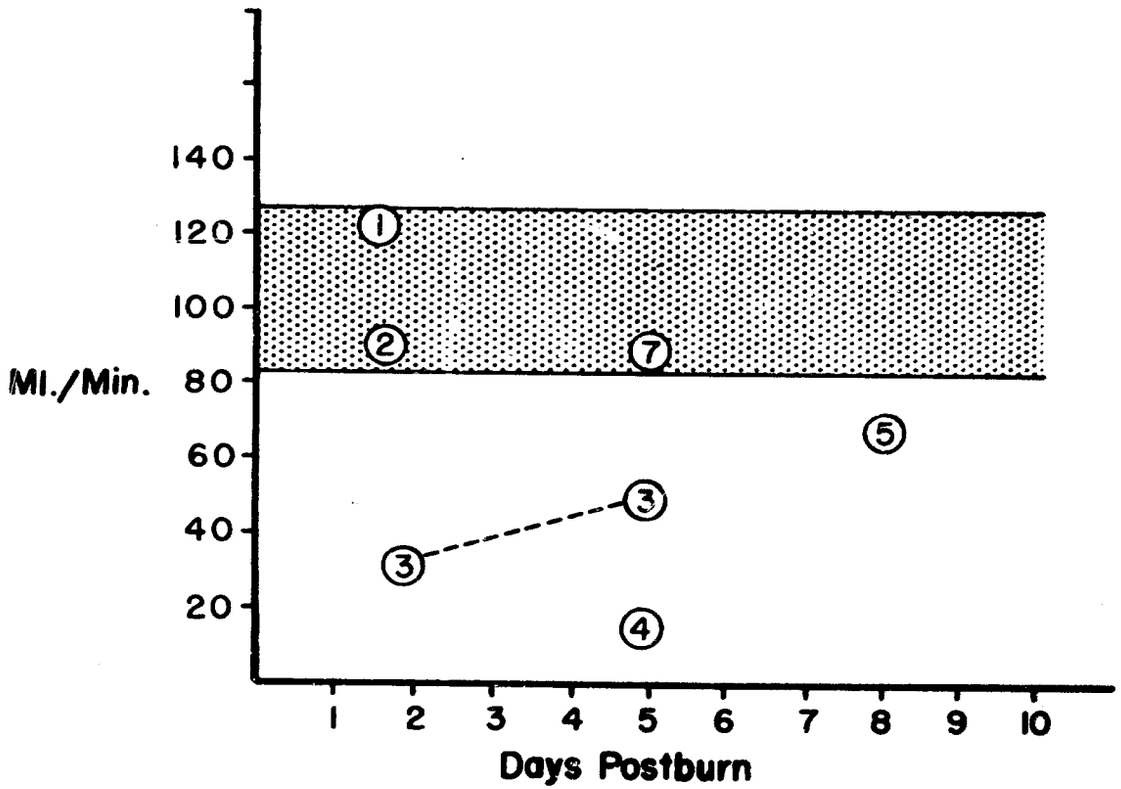

Fig. 5 ENDOGENOUS CREATININE CLEARANCE

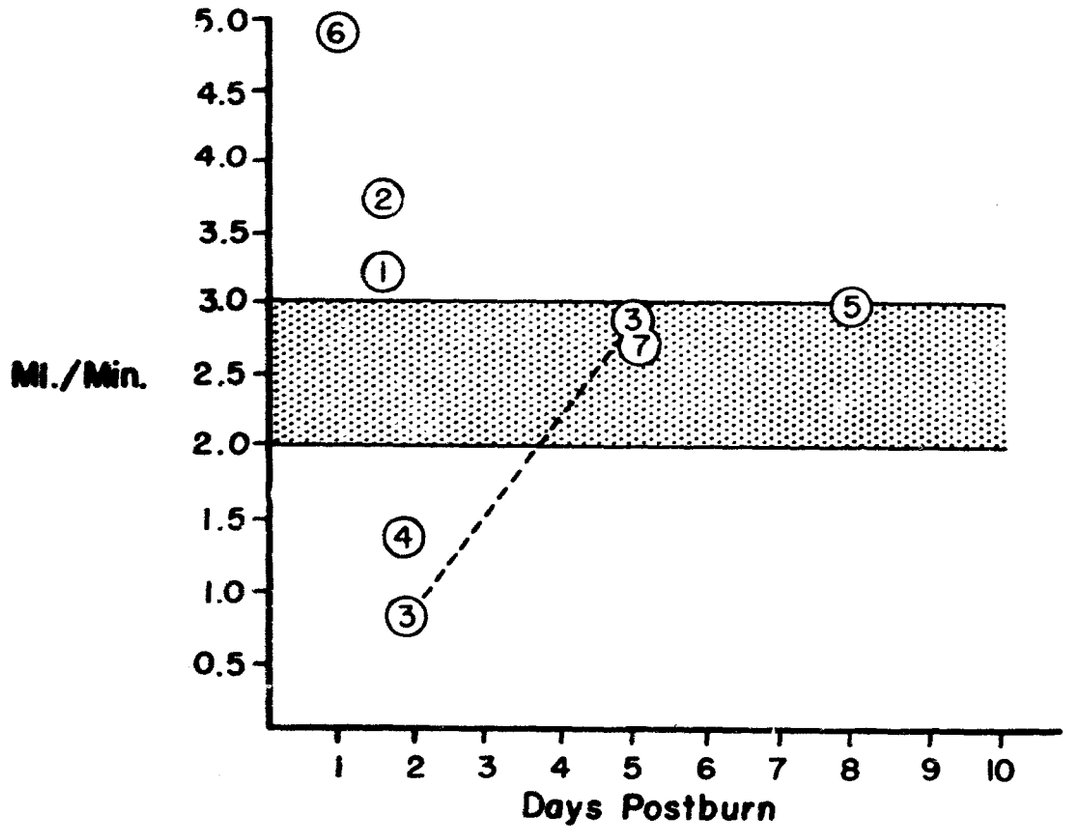

Fig. 6 OSMOLAR CLEARANCE

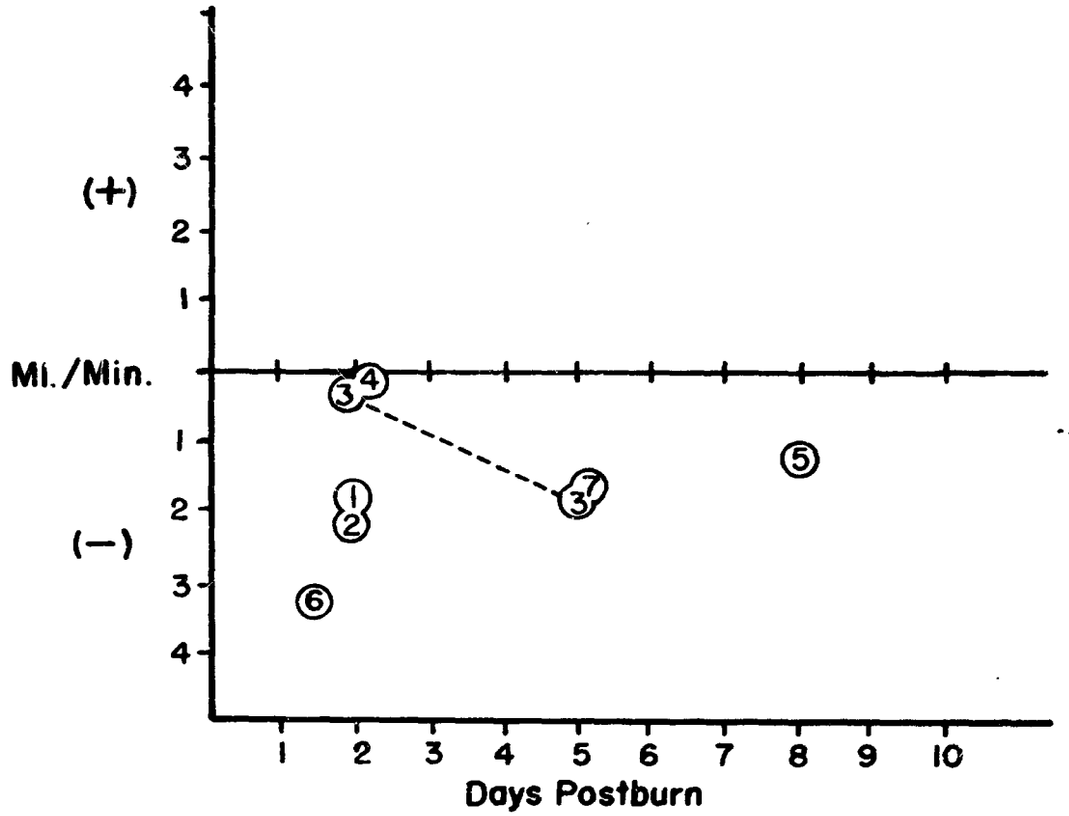

Fig. 7 FREE WATER CLEARANCE

Data are available on seven patients whose ages ranged from 20 to 40 years. All were males. Total burn ranged from 28 to 78 per cent, and burn index (third-degree burn plus one-half second-degree burn) ranged from 15 to 65 with an average of 36. One patient was studied at 36 hours postinjury, three patients at 48 hours, one at 48 hours and again at five days, one at five days, and one at eight days postburn. Only Patient #7 had evidence of anemia. He had a renal plasma flow of 430 ml/min, which is only slightly below the lower limits of normal.

Renal plasma flow (Fig. 1) was within the range of normal at 48 hours postburn in a young pilot with a burn index of 15 and another patient with an index of 24. The other five patients whose burn indices ranged from 20.5 to 65 had subnormal renal plasma flow as measured by clearance of PAH.

Glomerula filtration rates (Fig. 2) as measured by inulin clearance were below normal in three patients whose burn indices were 65, 47.5 and 47. One of the three approached normal glomerular filtration by the fifth day. Four patients with indices between 15 and 33.5 had normal glomerular filtration rates.

The filtration fraction (Fig. 3) was above normal in every instance.

Urea clearance (Fig. 4) was normal in three patients whose burn indices ranged from 15 to 24. Clearance of urea was below normal in the other four patients who had larger burns.

Endogenous creatinine clearance (Fig. 5) was normal in three patients with burn indices of 15, 20.5 and 33.5. It was subnormal in the other four patients.

Osmolar clearance (Fig. 6) was subnormal in one patient with a burn index of 47.5. It was depressed at 48 hours in the patient with a 65 index but rose to normal by five days. It was normal in the other five patients.

Clearance of osmotically free water (Fig. 7) was in the negative range in every instance.

The values obtained for serum and urine electrolytes are depicted in Table 2. Low urine sodium was the rule during the early postburn period unless large volumes of sodium-containing fluids were administered during the test period.

Comments

The most striking finding in this group of patients studied during the early postburn period was a marked decrease in renal plasma flow in the patients with burn indices over 24. This finding corresponds with the marked decreases in cardiac output and increases in peripheral resistance noted in patients and experimental animals with large burns.[7] It was also noted that severely burned patients might have normal blood pressure even in the face of markedly depressed cardiac output. In the same fashion, patients with clinically acceptable levels of urinary output may have markedly depressed renal blood flow. Decreased renal blood flow may be related to efferent glomerular constriction on the basis of a generalized increase in peripheral resistance and also to the decrease in cardiac output itself. Hence, it must be concluded that urinary output is not a uniformly reliable criterion for judging the adequacy of fluid resuscitation.

It is known that burned patients have increased secretion of adrenal steroids during the early postburn period, and these tend to increase both renal plasma flow and glomerular filtration.[2] None of the patients were hypotensive during the studies, a factor which leads to decreased renal flow. Also, none had body temperatures far from normal, so this was not a factor.[3] Although serum proteins were not routinely measured, it is probable that all patients had some decrease in total body proteins, a factor which leads to increased glomerular filtration but does not necessarily affect renal plasma flow.[8] Only one of the seven patients was anemic. His renal plasma flow was measured five days postburn, and the slightly depressed level might be accounted for on this basis.[1] The other four with subnormal renal plasma flow had hematocrits over 40%

Glomerular filtration rates were depressed in only the three patients with burn indices of 50 or more. Otherwise the patients had normal filtration rates even though renal plasma flow was below par.

The filtration fraction or inulin/PAH clearance ratio gives the fraction of plasma filtered at the glomerulus. All seven patients had normal or elevated percentages. This indicates that increased glomerular filtration, decreased renal plasma flow, or both, were operative in each instance.

Urea clearances tended to parallel the glomerular filtration rates except in patient #7, where the urea clearance was subnormal even though glomerular filtration was not. However, this may be due to a urine volume of less than 1.5 ml/min.

Endogenous creatinine clearance values were identical with glomerular filtration rates and indicate essentially the same thing.

The combined data of osmolar and free water clearances indicated that during the early postburn period an antidiuresis occurred. In virtually every instance, the urine carried a deficit of solute-free water, so that water balance was being maintained by the excretion of an osmotically concentrated urine. Whether this is due to the action of ADH alone on the basis of dehydration or possibly on the basis of release of histamine or other vasoactive substances is open to speculation. It is possible that other mechanisms may be involved as well.

Summary

Studies of renal function in seven burned patients during the early postburn period have been performed. The most striking finding was a depression of renal plasma flow without proportional decrements in glomerular filtration rates. An antidiuresis was also noted.

The decreases in renal flow are most likely accounted for on the basis of increased peripheral resistance and decreased cardiac output

Further studies combined with determinations of cardiac output are warranted and are in progress.

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Publications and/or Presentations

None.

GPO 770884

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6950	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501871R 01 191

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (U) Pathogenesis of Burn Wound Infection: Bacterial Flora of Burn Wounds with Sulfamylon Treatment (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. NUMBER	b. TYPE	c. DATE	d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House		NA			PRIOR FY 65	0	0
					CURRENT FY 66	2	15

19. GOVT LAB/INSTALLATION/ACTIVITY

NAME: Headquarters
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Washington, DC 20315

RESP. INDIV.: Kovaric, J.J., Lt Col
TEL.: 8 22 63737

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ADDRESS: Fort Sam Houston, Texas

INVESTIGATOR/ PRINCIPAL ASSOCIATE: Lindberg, R.B., Col, MSC
Contreras, A.A., MS

TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS: Burns, Infections

24. (U) Tech Objective - The control of invasive infection in severe burns has been accomplished by use of topical sulfamylon burn cream, but the mechanisms by which this effect is achieved are not well understood. It is assumed that quantitative and qualitative alteration of burn wound flora occurs with the treatment. The object of this study is to establish the relationship between the flora of the topically treated wound and healing and survival in severe burns.

25. (U) Approach - Serial quantitative and qualitative cultures of burn wounds will be taken at intervals, and examined for predominant and total flora. Comparison with pre-topical therapy results will be made. Isolates will be observed for susceptibility to sulfamylon, homogeneity of strain identities and virulence of selected strains.

26. (U) Progress (Jul 65-Jun 66) - Bacteremia appeared in 57% of patients admitted prior to start of sulfamylon, and in 9.7% of admissions in 1965, when topical treatment was used entirely. Mortality rate with bacteremia remained high, despite topical therapy. Pseudomonas and aerobacter predominated in blood cultures of sulfamylon-treated patients with bacteremia. Proteus spp. became more common. Burn wound flora showed a relative drop of 33% in Pseudomonas incidence, and a relative increase in Proteus and E. coli.

27. COMMUNICATIONS SECURITY	28.	29. OMD CODE	30. SUBJECT CODE
a. CRYPTO OR CRYPTO RELATED [X] b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. DEV. FUNDS (In thousands)	36.
CY 19	

DD FORM 1498

(Items 1 to 36 required in RDT&E Form 11-R1; see reverse side for items 37 to 40 and instructions.)

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: PATHOGENESIS OF BURN WOUND INFECTION: THE BACTERIAL FLORA OF BURN WOUNDS WITH TOPICAL SULFAMYLON TREATMENT

U.S. ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert B. Lindberg, Colonel, MSC
Anthony A. Contreras, M.S.
John A. Moncrief, Colonel, MC
Richard E. Jones, Sp5
Jose E. Carrillo, Pvt
David H. Andrews, M/Sgt

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: PATHOGENESIS OF BURN WOUND INFECTION: THE BACTERIAL FLORA OF BURN WOUNDS WITH TOPICAL SULFAMYLON TREATMENT

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert B. Lindberg, Colonel, MSC
Anthony A. Contreras, M.S.
John A. Moncrief, Colonel, MC
Richard E. Jones, Sp5
Jose E. Carrillo, Pvt
David H. Andrews, M/Sgt

6. Reports Control Symbol: RCS-MEDDH-288(R1)

A further comparison of wound and blood cultures, to determine the effect of Sulfamylon* on the wound flora, was carried out based on clinical culture specimens submitted from Ward 14A. Bacteremia continued to be relatively infrequent, in contrast to experience prior to topical therapy, and was associated with massive burns. Multiple blood stream infections appeared in one-half of the septicemias. Burn wound flora displayed a predominance of Pseudomonas, Aerobacter, and staphylococci.

(1) Burns (2) Infections

---

* Sulfamylon furnished by Sterling-Winthrop Research Institute, Rensselaer, New York.

PATHOGENESIS OF BURN WOUND INFECTION: THE BACTERIAL FLORA OF BURN WOUNDS WITH TOPICAL SULFAMYLON TREATMENT

The nature of the infectious process in the burned patient is reflected in the clinical bacteriologic culture data which is collected during any period of observation. Infection remains a major facet of burn wound management, even though effective control of burn wound sepsis has been achieved with topical sulfamylon.[1]* The demand for wound and blood cultures has, since the introduction of effective topical sulfamylon therapy, remained at one-half the level at which such requests occurred prior to the start of use of sulfamylon on burned patients. The reduction in the intensity of burn infection, and the virtual elimination of burn wound sepsis, have markedly altered the nature of the bacteriologic aspects of burn treatment.[2]

Bacteremia, since it is an obvious indicator of systemic invasion of the burned patient, is basically an indicator of the effectiveness of burn treatment. In contrast to surface cultures of burns, which always yield bacteria, blood cultures offer an index of burn wound invasion. The incidence of positive cultures is, of course, mediated markedly by the clinical signs and impressions; samples are usually collected only when there is a clinical indication for the procedure.

Table 1 shows the contrast between 1963, the last year prior to start of topical therapy, and 1965 when the therapeutic procedure was firmly established. It was evident that a great difference in incidence of septicemia appeared. In 1965, 174 patients, a new record, were admitted in contrast to 148 in 1963. Nevertheless, in 1963, 354 blood cultures were taken, as contrasted to 288 in 1965. Eighty-four patients, or 57% of admissions, had positive blood cultures in 1963. In 1965, 17, or 9.7% of the number admitted, developed positive blood cultures.

Prior to use of topical therapy, the greater part of the positive blood cultures showed but one strain of organism.

---

* Sulfamylon furnished by Sterling-Winthrop Research Institute, Rensselaer, New York.

Table 1. Patients with Positive Blood Cultures
1963 and 1965

Species	No. Patients with Positive Cultures 1963: 84	1965: 17
Pseudomonas aeruginosa	29	8
Staph. aureus, coag. pos.	19	3
Aerobacter-Klebsiella gp.	17	7
Escherichia coli	5	3
Mima group	5	1
Proteus sp.	6	5
Providencia gp.	1	3
beta-hemolytic strep.	4	0
alpha-hemolytic strep.	6	0
Bacillus	0	1
Staph. albins, coag. neg.	0	4
Serratia marcessens	0	1
Corynebacterium pseudo-diphtheriticum	0	1
Hafnia gp.	0	1
No. of patients with separate species	92	38
Total strains isolated	136	61
Total blood cultures taken	354	288
No. patients admitted	148	174

Thus in 1963, 84 patients yielded 92 strains in blood cultures. In 1965, 60 strains were recovered from 17 patients. Since the diagnosis of burn wound sepsis was virtually absent in 1965, the patients with positive blood cultures obviously were not so diagnosed, even though most of the positive cultures came from patients with massive burns. This is reflected in the fact that 14 of the 17 patients with positive blood cultures in 1965 died. These patients had burns with an average area of 67.4% of body surface. The three patients who survived episodes of bacteremia had somewhat smaller burns; their average of total body surface burned was 51%. During the final 48 hours of life in a patient with massive burns, episodes of bacteremia are not uncommon. Mixed blood stream infections were observed in 8 of the 17 patients with blood cultures positive in 1965. Seven of these yielded two strains present in a single culture at least once. One patient had three strains present, a Pseudomonas, Aerobacter, and an E. coli, in a single blood culture.

A reduction in incidence of Ps. aeruginosa in blood cultures would be expected when effective topical therapy was instituted, since it was the principal causative agent in burn wound sepsis. Staph. aureus, coagulase-positive, was unexpectedly infrequent; it occurred in only three patients. This was surprising in view of the fact that it is virtually ubiquitous on burn wounds. Four patients had coagulase-negative, non-pigmented strains in the blood. Such strains had been extremely rare in positive blood cultures in past years. Aerobacter-Klebsiella strains and Proteus spp.(both mirabilis and morganii strains) were relatively more common than they had been in the pre-sulfamylon period. Providencia strains were possibly more common than they were in 1963. In view of the small number of positive blood cultures, and the fact that the 1965 burns were either massive (so that survival was highly conjectural), or else complicated by other conditions not related to burn wound sepsis, it is possible to anticipate that with knowledgeable use of topical sulfamylon therapy, the incidence of septicemia,as a problem in burn treatment, will be held to a small fraction of the burns so treated.

Since the organisms have an immediate relation to a lethal threat, the biological behavior of blood stream isolates occasion greater interest than that of surface

wound flora. The antibiotic sensitivity of 27 strains of 9 genera of organisms recovered in blood cultures are summarized in Table 2. The relative resistance of Pseudomonas, Proteus and Aerobacter strains is notable. Colymycin was the drug of choice with these strains. With staphylococci, the penicillanic acid derivatives were the principal effective drugs, but the number of strains was too small to be significant.

The burn wound itself presents a complex problem in bacterial ecology. The topical treatment does not sterilize the wound, and with successful topical therapy wound cultures are done more in an experimental manner than as an actual guide to therapy. The proportion of species present is thus of more significance than actual totals. Table 3 shows the relative incidence of the wound surface flora in 1964 and 1965, compared to that of 1963 when topical therapy was not employed. This collection is somewhat biased since the strains listed include many cultures taken by swab on occasions where there was concern regarding the progress of the wound. A random sample would probably alter some of these proportions.

Pseudomonas aeruginosa remained less common when wounds were treated with sulfamylon than it was in the pre-sulfamylon era, but it was, in 1965, the most frequently encountered species with Aerobacter in second order of incidence. In contrast, in 1964, the Aerobacter incidence was by far preponderant.

Proteus spp (including principally P. mirabilis, with P. morganii also found), represented 9.3% of isolates. Despite the impression that has, at times, appeared that Proteus strains were assuming greater importance since they are highly resistant to sulfamylon, this does not appear to be the case. The other major group of wound bacteria were the staphylococci. It was of particular interest that the proportion of coagulase-negative staphylococci remained relatively high in the wounds. The fact that strains collected in 1965 showed a distribution quite similar to that in 1963 may reflect shifts in the microbial population not as yet understood. There is, however, a provocative fact, that the amount of Sulfamylon HCl burn cream used per patient, in 1965, was only about 60% of that used in 1964.

Table 2. Sensitivity of Blood Stream Isolates to Antibiotics

Antibiotic	Reaction	Pseudo-monas	Aero-bacter	Proteus	E.coli	Mima	Staph. coag. pos.	Staph. coag. neg.
Erythromycin	S*	0	1	0	0	0	1	2
	R*	3	4	5	2	1	1	0
Chloromycetin	S	0	4	1	1	1	1	0
	R	3	1	4	1	0	1	2
Terramycin	S	1	3	0	1	1	1	1
	R	2	2	5	1	0	1	1
Kantrex	S	1	1	1	1	0	3	2
	R	2	4	4	1	1	0	0
Polymyxin	S	0	1	0	0	0	1	
	R	3	2	4	1	1	0	
Colymycin	S	3	2	1	1	1	0	
	R	0	3	4	1	0	1	
Prostaphlin	S						2	2
	R						0	0
Lincocin	S						1	
	R						0	

Table 2. continued

Antibiotic	Reaction	Pseudo-monas	Aero-bacter	Proteus	E.coli	Mima	Staph. coag. pos.	Staph. coag. neg.
Staphcillin	S						1	0
	R						2	2

* S = sensitive
R = resistant

Table 3. Bacterial Flora of Burn Wounds
1963 - 1965

Species	Per Cent of Isolates		
	1963	1964	1965
Ps. aeruginosa	24.1	16.2	16.9
Aerobacter gp.	15.2	23.2	15.1
E. coli	5.8	9.0	8.2
Mima gp.	7.4	1.0	2.0
E. freundii	1.0	0.5	0.1
Proteus spp.	6.5	7.7	9.3
Providencia	1.2	1.5	4.8
Staph. coag. pos.	16.1	14.0	14.3
Staph. coag. neg.	7.2	13.9	7.1
Streptococci	9.7	4.3	4.8

As physicians have learned the technic of topical therapy, it has become evident that the amount of covering needed to prevent sepsis is less than was originally thought. With this lessened application, certain ratios of bacterial identity have returned to a pattern resembling that of 1963. The Ps. aeruginosa proportion, however, did not change.

Streptococci were not broken down into groups in previous years, but in 1965, presumptive bacitracin sensitivity screening was used to differentiate the Group A strains. Out of 39 strains recovered from wounds, 22 were Group A. This strain is principally a problem in infections of skin grafts. It responded promptly to penicillin therapy when such infection occurred.

Other species of interest included 11 strains of Candida albicans. One patient, a child of 18 months, arrived at the Surgical Research Unit after treatment for a week with soaks of neomycin. The wound was a pure confluent mass of Candida albicans. A burn cream of 10,000 units per gram of oral mycostatin was improvised, and in two days of topical treatment the Candida was gone, replaced by Pseudomonas aeruginosa. The latter responded to topical sulfamylon therapy. The full extent of infection risk due to Candida is not known.

In summary, the microbial flora of the blood stream in burned patients treated with sulfamylon cream during 1965 showed the same striking difference when compared with the pretopical treatment period that had been observed in 1964. The confirmation of the initial experience with the topical therapy supported the concept that the improvement in burn wound survival is real. The positive blood cultures seen in 1965 typify massive, lethal burns, but do not represent a consequence of wound sepsis.

In the case of the burn wound, the alteration in flora was not as notable as was the case with the blood stream. The bacterial population in sulfamylon-treated wounds was quantitatively reduced. Species present, however, were those also seen in unprotected wounds.

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Presentations

Lindberg, R.B.: The effect of Sulfamylon hydrochloride on the bacterial flora of the burn wound. Presented at AMA Society for Study of Trauma, Annual meeting, Philadelphia, Pa., 11-13 October 1965.

Publications

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25. (U) Approach - Tube dilution sensitivity tests determined degree and rate of sensitivity to drugs.

26. (U) Progress (Jul 65 - Jun 66) - Nine antibiotics, including previously untested Lincomycin and sodium nafcillin were assessed against 100 strains of Pseudomonas aeruginosa, Aerobacter, Proteus spp, staphylococci and non-group A streptococci. Pseudomonas were affected only by colistin sulfate; Aerobacter strains were extremely variable, with terramycin the most consistently useful antibiotic. Proteus spp were extremely resistant to all antibiotics. Staphylococci were relatively resistant to penicillanic acid derivatives.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: ANTIBIOTIC SENSITIVITY OF CURRENT BURN WOUND FLORA

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

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REPORTS CONTROL SYMBOL: RCS-MEDDH-288(R1)

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: ANTIBIOTIC SENSITIVITY OF CURRENT BURN WOUND FLORA

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

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6. Reports Control Symbol: RCS-MEDDH-288(R1)

Nine antibiotics, including for the first time lincomycin hydrochloride (Lincocin)* and sodium nafcillin (Unipen)/, were assessed for antibiotic activity against 20 recently isolated strains each of Pseudomonas aeruginosa, Proteus spp, Aerobacter-Klebsiella group, staphylococci and non-group A hemolytic streptococci. Pseudomonads were sensitive in general only to colistin sulfate (Coly-Mycin)**; the Aerobacter-Klebsiella group varied widely in sensitivity, with oxytetracycline hydrochloride (Terramycin)‡ the most consistently effective antibiotic; Proteus spp were virtually totally resistant, and the gram positive cocci showed wide variation in sensitivity. The staphylococcus strains were approximately 50 per cent resistant to the penicillanic acid derivatives.

(1) Burn wound flora (2) Antibiotic sensitivity

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* Lincocin furnished by Upjohn Company, Kalamazoo, Michigan.
/ Unipen furnished by Wyeth Laboratories, Philadelphia, Pa.
** Coly-Mycin furnished by Warner-Chilcott Labs., Morris Plains, New Jersey.
‡ Terramycin furnished by Chas.Pfizer & Co.,Inc., New York.

ANTIBIOTIC SENSITIVITY OF CURRENT BURN WOUND FLORA

In a burns unit concerned with treating injuries in which infection is a continuing problem, a surveillance of the sensitivity of burn wound flora is virtually a necessity. Burn wound sepsis has been successfully controlled in the Surgical Research Unit by use of topical sulfamylon, but it cannot be assumed that burn wound infection has been entirely eliminated. Burn wound infection has, during the past decade, seen emphasis shift from a predominant role of gram-positive cocci to a situation in which gram-negative bacilli are the problem organisms. Organisms presently regarded as benign or at worst readily managed may be supplanted by invasive strains which could alter the equilibrium between bacteria and the wound with dismaying rapidity.

In consequence of the dynamic nature of the balance between invasive wound infection and the therapeutic tools available, the investigation of new antibiotics and a continued review of the effectiveness of certain older forms are a continuing study. A collection of strains currently isolated from clinical material offered a suitable assortment for sensitivity tests to be used as a guide for therapy.

Test Antibiotics and Manufacturers

Oxytetracycline hydrochloride (Terramycin), Pfizer.
Sodium cephalathin (Keflin), Eli Lilly.
Colistin sulfate (Coly-Mycin), Warner-Chilcott.
Lincomycin hydrochloride (Lincocin), Upjohn.
Sodium nafcillin (Unipen), Wyeth.
Kanamycin sulfate (Kantrex), Bristol.
Sodium dimethoxyphenyl penicillin (Staphcillin), Bristol.
Sodium oxacillin (Prostaphlin), Bristol.
Sodium cloxacillin (Tegopen), Bristol.

Test Organisms

These strains were collected between November 1965 and March 1966. The sources of these collections are indicated.

Key No.	Pseudomonas spp.	Source	Aerobacter gp.	Source	Proteus spp.	Source
1	SRU 1-22-1(66)	Pustule,arm	SRU 1-29-12(66)	Stool	SRU 2-12-1(66)	Urine
2	SRU 1- 5-2(66)	Urine	SRU 1-27-4 (66)	Biopsy	SRU 2-28-37(66)	Eschar
3	SRU 8-19-13(65)	Burn,leg	SRU 1-18-4 (66)	Urine	SRU 2-23-5(66)	Urine
4	SRU 12-29-2(65)	Ear	SRU 3-1- 3 (66)	Blood	SRU 2-21-11(66)	Stool
5	SRU 12-12-1(65)	Burn, abd.	SRU 1-11-7 (66)	Burn, abd.	SRU 2-17-7 (66)	Biopsy
6	SRU 1-16-12(56)	Burn,chest	SRU 1-5-3 (66)	Burn,arm	SRU 2-17-4 (66)	Biopsy
7	SRU 10-29-16(65)	Autopsy	SRU 1-5-2 (66)	Urine	SRU 2-17-6 (66)	Biopsy
8	SRU 1-26-1 (66)	Ear	SRU 1-15-5 (66)	Burn	SRU 2-15-9 (66)	Eschar
9	SRU 3-21-2 (66)	Biopsy	SRU 12-6-20(65)	Burn	SRU 2-28-25(66)	Liver, Autopsy
10	SRU 12-3-28(65)	Burn,ankle	SRU 1-9-8 (66)	Burn,chest	SRU 2-15-8 (66)	Biopsy
11	SRU 3-28-3 (66)	Biopsy	SRU 1-31-4 (66)	Urine	SRU 2-21-19(66)	Homograft
12	SRU 1-22-3 (66)	Trachea	SRU 1-27-3 (66)	Biopsy	SRU 2-28-13(66)	Biopsy
13	SRU 11-16-2(65)	Urine	SRU 1-18-4 (66)	Urine	SRU 2-3-27 (66)	Biopsy
14	SRU 11-30-26(65)	Urethra	SRU 11-17-20(65)	Burn,arm	SRU 3-25-22(66)	Burn
15	SRU 12-15-6 (65)	Feces	SRU 1-7-3 (66)	Urine	SRU 2-3-6 (66)	Eschar
16	SRU 1-21-1 (66)	Urine	SRU 1-5-15 (66)	Whirlpool	SRU 2-17-17(66)	Urine
17	SRU 12-9-2 (65)	Mouth	SRU 1-25-2 (66)	Dialysis Tank	SRU 2-15-4 (66)	Burn
18	SRU [illegible]-2-21(65)	Trachea	SRU 1-18-4 (66)	Urine	SRU 2-15-14(66)	Urine
19	SRU [illegible]-23-19(65)	Burn,thigh	SRU 3-25-21(66)	Catheter	SRU 2-14-11(66)	Urine
20	SRU [illegible]-16-15(65)	Homograft	SRU [illegible]-1-66 (66)	Burn	SRU 2-3-26 (66)	Biopsy

Key No.	Staphylococci, coagulase-positive	Source	Beta hemolytic streptococci	Source
1	SRU 2-9-8 (66)	Sputum	SRU 2-14-16(66)	Sputum
2	SRU 2-9-1 (66)	Throat	SRU 2-12-12(66)	Eschar
3	SRU 2-24-7(66)	Autograft	SRU 2-11-24(66)	Biopsy
4	SRU 2-3-3 (66)	Burn	SRU 2-28-3 (66)	Biopsy
5	SRU 2-2-5 (66)	Burn	SRU 2-24-7 (66)	Autograft
6	SRU 2-2-6 (66)	Burn	SRU 2-18-7 (66)	Throat
7	SRU 2-14-16(66)	Sputum	SRU 2-18-4 (66)	Throat
8	SRU 2-14-9 (66)	Urine	SRU 2-10-2 (66)	Throat
9	SRU 2-14-8 (66)	Throat	SRU 2-9-1 (66)	Throat
10	SRU 2-14-7 (66)	Furuncle	SRU 2-2-2 (66)	Throat
11	SRU 2-14-18(66)	Furuncle	SRU 2-21-6 (66)	Throat
12	SRU 2-14-7 (66)	Lung, autopsy	SRU 2-21-13(66)	Throat
13	SRU 2-2-10 (66)	Burn	SRU 2-16-45(66)	Throat
14	SRU 2-17-17(66)	Urine	SRU 3-10-[illegible] (66)	Burn
15	SRU 2-5-1 (66)	Burn	SRU 3-11-[illegible] (66)	Throat
16	SRU 2-21-7 (66)	Throat	SRU 3-11-3 (66)	Throat
17	SRU 2-21-19(66)	Homograft	SRU 3-14-16(66)	Sputum
18	SRU 2-21-1 (66)	Wound	SRU 3-15-2 (66)	Sputum
19	SRU 2-17-18(66)	Trachea	SRU 3-15-7 (66)	Throat
20	SRU 2-17-15(66)	Throat	SRU 3-15-3 (66)	Throat

Methods

Aqueous dilutions of the test antibiotics[1] were made so that the final tube dilution contained the following concentration (µg/ml) 25.0, 12.5, 6.2, 3.1, 1.5, and 0.78. A representative colony of each test strain was inoculated in 7.5 ml trypticase soy broth (BBL) and incubated at 37° C for 4-6 hours. A 1:100 dilution of this seed culture was made in double strength trypticase soy broth to serve as the standard test inoculum.[2-4] The tube tests were performed by mixing 0.5 ml of each concentration of the aqueous antibiotics with 0.5 ml of each test inoculum. The 1.0 ml mixtures were then incubated at 37° C for 24 hours and visually examined for turbidity. The minimal inhibitory concentration (MIC) was designated as the last tube showing no growth.

Results

Table 1 shows the results of the sensitivity tests with Pseudomonas aeruginosa on oxytetracycline hydrochloride, sodium cephalathin, kanamycin sulfate, and colistin sulfate. For several years an arbitrary criteria of 12.5 µg/ml as the line of division between sensitive and resistant gram-negative bacteria has been used. Thus, 13 of 20 strains (65%) in Table 1 were sensitive to oxytetracycline hydrochloride. The Table shows that sodium cephalathin and kanamycin sulfate were not effective at 25.0 µg/ml. Colistin sulfate showed the greatest effectiveness, with 20 random strains being inhibited at 3.1 µg/ml (100% sensitive).

Table 2 shows the sensitivity tests of 20 strains of the Aerobacter-Klebsiella group against oxytetracycline hydrochloride, sodium cephalathin, kanamycin sulfate, and colistin sulfate. These strains were all sensitive to concentrations of oxytetracycline HCl below 12.5 µg/ml with 10 of 20 strains exhibiting a consistent sensitivity at 3.1 µg/ml. Sodium cephalathin was effective against 14 of 20 strains (70%) at 12.5 µg/ml to 6.2 µg/ml. Only 9 of 20 strains (45%) were sensitive to kanamycin sulfate. Colistin sulfate was effective at concentrations from 12.5 µg/ml (8/20) to 6.2 µg/ml (4/20) with a total of 13 of 20 strains (65%) sensitive.

Table 3 shows the results of tests on Proteus species with oxytetracycline HCl, sodium cephalathin, kanamycin sulfate, and colistin sulfate. The strains included

Table 1. Sensitivity by Tube Test of Twenty Strains of *Pseudomonas aeruginosa*

Antibiotic	MIC and Number Inhibited							Per Cent Sensitive
	> 25	25	12.5	6.2	3.1	1.6	0.78	
Oxytetracycline hydrochloride	0	7	13	0	0	0	0	65
Sodium cephalothin	25	0	0	0	0	0	0	0
Kanamycin sulfate	25	0	0	0	0	0	0	0
Colistin sulfate	0	0	0	0	20	0	0	100

Table 2. Sensitivity by Tube Test of Twenty Strains of Aerobacter Kiebsiella Group

Antibiotic	MIC and Number Inhibited							Per Cent Sensitive
	> 25	25	12.5	6.25	3.1	1.6	0.78	
Oxytetracycline hydrochloride	0	0	2	8	10	0	0	100
Sodium cephalathin	0	6	10	4	0	0	0	70
Kanamycin sulfate	3	8	9	0	0	0	0	45
Colistin sulfate	0	6	8	4	2	0	0	70

Table 3. Sensitivity by Tube Test of Twenty Strains of *Proteus* spp.

Antibiotic	MIC and Number Inhibited							Per Cent Sensitive
	> 25	25	12.5	6.25	3.1	1.6	0.78	
Oxytetracyclin hydrochloride	14	1	2	0	2	1	0	25
Sodium cephalathin	9	3	6	1	1	0	0	40
Kanamycin sulfate	13	6	0	0	1	0	0	5
Colistin sulfate	20	0	0	0	0	0	0	0

P. mirabilis, P. vulgaris, and P. morgani. Only 5 of 20 strains (25%) were sensitive to oxytetracycline hydrochloride, while 8 of 20 strains (40%) were sensitive to sodium cephalathin. Only one of 20 strains was sensitive to kanamycin sulfate, and none were inhibited by colistin sulfate.

Table 4 shows results of sensitivity studies with coagulase-positive staphylococci against a selected group of antibiotics regarded as particularly effective for this organism. Oxytetracycline hydrochloride, lincomycin hydrochloride, sodium cephalathin, sodium cloxacillin, methicillin, sodium oxacillin, and sodium nafcillin were used. Only 6 of 20 strains (30%) were sensitive to oxytetracycline HCl, and of these five had an endpoint at 0.78. Lincomycin HCl was effective in only 9 out of 20 strains (45%) tested. Sodium cephalathin was effective against 13 of 20 strains (65%) with the majority of strains inhibited by 0.78 µg. Sodium cloxacillin showed similar results with 14 of 20 strains (70%) sensitive. The results with methicillin showed that only 10 of 20 strains (50%) were sensitive. Experience has shown that this particular antibiotic may vary between lots, so that continuous monitoring is necessary to assure an accurate result in sensitivity tests. Sodium oxacillin, another penicillanic acid derivative, showed results similar to those with methicillin; strains were either very resistant or very sensitive. Sodium nafcillin exhibited an activity similar to the newer penicillins and at 12.5 µg inhibited 65 per cent of the test strains. The non-group A beta hemolytic streptococci, as gram-positive organisms, were also tested against the same antibiotics as the staphylococci. Table 5 shows these results. It was interesting to note that in test series a single strain out of 20 grew above the 6.2 µg/ml concentration; all others were consistently sensitive to less than 6.2, with the majority at 0.78 µg/ml.

Discussion

The 20 strains of recent isolates of organisms which are prominent in the burn wound flora were tested against antibiotics appropriate to the category of bacteria. Thus, Pseudomonas, Aerobacter, and Proteus strains were tested against oxytetracycline HCl, sodium cephalathin, colistin sulfate, and kanamycin sulfate. Staphylococci and hemolytic streptococci (primarily Group D) were tested with

Table 4. Sensitivity by Tube Test of 20 Strains of Staphylococci, Coagulase-Positive

Antibiotic	MIC and Number Inhibited							Per Cent Sensitive
	> 25	25	12.5	6.25	3.1	1.6	0.78	
Oxytetracycline hydrochloride	14	0	0	1	0	0	5	30
Lincomycin hydrochloride	11	0	0	0	0	7	2	45
Sodium cephalathin	2	4	1	2	0	1	10	70
Sodium cloxacillin	5	2	0	1	2	1	9	65
Methicillin	6	4	0	0	1	8	1	50
Sodium oxacillin	8	1	0	0	0	0	11	55
Sodium nafcillin	3	4	1	1	0	0	11	65

Table 5. Sensitivity by Tube Test of 20 Strains of Hemolytic Streptococci (Other than Group A)

Antibiotic	MIC and Number Inhibited							Per Cent Sensitive
	> 25	25	12.5	6.25	3.12	1.6	0.78	
Oxytetracycline hydrochloride	0	1	0	0	0	1	18	95
Lincomycin hydrochloride	0	1	0	1	2	3	13	95
Sodium cephalathin	0	0	1	2	1	5	11	100
Sodium cloxacillin	0	1	0	0	0	1	18	95
Methicillin	1	0	0	1	1	2	15	95
Sodium oxacillin	0	0	1	0	0	3	16	100
Sodium nafcillin	0	1	0	0	2	2	15	95

oxytetracycline HCl, lincomycin HCl, sodium cephalathin, sodium cloxacillin, methicillin, sodium oxacillin and sodium nafcillin.

Pseudomonas strains were essentially resistant to all antibiotics except colistin sulfate. The Aerobacter strains varied in their response to antibiotics but oxytetracycline HCl was the most consistently effective antibiotic. Proteus spp. were resistant to the entire spectrum of antibiotics tested.

Among gram-positive cocci, wide variations in response to test antibiotics were found. The staphylococci, all coagulase positive, were divided into relatively resistant and highly sensitive categories. Thus, with the penicillanic acid derivatives, strains were either highly resistant or very sensitive. This pattern was essentially the same for the whole spectrum of antibiotics tested.

The streptococci were, except for one strain, highly sensitive to the entire set of antibiotics tested. Sodium cephalathin was especially potent with this group of organisms.

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Presentations and/or Publications

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23. KEYWORDS: Pseudomonas, phage typing, burn wounds, topical chemotherapy

24. (U) Tech Objective - Determine the effect of topical therapy on phage type and comparison with other sources.

(U) Approach - Phage typing, using previously established typing system, was carrisd out on Pseudomonas aeruginosa strains collected from burn ward, renal ward, and from other hospitals.

(U) Progress (Jul 65-Jun 66) - It has now become apparent that Pseudomonas strains from a burn population intensively treated with Sulfamylon HCl acquire a significant increase in phage-resistance, as reflected in the continued presence of non typable (NT) strains comprising 1/4 of the strains collected on the burn ward. NT strains do not appear elsewhere in proportions above 4% to 6% of total strains. Type 21, 63, constituting 9% of the total tested, continued as most common type and thus indicates that a resistent predominant type can become established.

25.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: BACTERIOPHAGE TYPING OF PSEUDOMONAS AERUGINOSA IN BURNED PATIENTS: EPIDEMIOLOGY OF BURN WARD INFECTING STRAINS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

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Russell E. Brame, M.S.
Arthur D.Mason, Jr., M.D.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: BACTERIOPHAGE TYPING OF PSEUDOMONAS AERUGINOSA IN BURNED PATIENTS: EPIDEMIOLOGY OF BURN WARD INFECTING STRAINS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert B. Lindberg, Colonel, MSC
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Phage typing of Pseudomonas aeruginosa strains from the Burn Ward and Renal Sections, and from other hospital sources was carried out to delineate the precise nature of Pseudomonas epidemiologic status in the presence of continued sulfamylon therapy. It now appears that a resident predominant flora of phage type 21,68, and a large component of non-typable (NT) strains has become established during the past year. The NT population is unique to this unit in contrast to the typable strains found elsewhere, and appears to reflect the influence of sulfamylon on the strains. A group of seven severely burned individuals from a single accident introduced a distinctive phage pattern that furnished unique data on the relationship between exotic and resident flora of Ps. aeruginosa.

(1) Phage typing
(2) Pseudomonas aeruginosa

BACTERIOPHAGE TYPING OF PSEUDOMONAS AERUGINOSA IN BURNED PATIENTS: EPIDEMIOLOGY OF BURN WARD INFECTING STRAINS

The definitive phage typing system which has been developed in this laboratory has been applied in a continuing assessment of the Pseudomonas aeruginosa problem in the Burn Ward.[1] Epidemiologic considerations remain of importance despite the altered environmental factor that has been introduced with topical sulfamylon therapy. It has become evident that such therapy, while it may eliminate Pseudomonas from the wound during the period of intensive treatment, does not remove it from the ward population. Routine culturing of wounds showed, during 1964 and 1965, a one-third reduction in the incidence of Ps. aeruginosa.[2] The altered aspect of wound pathogenesis achieved with the control of burn wound sepsis has, however, been accompanied by an abrupt and apparently persistent alteration in the Pseudomonas flora of the burned patient. The organism is still found, but in a significant number of cases it appears to be altered in its phage reactions, which means that the genetic structure of the bacterial population has changed. The simultaneous presence of Pseudomonas in the Renal Section was modified during the year by a frequent influx of burned patients into the Trauma Ward, so that the Burn Ward flora was much more actively disseminated.[3]

Materials and Methods

The selection of phages for a typing system was accomplished two years ago. The propagation and identification procedure for the typing strains remains a critical aspect of this procedure. Identity patterns have been used at intervals to make sure the phages have not altered. This is particularly important with Ps. aeruginosa since these strains are so commonly lysogenic that any strain which appears not to be carrying prophage is scrutinized with great care to be certain it is indeed non-lysogenic.

Strains of Pseudomonas were not sought as industriously as in previous years from other hospitals. This activity, although interesting, had not produced a homogeneity of result that suggested a common pattern for virulent pseudomonads.

Observations

Pseudomonas Phage Types in Surgical Research Unit Burn

Ward Patients, 1965. Four hundred eighty-eight strains of Pseudomonas from burned patients were submitted to phage type identification in 1965. Fourteen of these were recovered from the blood stream of 10 critically ill patients. The remaining 471 were composed of specimens primarily from the burn wound, but also from urine, tracheal, catheter, and autopsy samplings. Pseudomonas strains were obtained from 105 of the 174 patients admitted to the Burn Ward during the year. Determination of the most prevalent types was based on the number of patients with a particular type rather than numbers of strains since a single patient with numerous strains of one given type can obscure the distribution picture.

The predominant types from all sources are listed in Table 1. The types listed represent 64.5 per cent (315 strains) of the total strains examined; the remaining 35.5 per cent (173 strains) were of 56 other phage types, no one type occurring in more than two patients. This multiplicity of types will be discussed later.

The predominant strain encountered was non-typable (NT) represented by 138 (28%) strains in 38 patients (36%). In 1964, an abrupt rise in the NT strains was noted; the per cent strains NT in previous years had been relatively low. In 1961, 6 per cent; 1962, 3 per cent; 1963, 5 per cent; however, in 1964, the percentage rose to 25 per cent, and for 1965 was slightly higher. The question of whether or not the extensive use of sulfamylon cream begun in January, 1964 has selectively altered the burn wound Pseudomonas population remains an unproven hypothesis. The possibility exists that (a) the NT strains are more resistant to sulfamylon, therefore, show a higher incidence; (b) sulfamylon may alter the phage susceptibility of typable strains so that they become NT; or that there has been (c) establishment and transmission of NT strains with no relation to topical therapy.

The most common type recognized was type 21,68. Nine per cent of all strains from 23 per cent of patients, were 21, 68. This type was the second most prevalent type in 1963, and the most common type in 1964. It appears to have become a well-established burned patient type. Such a phenomenon has not previously occurred.

Third in incidence were strains of phage type 21,31,68;

Table 1. Predominant *Pseudomonas* Phage Types in SRU Burn Ward Patients, 19[illegible]

Phage Type	No. of Strains	No. of Patients	Origin
NT	138	38	Observed in all years; relatively low incidence in '62 (2.8%) and '63 (4.8%) increasing in '6[illegible] (25%)
21,68	44	24	2d most prevalent type in '63; most common in '64
21,31,68	39	15	Predominant type in '62; 3d in '63; no. observed in '64
24	25	9	3d most prevalent in '61; very low incidence in '62, '63; not seen in '64
(M6)	12	5	2d most prevalent in '61; not observed in '62, '63; two strains in '64
2,7,21,68,119X	6		New
2,7,21,24,44,1214,352,119X,F7,M4	13	4	New
7,31	11		Low incidence in '63 (3/5); '64 (1/9
44,1214,M6	6		New
24,31,44,1214,352,119X,F7,M6	9	3	New
1214,68	5		New
2,7,73,119X,M4	4		Low incidence '60, '61, '64
2,7,21,44,1214,68,119X			New
(1214)			'64 (4/6)
119X,F7			New
31	3		Low incidence '60, '61; 2d most prevalent in '62; low incidence '63, '64

these constituted eight per cent of all strains, and were collected from 14 per cent of the patients. This strain shares two factor reactions with type 21,68, but is nevertheless a completely separate type. 21,31,68 strains were the most common in the unit during 1962, and were the third most common strain in 1963. They were present in that year only until June, however; they then disappeared for 18 months, to reappear in 1965. There is no obvious explanation for this disappearance of a predominant type for a period of 18 months, followed by its reappearance as an infecting strain. It remains a question: is it the same strain which has been present but not observed for an extended period of time, or is it a new strain of the same type which has been introduced into the ward from a new admission or perhaps a member of the staff? Presently there is no way to tell; future plans include preserving representative strains of predominant types so that a retrospective comparison could be made.

The fourth most common type during 1965 was a strain of type 24, which constituted five per cent of the strains from 8.6 per cent of the patients. This is a type that was observed in patients in previous years. It was the third most common strain in 1961, but was relatively rare in 1962 and 1963, and was not seen at all in 1964. Here, again, is a strain which disappeared from the ward, only to reappear after being absent for approximately one and one-half years.

Half of the strains typed during the year fell into one of the first four categories listed. The remainder of the 12 types listed, each occurred in no more than five patients. Seven of these were new types, that is, not previously observed in Surgical Research Unit burn wound flora; three, 7,31; 2,7,73,119X,M4; and (1214), have been seen infrequently, and two (M6) and 31 were prevalent types in prior years but are now uncommon.

There were 56 additional types seen during the year which could be added to this list. The multiplicity of types seen in the burned patient flora has been noted in previous years, but the diversity in this years' strains is much more extreme than was previously seen. Possibly the great increase in the number of patients was a factor, since it added new strains to a population of established strains.

This assumes that patients may be seeded or infected with Pseudomonas upon their arrival, which is not infrequently the case. It should be pointed out that, continuing the trend first noted in 1964, the phage types seen as most common are decidedly simpler in pattern than was the case in years before 1963.

Pseudomonas Phage Types in Blood Stream of Burned Patients, 1965. Blood stream invasion by Pseudomonas is associated with a high mortality. Blood stream strains hence merit special scrutiny.

During 1965, 14 strains were recovered from the blood stream of 10 patients. Three additional strains from two autopsies were listed as blood cultures, but of course come under the head of autopsy strains. These results are shown in Table 2. Type 21,68 was the type most common in blood cultures and appeared in five patients with five strains. The remaining nine strains included nine types; NT Pseudomonas appeared in only one patient. Two patients had two types isolated from the blood, while a third patient harbored three types. These 14 strains were of 10 phage types, which emphasizes the fact that no one type can be incriminated as the truly invasive strain in severe burn injury. This type is consistent with the great diversity of wound strains seen in 1965 in patients admitted to the Surgical Research Unit.

Chronologic Sequence of Phage Types in Burn Ward Patients, 1964 and 1965. The figure illustrates graphically the temporal distribution of the more prevalent types in burned patients during 1964 and 1965. As has been shown in prior years, certain types appear in the burned patient flora and persist for lengthy periods while others disappear or occur sporadically. It appears probable that with the addition of new patients, new types are constantly being introduced into the already diverse population of Pseudomonas strains. In the figure, the phage type appears at the left. The time during which it occurred is blocked in with the incidence (number of patients/number of strains).

Type 7,31 was first seen in three patients in 1963 and carried over in one patient to January 1964; it was not observed for 17 months and reappeared in four patients in 1965. Type 31 is a persistent strain seen in each year since 1960, almost always in low incidence. However, during 1962 it was

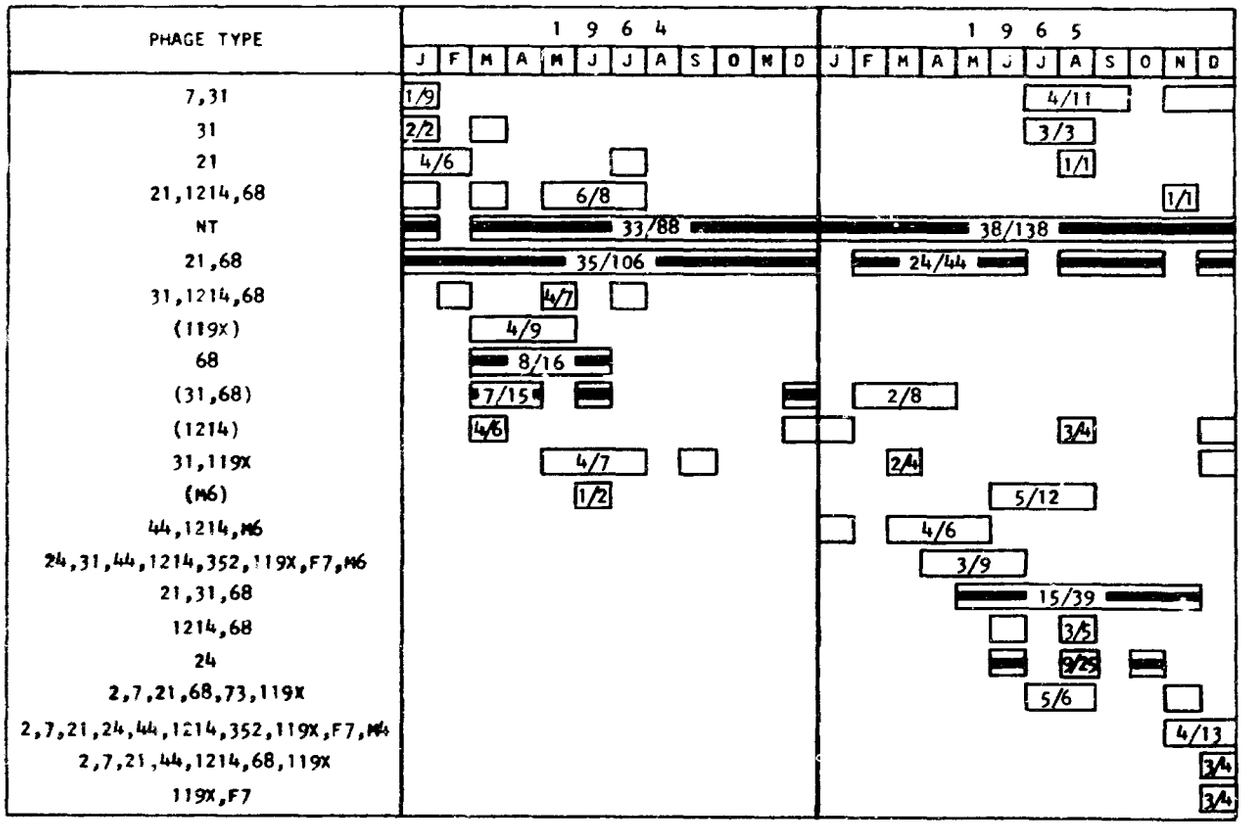
Table 2. Phage Types of Pseudomonas Recovered from Blood Cultures, 1965

Patient No.	% Body Surface Burned	No. of Strains	Phage Type
1	25*	1	(31,68)
2	65	1	109
3	85	1	44,1214
4	71	1	21,68
5	50	1	21,68
6	90	1	2,7,73
7	79	1	NT
8	79	1	2,21,68
		1	21,31,68
9**	50	1	21,68
		1	24
10	85	1	21,68
		1	2,7,21,24,44,1214,352, 119X,F7,M4
		1	2,7,21,44,1214,68,119X

* Patient recovered from burn; death due to acute pancreatitis.

** This patient survived. All others listed succumbed.

Pseudomonas Phage Types in the Surgical Research Unit Burn Ward:
Chronological Sequence, 1964, 1965



the second most prevalent type. It continues to reappear occasionally. Type 21 was the most common type in 1963. It was rare in 1964 and appeared in only one patient in 1965. Type 21,1214,68 was the fifth in prevalence in 1964, but only one strain was found in 1965. NT strains have been seen in all years, but there was a sharp increase in 1964 and 1965 as previously noted. Type 21,68 is almost unique in its persistence. It is, in this respect, as prominent as the NT strains. Type 31,1214,68 and (119X) were each observed in four patients during 1964. They did not appear in 1965. Type 68 was a common burn type in earlier years, and was the third most prevalent in 1964. It then disappeared; it has not been seen for one and one-half years. Type (31,68) was the fourth most prevalent in 1964, but appeared in only two patients in 1965. Type (1214) was relatively uncommon in both years. Type 31,119X has been a common burn type but appears sporadically and in relatively low incidence. Type (M6) was the fourth most prevalent type in 1960, second most prevalent in 1961, and was not seen again until 1964 in a single patient. It reappeared in 1965 in five patients. Types 44,1214,M6 and 24,31, 44,1214,352, 119X,F7,M6 are new types in 1965. They had never before appeared here. Type 21,31,68 was the most prevalent type during 1962, third most prevalent in 1963, and disappeared in 1964, to return in a high incidence in 1965. With the exception of type 24, the last six types listed were new types not previously observed here. Type 24 was the third most prevalent in 1961. It was very rare in 1962 and 1963, was not seen in 1964, and then appeared in nine patients in 1965.

These pattern distributions illustrate the problem of the epidemiology of Pseudomonas strains in a burn ward. Presently the population appears to be stabilized with the NT (if these are a type) and 21,68. Other types occur sporadically: 7,31; 31,21; 21,1214,68; (31,68); (1214); 31,119X; (M6); 21,31,68; and 24. These tend to reappear at intervals. Then, there are a constant succession of new types: 44,1214,M6; 24 ; 1214,68; 2,7,21,68,73; 119X; and 2,7,21,24,44,1214,352,119X, F7,M4. These will not, in the main, become established, in light of previous experience, but everyone of them is a potentially important pathogen. The disappearance of types 31,1214,68; (119X); and 68 has no explanation; they were there, then they were gone. During the period of predominance, some of these types seemed as firmly established as 21,68 is today.

Pseudomonas Phage Types in Physiotherapy Clinic, 1965. Many questions remain concerning the mode of transmission of Pseudomonas strains. The Hubbard tank in the Physiotherapy Clinic where patients continually undergo hydrotherapy treatment provide a constant site for transmission of Pseudomonas assuming the tanks are not properly disinfected before and after patient use. These strains are of interest because of their value in offering an insight into the mode of transmission of types and assessing the effectiveness of the disinfecting process.

There were 34 strains of Pseudomonas recovered from water in the Hubbard tanks. Most were taken after patient use. The types of these strains are shown in Table 3. Also listed are the dates on which they were isolated and number of strains of each type. The majority of these strains (24) were isolated during April. The predominant strain found, six strains, was NT, followed closely by 21,68 with five strains. This is consistent with the order of prevalence seen in burn wounds. Three strains each of type 31,M6 and 2,7,21 68,119X were found. These are burned patient types (two patients in 1965). There were two strains each of 24,31,44,1214,352,119X,F7,M6; 2,7,44,109, 352,F8; 2,7,21,31; and (M6). The first (three patients) and fourth (five patients) are burn types. Only one strain of each of the other nine listed types was found. Four of these are types that have not been found in burns: 2,7,21,119X; 2,7,21,44,68,109,352,119X,F8,M6; 2,7,21; and 2,7,31. Eight strains of six types could not be correlated with a history matching a "burned patient" type. These might be assumed to arrive via some outside source, or they might have been from burned patients from whom no strains had been cultured. Further, a patient could be cultured and yield a different type. The extreme complexity of the Ps. aeruginosa epidemiology is illustrated in these 34 strains, of 17 types, from the hydrotherapy equipment.

Pseudomonas aeruginosa in Renal Patients. The Renal Branch, Ward 13B, is physically separated from the Burn Ward, but traffic is heavy between the two, and more important, patients from the burn ward overflow increasingly into the Renal Branch. It would then be expected that we would seen strains from the Burn Ward in the Renal Branch.

Fifty strains of Pseudomonas aeruginosa were recovered from seven renal patients in 1965. The types are listed in Table 4.

Table 3. Phage Types of Pseudomonas Found in Hubbard Tank

Phage Type	Date							
	1-25	2-23	3-22	4-22	4-27	6-29	8-5	8-17
2,7,21,44,68,109,352,119X,M4,M6	1							
NT		1		3	1		1	
2,7,21,119X			1					
2,7,21,44,68,109,352,119X,F8,M6			1					
21,68				4	1			
31,M6				2	1			
2,7,21,68,119X				2		1		
44,1214,68,119X				1				
24,31,44,1214,352,119X,F7,M6				1	1			
2,7,44,109,352,F8				1	1			
(31,68)					1			
31,119X					1			
2,7,21,31					1	1		
2,7,21					1			
2,7,31					1			
(M6)						2		
7,31								1

Table 4. Phage Types of Pseudomonas from Renal Patient, Surgical Research Unit, 1965

Phage Type	Patient No. 1	2	3	4	5	6	7
2,7,31,119X,M4	7		16				
68				7		1	4
31,44,1214,352			1		1		
1214,M6					1		
(31,68)							1
31,119X	2						
21,31,68,119X				6			
21,M6						2	
NT		1					

Twenty-three strains came from two patients. This type, 2,7,31,119X,M4, appeared in the Burn Ward in six patients in 1962, and in one burned patient late in 1965. Type 68 was found 12 times in a total of three patients; it had been common until June of 1964, and has not been seen since. Type 31,44,1214,352, once each in two patients, has not been found in burns here. Type 1214,M6, occurred once in one patient, it was found in a burned patient who was also dialyzed. Type (31,68), found once in the Renal Branch, was prominent in burns in 1964 and in two patients in 1965. Type 31,119X had been a burn strain in 1964 and 1965. Type 21,31,68,119X, seen here, had only once before been seen in a burned patient in 1960. Type 21,M6 is a strain never recorded in burns. Thus, there is an indication that strains from the burn ward are getting into the Renal Branch in a much more noticeable way than was the case prior to 1965.

Six renal patients out of seven had two types in their specimens. Unlike the burn ward, the proportion of NT in renal patients was minimal. The absence of NT suggests that the renal population is definitely protected from indiscriminate seeding, since the two most common patterns, NT and 21,68, were not found on the Renal Branch.

Pseudomonas Phage Types from Other Installations. The identity of Pseudomonas strains from other parts of the country is of interest in several ways. It validates the thesis of the diversity of types which we see in the Surgical Research Unit; it confirms the adequacy of the typing system that has been developed here; and it validates the large NT population of strains that are being recognized in the Surgical Research Unit Burn Ward. Three sets of cultures were sent to this laboratory for typing.

The strains from the Henry Ford Hospital included especially strains from corneal ulcers and from ventricular fluid cultures in which indwelling catheters had led to contamination. The type distribution is listed in Table 5.

The diversity of strains is notable. Several of these strains have been seen at one time or another in this laboratory, but none is common. The interesting fact is noteworthy that only two NT strains were found; this experience reinforces our confidence in the adequacy of the Surgical Research Unit typing system.

Table 5. Phage Types of *Pseudomonas* Strains from Henry Ford Hospital, Detroit, Michigan

Source	Phage Type
Corneal Ulcer	2,7,21,31,68,73,119X 2,7,31,44,1214,352,119X,F8 2,7,31,73,119X,M4 2,7,31,1214,73,109 44,1214
Stool	2,7,31,119X,M4
Urine	31,68
Sputum	7,21,31,44,68,109,F8
Blood	M6
Ventricular Fluid	2,21,68,119X
C47	7,31
C51	7,21,F8
C62	44,119X,M6
C41	NT
C56	NT

Six strains from the University of Cincinnati presented an unexceptional picture of diverse types (Table 6). These strains were submitted with the assumption that they had a common source. This concept was obviously erroneous.

From Okinawa, the Army Hospital sent 10 strains reflecting their concern at a supposed cross contamination problem (Table 7). Except for strains of 21,68,119X with an accompaying F8 in the paired strains from humidifier and urine, the set was heterogeneous. Again, only two NT strains encourages the concept of the validity of our typing set.

Pseudomonas Infection on an Increment of Seven Patients Simultaneously Admitted to the Burn Ward. Seven persons were burned on November 24 in a fire at Keokuk, Iowa. They were brought to the Surgical Research Unit Burn Ward on Novembe 30, six days postburn. These patients were hospitalized at the same installation prior to their transfer to the Surgical Research Unit, and were transported to San Antonio simultaneously. The patients were cultured on their arrival to learn whether or not they were infected with Pseudomonas at that time, and if so, if there was a common type present. This could provide information on the mechanisms of cross-transmission and introduction of new types to the Surgical Research Unit Burn Ward. These findings are summarized in Table 8. This shows the "Date of Culture","Source", "Patient No." and under each, number of cultures "+ and/or -" for Pseudomonas and phage type of + cultures where performed with ( ) to indicate number of cultures examined for type. Thirty-three strains were phage typed. On the date of admission, all patients with the exception of Patient No. 2, showed + surface cultures. Four strains from Patient No. 1, three initial and one at six days post-admission, were of type A71. This type had not been on the ward earlier. Patient No. 2, although showing negative cultures through two days post-admission, appeared with type A71 on the third day and another at two weeks, and later a type F20. Patient No. 3 had a single type, F3, at three, six and nine days post-admission. Patient No. 4 had six strains of type A71 during the first week, a NT on the sixth day, and on the seventh, a type A72. Patient No. 5 showed primarily negative cultures until three weeks post-admission when he appeared with two types, D41 and A72 from the surface. Patient No. 6 expired 48 hours post-admission; eight strains from autopsy of viscera were of type D51. Patient No. 7,who expired six days post-admission, had two

Table 6. Phage Types of *Pseudomonas* Strains
University of Cincinnati Medical School

No.	Phage Type
F521	2,7,31,119X,M4
F525	21,68,119X,F7
F112b	44,1214
F274	(44)
F276	NT
F270	NT

Table 7. Phage Types of Okinawa *Pseudomonas* Strains

Source	Phage Type	No. of Strains
Humidifier	21,68,119X,F8	4
	(F8)	1
	NT	1
Urine	21,44,1214,68,109	1
	(F8)	1
	21,68,119X	1
Throat	NT	1
	7,21,31,44,1214,68,109,352	1
Ear infection	2,7,31,73,119X,M4	1
Foot wound	31,44,1214,119X	1

Table 8. *Pseudomonas* Phage Types and Occurrence in a Group of Seven Patients Burned in a Single Accident

Date	Source	Patient No. 1			2			3			4			5			6			7		
		+	-	Type	+	-	Type	+	-	Type	+	-	Type	+	-	Type	+	-	Type	+	-	Type
11-30	Surface	2				3		2			2			1	3		1	1		2		
	Urine	1				1			1						1							
	Throat	1		A71								1										
	Urethra	1		A71																		
	Catheter	1		A71																		
	Stool										1		A71									
	Blood														1			1			1	
12-1	Surface		2			2		2			1	3			2					2		
	Urine											1						1				
	Blood											1										
12-2	Surface	2				2			1		1	3			1					3		
	Catheter														1			1				
	Blood														1						1	
	Viscera																8	1	B51			
12-3	Surface				2		A71(1)	2		F 3(1)	4		A71(2)		2					2		A72(1)
	Trachea														1					1		A72
	Blood																				1	
12-4	Surface				1	1		1				3		2						2		
	Trachea											1								1		A72
	Blood					1						1										
12-5	Surface					1			1			2			2					2		
	Throat					1															1	
	Blood																					
12-6	Surface	2		A71(1)				1		F 3	2	1	A71(1)		1					1		
													NT									
	Blood																			2		A71
																						B41

Table 8. continued

Date	Source	Patient No. 1 +	1 -	1 Type	2 +	2 -	2 Type	3 +	3 -	3 Type	4 +	4 -	4 Type	5 +	5 -	5 Type
12-7	Surface										1		A71			
	Trachea										2		A71			
	Lukens												A72			
	Blood											1				
												1				
12-9,10	Surface		1		2	1		1	1	F 3		2				
12-13,14	Surface											1				
	Trachea										1				2	
	Throat				1		A71									
12-20 to 23,28,29	Surface				1		F20		1			1		2		A72
	Trach											1				D41
	Throat					1					1					
	Blood											2				

Phage Type

Type A71 - 2,7,21,24,44,1214,352,119X,F7,M4
Type A72 - 2,7,21,44,1214,68,119X
Type D41 - 21,68
Type F 3 - 31,119X
Type F20 - 119X,F7
Type D51 - 21,44,68

strains of type A72 on the third day, one strain of type A72 from the blood on the fourth, and on the sixth day, two types from the blood, A71 and D41.

This series of patients had two types in common. Four of the seven (Patient Nos. 1, 2, 4, and 7) had type A71 accounting for 13 strains. Type A72 was found in three patients (Nos. 4, 5, and 7) - five strains. Neither type was a recognized burn ward type. Patient No. 3 and 6 did not acquire either of these types. Though patient No. 3 had three strains of type F3, a type observed in Surgical Research Unit burned patient flora, it cannot be proved whether he acquired it after arrival. However, Patient No. 6 had eight strains of only one type, D51, which was definitely a nonburn ward type. Two patients, No. 5 and No. 7, had one strain each of type D41 which is a very prevalent type on the burn ward. Patient No. 2 had one strain of type F20, another burn ward type; and Patient No. 4 had one strain of NT Pseudomonas.

A cross-transmission of types A71 and A72 could be demonstrated in this series of patients since five of the seven had at least one strain of either type. This was not unexpected due to their close proximity prior to arrival, during being transported, and possibly after arrival on the Burn Ward. In a few instances, these patients did acquire some of the types commonly encountered in current burned patients. This was particularly noteworthy in their acquisition of D41, which designates 21,68, a common ward inhabitant. Note that only one patient from this group acquired a single NT strain, although NT's were common at the time. Referring to the Figure, the acquisition of the last three types on that listing can be ascribed precisely to the influx of this disaster group of patients. Interestingly, none of these strains have become established in the Ward, on the basis of observations extending into 1966.

Discussion

The phage typing system developed by this laboratory has made it possible to characterize the population of pseudomonads in the Burn Ward and the Renal Branch, and to trace the source of strains recovered in the Physiotherapy Section, where transmission of patients under treatment is a particular risk.

The nontypable component of strains, first recognized

in 1964, continued to be a conspicuous part of the flora. It has not been possible as yet to demonstrate the causal relationship, but the fact that these strains appeared only after sulfamylon therapy was introduced in a large scale, and that such strains have not been seen in populations not being treated with sulfamylon, suggests there may be a causal relationship.

No particular invasive type appeared during this period, but for the first time since this study was established, a single type appeared to have become predominant. This strain, designated 21,68, has been conspicuous in the ward bacterial population for two years. Strains have been distributed over the whole range of severity of infections. There has not, as yet, appeared evidence of any especial virulence in strains of this type. Type 21,68 strains exhibit in the rat model, a complete range of virulence from 100 per cent to zero lethality.

The experience of the admissions from a mass accident, with seven severely burned people admitted six days post-burn, heavily seeded with Ps aeruginosa, was unique in its demonstration of the potential of a Pseudomonas population to maintain its status on seeded individuals despite topical chemotherapy, hydrotherapy, and continuous exposure to a foreign bacterial population. The gradual replacement of the foreign strains by the resident flora of the Burn Ward was recognizable during the latter period of the hospital stay of this group.

References

1. Lindberg, R.B., Latta, R.L., Brame, R.E., and Moncrief, J.A.: A definitive bacteriophage typing system for Pseudomonas aeruginosa. Bact. Proc. 1963, p. 85.

2. Lindberg, R.B., Latta, R.L., Moncrief, J.A., and Brame, R.E.: Further observations on bacteriophage typing of Pseudomonas aeruginosa in burned patients: The effect of sulfamylon therapy on phage type and epidemiology. US Army Surgical Research Unit, Annual Progress Report, 30 June 1965.

Publications

Lindberg, R.B., Latta, R.L., Brame, R.E., and Moncrief, J.A.:

Alterations in Pseudomonas aeruginosa flora and phage type with topical sulfamylon treatment of severe burns. Bact. Proc. 1966, p.65.

Presentations

Lindberg, R.B.: Epidemiology of burn wound infections under treatment with topical sulfamylon. Dedication Symposium, Clinical Center, University of Alabama Medical School, Birmingham, Alabama, July 26, 1965.

GPO 770004

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6392	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"D. CHANGE" 27 08 65	U U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 127

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) The Effect of Sulfamylon on the Electrolyte Pattern of the Acute Burn (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 64	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	c. TYPE NA	PRIOR FY 65	4	15
		CURRENT FY 66	4	16

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas
INVESTIGATORS PRINCIPAL ASSOCIATE: Pruitt, B.A., Maj, MC; Switzer, W.E., Lt Col, MC
TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Sulfonamides, carbonic anhydrase, acidosis

24. (U) Tech Objective - The electrolyte changes in patients treated with topical sulfonamide cream are being studied to delineate the cause of these disturbances and their clinical significance. The extent and severity of electrolyte pattern changes in patients treated with either the hydrochloride salt or the acetate salt of one sulfonamide are being compared to determine whether the acid base changes can be modified by this means.

25. (U) Approach - Pre-application and serial post-application measurements of blood and urine volumes, chemistries and gases are performed in appropriate burned patients. Inability to clearly distinguish or eliminate the coexisting influence of hyperventilation has made interpretation of results in some study patients difficult. Work on this project has been decelerated by a recent marked increase in patient load.

26. (U) Progress (1 Jul 65 - 30 Jun 66) - A group of five patients treated with a hydrochloride salt and a group of seven patients treated with an acetate salt of a sulfonamide have been studied. The blood pH showed similar depression in both groups, but the average of the lowest recorded pH in each group remained in the physiologic range. Serum bicarbonate and blood pCO_2 showed a greater decrease in those treated with the hydrochloride salt. Blood levels of sulfonamide were similar in both groups. Urinary changes showed a marked carbonic anhydrase inhibitory effect which appears to be the principal cause of the "acidosis" observed in both groups of patients. Use of the hydrochloride salt seemed to result in greater disturbance of acid base equilibrium.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

36. EST. FUNDS (In thousands)	35.
(FY + 1)	

DD Form 1 AUG 64 1498

(Items 1 to 36 identical to NASA Form 1127) ARE OBSOLETE. USE PREVIOUS EDITIONS

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECT OF SULFAMYLON ON THE ELECTROLYTE PATTERN OF THE ACUTE BURN

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Basil A. Pruitt, Jr., Major, MC
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.
Walter E. Switzer, Lt. Colonel, MC
James P. Knochel, Major, MC
Charles R. Ritchey, Captain, MSC

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: THE EFFECT OF SULFAMYLON ON THE ELECTROLYTE PATTERN OF THE ACUTE BURN

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Basil A. Pruitt, Jr., Major, MC
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.
Walter E. Switzer, Lt. Colonel, MC
James P. Knochel, Major, MC
Charles R. Ritchey, Captain, MSC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

The electrolyte pattern of the acute burn is modified towards one of metabolic acidosis in burned patients treated with either Sulfamylon hydrochloride or Sulfamylon acetate. * The depression of blood pH from pre-application levels is similar in both groups, with the average lowest pH remaining within the physiologic range. Serum bicarbonate and blood pCO_2 decreased in both groups, with a greater decrease of both noted with Sulfamylon hydrochloride therapy. Blood levels of both treatment agents and para-carboxybenzene sulfonamide were similar.

Urinary changes showed increased urinary pH, decreased titratable acidity and ammonia excretion, and marked increase in urinary bicarbonate excretion consistent with a marked inhibition of carbonic anhydrase. This would appear to be the principal mechanism of the "acidosis" noted in both these groups of patients. The effect, is perhaps enhanced by the exogenous hydrochloride load in the group treated with Sulfamylon hydrochloride.

(1) Sulfonamides (3) Acidosis
(2) Carbonic Anhydrase

* Supplied by Sterling-Winthrop Research Institute, Rensslaer, New York

THE EFFECT OF SULFAMYLON ON THE ELECTROLYTE PATTERN OF THE ACUTE BURN

A preliminary survey of 15 months' experience with the topical chemotherapy of burns using Sulfamylon hydrochloride * revealed that approximately 25 per cent manifested some degree of metabolic acidosis. This was felt to be related to both the exogenous acid load imposed by the hydrochloride salt and the inhibition of carbonic anhydrase by both Sulfamylon and its degradation product, para-carboxybenzene sulfonamide, the latter effect having been demonstrated in vitro.

To further elucidate the clinical importance and the mechanism of development of this acidosis, the chemical and physical changes in the blood and urine consequent to Sulfamylon therapy are being studied. The hydrochloride salt of Sulfamylon is further being compared with the acetate salt in regard to the alterations of the electrolyte pattern of the acute burn. Patients admitted to this unit in the early postburn period, i.e., within five days of injury, with greater than 30 per cent total body surface burns who receive Sulfamylon therapy, have appropriate samples of blood and urine analyzed prior to Sulfamylon application and at 3, 8, 12, 24, 48, 72, 120 and 240 hours postapplication.

Progress

Two groups of patients have been studied. The first group consists of five patients who received twice daily Sulfamylon hydrochloride applications with an average burn of 45 per cent. The second group of seven patients who received similar therapy but with Sulfamylon acetate had an average burn of 53.5 per cent. At the time intervals noted above, blood volume determinations were made, as were measurements of electrolyte levels, pH, blood gases,

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* Supplied by Sterling-Winthrop Research Institute, Rensslaer, New York

creatinine, citrate, osmolality, serum protein levels, and levels of Sulfamylon and para-carboxybenzene sulfonamide. Assays of urine were also made at these times for pH, titratable acidity, ammonia, citrate, sodium, potassium, chloride, urine urea nitrogen, osmolality and bicarbonate levels. Tables 1 and 2 record the average pre-sulfamylon application values within each group for certain selected blood and urine determinations which were felt to show significance when compared to the average highest or lowest (depending upon direction of change) postapplication values which occurred during the 10-day study period.

Consideration of the data revealed a slight decrease in the blood pH in both treatment groups from the pre-application levels, with the lowest recorded pH usually occurring within 24 hours after initiation of therapy. The average of the lowest values recorded was, in both groups, within the physiologic range. The maximum decrease in pCO_2 from pre-application levels noted in both groups occurred earlier in the Sulfamylon hydrochloride-treated group than in the acetate-treated group, and in neither group did the time of maximum depression of pCO_2 correlate well with the time of lowest blood pH.

The highest osmolality recorded showed a similar increase over pre-application levels in both treatment groups, with the highest level generally corresponding with the highest recorded serum sodium level as would be expected.

The average highest serum chloride recorded in each group showed an increase of 11 and 8 mEq/L over the average pre-application level in the Sulfamylon hydrochloride and the Sulfamylon acetate groups respectively. The range in the Sulfamylon hydrochloride group was 111 to 121 mEq/L (average 115 mEq/L) and 105 to 121 mEq/L (average 114 mEq/L) in the Sulfamylon acetate-treated group. The time of maximum chloride elevation in each patient did not correlate well with the time of maximum pH depression. Average serum bicarbonate levels showed a greater decrease following treatment with Sulfamylon hydrochloride than with Sulfamylon acetate. The average decrease was approximately one-third and one-seventh of the pre-application levels in the Sulfamylon hydrochloride and Sulfamylon acetate groups respectively. This bicarbonate decrease bore no consistent relation to maximum pH depression nor to chloride elevation in the acetate group, although the maximum bicarbonate

Table 1. Blood Changes with Sulfamylon

Agent Applied	Average Levels									
	Cl (mEq/L)		HCO_3 (mEq/L)		pH		pCO_2 (mm)		Osm. (mOsm)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Sulfamylon Hcl	104	115	22	14	7.46	7.36	28.7	21.8	276	289
Sulfamylon Acetate	106	114	20	17	7.45	7.34	29.8	24.0	273	294

Table 2. Urinary Changes with Sulfamylon

Agent Applied	Average Levels											
	pH		Titr. Ac. (°)		Ammonia (mg%)		HCO_3 (mEq/L)		Osm. (mOsm)		Cl (mEq/L)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Sulfamylon Hcl	6.7	7.8	8.8	0	61.4	11.2	2.3	72.2	722	864	82	122
Sulfamylon Acetate	5.74	8.0	15.9	0	82.4	13.3	.65	61	634	776	66	69

and chloride changes in the hydrochloride group coincided in three of the five patients.

Maximum serum concentration of Sulfamylon and para-carboxybenzene sulfonamide ranged from 2.0 to 7.3 mg% (average 4.2 mg%) and 2.3 mg% to 4.5 mg% (average 3.4 mg%) respectively in the Sulfamylon hydrochloride-treated patients. In the Sulfamylon acetate-treated patients, the corresponding values were 3.6 to 12.3 mg% (average 5.8 mg%) and 3.5 to 10.5 mg% (average 6.2 mg%). The maximum level in each group and for each substance usually occurred within the first 48 hours after initial application.

Urinary changes revealed a marked carbonic anhydrase inhibition effect which occurred promptly and usually persisted in both groups. This was manifested by an increase in urinary pH; a decrease of titratable acidity reaching the zero level in all cases in both groups; a marked decrease in urinary ammonia excretion with maximum depression occurring at or before 24 hours postapplication, and a marked increase in urinary bicarbonate excretion (35-fold increase in the hydrochloride-treated group and 100-fold increase in the Sulfamylon acetate-treated group).

As was to be expected, the hydrochloride-treated group showed an average maximum increase in urinary chloride excretion of 48 per cent while that of the Sulfamylon acetate-treated group remained essentially at the pre-application level.

Summary

Electrolyte changes in the early postburn period have been noted subsequent to therapy in both a Sulfamylon hydrochloride and a Sulfamylon acetate-treated group of burned patients, with a trend in the direction of metabolic acidosis in both groups. With both Sulfamylon hydrochloride and Sulfamylon acetate, blood pH decreases from pre-application levels although the average of the lowest levels recorded remains in physiological ranges in both groups. The pCO_2, depressed in both groups initially, showed further decrease with both agents, the depression being somewhat greater with the Sulfamylon hydrochloride.

The range and average of serum chloride elevation are quite similar in both groups, but a greater (33% versus 15%) average decrease in serum bicarbonate is noted in the Sulfamylon hydrochloride-treated group. The average highest concentrations of Sulfamylon and para-carboxybenzene sulfonamide are 5.8 mg% and 6.2 mg% respectively in the Sulfamylon acetate-treated group, and 4.2 mg% and 3.4 mg% respectively in the Sulfamylon hydrochloride-treated group.

Urinary changes indicative of a marked carbonic anhydrase inhibition occurred promptly after application in both groups and in general persisted throughout the study period, although in a few patients "escape" was noted at or after 72 hours following initiation of therapy. This urinary effect was manifested by an increase in urinary pH, a decrease in titratable acidity and urinary ammonia excretion, and a marked increase in urinary bicarbonate excretion.

The primary acidifying action of Sulfamylon thus appears to be mediated by the influence of carbonic anhydrase inhibition on renal mechanisms of urinary acidification. In the Sulfamylon hydrochloride-treated group, the exogenous chloride load seems to further depress serum bicarbonate, although in the patients studied there was no significant difference between groups in the average lowest pH recorded.

Publications and/or Presentations

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6961	CSCRD - 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY INPT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 202

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (11) Systemic Absorption of Topical Xylocaine from Second Degree Thermal Burns (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	2

19. GOVT LAB/INSTALLATION/ACTIVITY
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RESP. INDIV.: Kovaric, J.J., Lt Col
TEL.: 8 22 63737

20. PERFORMING ORGANIZATION
NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas
INVESTIGATORS PRINCIPAL ASSOCIATE: Gipson, E.M., Capt, MC; Summerlin, W.T., Capt, MC
TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Xylocaine, Sulfamylon, second degree burns

24. (U) Tech Objective - To determine if this Xylocaine-Sulfamylon cream can decrease pain. To determine the absorption of Xylocaine systemically. To determine how large a second degree burn can be treated.

29. (U) Approach - Plan is to apply this cream to 25-50 second degree burns (small areas) and measure Xylocaine levels of control and post application serum (up to 120 minutes). To measure amount of Xylocaine applied to wound via weighing container before and after applications.

(U) Progress (Jul 65 - Jun 66) - Six patients studied so far show Xylocaine to be absorbed in less than toxic levels systemically and that Xylocaine peak in blood stream is approximately 10-30 minutes post application. Approximately 50-60% of amount Xylocaine applied is being absorbed.

30.

27. COMMUNICATIONS SECURITY	28.	29. DoD CODE	30. BUDGET CODE
☐ a. CLASSIFIED ☒ b. NOT CLASSIFIED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.

DD FORM 1 JAN 66 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: SYSTEMIC ABSORPTION OF TOPICAL XYLOCAINE FROM SECOND-DEGREE THERMAL BURNS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Eddie M. Gipson, Captain, MC
William T. Summerlin, Captain, MC
Walter E. Switzer, Lt. Colonel, MC
John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: SYSTEMIC ABSORPTION OF TOPICAL XYLOCAINE FROM SECOND-DEGREE THERMAL BURNS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Eddie M. Gipson, Captain, MC
William T. Summerlin, Captain, MC
Walter E. Switzer, Lt. Colonel, MC
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Pain in the immediate postburn period is characteristic of second-degree burn. Pain is also associated with application of topical Sulfamylon* to areas of second-degree burn, but not normally in third-degree areas. Intensity and duration of pain varies between patients and with the time postburn. In some individuals, mild sedation is adequate while in others narcotics, even in moderate quantities, leave much to be desired. With this study, an effort has been made to quantitate the absorption of topical Xylocaine from second-degree burns and also to decrease subjective pain caused by the application of Sulfamylon.

The following questions provided the basis for this investigation:

(1) Can Xylocaine-Sulfamylon mixture actually decrease pain?

(2) What is the peak venous blood level of Xylocaine when applied to second-degree burns as a 0.5% concentration in a water-soluble base?

* Supplied by Sterling-Winthrop Research Institute, Rensslaer, New York

3. How large a second-degree burn area can be treated without the appearance of toxic signs secondary to high venous Xylocaine blood levels?

Patients were picked with approximately 10 per cent burns, predominantly second degree, that had no other major complications and had an accessable vein for venous sampling. An accurate history of weight, sex, age, past history, and drug intake was taken. The percentage of burned area was recorded as accurately as clinically possible, and a mixture of 0.5% Xylocaine and 10% Sulfamylon was applied to the burned area. This was followed in 30 minutes with a thick layer of plain 10% Sulfamylon. In spite of the subjectivity and biologic variations present, only patients who had previously complained of severe pain upon application of Sulfamylon were used, and were evaluated for pain relief as accurately as possible by both nursing staff and attending physicians. In the six patients studied to date, analgesia has been considered excellent by gross criteria as stated. There have been no toxic symptoms or signs in these few patients, and sedation has not been observed in doses up to 500 mg of topical Xylocaine. We have considered that the minimum venous level for toxic signs or symptoms to be approximately 5 mica per milliliter. It was pleasing to note that in this series the maximum level was 3 micra per milliliter, which occurred 30 minutes post-buttering. In this small series, it appears that absorption of Xylocaine from second-degree burns falls in a range somewhere between intravenous administration and intramuscular administration of plain Xylocaine without epinephrine. This also indicates that peak blood levels could probably rise above 5 micra per milliliter without causing signs of toxicity.

In summary, it appears that 0.5% Xylocaine and 10% Sulfamylon can provide topical adequate local analgesia without causing cardiovascular or respiratory depression.

(1) Topical Xylocaine
(2) Sulfamylon

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOV'T ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6955	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 196

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (U) Clinical Evaluation of Freeze-Dried and Fresh Frozen Homograft in Burn Patients (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	11 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	c. TYPE NA	PRIOR FY 65	0	0
		CURRENT FY 66	2	4

19. GOV'T LAB/INSTALLATION/ACTIVITY

NAME: Headquarters
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20. PERFORMING ORGANIZATION

NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas

INVESTIGATORS PRINCIPAL ASSOCIATE: Pruitt, B.A., Jr, Maj, MC
O'Neill, J. ., Jr, Capt, MC
TEL. / TYPE

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Homograft, burns, heterograft

24. (U) Tech Objective - Lyophilized homograft and fresh frozen homograft are to be evaluated as materials for covering both second degree and open third degree wounds in burn patients. The advantages of these materials in burn care would be (1) potential ready availability, (2) easy preparation of the material at the user level, and (3) indefinite shelf life.

25. (U) Approach - The lyophilized homograft and the fresh-frozen homograft are to be compared with fresh heterograft, formalinized homograft and fresh homograft with regard to adherence of these materials to the burn wound as used in (1) preparation of the burn wound, (2) "test" material prior to autografting, and (3) coverage of second degree burns. The bacterial count present on the burn wound at time of removal of each material is to be recorded, as is the appearance of the wound beneath each material and its acceptance of autograft.

26. (U) Progress (Nov 65-Jun 66) - Thirty-two patients have had 177 bottles of lyophilized homograft applied to date. Adherence has been somewhat less and bacterial count somewhat higher in areas of freeze-dried homograft application as compared to fresh homograft. The burn wounds, however, appeared identical beneath these two materials. The lyophilized homograft appears clearly superior to both heterograft and formalinized homograft. Freeze-dried homograft, thus, in these preliminary studies appears to be a satisfactory substitute for fresh homograft with regard to preparation of the burn wound prior to autografting and for coverage of second degree burns.

27. COMMUNICATIONS SECURITY	28.	29. GSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CY:	

DD FORM 1 JAN 66 1498 (Items 1 to 36 identical to AMXRA Form 77331. Use previous editions until exhausted.)

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CLINICAL EVALUATION OF FREEZE-DRIED AND FRESH FROZEN HOMOGRAFTS IN BURNED PATIENTS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

15 November 1965 to 30 June 1966

Investigators:

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Paul Lotke, Lt. MC, USNR*
Kenneth Sell, Lt. Commander, MC, USN*
James A. O'Neill, Jr., Captain, MC
Robert B. Lindberg, Colonel, MSC
John A. Moncrief, Colonel, MC
Walter E. Switzer, Lt. Colonel, MC

* From the National Naval Medical Center Tissue Bank, Bethesda, Maryland

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: CLINICAL EVALUATION OF FREEZE-DRIED AND FRESH FROZEN HOMOGRAFT IN BURNED PATIENTS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 15 November 1965 to 30 June 1966

5. Investigators: Basil A. Pruitt, Jr., Major, MC
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6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Lyophilized homograft is being compared with heterograft, formalinized homograft and fresh homograft as a "physiologic dressing" in burned patients. As used for both preparation of the wound bed and as a "test" material prior to autografting, the freeze-dried material shows only slightly less adherence to the wound bed than does fresh homograft but considerably better adherence than either heterograft or formalinized homograft. Bacterial counts at the time of removal of the grafts were lowest beneath fresh homograft but only slightly lower than under the freeze-dried material. The wound beds beneath lyophilized homograft were in general identical to those beneath fresh homograft and accepted autograft equally well at the time of definitive grafting. Similarly, lyophilized homograft appears to be a suitable substitute for fresh homograft as an immediate covering material for second-degree burns.

(1) Homograft
(2) Heterograft
(3) Burns

CLINICAL EVALUATION OF FREEZE-DRIED AND FRESH FROZEN HOMOGRAFT IN BURNED PATIENTS

The use of fresh homograft and heterograft combined with topical chemotherapy in the treatment of burned patients has resulted in virtual elimination of burn wound sepsis and improved survival. With this expanded use of homograft, the supply of homograft frequently fails to keep pace with need. Lyophilized homograft as supplied by the Tissue Bank of the National Naval Medical Center is being evaluated for use in burned patients. The material is being compared with fresh heterograft, fresh homograft and formalinized homograft as applied to burns for:

(1) Debridement of "untidy" wounds immediately after eschar separation.

(2) Promotion of healing of deep second-degree burns.

(3) Temporary, immediate wound coverage following excision.

(4) Coverage of granulation tissue between "crops" of autograft in the larger burns.

(5) "Test" material prior to autografting.

(6) Immediate coverage of superficial second-degree burns.

(7) Coverage of any other open surgical wound.

Comparisons are made between materials applied in alternating strips or on similarly burned extremities in the same patient and expressed as the per cent adherence observed for each material. The appearance of the wound pre- and postapplication is also noted. A comparison of the type of bacteria and the colony count pre- and postapplication of each material is also being made.

Progress

To date, lyophilized homograft has been applied to

32 burned patients. This represents 177 bottles of freeze-dried skin as prepared by the National Naval Medical Center Tissue Bank. It has appeared that the per cent adherence of lyophilized homograft is somewhat less than that of fresh homograft but much better than that of either heterograft or formalinized homograft. The bacterial studies, although incomplete at this time, have shown a one to two "log" greater count beneath the lyophilized homograft as compared to fresh homograft at the time of removal of the grafts from the burn wounds. In general, the wound bed beneath the lyophilized homograft was identical in appearance to that beneath fresh homograft and equally receptive of autograft.

The initial lyophilized material tended to become macerated after 48 to 72 hours, with the epidermis being easily elevated by fluid-filled blebs exposing the dermis to dessication. This has been largely eliminated by use of thicker, more uniform freeze-dried homograft.

When available from the National Naval Medical Center Tissue Bank, fresh-frozen homograft is to be evaluated in similar fashion.

Summary

Lyophilized homograft appears to be an adequate substitute for fresh homograft as used for debridement in preparation of the burn wound, as well as for coverage of second-degree burns. The freeze-dried material further seems to have the advantages of (1) potential ready availability at even smaller installations, (2) easy preparation at the user level, and (3) indefinite shelf life.

Publications and/or Presentations

None.

GPO 779884

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RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 08 6956	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY APT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

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11. TITLE:
(U) Immediate Homografting of Second Degree Burns (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	06 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE b. NUMBER c. TYPE d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YRS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	1	1
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19. GOV'T LAB/INSTALLATION/ACTIVITY
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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Homograft (fresh), second degree burn, evaporative water loss, histopathology

24. (U) Tech Objective - To determine the effect of fresh homograft on 2nd degree burn when applied within the first 48-72 hours post injury. Both a clinical and histologic study.

25. (U) Approach - (1) Weighing patients before and after homografting on metabolic scale to determine actual amount of evaporative water loss. (2) Alpha globulin studies to document immunologic aspects. (3) Clinical and micro photographs to document course of homografting. (4) Biopsies of homograft and unhomografted natural skin areas at regular intervals. (5) Major technical problem (tending to decelerate work) availability of fresh homograft. (6) Histology supports mechanical separation of homograft via reepithelialization.

26. (U) Progress (Jul 65-Jun66) - Seventeen patients studied (10 completely). (1) Decrease pain second degree burn. (2) Increase mobility of burned areas. (3) Decrease evaporative water loss by Y3-Y2. (4) No alpha globulin response. (5) Rate healing.

27. COMMUNICATIONS SECURITY	28.	29. DOD CODE	30. BUDGET CODE
☐ a. CONSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. $ (In thousands)	36.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: IMMEDIATE HOMOGRAFTING OF SECOND DEGREE BURNS. I. CLINICAL FINDINGS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

William T. Summerlin, Captain, MC
Franklin D. Foley, Captain, MC
Lawrence H. Foster, Jr., Major, MC
Elinor Pearson, Major, AMSC
Timothy A. Miller, Captain, MC
Walter E. Switzer, Lt Colonel, MC
John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: IMMEDIATE HOMOGRAFTING OF SECOND DEGREE BURNS. I. CLINICAL FINDINGS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: William T. Summerlin, Captain, MC
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6. Reports Control Symbol: RCS-MEDDH-288(R1)

Medical literature fails to reveal a fair evaluation of the effect of normal fresh homograft upon the healing of the second degree burn; therefore, we desired to study various clinical and histopathologic parameters in evaluation of the efficacy and advisability of this proposed therapy. To date, 10 patients have been completely evaluated and there is a significant and immediate decrease in the subjective pain along with a concomitant and impressive increase in the objectively evaluated amount of early mobility of the burned and homografted extremity. There has been no significant alpha-globulin response in these patients. Evaporative water loss studies have revealed that prehomograft control values are reduced by one-third to one-half in the first 24 hours post-homografting; during hospitalization, these patients are never quite able to decrease to the normal range of evaporative water loss in humans.

There has been an apparent increase in the rate of healing of the second degree burn, such that complete re-epithelialization is accomplished in approximately 14 days (plus or minus three days); unhomografted areas healed completely in approximately 21 days (plus or minus two days).

There has been a decrease in the thickened-reddened appearance of the healed second degree burn along with a reduction of the susceptibility of the healed burn areas to trauma and vesiculation.

Thus, this preliminary study suggests that this homografting process appears responsible for both cosmetic and physiologic benefit. The healing process in these patients has been followed closely with photographs, clinically, and by sequential biopsies.

(1) Homograft (fresh)
(2) Second degree Burn
(3) Evaporative water loss
(4) Histopathology

IMMEDIATE HOMOGRAFTING OF SECOND DEGREE BURNS. I. CLINICAL FINDINGS

A review of the medical literature covering the past decade has failed to reveal a thorough evaluation of the effect of normal fresh homograft upon the healing of a second degree burn wound. This study was undertaken with this in mind.

The question of a strict definition of "immediate" arises. In deciding which second degree burns are too late to be homografted, the decision has been made on a clinical rather than a temporal basis; i.e., the second degree burned patient that has already developed the familiar crust that cannot be easily debrided should be excluded from the study. Otherwise, patients with minimal crust that can be easily debrided or patients with no crust development at all should be included in the study.

The perennial question of determining depth of second degree burn is also a consideration. It is presumed that second degree burns of varying depth respond somewhat differently to immediate homografting. This area of evaluation is most assuredly subject to the weaknesses of quantitative subjective observation.

It was planned that patients with some elements of third degree burns be included in this immediate homografting of second degree burns, for it was not felt that this would interfere with the study of the second degree burn areas.

There was interest also in the effect that early coverage of the second degree burn wound would have on the evaporative water loss through burns of this depth. It is known that each gram of evaporative water removes approximately 0.580 kilocalories of heat from the body.[1] It has been shown that heat production increases as evaporative heat loss and severity of injury increases, and that total body water losses are many times much higher than commonly expected to be by standard replacement formulae.[2] It has also recently been pointed out that the diffusion barrier is destroyed in burned skin, and only partially replaced after the healing of partial-thickness burns.[3]

This project was designed to: (1) study the clinical appearance of second degree burns having healed while

covered with the physiological dressing of homograft and to observe the clinical parameters of subjective pain and objective burn area motility; (2) quantitate pre- and post-homografting evaporative water loss in these patients, as well as alpha-globulin response; (3) follow up the significance of homograft influence on eventual scar formation and the reaction of second degree burn, healed under homograft, to trauma and vesiculation; and (4) evaluate the histopathologic features which characterize the second degree burn covered with homograft.

Methods

Immediately upon admission, all second degree burned patients have been evaluated as possible candidates for this study. If chosen, their pre-homograft evaporative water loss study is begun some two hours post-initial debridement. After this initial four hour weight study, the patients are homografted; the first post-homograft evaporative water loss weight study is then initiated sometime in the first 24 hours post-homograft. In the pre-homograft period, control alpha-globulin blood studies are drawn and are followed on a weekly basis thereafter in the post-homograft period.

The healing process is followed closely with photographs and movies of the pre- and post-homograft period, with particular attention to the clinical appearance of the burned areas. Biopsies of various burned areas with homograft are taken at post-homograft intervals of from 48 to 72 hours through 30 to 60 days. Photomicrographs document the results seen here (see Part II this report, "Histopathological Findings").

Results

Although 17 patients have been included in this study to date, because of various technical and/or patient care complications only 10 studies were completed. Clinically, the patients have shown a great decrease in the amount of pain felt with their second degree burn areas, beginning immediately after homograft application and continuing so until healing occurs. There has also been an impressive increase in the early and continued mobility of homografted extremities with second degree burns. There has been no significant alpha-globulin response in these patients.

The four-hour evaporative water loss studies have been carried out on a Brookline metabolic scale,* which measures to the nearest gram. The patients were studied immediately pre-homografting as a control and were free from water bath or heat lamp variables for at least two hours prior to weighing. They were studied again in the first 24 hours post-homografting, and after this were followed weekly during their hospitalization. Respiratory water loss was considered and measured as a part of the study. Studies in the first 24 hours post-homografting showed that control evaporative water loss values were decreased by one-third to one-half in each of the patients studied. From this point on, the losses gradually decreased with each week's measurement, approaching but never reaching the upper limits of normal human evaporative water loss.

There has been an apparent increase in the rate of healing of the second degree burn treated with immediate homografting. Complete reepithelialization is accomplished on the average at approximately 14 days (plus or minus three days); unhomografted areas healed completely in approximately 21 days (plus or minus two days) on the average. There has been a definite decrease in the thickened erythematous appearance of the healed second degree burn along with a reduction in the susceptibility of the healed burn area to trauma and vesiculation. Followup on several patients has shown that this contrast between homografted and unhomografted second degree areas is most apparent during the first month post-reepithelialization, and becomes less and less detectable clinically as the unhomografted healed second degree areas apparently develop and mature. The only difference seen in those deeper second degree burns studied has been the extra 48 to 96 hours required to complete the reepithelialization and homograft rejection.

In spite of the fact that this is a preliminary report, it can be said that this homografting process appears responsible for both cosmetic and physiologic benefit.

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* Brookline Instrument Company, Brookline, Massachusetts.

References

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Presentations and/or Publications

None.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: IMMEDIATE HOMOGRAFTING OF SECOND-DEGREE BURNS. II. HISTOPATHOLOGICAL FINDINGS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Franklin D. Foley, Captain, MC
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John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: IMMEDIATE HOMOGRAFTING OF SECOND-DEGREE BURNS. II. HISTOPATHOLOGICAL FINDINGS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Franklin D. Foley,Captain, MC
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Timothy A. Miller, Captain, MC
Walter E. Switzer, Lt. Colonel, MC
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

The various histopathological changes which occur in homograft covering partial-thickness burns are described. The microscopic basis for cosmetically superior repair in homograft covered as compared to uncovered second-degree burns is defined and analyzed in relation to earlier biopsies. Immunological phenomenon may be responsible for homograft rejection from second-degree burns in a small number of cases, but definitive studies are required in this regard.

(1) Homografting
(2) Histopathology

IMMEDIATE HOMOGRAFTING OF SECOND-DEGREE BURNS
II. HISTOPATHOLOGICAL FINDINGS

The sequential histopathological features which occur in homograft covering a partial-thickness cutaneous burn have not been previously studied and form the basis of this report.

Material

A total of 32 biopsies from eight patients were available for study. The biopsies were taken at post-grafting intervals from 3 to 38 days and consisted, in various instances, of homograft alone, homograft and recipient together and junctions of homograft covered and uncovered second-degree burns before and after graft separation. Biopsy sections were stained with H&E and a modified buffered Giemsa stain. Two hundred serial sections of selected biopsies, mounted on 35 mm film strip, were studied to follow the direction of regeneration from epithelial appendages in homograft.

Results

Histology of Non-accepted Homograft

Homograft covering partial-thickness burns frequently did not "take", i.e., rapid epidermal repair occurred in the recipient and this undermined the homograft and presumably prevented it from acquiring vascular connections and circulation. In these instances, the homograft dried out and peeled parchmentlike during the first week. Biopsy of this material revealed a mummified split-thickness graft.

The earliest biopsies of recipient at three days occasionally demonstrated epidermal covering of the wound in superficial partial-thickness burns, particularly in children, and in all cases there was some evidence of epidermal regeneration at this time. By two weeks, reepidermidization of all wounds biopsied was complete. Biopsies of uncovered second-degree burns during the first week showed a crust of fibrin, neutrophils and cellular debris, covering a dermis which was infiltrated with neutrophils. The crust and dermal inflammation were absent from adjacent homograft covered burns of comparable depth.

Histology of Accepted Homograft

In other patients with deeper second-degree and focal full-thickness, am t partial thickness injury, the homografts became pink thin two days postgrafting, indicating a "take." Biopsies ring the first week in these instances revealed norm l homograft skin cut through the reticular dermis and closely apposed to recipient. The apposition was readily recognized and adjoining for the most part, but in a few foci, a 5-15 micra, empty or serous filled cleft occurred at the graft-recipient junction. Hyperplasia of homograft basal cells and appendages occurred in biopsies during the first week and regenerating epidermal cells were often found filling the cleft described above. Study of serial sections indicated that the epidermal cells contained in the cleft may originate from the transected ends of homograft appendages as well as from regenerating recipient epidermal appendages. In the former situation, the regenerating homograft epidermal cells covered the undersurface of the graft and occasionally bridged the cleft and covered small portions of the recipient dermis. Also, in a few biopsies it appeared that the cleft was filled with epidermal cells rom both homograft and recipient. The former covered the roof of the cleft where the epidermal regeneration presented a reversed polarity, i.e., keratinization and maturity proceeded inward while the recipient epidermal regeneration covering the base of the cleft retained the usual polarity of keratinization and maturation from basal cells, which proceeded outward.

During the first week the vessels in the homograft dermis were intact and contained normally appearing erythrocytes. In many serial sections studied we could not find vessels crossing the graft-recipient junction.

Histology of Homograft Rejection

At eight days posthomografting, edema, congestion and microscopic perivascular hemorrhage occurred in the homograft dermis. This represented the first histological evidence of graft rejection. Biopsies at 11 to 14 days posthomografting also showed congestion and focal hemorrhage and, in some biopsies, the vessels had undergone fibrinoid necrosis with a perivascular infiltrate of lymphocytes and monocytes.

Histology After Homograft Separation

The most striking histological findings were in the

biopsies at the junction of homograft covered and uncovered second-degree burns, taken two and three weeks after homograft separation. A difference in healing and repair of second-degree burns was found in these biopsies and was also noted in separate paired biopsies of homograft covered and uncovered second-degree burns. The epidermis of the healed, uncovered burn was dysplastic with foci of atrophy alternating with hyperplasia. There was no orderly maturation sequence of epidermis from basal cells towards keratinization. The dermis consisted of young, vascular and edematous connective tissue, evolving from granulation tissue.

In contrast, the healed homograft covered burn had a mature epidermis of uniform thickness with an orderly progressive maturation of epidermal cells. The dermis was composed of normally arranged mature collagen fibers in an organized papillary and reticular dermis.

The latest biopsy available was taken 38 days posthomografting and the epidermis in the previously homograft covered and uncovered areas was indistinguishable. The dermis, though, still consisted of immature connective tissue with an edematous stroma and focal chronic inflammation in the portion of the biopsy which had not been covered with homograft. The dermis in the site which had been covered with homograft consisted of normal collagen fiber maturity and arrangement.

Discussion

The apparent difference in healed, homograft covered and uncovered second degree burns is evident histologically as well as grossly. In general, the microscopic appearance of healed, homograft covered second degree burns resembles normal epidermis and dermis more so than temporally paired biopsies from healed, uncovered second degree burns. The vascular and edematous connective tissue is probably responsible for most of the gross redness and textural abnormality observed in healed, uncovered second degree burns. The immature type of connective tissue just beneath the recently regenerated epidermis apparently evolves from the inflammation and crust formation that was seen in earlier biopsies of uncovered second degree burns. In contrast, a normal pattern of mature dermal collagen bundles is maintained in homograft covered second-degree burns and consequently, when reepidermidization occurs, the tone and texture of normal skin is maintained. Homograft,

therefore, contributes to repair of partial thickness thermal injury by prevention of crust formation and trauma, with its subsequent inflammation and granulation tissue. In addition, regeneration in epidermal appendages of homograft has been observed to cover portions of recipient. If this vital phenomenon also improves repair of partial-thickness burns by facilitating recipient epidermal recovery, then the use of dead, freeze-dried homograft should not yield similar results. Observations and biopsies will be made in this regard.

The structural details of homograft vascularization are obscure. Stereomicroscopic *in situ* observations of vital homograft have demonstrated that circulation occurs, in fact, circulatory arrest within homograft is considered the most reliable temporal indication of homograft rejection.[1,2] Other features of homograft rejection, *e.g.*, discoloration, hemorrhage, or sloughing depend on variables other than the onset of immunological rejection. We were not able to demonstrate vascular communication across the graft-recipient junction in the above patients. Apparently, the homograft is not diffusely vascularized by granulation tissue proliferating in the graft bed. If vascularization occurs focally in partial-thickness injury, it could easily be missed in serial sections but might be readily detected by vascular injection studies with radiopaque media and roentgenograms of homograft and recipient removed en bloc. Studies are underway in this regard.

Numerous histological studies of the homograft reaction in experimental animals are available, but most of these employ full-thickness homograft covering a surgically prepared bed, also excised to full thickness, rather than split-thickness homograft overlying thermally injured skin. Medawar was among the first to describe the histopathology of cutaneous homograft acceptance and rejection.[3,4] He also studied sequential biopsies in a patient who had sustained a large, full-thickness burn which was subsequently covered with pinch homografts.[5] The morphological features of homograft rejection which he described resemble those which occurred in some of our patients. The histological reaction of homograft covering a partial-thickness burn also does not differ markedly from that which occurs in homograft set in an excised wound. Nevertheless, the

presence of viable epidermal appendages transected in the split-thickness homograft and remaining in the dermis of partial-thickness cutaneous burns might be expected to modify certain features in the histopathological sequence as described by Medawar and perhaps alter the interpretation thereof. It is conceivable that because of epidermal regeneration beneath the homograft, the acceptance and rejection of cutaneous homograft in patients with second-degree burns does not represent an immunological reaction in many cases.

Clearly, the rapid peeling of homograft that never acquires a circulation, but adheres briefly to recipient, is the prototype of mechanical rejection. The question concerns those homografts that have acquired a circulation, but are rejected during the second week. The histopathology suggests an immunological phenomenon in those biopsies that demonstrate perivascular hemorrhage with lympho-monocytic infiltration; however perivascular hemorrhage, indicative of circulatory stasis, which is the earliest sign of homograft rejection, has been noted in the absence of a leukocytic infiltrate. Does this reflect humoral rather than cellular antibody effect, or is this the response that occurs when vessels at the graft-recipient junction are narrowed or closed by epidermal recovering of the partial-thickness burn? If a non-immunological basis exists for second-week homograft rejection overlying second-degree burns in some instances, then it should be demonstrable by utilizing burned animals and homograft from inbred rat colonies. A future study is planned in this regard.

Summary and Conclusions

The various histopathological changes which occur in homograft covering partial-thickness burns are described. The microscopic basis for cosmetically superior repair in homograft covered as compared to uncovered second-degree burns is defined and analyzed in relation to earlier biopsies. Immunological phenomenon may be responsible for homograft rejection from second-degree burns in a small number of cases, but definitive studies are required in this regard.

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Publications and/or Presentations

None.

GPO 770864

13

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
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4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	(U) U (WORK)	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B91R 01 166

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (U) Control of Blood Loss from Donor Sites by the Addition of Epinephrine to Subcutaneous Saline Infusions (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE b. NUMBER c. TYPE d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	10

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TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS: Epinephrine, donor sites, hemorrhage, saline infusion

24. (U) Tech Objective - To decrease blood loss from skin donor sites during operative procedures.

(U) Approach - Subcutaneous infusion of saline containing 1 mcg per cc of epinephrine subjacent to skin graft donor sites.

25. (U) Progress (Jul 65-Jun 66) - Patients with extensive thermal injuries who require skin grafting procedures often sustain blood loss from skin donor sites of a magnitude necessitating replacement therapy in the operating room and under anesthesia. To decrease this operative blood loss, the potent vasoconstrictor effects of epinephrine were employed by adding 1 mg of epinephrine to 1,000 cc of normal saline solution infused subcutaneously in anatomical areas utilized as skin donor sites. With this drug concentration, the systemic physiological responses to the drug were minimal, while achieving a marked decrease in donor site bleeding. No postoperative complications which could be attributed to this technique were observed in 35 patients who underwent removal of split-thickness skin grafts for autografting procedures.

27. COMMUNICATIONS SECURITY	28.	29. DDD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
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FINAL REPORT

PROJECT NO. 3A014501871R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CONTROL OF BLOOD LOSS FROM DONOR SITES BY THE ADDITION OF EPINEPHRINE TO SUBCUTANEOUS SALINE INFUSIONS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

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Walter E. Switzer, Lt Colonel, MC
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Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: CONTROL OF BLOOD LOSS FROM DONOR SITES BY THE ADDITION OF EPINEPHRINE TO SUBCUTANEOUS SALINE INFUSIONS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Eddie M. Gipson, Captain, MC
Walter E. Switzer, Lt Colonel, MC
Basil A. Pruitt, Jr., Major, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

Patients with extensive thermal injuries who require skin grafting procedures often sustain blood loss from skin donor sites of a magnitude necessitating replacement therapy in the operating room and under anesthesia. To decrease this operative blood loss, the potent vasoconstrictor effects of epinephrine were employed by adding one mg of epinephrine to 1,000 cc of normal saline solution infused subcutaneously in anatomical areas utilized as skin donor sites. With this drug concentration, the systemic physiological responses to the drug were minimal, while achieving a marked decrease in donor site bleeding. No postoperative complications which could be attributed to this technique were observed in 35 patients who underwent removal of split-thickness skin grafts for autografting procedures.

(1) Epinephrine
(2) Donor sites
(3) Hemorrhage
(4) Saline infusion

CONTROL OF BLOOD LOSS FROM DONOR SITES BY THE ADDITION OF EPINEPHRINE TO SUBCUTANEOUS SALINE INFUSIONS

Epinephrine is probably one of the oldest and most thoroughly studied drugs in present-day usage. Its potency is such that intravenous injection of only a few micrograms per minute produces a marked physiologic response consisting of an increase in systolic blood pressure, a decrease in diastolic blood pressure, vasoconstriction in the skin, increase in heart rate, and a slight increase in respiratory rate and depth. This "fight or flight" phenomenon apparently activates the animal for increased motor activity by vasoconstriction of the skin, vasodilation in muscle, and an alerting effect on the central nervous system. While the intravenous route of administration produces immediate effects, subcutaneous injection results in vasoconstriction which decreases and slows drug absorption. Because of this response, much larger doses of the drug are tolerated subcutaneously than by intravenous administration.

Epinephrine has long been used to decrease operative blood loss from surgical procedures by local injection techniques. In the usual concentration of 1:50,000, there is often a reactive vasodilation in the postoperative period with an associated hemorrhage. Until recently, epinephrine has seldom been used with general anesthesia, since its combination with most anesthetic agents may produce myocardial sensitization and ventricular fibrillation. However, two general anesthetic techniques currently available and compatible with exogenous epinephrine are nitrous oxide-curare and methoxyflurane (Penthrane*).

The present study evolved from two observations: first, that large amounts of split-thickness skin autograft could be obtained from difficult anatomical areas after the infusion of sufficient volumes of saline solution in the subcutaneous tissues to obscure bony landmarks, and second, that the addition of only one mg of epinephrine to 1,000 cc of normal saline solution injected subcutaneously resulted in minimal changes of vital signs while producing a marked vasoconstrictive effect with little tendency for postoperative hyperemia. The combination of these two techniques permitted

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* Abbott Laboratories.

removal of skin grafts from almost any surface area with inconsequential blood loss.

This regimen was used in a series of 35 patients, both children and adults, with no change of statistical significance in the anesthetic records which were monitored for pulse, ventricular arrhythmias, respiration and occasionally blood pressure. Blood loss from the skin donor sites was consistently small, approximating 5 to 10 per cent of that ordinarily expected from split-thickness skin graft excision procedures. In the postoperative period, the donor sites were managed by covering with a single layer of fine-mesh gauze and exposure. No drying lamps were used, to avoid reactive hyperemia and blood loss through the interstices of the fine-mesh gauze. Healing rates of donor sites injected with the epinephrine-saline solution appeared to be identical with those infused with the saline solution alone.

Appropriate photographs of control and treated donor sites are available in the files of the Surgical Research Unit.

Presentations and/or Publications

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6389	CSGRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A, NEW"	U U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A0145018718 01 180	NONE

11. TITLE: (U) Evaluation of the New Cyanoacrylate Tissue Adhesives Prepared by USAMBRL for Fixation of Immediate Homografts on Partial-thickness Burns (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	01 66	NA	Other DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	1

19. GOVT LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME: Headquarters	NAME: US Army Surgical Research Unit
ADDRESS: US Army Medical Res & Dev Command, Washington, DC 20315	ADDRESS: Fort Sam Houston, Texas
RESP. INDIV.: Kovaric, J.J., Lt Col	INVESTIGATORS PRINCIPAL: Foster, L.H., Jr, Maj,MC
TEL.: 8 22 63737	ASSOCIATE: Poni, K.C., M.D.
	TEL.: 512 - CA1 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS: Cyanoacrylates, adhesives, homograft, autograft, donor sites, polymers, monomers

24. (U) Tech Objective - The objectives are to evaluate all possible applications in burn surgery of the new, less toxic cyanoacrylate tissue adhesives prepared by USAMBRL.

25. (U) Approach - Laboratory evaluation was made on split-thickness skin grafts in rats, burn wound sepsis rats and agar plate bacterial sensitivities. Clinical evaluation is progressing with applications as second-degree burn dressing, granulation bed dressing, donor site dressing, homograft dressing, homograft bed adhesive, and autograft suture substitute.

(U) Progress (Jan 66 - Jun 66) - All clinical applications have been made. The only encouraging results are applications as second-degree burn dressing, second-degree wound homograft adhesive, and as a donor site dressing.

26.

27. COMMUNICATIONS SECURITY	28	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
FY + 1	

DD FORM 1498 (Items 1 to 36 identical to NAVY Form 3892) OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EVALUATION OF THE NEW CYANOACRYLATE TISSUE ADHESIVES PREPARED BY USAMBRL FOR FIXATION OF IMMEDIATE HOMOGRAFTS ON PARTIAL-THICKNESS BURNS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

13 January to 30 June 1966

Investigators:

Lawrence H. Foster, Jr., Major, MC
K.C. Poni, M.D. *
Fred Leonard, Ph.D. *
Paul E. Teschan, Lt. Colonel, MC †
Franklin D. Foley, Captain, MC

* From the US Army Medical Biomechanical Research Laboratory, Walter Reed Army Institute of Research, WRAMC, Washington, DC

† From Department of Surgical Physiology, Walter Reed Army Institute of Research, WRAMC, Washington, DC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: EVALUATION OF THE NEW CYANOACRYLATE TISSUE ADHESIVES PREPARED BY USAMBRL FOR FIXATION OF IMMEDIATE HOMOGRAFTS ON PARTIAL-THICKNESS BURNS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 13 January to 30 June 1966

5. Investigators: Lawrence H. Foster, Jr., Major, MC
K.C. Poni, M.D. *
Fred Leonard, Ph.D. *
Paul E. Teschan, Lt. Colonel, MC †
Franklin D. Foley, Captain, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Six cyanoacrylate monomers are being studied in the following applications: (1) as a graft bed adhesive in rats; (2) as a wound dressing in the inoculated burn wound sepsis rat; (3) agar plate bacterial sensitivities; (4) as a sole cover for clinical second-degree burns; (5) as a graft bed adhesive for immediate homografting of second-degree burns; (6) as a topical spray dressing over homografted second-degree burns; (7) as a sole covering over clean granulations; (8) as a graft bed adhesive for homografting clean granulations; (9) as a suture substitute under the overlapped edge of split-thickness skin graft; (10) as a hemostatic, analgesic dressing for donor sites.

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* From the US Army Medical Biomechanical Research Laboratory, Walter Reed Army Institute of Research, WRAMC, Washington, DC

† From The Department of Surgical Physiology, Walter Reed Army Institute of Research, WRAMC, Washington, DC

Preliminary results warrant further clinical evaluation of applications as a sole covering for second-degree burns, as a graft bed adhesive for immediate homografting of second-degree burns and as a hemostatic, analgesic donor site dressing.

(1) Adhesives
(2) Cyanoacrylates
(3) Homograft
(4) Autograft
(5) Donor Sites
(6) Polymers
(7) Monomers

EVALUATION OF THE NEW CYANOACRYLATE TISSUE ADHESIVES PREPARED BY USAMBRL FOR FIXATION OF IMMEDIATE HOMOGRAFTS ON PARTIAL-THICKNESS BURNS

The US Army Medical Biomechanical Research Laboratory has recently synthesized a series of new cyanoacrylates. This study is designed to evaluate these materials as tissue adhesives for the fixation of skin grafts.

Methyl-2-cyanoacrylate is a clear liquid which rapidly polymerizes on contact with moisture. It is marketed by Eastman Kodak as E-910 and by Borden as Ad/Here and is licensed by the FDA for investigational use. There are more than 50 reports in the literature of extensive clinical and laboratory trials of such diverse applications as cornea, dura, bronchial stump, duodenal stump, pancreatic resection edge, liver resection edge, blood vessel anastomoses, intestinal anastomoses, bone grafts and skin wounds.[1-8] Laboratory studies have also included absorption, excretion, breakdown, carcinogenesis, tissue reaction and new methods of application.

Ivalon sponges impregnated with 0.5 cc of C^{14}-labeled E-910 implanted subcutaneously in rats lose 50 per cent of the C^{14} in 30 days and 100 per cent in six months; 46 per cent is excreted in the urine, 5.5 per cent in the feces and 40 per cent in expired CO_2. None was ever recovered from fat, muscle, bone, liver, spleen, kidneys or brain. There is no evidence of carcinogenic activity and no suggestion of bone marrow suppression even when the polymer is injected into the femoral medulla. Local tissue toxicity is, however, a problem, as evidenced by acute inflammation and a thin layer of necrosis adjacent to all brushed on monomer implants. Skin grafts slough where monomer is brushed on the graft bed but not where the monomer is applied over the external graft surface. No necrosis is seen on implants and graft beds, however, when very thin layers are sprayed on. This local tissue toxicity may be due to the heat of polymerization (maximum temperature increase is 4°C.) and/or to the formaldehyde released by water degradation (maximum 27 per cent by weight converted to formaldehyde).

In a search for less toxic monomers, several higher carbon homologues were synthesized at the US Army Medical Biomechanical Research Laboratory. These are ethyl, propyl, butyl, isobutyl, amyl, hexyl, heptyl, octyl, nonyl, and decyl 2 alpha cyanoacrylates. Several of these produce less heat of polymerization, much less formaldehyde (1/20th as much as methyl) and much less tissue reaction. A new method of application was also developed (spray with nitrogen or freon vehicle) which results in a smaller dose in a more even layer than previous dab-on and brush-on techniques. All of these new monomers were sprayed onto 1 cm^2 areas of rat bowel serosa. The rats were sacrificed at weekly intervals and the bowel biopsied, with the finding of acute inflammatory response with methyl, chronic inflammation with ethyl and propyl, and only slight foreign body reaction with butyl, amyl and heptyl. Application of these higher homologues underneath split-thickness skin grafts and homografts resulted in a high incidence of takes with an unexplained survival of some of the homografts for prolonged periods.

Progress

Since receipt of six monomers in January 1966, the following experience has been accumulated:

1. Skin Grafts in Rats

Four rats had split-thickness skin grafts replaced on the dermal donor site after the site was sprayed with the butyl monomer in two of the animals and the propyl monomer in the other two animals. A 50 per cent take resulted, with the remainder of the graft elevated by regrowth of hair in the donor site.

Four rats had split-thickness skin grafts replaced on the donor site after the dermis was excised and the panniculus sprayed with butyl monomer in one, graft edges only with butyl monomer in two, and propyl monomer in one. A 75 per cent take was obtained where the graft bed was sprayed and a 100 per cent take where the edges only were sprayed.

2. Burn Wound Sepsis in Rats

Fifteen rats received a standard 30 per cent third-degree scald wound and were inoculated with Pseudomonas strain SRU 12-4-4(59). Five controls had no cyanoacrylate, five were sprayed with propyl before inoculation and five after inoculation. Four survivors at two weeks included two control animals, one animal sprayed before inoculation and one sprayed after inoculation.

3. Bacterial Sensitivity

Twenty plain agar plates were streaked and six monomers dropped on each plate and read as disc sensitivites at 48 hours as follows:

Five different strains of Stapylococcus aureus
Five different strains of Pseudomonas
Five different strains of Proteus
Five different strains of Aerobacter

Two to 10 millimeter zones of inhibition were seen around the drops, the largest zones associated with the methyl, propyl and octyl monomers.

4. Clinical Applications

a. Immediate spray application of the propyl monomer to two small areas of fresh second-degree burns has been done. One patient reported a mild transient burning sensation; the other said it was painless. There was no gross difference in the healing rate or result as compared to adjacent areas treated by standard methods.

b. Spray application of the propyl monomer beneath a small area of homograft over a fresh second-degree burn in one patient resulted in transient burning, good adherence, and no gross difference in healing rate or result.

c. Topical spray application of the propyl monon over a homografted second-degree burn in one patient did not improve homograft adherence.

d. Spray application of the propyl monomer over a small area of clean granulation tissue in two patients was painless but resulted in superficial necrosis and accumulation of purulence.

e. Spray application of the propyl monomer on a small granulating bed prior to homografting in two patients was painless but resulted in graft necrosis.

f. Dab-on application under overlapped edges of problem areas was done on four homograft patients and four autograft patients, with five poor results and three fair results.

g. Donor site hemostasis was achieved with a thin layer spray of propyl, hexyl, heptyl and octyl in 13 patients, with good to excellent results in all. On adequately covered donor sites, there was no postoperative weeping, pain or limitation of motion as seen with the fine-mesh gauze technique. Separation of the polymer crus frequently took several days longer, but there was no gross difference in the final healed result. Down-side donor sites still tend to become moist and stick to the bedding.

Further experience will be accumulated with application over fresh second-degree burns and under overlapped edges of homografts and autografts; a 16 mm film will be made of the application, healing and result of application to split-thickness skin graft donor sites.

Summary and Conclusions

Six cyanoacrylate monomers are being studied in the following applications:

1. As a graft bed adhesive in rats.

2. As a wound dressing in the inoculated burn wound sepsis rat.

3. Agar plate bacterial sensitivities.

4. As a sole cover for clinical second-degree burns.

5. As a graft bed adhesive for immediate homografting of second-degree burns.

6. As a topical spray dressing over homografted second-degree burns.

7. As a sole covering over clean granulations.

8. As a graft bed adhesive for homografting clean granulations.

9. As a suture substitute under the overlapped edge of split grafts.

10. As a hemostatic, analgesic dressing for donor sites.

Preliminary results warrant further clinical evaluation of applications as a sole covering for second-degree burns, as a graft bed adhesive for immediate homografting of second-degree burns, and as a hemostatic, analgesic donor site dressing.

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4. Jesse, R.H., Anderson, B.C., and Healey, J.E., Jr.: Fixation of split-thickness skin grafts with adhesive. Plast. & Reconstr. Surg. 33:272, 1964.

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Publications and/or Presentations

None.

GPO 770984

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 08 6391	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	RPT U MRK	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 182

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (U) Cause of Death in the Burn Patient (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (in thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	8

19. GOV'T LAB/INSTALLATION/ACTIVITY
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TEL.: 8 22 63737

20. PERFORMING ORGANIZATION
NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas
INVESTIGATORS PRINCIPAL ASSOCIATE: Foley, F.D., Capt, MC
TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Burn, septicemia, respiratory, gastrointestinal, renal failure

24. (U) Tech Objective - A clinico-pathologic mortality study was undertaken to update the major causes of death in the burn patient.

(U) Approach - Clinical summaries, histological sections and autopsy protocols were reviewed prior to classifying burn deaths.

25. (U) Progress (Jul 65 - Jun 66) - Review covered burn deaths between 1960-65 and was completed. Data indicate respiratory disease has replaced burn wound sepsis as major cause of death.

26.

27. COMMUNICATIONS SECURITY	28.	29. DDC CODE	30. SUBMIT CODE
☐ a. COMSEC or COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY 1	

DD FORM AUG 64 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CAUSE OF DEATH IN THE BURNED PATIENT, 1960-1965

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigator:

Franklin D. Foley, Captain, MC

Reports Control Symbol: RCS-MEDDH-208 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: CAUSE OF DEATH IN THE BURNED PATIENT, 1960-1965

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigator: Franklin D. Foley, Captain, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

The autopsy records and histological sections from burn deaths between 1960-1965 are reviewed and the causes of death classified. Respiratory disease has replaced burn wound sepsis as the major cause of death

(1) Respiratory Disease
(2) Septicemia
(3) Burn Deaths

CAUSE OF DEATH IN THE BURNED PATIENT
1960-1965

During 1965, a system for cross-indexing of pathological diagnoses was instituted for all burn autopsies performed at the Surgical Research Unit since 1960. The histologic sections, autopsy protocol and clinical summary were reviewed from each autopsy prior to filing of diagnoses. The review provided a unique opportunity for a clinico-pathological mortality study of burn deaths. The data collected during this period are unique in that it probably represents the largest group of burn deaths studied, consists of autopsies done by pathologists who autopsied burned patients almost exclusively and were therefore experienced in the peculiarities of the burn autopsy, and thirdly, is composed of patients who, because of their youth, died of burns and their complications rather than from burns plus preexistent disease as might be expected in an older age group. The latter feature permits one a more uncluttered view of the pathogenesis of death in the burned patient.

The cause of death, in addition to the burn, is listed in the accompanying table. Although a single cause of death was the desideratum, it will be noted that more than one cause is given in a few instances. In no patient was it found necessary to list more than two causes of death, in addition to the burn.

Discussion

Septicemia has decreased markedly because of the elimination of burn wound sepsis as a major cause of death. Three deaths due to burn wound sepsis are recorded in 1965. One patient was received two weeks post-burn with an established wound infection and died of septicemia. Two plausible origins for septicemia were found at autopsy, viz., small areas of burn wound sepsis and suppurative phlebitis at a cutdown site. Another patient developed sepsis beneath large areas which were covered with homograft. The third patient had infection of an amputation stump in a burned area, in addition

to renal failure. This last patient is similar to one of the three patients who died from burn wound sepsis in 1964, whose sepsis originated from infection of two amputation stumps, both in burned areas, and who also had renal failure listed as a second cause of death. These are not typical cases of burn wound sepsis. The first patient autopsied in 1964 (A-1-64) with burn wound sepsis never received Sulfamylon therapy and the third burn wound sepsis death in 1964 died 24 days after Sulfamylon was discontinued because of allergic manifestations.

The overall decline in deaths due to septicemia may not be so marked as the table indicates since antemortem blood cultures are not taken so frequently in patients with pulmonary complications as they were in patients with suspected burn wound sepsis. More frequent antemortem blood cultures would be helpful in defining the pulmonary complications in our burned patients as infectious lower respiratory disease, especially when combined with postmortem pulmonary bacteriological studies. An amplification of respiratory deaths, including the complete spectrum of pulmonary pathology in the burned patient, is included in a separate report.

Suppurative phlebitis at cutdown sites remains an occasional cause of septicemic deaths. Again, the actual incidence of this complication in the burned patient is grossly underestimated by the mortality table indicating that this life threatening complication is recognized and successfully treated in most patients. Septicemia of miscellaneous origin includes one patient with empyema of the gallbladder and one with a mandibular abscess. Patients who died with septicemia which had been documented clinically and pathologically, in whom necropsy did not disclose a satisfactory origin of infection, were categorized as septicemic deaths of uncertain origin.

Gastrointestinal hemorrhage is the common mechanism of death in patients who die of Curling's ulcers, but one patient in this category died of a perforated duodenal ulcer with peritonitis.

1960 - 1965, CAUSES OF DEATH

	1960	1961	1962	1963	1964	1965
Septicemia, origin, burn wound sepsis	18	15	31	34	3	3
origin, respiratory infection	(3)	(5)	(3)*	(1)*	(2)	(6)
origin, phlebitis	-	1	-	2	1	2
origin, miscellaneous	-	1	1	-	-	-
origin, uncertain	2	3	4	2	-	1
Respiratory disease	5	5	3	6	12	13
Gastrointestinal ulceration	-	-	3	2	2	1
Renal failure	-	-	2	2	2	2
Miscellaneous	1	1	2	8	9	4
Cause of death, uncertain	4	4	9	7	7	11
No. of patients	96	88	142	148	184	159
No. of deaths	30	31	54	57	37	34
% mortality	31.3	35.2	38.0	38.5	20.1	21.4
No. of autopsies	30	30	51	56	34	32
No. of causes of death	30	30	55	63	36	37

( ) already listed under respiratory disease.
* also had other possible causes for sepsis.

Renal failure is listed as a mortal complication in eight patients over the six-year period when it was both a clinical and pathological cause of death. Patients with terminal oliguria, often seen in deaths due to burn wound sepsis, are not included in this group. All deaths attributed to renal failure occurred within nine days postburn and were due to unsuccessful resuscitation in the early postburn period. The mechanism of death was either cardiac arrhythmia with a rapidly rising serum potassium or overhydration in a belated effort to increase urinary output after admission to the Surgical Research Unit.

Additional deaths due to inadequate resuscitation manifested themselves by peripheral vascular collapse and are classified in the miscellaneous category under shock (5). The total deaths in these two groups (13) could be combined and considered as deaths due to inadequate resuscitation.

In the category, cause of death uncertain, are all patients dying within the first few days postburn with pulmonary edema and congestion as the sole finding at autopsy. Many of these patients suffered a cardiac arrest in turning or while being intubated. Also in this group are patients whose death was genuinely a puzzle to both clinicians and to the pathologist although there may have been no lack of pathological diagnoses.

In the miscellaneous category were attributed deaths due to shock in the early postburn period (5), coronary heart disease (4), acidosis (3), adrenal infarcts (2), pancreatitis (2), pulmonary embolus (2), peritonitis (2), cerebral vascular accident (1), hepatic cirrhosis (1), subacute bacterial endocarditis (1), aortic esophageal fistula (1), and retroperitoneal hemorrhage (1).

Summary and Conclusions

A clinico-pathological study of burn deaths between 1960-1965 indicates that respiratory disease has replaced

burn wound sepsis as the major cause of death. Other lethal complications during this period have also been classified. It should be noted that a study of this sort indicates the frequency of mortal complications in the burned patient but tends to minimize nonlethal complications. The latter would not be listed in such data although they might have been directly or indirectly responsible for the subsequent development of the complications to which death was attributed.

Publications and/or Presentations

None.

RESEARCH AND TECHNOLOGY RESUME			2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
				DA OA 6982	CSCRD-103
4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / RMK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER CODE: 61145011 3A014501B71R 01 167

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) Pulmonary Pathology in the Burn Patient (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	2	10

19. GOVT LAB INSTALLATION/ACTIVITY
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Moncrief, J.A., Col, MC
TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Burn, lung, respiratory, pneumonia

24. (U) Tech Objective - More burn patients died of respiratory complications in 1964-65 than in previous years. The present study defines the pulmonary complications.

(U) Approach - Clinico-pathological review of all burn autopsies (233) between 1960-65 was performed and the data analyzed.

25. (U) Progress (Jul 65-Jun 66) - Deaths due to respiratory disease in the burn patient consist mainly of bronchopneumonia with tracheostomy complications a major factor.

26.

27. COMMUNICATIONS SECURITY	28.	29. OBO CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY + 1	

DD FORM 1 AUG 66 1498 (Items 1 to 36 identical to NASA Form 1121? REPLACES DD FORM 613 & 613C WHICH ARE OBSOLETE.)

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: PULMONARY PATHOLOGY IN THE BURNED PATIENT

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Franklin D. Foley, Captain, MC
John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: PULMONARY PATHOLOGY IN THE BURNED PATIENT

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Franklin D. Foley, Captain, MC
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

A clinico-pathological study of 233 burn autopsies between 1960-1965 was performed to define the pathology of the lung in the burned patient and to investigate the recent increase in deaths due to respiratory complications. Respiratory deaths were found to be mainly due to pneumonia and the complications of tracheostomy. The increase in deaths due to pneumonia in 1964-1965 compared to 1960-1963 is statistically significant. The precise factors responsible for this increase are unknown.

(1) Pneumonia
(2) Burn

PULMONARY PATHOLOGY IN THE BURNED PATIENT

In 1964 and 1965 more burned patients died of respiratory complications at the US Army Surgical Research Unit than in previous years. The present report classifies the respiratory pathology in these patients and summarizes the pathological findings in the lungs of all burned patients autopsied at the Surgical Research Unit since 1960.

Material

All autopsy protocols, clinical summaries and tissue sections from burn autopsies between 1960-1965, inclusive, were studied. There were 233 autopsies out of 243 burn deaths during this period, involving 1821 sections taken from the respiratory system, an average of 7.8 per autopsy. The pathological diagnoses in the respiratory system were listed after review of the clinical and gross autopsy data and personal review of all of the sections. Approximately 30 paraffin blocks required recutting and restaining and additional special stains were performed in several cases. The cause of death was ascertained from the clinical and pathological material and in no case was it necessary to list more than two causes of death, in addition to the burn. Major factors contributing to demise, in addition to the cause of death, were arbitrarily listed when they were prominent in the clinical course or of unusual pathological extent.

Discussion

The accompanying table indicates that pneumonia, although annually noted in burn autopsies as the leading cause of respiratory deaths has increased in frequency as a cause of death. In fact, pneumonia has replaced burn wound sepsis as the major cause of death in the burned patient in 1964-1965. In view of the decline in deaths due to burn wound sepsis, the increase in pneumonia as a cause of death might appear to be for a lack of a better cause. This is unlikely since the extent of the

PULMONARY PATHOLOGY, USASRU, 1960-65

	1960			1961			1962			1963			1964			1965		
	TOTAL	COD*	MF**	TOTAL	COD	MF	TOTAL	COD	MF	TOTAL	COD	MF	TOTAL	COD	MF	TOTAL	COD	MF
Pneumonia, airborne with hyaline membrane	7 (2)	4	1	8 (2)	4	1	11 (1)	2	1	13 (2)	6	-	15 (9)	9	-	15 (9)	12	-
Pneumonia, embolic	5			11			25			11			1			1		
Tracheitis, erosive with tracheostomy	17 (14)	1	4	20 (19)	1	1	18 (18)	1		24 (20)			13 (13)	2	1	17 (15)	1	
Pulmonary thromboemboli, non-infected with infarction	13 (6)	1	.	9 (2)			8 (2)			16 (1)			5 (3)	1		8 (1)		
Pneumothorax tracheostomy complication spontaneous, traumatic or post-thoracentesis	2 (1) (1)			3 (3)			2 (2)			2 (1) (1)			2 (2)	1		2 (1) (1)		
Preexistent pulmonary disease	2			3						2			6		1	3		1
Miscellaneous: Pulmonary edema & congestion Atelectasis Pulmonary megakaryocytes etc. - see discussion	15 3			20 9			32 5 11	?1	?1	42 1 13		?1	26 2 2			22 11		

*COD - Cause of death
**MF - Major factor

pulmonary inflammatory response necessary to assign it as a cause of death appears to have remained the same over the years. In corollary, the pathological diagnosis of small foci of acute bronchopneumonia is still made as an incidental finding of no major importance in some 1964-1965 autopsies as it was in autopsies during 1960-1963. Also, the increase in pneumonia as a cause of death in 1964-1965, compared with 1960-1963, in reference to total deaths during each period is statistically significant ($p < 0.001$).

The pathological features in lungs from burned patients who have died of pneumonia are essentially unchanged over the years and except for extent of involvement, the histopathology is also similar in those patients in whom pneumonia was an incidental autopsy finding. In almost all instances, the pulmonary response was bronchopneumonic with occasional examples of lobar pneumonia. All such deaths presumably developed via airborne infection as the histopathology was not that of embolic pneumonia as described below. Bacteria were isolated in significant amounts from quantitative postmortem cultures of the lower respiratory system in all instances, when searched for. The cultures yielded more than one organism as a rule, suggesting a mixed infection. The common pathogens isolated from patients dying from lobar or bronchopneumonia were in the coliform group, with Aerobacter species predominating; however Pseudomonas species were also commonly isolated. The increase in hyaline membrane formation, which has been almost invariably associated with inflammatory pulmonary disease is currently under study but is thought to be due to more frequent use of positive pressure ventilation with oxygen.

Although the mortality table reveals that pneumonia is responsible for the increase in respiratory deaths in the burned patient during 1964-1965, it does not disclose the reasons for the increase. It appears from the clinical sequence of events that many patients who succumb with pneumonia have experienced hyperventilation, due to the hyperchloremic acidosis of Sulfamylon therapy

for a day or more prior to the development of infectious lower respiratory disease. Tracheostomy and prolonged hyperventilation may quite plausibly increase the susceptibility of the lung to infection by dehydration of the mucus blanket and consequent ciliary inefficiency. In fact, however, the precise reasons for the increase in deaths due to pneumonia in 1964-1965 are unknown.

No deaths are attributed to embolic pneumonia, but rather to the original complication which produced hematogenous dissemination of infection to the lung, e.g., burn wound sepsis or suppurative phlebitis. Due to the decline in progressive burn wound sepsis, there has been a proportionate decline in embolic pneumonia. Embolic bacterial pulmonary disease presents a unique pathological picture with discrete subpleural hemorrhagic lesions usually swarming with bacteria. Pseudomonas species were commonly isolated from these areas in large numbers. Embolic pneumonia was found in one patient in 1964 who had progressive bacterial infection of burned amputation stumps. One patient in 1965 had multiple embolic pneumonic foci which were classified of uncertain origin, although the source was probably an infected phlebotomy site. Except for these isolated instances, embolic pneumonia has virtually disappeared from our autopsied patients.

Erosive tracheobronchitis is almost invariably a tracheostomy complication initiated by abutment of the tracheostomy tube on the anterior tracheal wall inferior to the tracheostomy stoma. Over the six-year period it has been a direct cause of death in six instances. In two patients, the erosion was deeply invaded with bacteria with peritracheitis and septicemia presumably on this basis. One patient developed mediastinitis via bacterial invasion of the eroded trachea. One patient had diffuse invasive bacterial tracheobronchitis which originated in the tracheal ulcer. Two others died with massive hemorrhage from the anterior tracheal erosion. Tracheostomy has also been indirectly responsible for other deaths, although not listed as a cause of death in the accompanying table, for example, patients who

die of pneumonia, subsequent to temporary obstruction of a main stem bronchus by the tracheostomy tube earlier in their course, or patients who develop cardiac arrest during tracheostomy or who develop pneumothorax as a tracheostomy complication. In the latter instances, demise may be directly related to the pneumothorax or to the subsequent development of pneumonia. Also, bacterial infection of tracheal erosions might conceivably represent a nidus for the airborne dissemination of lower respiratory infection. In any case, the hazards of tracheostomy in the burned patient are grossly underestimated by the accompanying table.

The frequency of tracheobronchitis in the burned patient is partly responsible for the controversy regarding inhalational injury. Tracheobronchitis in our autopsies has been focal in distribution and almost invariably associated with tracheostomy. In those patients who have extensive tracheobronchitis at autopsy, different prosectors, experienced in the burn autopsy, have consistently noted the normality of the respiratory tract above the tracheostomy stoma. In the small percentage of cases who had tracheobronchitis, without tracheostomy, the lesions retained their focal character and histopathologically resembled the lesions which were associated with tracheostomy. Erosive tracheobronchitis in these few patients without tracheostomy is presumably due to the trauma of endotracheal suctioning or intubation. For the above reasons, the hypothesis of inhalational tracheobronchitis incurred at the time of thermal injury is not supported by our pathological observations, nor is it controverted by these same observations.

Pulmonary thromboemboli are commonly found in lungs of burned patients at autopsy; however they are usually small, often microscopic, and few in number in relation to the total cross-sectional area of the pulmonary vasculature. In 1960, one patient expired with massive hemoptysis from a large pulmonary infarct following major thromboemboli, and main pulmonary artery thromboemboli without infarction were thought to be a direct cause of death in a single burned patient in 1964. These two patients were not classified as dying from a respiratory disease.

It will be noted from the table that very few burned patients develop pneumothorax either from chest trauma, post-thoracentesis, or on a spontaneous basis. Pneumothorax in patients received at the Surgical Research Unit is most frequently the result of a tracheostomy performed too low in the neck. The single patient who died directly from bilateral pneumothorax was a 30-month-old child whose tracheostomy, which had been performed elsewhere, was between the seventh and eighth tracheal rings. He, like others, was initially felt to be suffering pulmonary complications of thermal injury per se. Many more patients, especially children, are seen at autopsy with mediastinal emphysema since this is the avenue for the subsequent development of pneumothorax. Mediastinal emphysema is easily missed at autopsy unless carefully searched for, since removal of the anterior chest cage, as routinely practiced in postmortem exposure of the thorax, customarily introduces air into the soft tissue of the anterior mediastinum. In fact, artifactual mediastinal emphysema is always present when the anterior chest cage is so removed. A careful examination following lateral exposure is necessary for the necropsy diagnosis of mediastinal emphysema. It may also be noted that the autopsy diagnosis of pneumothorax also requires special care, either by direct postmortem manometric measurements or simply by incising the parietal pleura under a small pool of water. These complications of tracheostomy, therefore, may be more frequent at autopsy than realized.

Few of our burned patients have pre-existent pulmonary disease at autopsy and it has been a significant factor in mortality in only two patients over the six-year period. One patient was 61 years old, with congestive heart failure secondary to coronary heart disease, in whom a pneumonectomy had been performed for bronchogenic carcinoma. His opposite lung was emphysematous with acute tracheobronchitis and metastatic carcinoma was present in the periaortic lymph nodes. The second patient was 75 years old and died of coronary heart disease with a recent myocardial infarct and also had acute bronchopneumonia superimposed on chronic bronchitis and emphysema. The problems in evaluating burn concepts

in these patients are obvious since their clinical course and demise are too frequently multifactorial.

Pulmonary edema and congestion, by far the most common autopsy finding in the lungs of burned patients, is a meaningless diagnosis in itself. It frequently is the only finding in patients with extensive burns who die in the early postburn period. No effort was made to isolate examples of overhydration, renal failure, cardiac arrest with or without arrhythmias, or other clinical events that might plausibly relate to pulmonary edema and congestion since retrospective autopsy material is incomplete and arbitrary in this regard and since pulmonary edema and congestion may develop rapidly as an agonal phenomenon. Atelectasis is another autopsy diagnosis of uncertain significance since the normal state of the lung at autopsy is one of deflation when the pleural cavity is opened. The diagnosis of "congestive atelectasis" would appear to be one of composite incomprehension.

Pulmonary megakaryocytes are found in increased numbers in burned patients' lungs. This probably reflects the general posttraumatic response in which megakaryocytic mobilization from bone marrow is increased and entrapment in the pulmonary capillaries is an accepted phenomenon.

Thus far, the important pathological diagnoses in the lungs from burn autopsies performed at the Surgical Research Unit over a six-year period have been outlined. Other features mentioned below could not be evaluated or were thought not to be of any unique interest, e.g., pulmonary septal macrophages are prominent in almost all lungs of burned patients; however this response is probably too nonspecific to warrant any meaningful interpretation. The frequency of pulmonary fat emboli was not evaluated since the tissue available was paraffin embedded and had therefore been processed through fat solvents. A few instances of incidental microscopic bronchiolitis were uncovered. Focal pleuritis overlying pneumonic areas and pleural fibrosis were simply recorded. Some patients had minor foci of granulomatous pulmonary disease

in various stages of repair, and these were considered irrelevant. Other findings, e.g., pleural effusion and focal intra-aveolar hemorrhage apparently relate to pulmonary edema and congestion and were not individually analyzed.

Summary

A clinico-pathological study of 233 burn autopsies between 1960-1965 was performed to define the pathology of the lung in the burned patient and to investigate the recent increase in deaths due to respiratory complications. Respiratory deaths were found to be mainly due to pneumonia and the complications of tracheostomy. The increase in deaths due to pneumonia in 1964-1965 compared to 1960-1963 is statistically significant. The precise factors responsible for this increase are unknown.

Publications and/or Presentations

None.

GPO 710804

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6985	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 01 170	NONE

11. TITLE:

(U) Massive Adrenal Hemorrhage following Thermal Injury (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	03 66	NA	Other DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	c. TYPE NA	PRIOR FY 65	0	0
		CURRENT FY 66	0	1

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS

Adrenal, burn, shock

24.

(U) Tech Objective - Describes unusual complications leading to death in the burned patient from data accumulated on autopsy material from 1959-66 with view to improvement in burn mortality.

(U) Approach - Study was clinico-pathological correlation concerning a unique cause of death in the burn patient.

25.

(U) Progress (Jul 65 - Jun 66) - Reporting interval covered 1959-66 from which period only four instances of death due to massive adrenal hemorrhage were observed. Report was initiated 1 March 66 and review completed 15 April 66.

26.

27. COMMUNICATIONS SECURITY	28.	29. DDC CODE	30. BUDGET CODE
☐ a. CLASSIFIED COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY 1	

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(Items 1 to 36 SECURITY CLASSIFICATION. USE UNCLASSIFIED WHERE POSSIBLE. IF CLASSIFIED, USE CODES AS PRESCRIBED IN AR 380-5. TYPE UNDER EACH ITEM. COMPLETE ALL.)

FINAL REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: MASSIVE ADRENAL HEMORRHAGE FOLLOWING THERMAL INJURY

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Franklin D. Foley, Captain, MC
Basil A. Pruitt, Jr., Major, MC

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: MASSIVE ADRENAL HEMORRHAGE FOLLOWING THERMAL INJURY

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Franklin D. Foley, Captain, MC
Basil A. Pruitt, Jr., Major, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

Over a six-year period there have been four instances of massive adrenal hemorrhage in 238 burn autopsies. The clinical and pathological features in these patients are described and discussed in relation to adrenal apoplexy in the non-burned patient. In none of the four patients was the diagnosis suspected during life.

(1) Adrenal
(2) Burn
(3) Shock

MASSIVE ADRENAL HEMORRHAGE FOLLOWING THERMAL INJURY

Small foci of adrenal hemorrhage and necrosis are frequently found at autopsy in the burned and non-burned patient. This is not customarily thought to be of any functional significance in view of the limited extent of the lesions and the magnitude of functional adrenal reserve. Also, these microscopic lesions may be interpreted as an agonal phenomenon and again of no essence. Massive adrenal hemorrhage and necrosis is a rare cause of death in the burned patient. Since 1959 there have been four patients whose demise was thought to be due to acute adrenal cortical insufficiency on this basis.

Case No. 1

A nine-year old white female sustained 78.5 per cent total, 65 per cent third degree, flame burns while attempting to light a wood stove with kerosene. Resuscitation was satisfactory and oral fluids were begun on the third postburn day (PBD). On the fifth PBD she became febrile, and a blood culture yielded Aerobacter-Klebsiella. She became oliguric and hypotensive in spite of intravenous Levophed and died on the fifth postburn day. At autopsy the combined adrenal weight was 12 gm. Most of the adrenal medulla and cortex was grossly necrotic, and microscopically there was prominent separation of cortical cords with hemorrhage. Many necrotic foci were infiltrated with neutrophils and the medulla was densely infiltrated with lymphocytes, plasma cells and eosinophils. No adrenal thrombi were noted. No infection of the burn wound was found.

Autopsy Cause of Death

1. 78.5 per cent total body burns, 65 per cent third degree.

2. Adrenal cortical hemorrhage and necrosis, diffuse, bilateral.

3. Pulmonary edema and congestion.

Case No. 2

A 49-year old white female sustained 67.5 per cent total, 36 per cent third degree, flame burns after a butane explosion. Resuscitation was satisfactory; on the fifth PBD she became hypothermic and intermittently remained so until her demise. Blood cultures 8 and 12 days prior to death yielded Staphylococcus aureus and Flavobacterium aquatile, respectively, but these were believed to be contaminants. Abdominal distention occurred terminally and she became oliguric and disoriented and expired on the 20th PBD. At autopsy the adrenals together weighed 16 gm and the left adrenal artery was occluded by thrombus. Both adrenals were the site of large areas of pale necrosis alternating with hemorrhage. Microscopically, multiple foci of hemorrhagic infarction were found with recent thrombus in the central adrenal vein, bilaterally. No burn wound infection was found.

Autopsy Cause of Death

1. 67.5 per cent total body burns, 36 per cent third degree.

2. Adrenal insufficiency due to adrenal infarction, focal, extensive, bilateral.

Case No. 3

A five-year old white female sustained 40.5 per cent total, 28 per cent third degree, flame burns when her clothes ignited from an open heater. Her initial course was excellent, except for a single staphylococcal bacteremic episode presumably related to an infected cut down. Shortly thereafter, her wounds deteriorated and multiple blood cultures yielded Pseudomonas aeruginosa. She pursued a septic course and expired in the 30th PBD. At autopsy the left adrenal weighed 11 gm and the right, 10 gm. Diffuse subcapsular and medullary hemorrhage was noted grossly. Microscopically, there was virtually complete cortical necrosis and infarction without bacteria. Fibrin thrombi were noted in the subcapsular sinusoid and pericapsular vessels. The central vein was free of thrombus. Invasive burn wound infection was found at autopsy.

Autopsy Cause of Death

1. 40.5 per cent total body burns, 28 per cent third degree.

2. Burn wound sepsis.

3. Adrenal cortical necrosis, diffuse, bilateral.

Case No. 4

A 51-year old white male sustained 43 per cent total, 24 per cent third degree, flame burns while attempting to light a wood stove with kerosene. His resuscitation and early course were satisfactory until the fifth PBD when cellulitis developed in the right leg. Shock and oliguria occurred rather unexpectedly 24 hours prior to death and he expired in the 10th PBD. At autopsy the right adrenal weighed 30 gm and the left, 19 gm. There was interstitial pericapsular and diffuse medullary hemorrhage, particularly on the right. A 1 mm rim of cortex was visible. The central vein within both adrenals was occluded with recent thrombus. Microscopically, there was diffuse cortical and medullary necrosis with extensive hemorrhage bilaterally. Fibrin thrombi were noted in the capsular vessels. No bacteria were found in the adrenal sections. There was no burn wound sepsis and the cellulitis was resolving.

Autopsy Cause of Death

1. 43 per cent total body burns, 24 per cent third degree.

2. Adrenal hemorrhage and necrosis, diffuse, bilateral.

Discussion

The gross and histopathological features in the four patients reported above, including the patient with obvious burn wound sepsis, are not those found in the Waterhouse-Friederichsen syndrome. They represent, instead, the lesion referred to as adrenal infarction, massive intra-adrenal hemorrhage, adrenal hematoma or adrenal apoplexy.

Greendyke[1] recently collected 33 cases of gross intra-adrenal hemorrhage from the records of 21,000 autopsies performed over a 38-year period. This fatal complication usually occurred in patients with a variety of preexistent disease, including cardiovascular disease (6), perforated viscus with peritonitis (7), pneumonia (3), malignancy (4), renal failure (3), and severe trauma (7). Sepsis may have been present in 12 patients and six patients had undergone major surgery prior to their death. Other reports also point out the association of adrenal apoplexy with infections, trauma, pregnancy, ACTH, and anticoagulant therapy.[2-7]

To this list may be added the four patients with gross intra-adrenal hemorrhage described above. Sevitt reported two of 82 burned patients who died with extensive adrenal hemorrhage and necrosis at the Birmingham Accident Hospital.[8] The infrequency of this occurrence in our experience parallels Sevitt's since the four patients presented herein are collected from 238 consecutive burn autopsies at the Surgical Research Unit, since 1959. The 50-year-old observation of Weissketten that focal adrenal hemorrhage and necrosis is common in burn fatalities is not in disagreement with our observations concerning the infrequency of extensive adrenal hemorrhage.[9]

The clinical course in patients with gross adrenal hemorrhage has been described and is consistent with acute adrenal insufficiency. In a review of 24 patients (one with burns), abdominal pain was the most frequent complaint, occurring in the epigastrium, upper quadrants, generalized or lumbar, in that order of frequency, usually accompanied by tenderness and spasm.[10] Pallor, prostration, cyanosis and hypotension were commonly present. In contrast to the Waterhouse-Friederichsen syndrome, purpura is usually absent. The hemorrhage usually involves both adrenals, however, it may be unilateral. In two patients with unilateral disease, adrenalectomy was performed with eventual recovery.

Pathologically, in addition to the necrosis and hemorrhage, thrombus may be found in the adrenal arteries or vein, but is not a consistent finding. The hemorrhage may infiltrate the perirenal and retroperitoneal fat and in rare instances intraperitoneal hemorrhage may occur. The intra-adrenal hemorrhage may be extensive, as up to 1000 ml has been described with adrenal remnants contained therein.

No bacteria have been found in adrenal sections of patients who died with adrenal hemorrhage of this type.

The four patients described were not known to have sustained direct trauma in the region of the adrenals nor was there evidence of a general hemorrhagic diathesis. The clinical and pathological features were not those of the Waterhouse-Friederichsen syndrome and there was no support for the necropsy diagnosis of vasculitis or the generalized Schwartzman reaction.

Summary

Over a six-year period there have been four instances of massive adrenal hemorrhage in 238 burn autopsies. The clinical and pathoiogical features in these patients are described and discussed in relation to adrenal apoplexy in the non-burned patient. In none of the four patients was the diagnosis suspected during life.

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Presentations and/or Publications

None.

GPO 770804

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6385	CACRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 01 176	NONE

11. TITLE:
(U) Alterations of the Immune Response Following Severe Thermal Injury (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	09 64	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	1	4
		CURRENT FY 66	3	20

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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TEL. 512 - CAI 2720 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS: Burns, immunity, serum protein, immunodiffusion, tetanus, immune response, electrophoresis

24. (U) Tech Objective - To study immunological lesions that develop following severe thermal injuries. Subordinate objectives were a study of serum protein changes in burns with and without infection and development of a new simple double-diffusion technic in agar gel for quantitation of tetanus antitoxin levels.

25. (U) Approach - Primary and secondary antigenic response was investigated in adult patients sustaining greater than 30% total body surface burns and in experimental animals. The gel-diffusion method for measurement of tetanus antitoxin was developed to monitor the changes in tetanus antitoxin levels in burned patients. Multiple serum electrophoretic studies were done, in both patients and experimental animals, to determine what influence infection had upon the changes following thermal injury.

26. (U) Progress (Jul 65-Jun 66) - An immunodiffusion technic for quantitation of tetanus antitoxin levels in patient sera has been developed which should find wide usage in commercial drug houses and in investigative centers. It has been shown that alterations produced by infection contribute significantly to the changes in electrophoretic pattern found following thermal injury, but since infection exists in nearly every burn wound, electrophoresis has little clinical application. The most important finding was the demonstration of a depressed primary immune response to heterologous erythrocytes in both experimental animals and in patients. This has been related to time of administration of the antigen following burn injury and also to the size of burn injury. Further investigations are being carried out to determine if the nature of the antigen is critical and why such a deficit exists. The finding that the host is unable to cope with cellular antigen may largely explain the increased susceptibility of burned individuals to bacterial infection.

27. COMMUNICATIONS SECURITY	28.	29. DDC CODE	30. SUMMARY CODE
☐ a. CONSEC OR CRYPTO RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
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PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: ALTERATIONS OF THE IMMUNE RESPONSE FOLLOWING SEVERE THERMAL INJURY

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

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The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

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ABSTRACT

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This series of experiments has been performed to study the alterations of the immune response following severe thermal injury since such alterations may play an important role in the increased susceptibility to infection which follows burn injury. The anamnestic response to an antigenic stimulus, in this case tetanus toxoid, was found to be of greater magnitude in patients with severe burns than in normal individuals: the average titer rose to a much higher level, and the peak occurred four days earlier. Tetanus antitoxin levels in two patients who did not receive a booster injection of toxoid tended to parallel their gamma globulin levels. In the burned patients given a primary antigenic stimulus, a deficient response occurred which was reflected in an inability to produce humoral antibodies against injected erythrocytes from Cayman sclerops and also in a prolongation of the survival time of skin homografts which were allowed to remain in place to the time of rejection.

Several studies in experimental animals were made to obtain a clearer understanding of the immune deficit to a primary antigenic stimulus. It was found that a third degree burn involving 20% of the surface area of a rat failed to produce a significant inhibition in the response to injected human erythrocytes. When the size of the burn was increased to 30% however, a substantial inhibition of the production of specific humoral antibody was found. The greatest inhibition of the immune response was seen when the antigen was given one and four days

following thermal injuries with lesser, but nevertheless significant, degrees of inhibition of the response seen when the antigen was given one, two,and three hours following injury. It was found that the production of severe infection did not create a diminished response to the injection of erythrocytes in the animals with 20% burns. The site of the immunologic lesion produced by thermal injury has not been determined by these studies and could lie anywhere from the process of phagocytosis to the coding process for antibody synthesis.

The impairment of the ability to produce specific antibodies to a primary antigenic stimulus is felt to be a significant contributing factor to the development of septic complications in severely burned patients.

(1) Burns
(2) Immunity
(3) Tetanus

ALTERATIONS OF THE IMMUNE RESPONSE FOLLOWING SEVERE THERMAL INJURY

Severe thermal injury results in a susceptibility to infection which continues to be the greatest single cause of death in those patients surviving the initial shock phase. Numerous conditions may contribute to this susceptibility such as the presence of an open, contaminated wound which serves as a pabulum for bacterial growth, increased metabolic requirements with a decreased nutritional intake, loss of plasma proteins into the burned area, and suppression of the patient's defense mechanisms. The last of these possibilities has been the object of several studies within the past few years, but as yet the problems have been incompletely explored.

This investigation was undertaken to delineate some of the alterations of the immune response following severe thermal injury in both patients and experimental animals. For this purpose, the development of specific antibodies to both primary and secondary antigenic stimuli was studied in patients who had sustained greater than 30% thermal injuries, and the response to a primary antigenic stimulus was studied in the burned experimental animal.

Materials and Methods

The patients used for these studies were all treated at the US Army Surgical Research Unit, Fort Sam Houston, Texas, although their initial therapy was usually given elsewhere.

Adult, male Sprague-Dawley rats were used for all of the studies in Experiments IV - VIII except in Experiment VII where female rats of the same strain were used. The bacteria for contamination of the burn wounds and subcutaneous inoculation were prepared from a single strain of Pseudomonas aeruginosa by incubation of an inoculated tube of trypticase soy broth for 18 hours. Standard full-thickness burn injuries were produced on the backs of the experimental animals with the use of a mold by immersion of the clipped area to be burned for 10 seconds in water heated to 100° C. This technic is described in detail elsewhere.[1]

All agglutination studies were done with doubling or tripling dilutions of sera and saline washed and suspended erythrocytes from the appropriate species using standard technics.

Experiment I. The anamnestic response to an injection of tetanus toxoid was measured in 23 patients and eight volunteers utilizing the method of quantitative immunodiffusion for tetanus antitoxin levels described by Alexander and Moncrief.[2] All of the patients had had a full course of immunization with tetanus toxoid with the last booster being given from a period of three weeks to 11 years prior to the time of burn injury (average 3.34 years). Their average age was 29.6 years, and the average size of their burn was 51.4% with 24.2% third degree. Each patient who received tetanus toxoid at our institution was given a single injection of 3.5 Lf (0.5 ml) subcutaneously. Approximately the same dose was given to patients first seen at other institutions.

Eight normal adult volunteers, all of whom had been immunized previously for tetanus and who had received their last booster injection of toxoid ranging from three months to five years previously (average 2.6 years), were given a single injection of 3.5 Lf of tetanus toxoid subcutaneously. Their average age was 28.6 years.

The measurement of tetanus antitoxin levels was made at daily to biweekly intervals following the booster injection, and both patients and control subjects were followed for a period of at least 20 days or until the time of death, which occurred sooner in four patients. Approximately 400 individual immunodiffusion tests were used to determine the tetanus antitoxin levels for both groups of individuals.

Serial tetanus antitoxin and gamma globulin levels were measured in two additional immunized patients with 40% and 18% burn injury from whom the booster injection of tetanus toxoid was withheld. Antitoxin levels were also measured in three patients who had received no prior immunization but who were given 3.5 Lf of tetanus toxoid upon admission.

Experiment II. The primary immune response to alligator erythrocytes was studied in 12 patients and eight normal adult volunteers who served as controls. Ten of the patients and six of the control individuals received a single injection of 1.0 ml of a saline suspension of washed erythrocytes from the species Caiman sclerops, and two patients and two control individuals received an injection of 1.0 ml of a saline suspension of washed erythrocytes from the species Alligator mississippians. The

patient and control groups were run in parallel, and agglutinin titers were determined daily for a period of three weeks following the injection. The patients receiving Cayman cells had burn injuries ranging from 31% to 65%, and the two individuals who received cells from Alligator mississippians had burns of 30% and 71%, respectively. Each was given the antigen from the second to the 27th postburn day, usually during the first week following injury.

Preinjection samples showed that no agglutination occurred with cells from either Alligator mississippians or Cayman sclerops, but full strength sera from both the patients and the controls had slight hemolytic activity on the cells of Alligator mississippians.

Experiment III. Skin homografts with good takes were allowed to remain until rejected in five patients because of lack of available donor areas in these individuals. The period midway between the beginning of rejection and its completion was taken as the rejection time.

Experiment IV. This experiment was performed to determine whether or not burn injury, burn plus severe infection, or severe acute infection by itself would result in a diminution of the response to a primary antigenic stimulus given prior to the injury. For this purpose, the experimental animals were divided into four groups.

Group A had 21 animals who served as controls. Three animals were sacrificed at the time of burn and/or infection in the other groups, and three were sacrificed every other day following that time.

Group B had 18 animals who received 20% third degree burn injuries. Three animals were sacrificed every other day starting on the second day following the burn injury.

Group C had 18 animals who received 20% third degree burns, and their burn wounds were seeded with 0.1 ml of an 18-hour broth culture of a single strain of Pseudomonas aeruginosa, SRU 12-4-4(59), approximately 10^8 organisms. Three animals were sacrificed every other day beginning on the second day.

Group D had 18 animals who were not burned, but an abscess was created in each animal by the subcutaneous inoculation

of an 18-hour broth culture of Pseudomonas, SRU 12-4-4(59). Six animals received 0.5 ml of the culture, six received 1.0 ml, and six 1.5 ml. One animal from each subgroup was sacrificed every other day beginning on the second day.

All of the animals were given an antigenic stimulus of 0.5 ml of a two per cent suspension of washed human O-positive erythrocytes intraperitoneally six days prior to the burn and/or infection. Equal volumes of sera from each of the three animals sacrificed at each interval from day two through 12 were combined to determine an average agglutinin titer for each group. All agglutinations were done with fresh, washed human type O-positive erythrocytes from the same person who donated the antigen.

Experiment V. This experiment was done to determine the response to a primary antigenic stimulus given one day following thermal injury and/or infection. One day following thermal injury and/or infection, all test animals in the experiment were given an intraperitoneal injection of 0.5 ml of a two per cent saline suspension of washed type O-positive human erythrocytes. Three animals from each group were sacrificed on postinjury day 2, 4, 6, 8, 10, and 12, and equal quantities of their sera were pooled and agglutinin titers determined on the pooled sera. Four equal groups of 18 animals were studied.

Group A. These animals served as controls and received no thermal injury or infection.

Group B. Animals within this group received a 20% third degree burn and were not infected.

Group C. These animals received a burn similar to that given in Group B, but the burn wound was contaminated with 0.1 ml of an 18-hour broth culture of Pseudomonas, SRU 12-4-4(59).

Group D. All animals received a subcutaneous injection of 1.0 ml of an 18-hour broth culture of Pseudomonas, SRU 12-4-4(59) which was given at the same time of the burn injury in Groups B and C.

Experiment VI. This experiment was performed to determine the degree of difference in response to a primary antigenic stimulus of 0.5 ml of a 0.02 per cent saline suspension of human erythrocytes four days after 20% third degree burn

injury with and without infection.

Group A. Eight normal animals served as controls.

Group B. A 20% third degree burn injury was produced in eight animals. The wounds were not contaminated.

Group C. In eight animals, a 20% third degree burn injury was produced, and the wounds were seeded with 0.1 ml of an 18-hour broth culture of Pseudomonas, SRU 12-4-4(59).

A 1.0 ml sample of blood was drawn by cardiac puncture from each of the animals on the third, fifth, seventh and ninth days after the antigenic stimulus was administered. An average of the titers was obtained, and the groups were compared statistically by a ranking test.

Experiment VII. This experiment was done to determine if a larger degree of thermal injury would cause more significant alterations of the primary immune response than did a smaller burn. All animals were given an antigenic stimulus intraperitoneally of 0.5 ml of a 0.02 per cent saline suspension of human O-positive erythrocytes. A 1.0 ml sample of blood was drawn by cardiac puncture for determination of agglutinin titers seven days and 14 days after administration of the antigen. All test animals were given a 32% third degree burn injury.

Group A. Fifteen animals served as controls and received no injury.

Group B. The antigenic stimulus was given 24 hours prior to burn injury in seven animals.

Group C. The antigenic stimulus was administered three hours following thermal injury in seven animals.

Group D. Seven animals received the antigen 24 hours after burn injury.

Group E. The antigen was given to seven animals four days following thermal injury.

Experiment VIII. This experiment was undertaken to determine more clearly the time of onset of the immune deficit following thermal injury. A 30% third degree burn was inflicted

upon each of the animals in the test groups. All of the animals, including the controls, were given an antigenic stimulus of 0.5 ml of a 0.02 per cent saline suspension of human O-positive erythrocytes which were given intraperitoneally. A 1.0 ml sample of blood was drawn by cardiac puncture on the 7th, 14th, and 21st days following the administration of the antigen.

Group A. Ten animals served as controls with no thermal injury.

Group B. The antigen was given one hour following burn injury in each of 10 animals.

Group C. Ten animals received the antigen two hours following burn.

Group D. Five animals were given the antigenic stimulus three hours following burn injury.

Results

Experiment I. (The anamnestic response to tetanus toxoid in burned patients). Four of the 23 patients known to have had previous active immunization with tetanus toxoid failed to live for as long as one week, but two of them had obtained measurable titers by the fourth and sixth postburn days, respectively. The other two failed to develop titers greater than 0.5 unit per milliliter at the time of death on their fifth postburn day. Of the remaining 19 patients, 18 developed titers which measured 8 units per milliliter to greater than 128 units per milliliter at their highest level. Patients with measurable titers who were studied early in their course had a drop in the circulating titer of tetanus antitoxin during the first to the fifth postburn day concomitant with a decrease in total circulating gamma globulin. There was a rise in titer beginning between the fourth and fifth postburn days which rose to an average height of 68 units per milliliter 12 days after the injection of toxoid. Seven of the patients attained a titer of 128 units per milliliter or greater. One patient did not develop a measurable titer of greater than 0.5 unit per milliliter. It was doubtful, however, that this individual had received a booster injection of tetanus toxoid, and it had been at least five years since he had received his last known booster injection. He died from a septicemia on his 54th postburn day. None of the three patients who had no prior

immunization developed a titer of greater than 0.5 unit per milliliter during the 20-day period of observation.

Of the eight normal controls, seven developed measurable titers of tetanus antitoxin using the technic of immunodiffusion during the 20-day period of observation. The onset of rise in titer was between the second and ninth day following the booster injection, generally proportioned to the period of time since the last booster. The average titer, 16 units per milliliter (range eight to 32 units per milliliter), reached a peak on the 16th day following the booster injection. The normal control who failed to produce a measurable precipitating antibody of greater than 0.5 unit per milliliter by the immunodiffusion test was shown to have a titer of four units per milliliter 14 days after the administration of toxoid when assay was done by mouse protection test. Spot checks of several other serum samples from both burned and control individuals showed excellent correlation between immunodiffusion and mouse protection tests.[2] It appears that this individual responded with a different type of antibody than did the others for reasons which are as yet unclear. The average titers of both burned patients and the normal controls are shown in Figure 1.

Two individuals who had received their last booster shot of tetanus toxoid four months and nine months prior to burn injury received no toxoid following the burn injury. Tetanus antitoxin and gamma globulin levels were followed for three and four weeks, respectively, to determine their relative change during this period. The results, which are shown in Figure 2, show that the change in specific tetanus antitoxin titer tended to parallel the change in total circulating gamma globulin.

The findings in this experiment indicate that patients with severe thermal injury respond to an anamnestic stimulus to a much greater degree than do normal individuals since the average titer of tetanus antitoxin in the burned patients rose to a much higher level, and the peak of the titer occurred at an earlier time than in the normal controls.

Experiment II. (The primary immune response to alligator erythrocytes in burned patients). None of the patients who were given an injection of Cayman cells developed a measurable agglutinin titer during the three-week period of observation. On the other hand, each of the normal control individuals

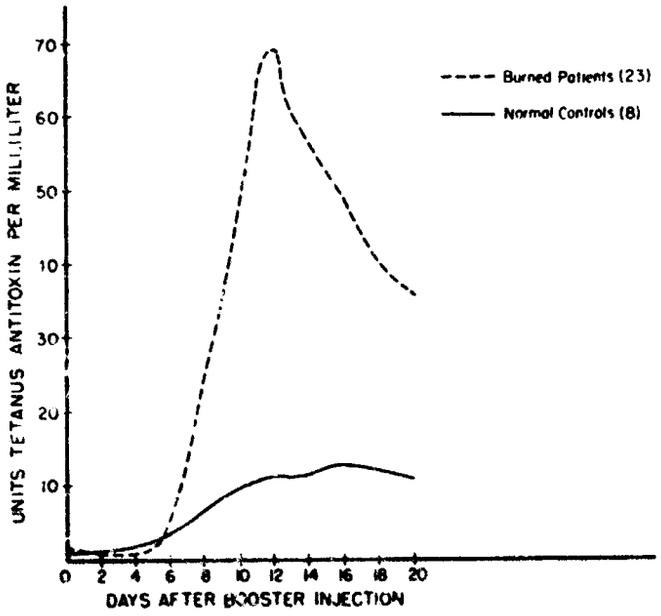

Figure 1. Anamnestic response to tetanus toxoid in burned patients and normal controls.

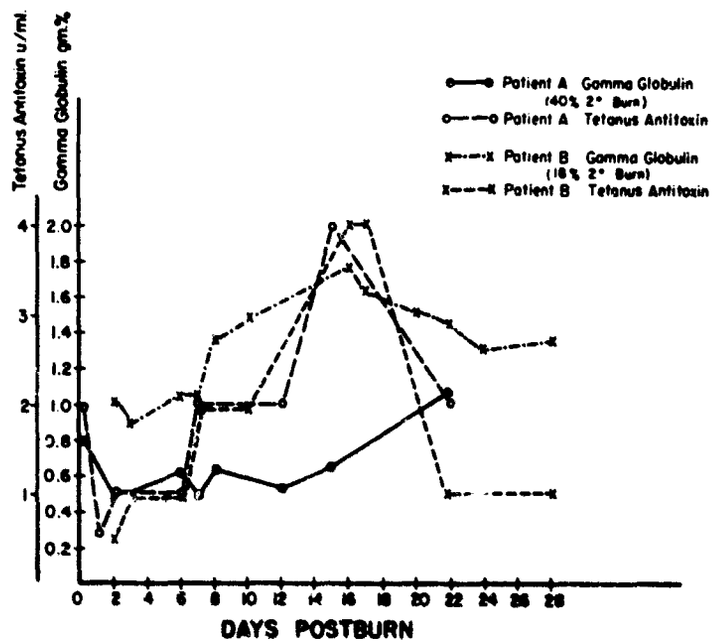

Figure 2. Tetanus antitoxin and gamma globulin levels in two patients to whom no tetanus booster was given.

developed measurable agglutinin titers which were first detectable between the sixth and the 12t' day after injection. These rose to a high of 1:6 to 1:24.

The two patients who were given erythrocytes from Alligator mississippians also developed measurable agglutinin titers for the first time on the 12th and 13th day after injection, each of which rose to a high of 1:8. The two normal controls, who were run in parallel, first developed measurable titers on the 11th and 12th days following injection which rose to a high of 1:12 and 1:16.

The results of this experiment indicate that individuals with severe thermal injuries have a deficit in the ability to produce humoral antibodies to a primary antigenic stimulus, in this case alligator erythrocytes.

Experiment III. (Measurement of homograft survival in patients). The sex, age, size of burn, and length of homograft survival for the five patients studied are as follows:

1. Male, age 14, total burn 61% with 48% third degree; homograft survived four and one-half months.

2. Male, age 9, 57% burn with 33% third degree; homograft survived seven weeks.

3. Male, age 51, 38% burn with 30% third degree; homograft survived four weeks.

4. Female, age 16, 55% burn injury with 5% third degree; homograft survived three weeks.

5, Female, age 5, 58% burn injury with 29.5% third degree; homograft survived three weeks.

These represent prolonged homograft survival times in each case.

Experiment IV. (Primary immune response in rats with 20% burn and/or infection. Antigen given six days prior to injury). The titers of the combined sera for each group of animals are shown in Figure 3. Three animals in the burn-infected group died (Group C) as did one in the infected only group (Group D).

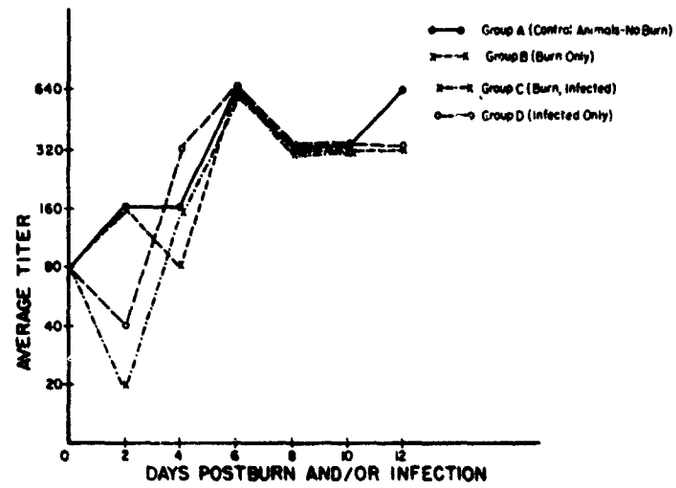

Figure 3. Primary immune response in rats with 20% third degree burn and/or infection. Antigen given six days prior to injury.

The last determination for the infected only group (Group D), therefore,contains only two animals, and no determination could be made at 12 days after burn for the burn-infected group (Group C). It is readily apparent that there was no significant difference among any of the groups. The burn-infected and infected only groups (Groups C and D) did show an initial drop in titer which was probably concomitant with a decrease in the total circulating gamma globulin on the second day following burn injury and/or the introduction of a Pseudomonas abscess.

Experiment V. (Primary immune response in rats with 20% burn and/or infection. Antigen given one day following injury). The titers are shown in Figure 4, with each point representing a mean of three animals with the exception of the last point recorded for Group D, which represents only two animals. Four animals in Group D died before the experiment's termination.It is apparent from the graph that no significant difference in the titers of anti-human erythrocyte antibody occurred among the four groups when the antigen was administered 24 hours following thermal injury with or without infection in animals with a 20% third degree burn, nor was a difference apparent in those animals with a severe abscess caused by Pseudomonas aeruginosa.

Experiment VI. (Primary immune response in rats with 20% burn with and without infection.Antigen given four days following burn injury). The average of the individual titers taken for each group in this experiment is depicted graphically in Figure 5. All of the control animals lived to the termination of the experiment, but a number of animals in both the burn and the burn-infected group died prior to the termination of the experiment. The numbers of remaining animals upon which titers were done are shown in parentheses in the graph. Although there is some apparent difference in the average titer among these three groups, when a comparison of means was done by ranking, the difference was insignificant ($p > 0.05$). This experiment suggests that there may be a diminished response to a primary antigenic stimulus under the chosen experimental conditions, but the results are by no means conclusive.

Experiment VII. (Primary immune response in rats with 32% third degree burn. Antigen given at various intervals before and after burn). The averages of the individual titers are shown graphically in Figure 6. The number of surviving animals in each group is shown in parentheses for each

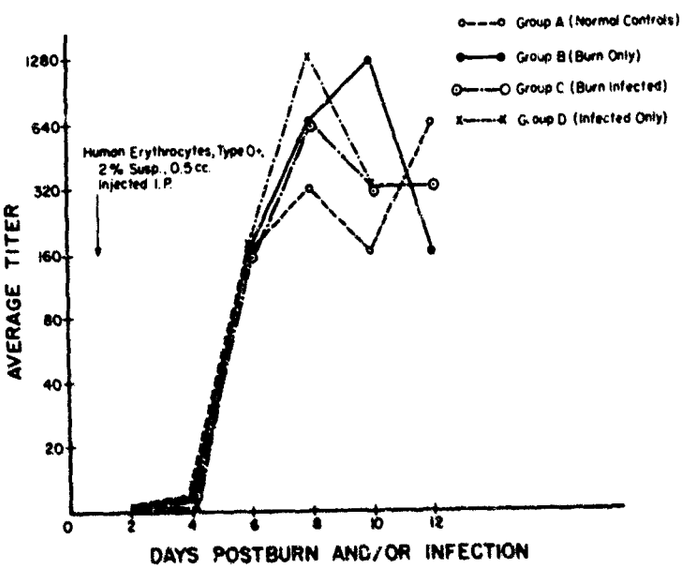

Figure 4. Primary immune response in rats with 20% third degree burn and/or infection. Antigen given one day following injury.

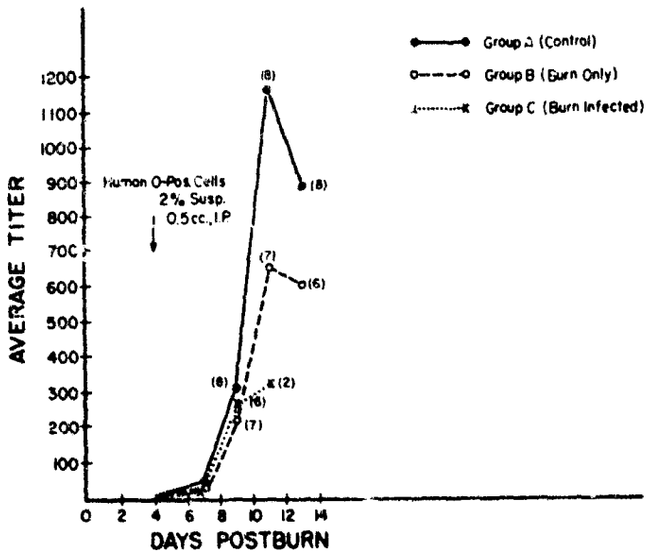

Figure 5. Primary immune response in rats with 20% third degree burn with and without infection. Antigen given four days following injury. The numbers of surviving animals are shown in parentheses.

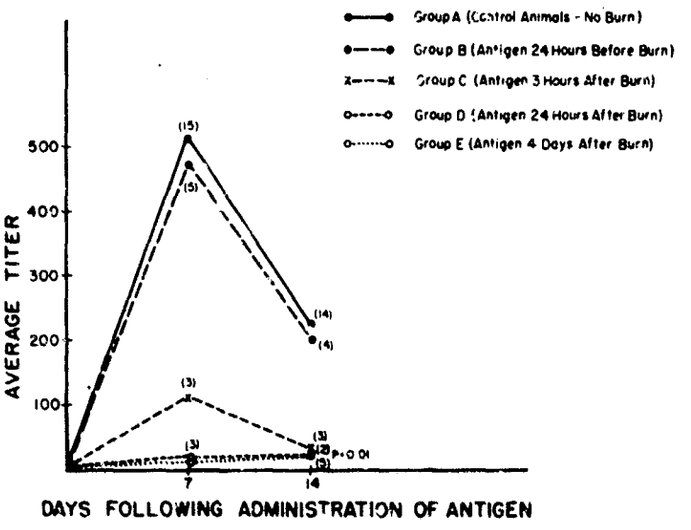

Figure 6. Primary immune response in rats with 32% third degree burn. Antigen given at intervals indicated. The numbers of surviving animals are shown in parentheses.

series of determinations. When the antigen was administered 24 hours before the burn injury (Group B), there was no significant difference from the control animals. There is a difference, however, in the remaining three groups, with the most striking inhibitions of the primary response being seen when the antigen was given 24 hours and four days following thermal injury. The average titer in Group IV was significantly different from the controls ($p = 0.01$). The small number of survivors in the other groups did not permit accurate statistical evaluation, but again there is an apparent difference.

It was concluded that this larger thermal injury produced a significant deficiency in the response to a primary antigenic stimulus when it was administered following thermal injury. No deficit occurred when the antigen was given prior to thermal injury.

Experiment VIII. (Primary immune response in rats with 30% third degree burn. Antigen given one, two, and three hours after burn). Figure 7 shows graphically the average of the individual titers for each of the four groups in this experiment. The number of surviving animals in each group is given in parentheses at each point, and the probability of the difference between the test group and control group is given for each point. From these data, it is apparent that a thermal injury of 30% third degree burn produced a measurable and significant deficit in the immune response to an antigen administered one or two hours following the burn. This deficit was of even greater magnitude when the antigen was administered three hours following injury. It was thus concluded that the deficit in the immune response following thermal injury becomes more pronounced as the interval following injury increases.

Discussion

A number of independent variables may contribute to the increased susceptibility to infection that occurs in patients who have received severe thermal injury. Probably the most important of these is an increase in the opportunity for bacterial colonization of the burn wound itself, which favorably supports bacterial growth. Of almost equal importance is the lack of ability of the patient to combat invasive infection once colonization has occurred. It has been shown by Liedberg[3] and by McRipley and Garrison[4] that thermal injury markedly increases the susceptibility to infection and that there is a

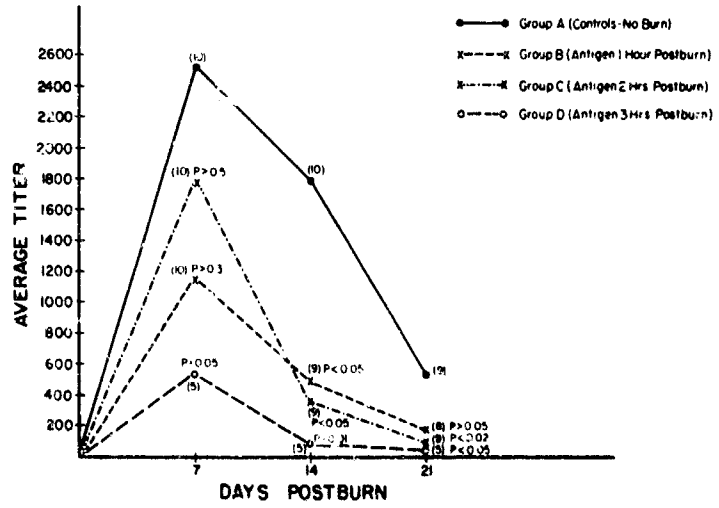

Figure 7. Primary immune response in rats with third degree burns. Antigen given one, two and three hours after burn. The numbers of surviving animals and probabilities of difference from the control group are shown in parentheses.

decreased clearance of organisms from the blood stream of burned experimental animals. Balch[5] and McRipley and Garrison[4] have shown that the nonspecific bactericidal effect of the plasma and serum of clinical patients and experimental animals does not contribute to this susceptibility since it is increased for a short period following thermal injury and later returns to normal values.

The ability of the host to produce specific antibodies has been poorly studied even though active immunity to certain bacteria has been shown to prevent progressive invasion of the wounds in experimental animals. A number of investigators, such as Jones, et al.,[6] have shown that there is an increase of the antibody titer to bacteria which colonize the burn wound, such as Staphylococcus aureus and Pseudomonas aeruginosa, in surviving burned patients. Magovern and Harrison[7] studied the immune response to I^{131} labeled gamma globulin in rabbits with third degree hot water burns of 15% to 20%. They found that there was a slight prolongation of the time for the beginning of antibody formation in burned animals and that there was a slight decrease in the rate of degradation of the circulating antigen. Their findings were inconclusive, however, and they felt there was no significant difference between the experimental and control groups.

The experimental studies presented herein demonstrate that several significant alterations of the immune response occur following thermal injury. In Experiment I, it was demonstrated that the anamnestic response to a booster injection of tetanus toxoid in patients who had been immunized previously was far in excess of the response seen in the normal individual. The responses seen in our controls were similar to the values reported by Goldsmith, et al.,[8] and King, et al.,[9] The difference in response is highly significant, and there is little question that not only is there no impairment of the response to a secondary antigenic stimulus in severely burned patients, but there is actually an increased production of specific antibody. On the other hand, when many of the same patients were given an antigenic stimulus with which they had had no previous experience, a marked deficit in the production of humoral antibodies was found. None of the 10 patients injected with Cayman sclerops erythrocytes developed detectable antibodies whereas all of six controls did so. This represents a highly significant difference between the two groups. The erythrocytes of Cayman

sclerops were selected as the primary antigen to avoid antigenic materials with which humans may have had previous experience, such as the bacterial antigens. It is also quite unlikely that individuals who received the antigen will be exposed to it again, thus avoiding hypersensitivity reactions. In Experiment III, homograft survival was found to be prolonged in five patients, indicating that not only humoral but cellular antibody formation was depressed to a primary antigenic stimulus. This was not an unexpected finding inasmuch as Kay[10] reported one patient that retained a homograft from a related individual for an eight-month period; and Rapaport, et al.,[11] in an experimental study in rats with 30% third degree burns, found a prolongation of survival time of homografts placed in unburned areas. No controls were done for the patients in our series, but homograft survival was longer than in persons who have been studied by other investigators. Gardner and Preston,[12] in 1962, reported that the survival of small homografts in 18 normal individuals ranged from 8 to 25 days with a mean of 12.4 days; and Graham and Petersons,[13] in 1963, reported that only 10 of 110 untreated patients with cervical cancer had survival of skin homografts for longer than two weeks.

Our studies in experimental animals indicate that the degree of inhibition of the primary immune response somewhat parallels the degree of thermal injury inasmuch as rats receiving a 20% third degree burn showed only a slight inhibition of the primary immune response, while the inhibition in rats with a 30% third degree burn was much more striking. The studies also indicate that the degree of inhibition of the primary immune response is somewhat dependent upon the temporal relationship at which the antigen is given following the thermal injury. A progressive inhibition of the response was seen as the interval between burn injury and antigen administration increased. No inhibition of the immune response was found when antigenic material was administered prior to the thermal injury. The complication of a severe, ultimately lethal infection with a gram negative organism in Experiments IV, V, and VI did not significantly inhibit the response to an unrelated primary antigenic stimulus. These studies show that once antigenic information is assimilated, the production of humoral antibodies is not impaired. The decreased titers in all of the experimental groups of Experiment VII were felt to be related to the fact that female animals were used rather than male animals and that the cells used for injection were somewhat older than those used in the other experiments.

The immune deficit to a primary antigenic stimulus following thermal injury is not likely to be on the basis of a nutritional deficiency since the patients and experimental animals were not malnourished when studied; and, furthermore, malnutrition and protein deficiency, unless extremely severe, have been found to be of only slight importance in the response to a primary antigenic stimulus in both experimental animals[14] and man.[15,16] The assimilation of material from particulate antigens, such as erythrocytes, may be somewhat inhibited inasmuch as it has been shown experimentally by McRipley and Garrison[4] that phagocytosis of bacteria was decreased in certain organs following 30% total body surface burns in rats, although this was not so with other organs. The poor response to a primary stimulus may be related to the phenomenon of competition of antigens,[17] but it is difficult to believe that this occurs so quickly following burn injury, and, if such were the case, a greater difference should be seen in those animals who had both burn and infection. A block in the coding process for antibody synthesis within the immune competent cells could undoubtedly be contributory. The finding that no deficit in the production of antibody occurred when the antigen was given 24 hours before burn indicates that the immunologic lesion did not appear once antigenic information had been assimilated.

Summary and Conclusions

This series of experiments has been performed to study the alterations of the immune response following severe thermal injury since such alterations may play an important role in the increased susceptibility to infection which follows burn injury. The anamnestic response to an antigenic stimulus, in this case tetanus toxoid, was found to be of greater magnitude in patients with severe burns than in normal individuals; the average titer rose to a much higher level, and the peak occurred four days earlier. Tetanus antitoxin levels in two patients who did not receive a booster injection of toxoid tended to parallel their gamma globulin levels. In the burned patients given a primary antigenic stimulus, a deficient response occurred which was reflected in an inability to produce humoral antibodies against injected erythrocytes from Cayman sclerops and also in a prolongation of the survival time of skin homografts which were allowed to remain in place to the time of rejection.

Several studies in experimental animals were made to obtain

a clearer understanding of the immune deficit to a primary antigenic stimulus. It was found that a third degree burn involving 20% of the surface area of a rat failed to produce a significant inhibition in the response to injected human erythrocytes. When the size of the burn was increased to 30%, however, a substantial inhibition of the production of specific humoral antibody was found. The greatest inhibition of the immune response was seen when the antigen was given one and four days following thermal injuries with lesser, but nevertheless significant, degrees of inhibition of the response seen when the antigen was given one, two, and three hours following injury. It was found that the production of severe infection did not create a diminished response to the injection of erythrocytes in the animals with 20% burns. The site of the immunologic lesion produced by thermal injury has not been determined by these studies and could lie anywhere from the process of phagocytosis to the coding process for antibody synthesis.

The impairment of the ability to produce specific antibodies to a primary antigenic stimulus is felt to be a significant contributing factor to the development of septic complications in severely burned patients.

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Alexander, J.W., and Moncrief, J.A.: Alterations of the immune response following severe thermal injury. Arch. Surg. (In press).

Presentations

Alexander, J.W.: Alterations of the immune response following severe thermal injury. Presented at the Surgical Seminar, University of Cincinnati Department of Surgery, Cincinnati, Ohio, November 3, 1964.

Alexander, J.W.: Alterations of the immune response following severe thermal injury. Presented at the Central Surgical Association meeting, Chicago, Illinois, March 3-5, 1966.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: USE OF A SIMPLE DOUBLE-DIFFUSION TECHNIC IN AGAR GEL FOR THE QUANTITATION OF TETANUS ANTITOXIN LEVELS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

J. Wesley Alexander, Captain, MC
Beverly L. Worley, B.S.
John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288(R 1)

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: USE OF A SIMPLE DOUBLE-DIFFUSION TECHNIC IN AGAR GEL FOR THE QUANTITATION OF TETANUS ANTITOXIN LEVELS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: J. Wesley Alexander, Captain, MC
Beverly L. Worley, B.S.
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

A new test for the semi-quantitative measurement of tetanus antitoxin levels is described which utilizes a micro-technic of double-diffusion in agar gel. This method was designed to provide a reliable but inexpensive method for the estimation of antitoxin levels in large numbers of individual sera. The quantitative gel-diffusion technic for measurement of tetanus antitoxin levels is felt to supplement the in vivo assays by mouse protection and the in vitro passive hemagglutination technics. The latter tests are quite sensitive for low levels of tetanus antitoxin which cannot be measured by gel-diffusion, but are less reliable for measurements of higher concentrations.

Perhaps of greater importance, the gel-diffusion test can be used to determine the prior immune status of individuals against tetanus by measurement of the antitoxin level two weeks after a single injection of toxoid. If less than one-half unit per milliliter is obtained at that time, the patient may be considered to have received either inadequate or no prior tetanus immunization. Falsely positive precipitin bands have not been obtained thus far in individuals without prior immunization.

(1) Tetanus
(2) Immunity
(3) Burns

USE OF A SIMPLE DOUBLE-DIFFUSION TECHNIC IN AGAR GEL FOR THE QUANTITATION OF TETANUS ANTITOXIN LEVELS

Severe and often fatal illness from acute clinical tetanus continues to be a recurring problem in almost every major medical center despite the effectiveness of active immunization for tetanus and its rather widespread application. Surveys by Levine and Wyman[7,8] indicate that immunity of the population in widely scattered areas is generally increasing, but nonimmunity continues in certain populations, particularly in women over the age of 20 and men without prior military service. Even among those young and middle-aged adults who have had prior immunization, it is often difficult to establish the fact because of lack of information from the patient or lack of adequate clinical records. All too frequently, young adults requiring hospitalization for traumatic injury or burns are unable to furnish information concerning prior tetanus immunization as a youngster. Only persons having had prior military service can be considered to have received adequate tetanus immunization in the absence of clinical records.

The use of homologous tetanus antitoxin has proved to be safer by far than the administration of heterologous antitoxin, and allergic reactions are indeed infrequent. Because of the expense and limited availability, however, administration of homologous antitoxin is unwarranted in those cases in which patients have had prior immunization, especially since protective levels of antitoxin are elicited by toxoid for as long as 19 - 21 years after primary immunization.[4,6,10] A simple but satisfactory *in vitro* test for the determination of prior immunization would be helpful in those cases where continued tetanus prophylaxis is indicated. *In vivo* tests and *in vitro* passive hemagglutination tests,[1,9] while accurate, are both expensive and complicated for this purpose.

This report deals with an inexpensive *in vitro* method using double-diffusion in agar gel for estimating specific levels of tetanus antitoxin. Its simplicity and low cost would allow its performance in most clinical laboratories. Serum antitoxin levels of 0.5 unit per milliliter or greater may be measured with reasonable accuracy using this technic. It has also been found to be particularly useful in following the secondary (anamnestic) response to tetanus toxoid in human patients.

Materials and Methods

The gel-diffusion reactions were accomplished in one per cent agar gel with the aid of a plexiglass template on a microscope slide. Pre-cleaned 1 x 4-inch glass microscope slides were coated on one side with a thin layer of 0.25 per cent agar, which was allowed to dry into a hard film. Two layers of plastic tape (Sears, all-purpose plastic tape, 34-5995) were placed approximately three-quarters of an inch apart on the top of the slide so that a one-inch by one-inch plexiglass template could be bridged across the doubled layers of tape. This resulted in a satisfactorily uniform depth between the bottom of the template and the microscope slide. The template consisted of a one by one inch square of clear plexiglass, 3/32 of an inch in thickness, with a central well 3/16 inch in diameter. Eight peripheral wells 1/16 inch in diameter were placed circumferentially around the center well at a radius of one-fourth inch center to center. These templates were placed upon the slide, and the area between the template and microscope slide was filled with molten, one per cent, laboratory grade agar (Bacto-agar) which had been freshly dissolved in barbital buffer. The barbital buffer, which had a pH of 7.4 and an ionicity of 0.15, was prepared by dissolving 6.0 gm sodium chloride, 6.98 gm sodium barbital, and 22.7 ml 1.0 normal hydrochloric acid in deionized water q.s. to 1000 ml. The agar on the slide was allowed to solidify at room temperature in a petri dish which was humidified by the addition of a water-saturated sponge (Fig. 1). A commercial tetanus toxoid containing 7.5 Lf toxoid per milliliter (Lederle Laboratories, Lot No. 032-579) was diluted 1:10 in normal saline and used for the antigen in each case. This was used to fill the center well. Undiluted serum to be tested was placed in the first peripheral well, with serial two-fold dilutions being placed in subsequent peripheral wells. Approximately 0.0047 ml of material filled each of the peripheral wells, and 0.042 ml filled the center well, each being filled to capacity with a flat meniscus.

The gel-diffusion slides were placed in individual petri dishes wrapped with a plastic film in groups of five or six, and placed in the refrigerator at 4° C for 72 to 96 hours. It was necessary to encase the diffusion tests to insure proper humidification and prevent drying of the agar during this period of incubation. A humidity box would serve equally satisfactorily. After incubation, the plexiglass templates

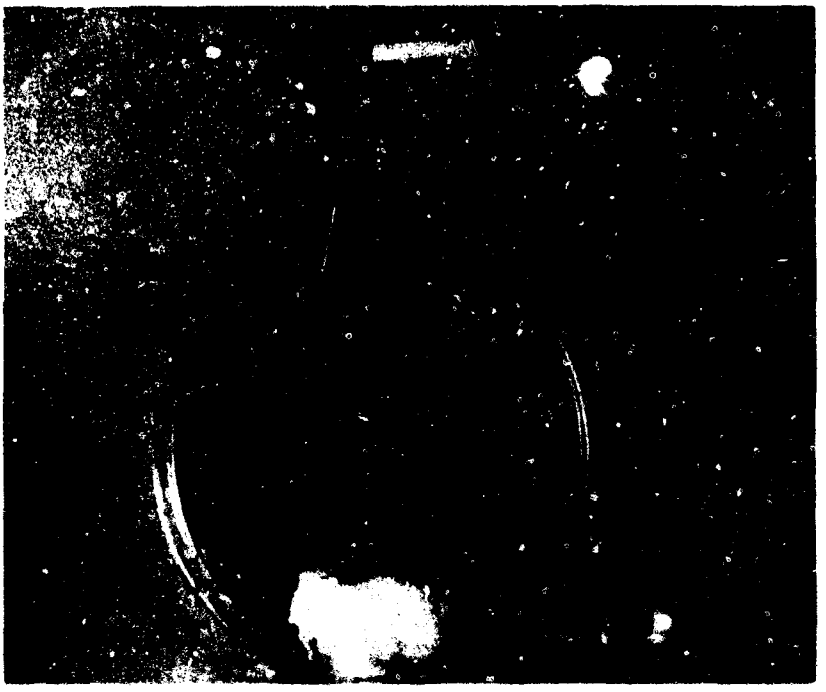

Figure 1. Preparation of slides for diffusion.
A. Molten agar is introduced through the center well into the space beneath the template.

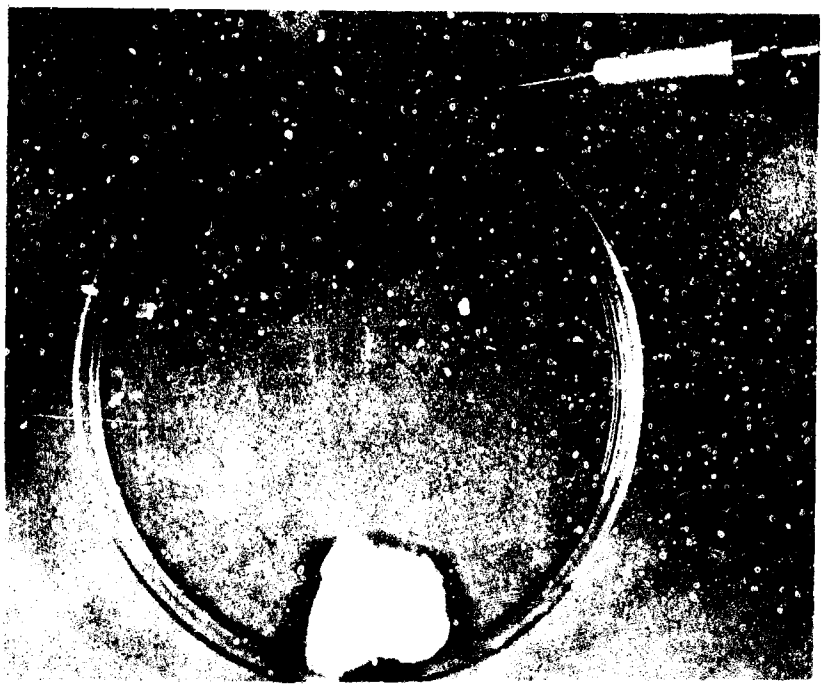

Figure 1. Preparation of slides for diffusion. B. After the agar has gelled firmly, the reactants are placed into the wells. Care must be taken to avoid the formation of bubbles in the wells.

could be lifted easily from the cold agar by elevating one corner with a sharp instrument. The plastic tapes were then removed, and the agar washed in the petri dishes. To remove uncomplexed proteins, the slides were flooded with barbital buffer, pH 7.4, ionic strength 0.15, for 24 hours with a change of buffer after the first two hours. Two per cent acetic acid was used for 10 minutes for fixation, and the slides were stained for 10 minutes with one per cent Thioflavine-S in distilled water. Differentiation was accomplished by two 10-minute washes with two per cent acetic acid. The antigen-antibody precipitin bands could be seen easily by visualization of the slides by indirect lighting from the bottom. Records may be made photographically or the slides preserved simply by drying at room temperature. The end point was considered to be the highest serum dilution at which a definite precipitin band could be visualized. This technic was essentially a modification of that described by Crowle[2] for the quantitative measurement of specific antibody. It has been found to be accurate and duplicable when properly performed.

All of the tests on clinical patients were done on sera from 28 patients sustaining 30% or larger total body burns, who were admitted to the US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas. In addition, eight normal individuals having had prior tetanus immunization were used for controls. Twenty-five of the burned patients had known prior immunization. There were two patients who had no prior tetanus immunization and one patient in whom the status of immunization was unknown. A booster shot of tetanus toxoid (3.75 Lf) was given to the eight normal controls and to 25 of the patients, either upon their admission to the Surgical Research Unit or shortly after the time of their burn. In one patient with prior immunization it could not be determined whether or not a booster injection of tetanus toxoid had been given, and in two other patients the booster injection was purposely withheld to follow specific antibody titers following the burn injury. Sera for titers were drawn at daily to biweekly intervals. A standard serum was run as a control with each of approximately 20 sets of tests, each set averaging 20 individual diffusion tests. This serum contained approximately four units of antitoxin per milliliter and produced the last precipitin band at a dilution of 1:8 (Fig. 2). There was no variation in the location of the last precipitin band in any of the controls when a technically satisfactory diffusion was accomplished.

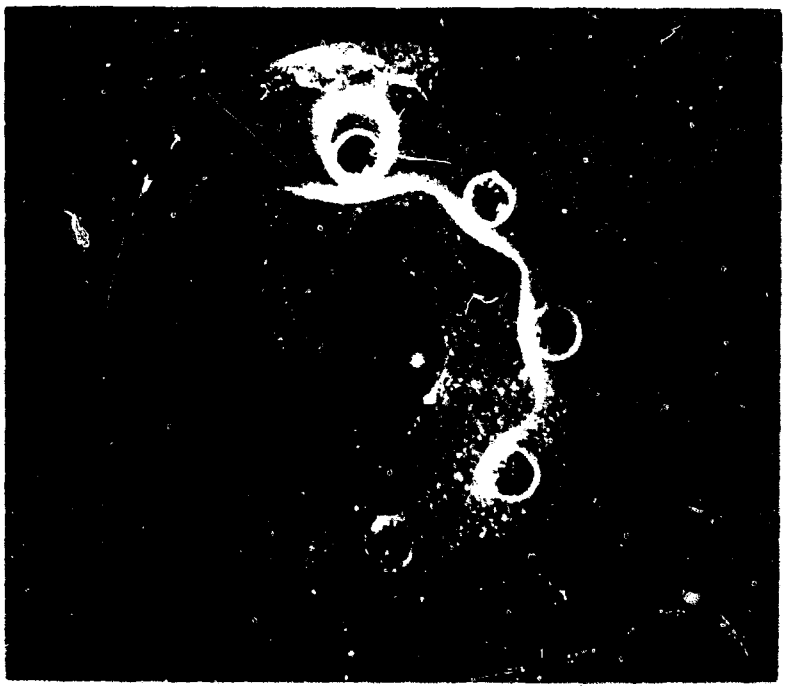

Figure 2. Photographs of two diffusion tests. A. The last visible band is seen at well Nr. 4 which contains a serum dilution of 1:8. This is the standard antiserum used with each set of tests and contains four units of tetanus antitoxin per milliliter. Note the three precipitin lines at well Nr.1. The lighter bands blend with the heavier one as the serum is diluted. The ring of precipitated lipoprotein around well Nr. 1 is sometimes seen with undiluted sera and should not be confused with specific antigen-antibody complexes.

Figure 2. Photographs of two diffusion tests. B. The last visible precipitin band is seen at well Nr. 5 which contains a serum dilution of 1:64. The serum thus contains approximately 32 units of tetanus antitoxin per milliliter.

Five antisera with titers determined by mouse protection tests were obtained from Parke, Davis and Company for standardization of the technic. An additional five sera selected from the patients and controls were titered by the mouse protection assay.

Results

A precipitin band could be detected within the agar gel with a serum dilution which contained approximately one-half unit of tetanus antitoxin when the above technic was utilized with known antisera. One major and two minor bands were often seen in the undiluted serum (Fig. 2).

A comparison between the level of tetanus antitoxin as estimated by the gel-diffusion test and as measured by the mouse protection assay was made with 10 sera and is graphically shown in Figure 3. Excellent correlation was obtained, well within the limits of error for a doubling dilution.

In each of the patients with known prior tetanus immunization to whom a booster injection of toxoid was given, and who survived longer than one week, there was a definite rise in titer which measured from eight units per milliliter to 128 units per milliliter. The peak of the response was 11 to 14 days after the booster. Four patients died during the first week after burn injury. Two of these developed rising titers before the time of death, whereas the other two failed to develop titers above 0.5 unit per milliliter during that period. In the two patients in whom there had been no prior immunization, there was no detection of tetanus antibody by this method. One patient whose immune status could not be determined by history developed a detectable titer of greater than one-half unit per milliliter for the first time on her 25th post-injection day, indicating that this was a primary immune response rather than a secondary one. The patients in this series who were previously immunized had received their last booster injection of tetanus toxoid ranging from one month to 11 years before their burn injury.

In eight normal individuals, 98 separate tests were performed to determine the specific titers at various intervals following the booster injection of tetanus toxoid. All individuals had been immunized previously. One of these individuals, however, did not develop a titer greater than 0.5 unit per

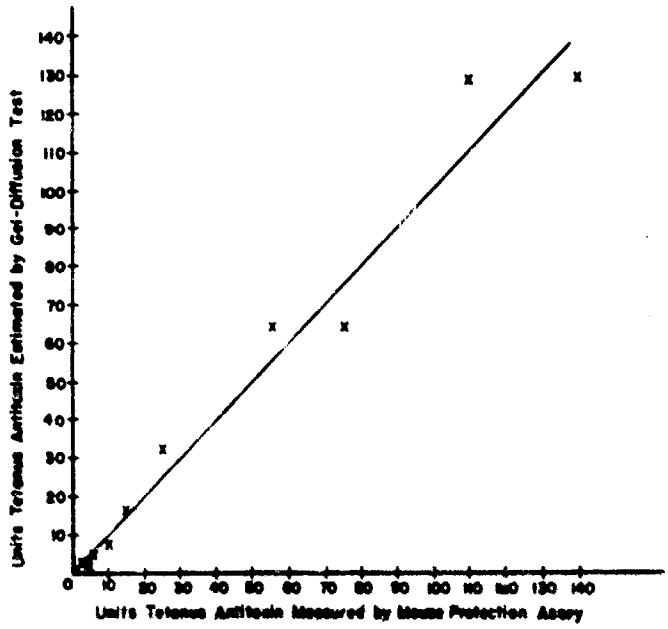

Figure 3. Comparison of the tetanus antitoxin concentration as measured by the gel-diffusion test and by mouse protection assay in 10 individual sera. Excellent correlation was obtained.

milliliter until the 43rd post-injection day, as measured by the gel-diffusion technic. The serum titer, as measured by the mouse assay technic, rose to five units per milliliter, indicating that an antibody had been formed which did not produce a good precipitin complex with the toxoid. This is the only individual in which this was found. Six of the controls had titers ranging from 0.5 to 2 units per milliliter prior to their booster injection. Two had titers of less than one-half unit per milliliter. The last booster injections ranged from three months to five years. Onset of the rise in titer after the booster injection was from the second to the ninth day, with the shorter responses being present in those individuals in whom recent booster injections had been given. The peak titer ranged from eight units per milliliter in two individuals to 32 units per milliliter in one individual. These titers are essentially the same as those found by Goldsmith, et al.,[3] using mouse protection tests for assays of the booster response in 55 patients who had been previously immunized, and by Kaiser, et al.,[5] in studies of 41 World War II veterans.

The patients with severe burn injury generally responded more quickly with an anamnestic response to a booster injection of tetanus toxoid than did normal individuals, and the titers rose to much higher levels. The significance of this and further details of the immune response in burned patients will be reported more completely in a subsequent paper.

Discussion

This double-diffusion technic in agar gel for the quantitation of tetanus antitoxin levels has proven to be a simple, accurate, and inexpensive method for the estimation of tetanus antitoxin levels in patient sera. The sensitivity for titers ranging above 0.5 unit per milliliter has been quite satisfactory. By comparison, the passive hemagglutination technic appears to be quite adequate for measurement of smaller concentrations of tetanus antitoxin but is not as accurate at higher levels, and is also more complicated to perform. In vivo protection studies are both time-consuming and expensive.

Both in military and civilian practice, patients are encountered frequently who require continued tetanus prophylaxis. With many of the patients, however, the status of prior immunization is difficult, if not impossible, to obtain. To insure protection in such patients, it would be necessary to give

rather large quantities of either homologous or heterologous antitoxin. The former is expensive whereas the latter is dangerous because of serum reaction. The test described in this paper can be used to determine the immune status of a patient against tetanus in such instances where continued tetanus prophylaxis is indicated. An initial injection of tetanus toxoid should be administered to every tetanus-prone patient upon hospital admission. If the patient has received adequate prior immunization for periods ranging up to 21 years before the injury, he will develop a tetanus antitoxin titer greater than one-half unit per milliliter by the 14th day after booster injection, thus making further tetanus prophylaxis unnecessary.[4] This proved to be the case in each of the patients and normal individuals studied in this series who definitely had received a booster shot of tetanus toxoid. In the series reported by Goldsmith, et al.,[3] 96 per cent of 55 patients had a titer greater than one unit 14 days after booster injection, and 100 per cent had a titer greater than one unit 21 days after booster injection.

Strict adherence to the details of the double-diffusion test should be observed if accurate results are to be obtained. Using this micro-technic, the drying attendant with incubation at room temperature was prohibitive to a good diffusion. Control of the humidity was of vital importance, and it was necessary to place the diffusion slides not only in petri dishes with water-soaked sponges in the dish, but also to wrap the plates so that an almost air-tight seal was obtained. For this purpose, a plastic film wrap proved to be easily available and satisfactory. As Crowle[2] has pointed out in his text on immunodiffusion technics, the use of clean, grease-free slides is essential. The templates should be polished with silicone fluid prior to their use. Constant temperature control insures sharp precipitin bands. In our tests, barbital buffer resulted in considerably better precipitin bands than did phosphate buffer. Tris buffer was not evaluated. Fresh agar should be prepared before each set of tests since the quality of the precipitin bands decreases as the agar ages, even when it is stored at 4° C. Preservatives to prevent bacterial growth were found not only to be unnecessary but undesirable. Merthiolate inhibited the density of the precipitin bands to some degree in the buffered agar in these experiments. The use of laboratory-grade agar has proven to be considerably more satisfactory than the purified or special agars, such as Noble agar.

Several stains have been described for staining the precipitin

bands in gel-diffusion technics, but many of these have proved unsatisfactory. After evaluating a number of stains, one per cent Thioflavine-S in distilled water proved to be the most satisfactory and gave considerably better results than did Nigrosin, Aminidoschwartz and Crocein Scarlet MOO, as well as several others.

Permanent records may be made either by air drying of slides at room temperature or by photographic recording. Pretreatment of the slides with glycerol has been found unnecessary before air drying.

After the molten agar has been placed beneath the template with a tuberculin syringe, it is essential to allow the agar to gel firmly before the test is performed. Significant and undesirable drying of the agar can occur, however, if too long a period lapses before the introduction of the reactants. It has been found that micro-pipettes to fill the peripheral wells may be made easily by drawing tips on 1.85 mm capillary tubing. Hundreds of individual pipettes may be made in a short period of time and are thus inexpensive and disposable. Dilution of the serum to be tested can be accomplished with standard technics.

Summary and Conclusions

A new test for the semi-quantitative measurement of tetanus antitoxin levels is described which utilizes a micro-technic of double-diffusion in agar gel. This method was designed to provide a reliable but inexpensive method for the estimation of antitoxin levels in large numbers of individual sera. The quantitative gel-diffusion technic for measurement of tetanus antitoxin levels is felt to supplement the *in vivo* assays by mouse protection and the *in vitro* passive hemagglutination technics. The latter tests are quite sensitive for low levels of tetanus antitoxin which cannot be measured by gel-diffusion, but are less reliable for measurements of higher concentrations.

Perhaps of greater importance, the gel-diffusion test can be used to determine the prior immune status of individuals against tetanus by measurement of the antitoxin level two weeks after a single injection of toxoid. If less than one-half unit per milliliter is obtained at that time, the patient may be considered to have received either inadequate or no prior tetanus immunization. Falsely positive precipitin bands have not been obtained thus far in individuals without prior immunization.

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Alexander, J. W., and Moncrief, J.A.: Use of a simple double-diffusion technic in agar gel for the quantitation of tetanus antitoxin levels. J. Trauma (In press).

Presentations

None.

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Drs. H. B. Devlin and M. W. Fisher of the Research Laboratories, Parke-Davis and Company, graciously performed the mouse protection assays and provided several sera of known tetanus antitoxin titer. Dr. A. J. Crowle was kind enough to give his personal advice concerning the performance of the immunodiffusion tests.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE INFLUENCE OF INFECTION UPON SERUM PROTEIN CHANGES IN SEVERE BURNS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

J. Wesley Alexander, Captain, MC
Wanda L. Brown, M.S.
Beverly Worley, B.S.
Arthur D. Mason, Jr., M.D.
John A. Moncrief, Colonel, MC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: THE INFLUENCE OF INFECTION UPON SERUM PROTEIN CHANGES IN SEVERE BURNS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

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Arthur D. Mason, Jr., M.D.
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

A parallel study comparing the electrophoretic patterns in two groups of comparable severely-burned patients, where fatal septic complications were seen frequently in one group and much less frequently in the other, was made to determine what influence infection had upon the electrophoretic patterns following severe thermal injury. A second study in experimental animals compared the serum protein changes with burn, burn plus infection, infection associated with abscess formation, and the injection of bacterial extracts with neither burn injury nor infection.

Those patients dying with septicemia caused by Pseudomonas aeruginosa or Staphylococcus aureus definitely tended to have a marked terminal depression of albumin with elevated gamma globulin and alpha-one globulin levels. With both moderate and severe infections, the alpha-two globulin levels tended to be high, but these were usually depressed terminally with fatal infections, possibly because of hemolysis with haptoglobin combining with free hemoglobin. Generally, however, the electrophoretic patterns for both groups were quite similar except at the time of actual septicemia.

Infection of the burn wound in the experimental animal caused a characteristic increase in the alpha-one and alpha-two globulin and beta-globulin, a mild depression of the gamma globul

and severe depression of the albumin, with exceedingly low values found terminally. The induction of an abscess in itself caused similar changes which were even more marked at an earlier time. The changes in the electrophoretic patterns of severely burned patients in both groups tended to more closely resemble those of experimental animals with both burn injury and infection rather than those with burn injury without infection.

It is concluded that the effects of infection may contribute significantly in producing alterations of the serum protein fractions following severe thermal injury. However, the determination of serum protein patterns by electrophoresis has not been found to be a useful tool in clinical management.

(1) Burns
(2) Serum Protein
(3) Electrophoresis

THE INFLUENCE OF INFECTION UPON SERUM PROTEIN CHANGES IN SEVERE BURNS

Thermal injury of sufficient magnitude results in marked alteration of the serum protein pattern as determined by paper electrophoresis. These changes have been attributed primarily to protein shifts into the extravascular fluid compartment during the first few days after burn injury, but striking deviations from the normal pattern remain for long periods in those patients with large burns. These changes may be a result of both increased production of specific proteins as well as an increased catabolism[2,5,6,10] and continued loss from the burn wound.[15]

The relationship of septic complications to alterations in the serum protein pattern has not been studied extensively although a few investigators have suggested that hypogammaglobulinemia during the initial phase of burn injury may contribute to the development of sepsis.[1,9,12] The routine use of topical Sulfamylon therapy at this institution has significantly reduced septic complications following thermal injury,[13] producing an opportunity to compare the serum protein alterations in severe burns in two groups of patients, one in which fatal septic complications were common, and the other in which they were much less frequent. This investigation was undertaken to make such a comparison with additional information obtained from animal experiments.

Materials and Methods

Experiment 1 - Comparison of Patient Groups. The patients reported in this study were treated by the Clinical Division of the US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas, during two separate periods: 1958 to 1959 and 1964 to 1965. All had thermal injuries involving 30 per cent of the total body surface or greater. They were treated by the conventional regimen used at this institution during the particular period involved, the primary difference in treatment being the use of topical chemotherapy (Sulfamylon) in every patient in the latter group. None of the patients in the first group received topical chemotherapy, although many were treated with systemic antibiotics for specific infections. Also, better serum albumin replacement, in the form of plasma protein fraction (Plasmanate) was accomplished in Group B.

Group A. The 25 patients in this group were studied during the period 1958 to 1959. The average size of their burn injuries was 56 per cent with 33 per cent third degree. Serum electrophoretic patterns were determined by electrophoresis on a Spinco Model R Electrophoresis Unit for 16 hours using Whatman 3 mm electrophoresis paper and barbital buffer, pH 8.6, ionic strength 0.075. Aqueous bromophenol blue was used as a stain, and the strips were read with a Spinco Analytrol apparatus. Ninety-eight separate electrophoretic determinations were done on sera drawn between the time of injury and the 32nd day following injury. Total protein determinations were done by the Biuret method, and the serum protein constituents were calculated in grams of protein per 100 ml serum. Averages for all of the determinations on each postburn day were obtained.

Group B. The 22 patients in this group were treated during the period 1964 to 1965. The average size of their burn injuries was 54.1 per cent with 25 per cent third degree. One hundred seventy-nine separate electrophoretic determinations were done from the first through the 32nd day, with serial daily to biweekly determinations in most surviving patients. All of the patients in this group were treated with topical Sulfamylon therapy. Electrophoreses were done as described above with the exception that S & S 2043A paper was used, and the strips were stained with alcoholic bromophenol blue before being read. The serum protein components were expressed similarly as grams per cent, and the averages for all determinations taken on a given day were made and recorded.

Experiment 2 - Studies in Experimental Animals. Healthy, young, adult Sprague-Dawley rats weighing 180 gm to 200 gm were used throughout this experiment. They were individually caged and given Raulston Chow and water ad libitum. Blood for serum electrophoresis was obtained by exsanguination from cardiac puncture. Total protein determinations were done by the Biuret method, and electrophoreses were performed for 16 hours using a Spinco Model R Electrophoretic Unit, barbital buffer pH 8.6, ionic strength 0.075, and S & S 2043A paper. The strips were stained with alcoholic bromophenol blue and read on a Spinco Analytrol Unit. All of the infections produced in Groups C and D were made by inoculation with an 18-hour trypticase soy broth culture of a single strain of Pseudomonas aeruginosa (SRU-12-4-4(59)). The experimental

animals were divided into seven groups.

Group A. Thirty-six animals served as controls to establish normal values for the serum proteins. Six animals were bled every other day at the same time as the animals in Groups B, C, and D.

Group B. A 20 per cent full-thickness burn was inflicted on each of these 36 animals under pentobarbital anesthesia with the use of a mold by immersion in hot water at 100° C for 10 seconds. This technic is described in detail elsewhere.[18] The burn wounds were not treated otherwise. Six animals from this group were sacrificed every other day following burn injury through the 12th postburn day. Determinations were done for each individual serum, and an average value was obtained for each day.

Group C. Thirty-six animals received 20 per cent full-thickness burns using the technic described above. The burn wounds were seeded with 0.1 ml of an 18-hour broth culture of Pseudomonas strain SRU-12-4-4(59) (approximately 10^8 organisms) immediately after the burn injury. Six animals were sacrificed every other day following burn injury, and individual determinations made on their sera. An average was obtained for each day.

Group D. A subcutaneous abscess was produced in each of 36 animals by the injection of an 18-hour broth culture of Pseudomonas strain SRU-12-4-4(59). Six animals were injected with 0.5 ml of the bacterial suspension; an additional six were injected with 1.5 ml, and 24 animals were injected subcutaneously with 1.0 ml of broth culture. One animal that was inoculated with 0.5 ml broth culture, one inoculated with 1.5 ml, and four inoculated with 1.0 ml were sacrificed every other day through day 12. Determinations were made for each individual serum, and an average was obtained for each subgroup.

Group E. A saline extract was made from Pseudomonas strain SRU-12-4-4(59) organisms which had been harvested after growth on trypticase soy agar and disrupted in the frozen state with a Hughes press. The extract was sterilized by filtration through a sintered glass filter and stored in a dry ice chest until ready for use. One-half ml of this extract was injected intraperitoneally daily into each of five animals. Two animals were sacrificed by exsanguination on the

second day after the injections were started, and three were sacrificed on the fourth day after injections were started. Determinations of the total serum proteins and serum electrophoreses were done on each, and averages obtained.

One ml of the same extract was given intraperitoneally daily to four additional animals. Two animals were sacrificed on the second day after the injections were started, and two on the fourth day. Serum protein determinations and electrophoretic studies were carried out on each, and an average was obtained for each period of scrifice.

Two ml of the extract were given as a single intraperitoneal injection to two animals, and both of these were sacrificed by exsanguination two days later. Serum protein determinations and electrophoretic studies were carried out and an average obtained.

Results

Experiment 1 - Comparison of Patient Groups. The total serum proteins, albumin, gamma globulin, beta globulin, alpha-one globulin, and alpha-two globulin are shown graphically for both groups of patients in Figure 1. The changes are self-evident and need no verbal description. Average values are expressed since individual determinations varied considerably.

Group A patients were divided further into those having septic deaths and the remainder in whom there was either survival or death due to non-septic causes. Although the differences were not great, it was found that albumin levels tended to be lower in those patients with septic deaths, and alpha-one globulin levels tended to be higher. When individual patients having Pseudomonas or Staphylococcal sepsis were considered (Table 1), there was often a profound decrease in the serum albumin levels terminally with a depression of the total serum protein, and, occasionally, the alpha-two and beta globulins. The gamma globulin tended to remain at normal levels or above without a terminal drop in these particular patients. Alpha-one globulins tended to rise terminally in the majority of septic patients.

Experiment 2 - Studies in Experimental Animals. The mean concentrations of total serum protein, albumin, beta globulin, gamma globulin, and alpha-one and alpha-two globulins are

Legend for Figure 1.

Average values for serum protein fractions in patients with burn injuries of greater than 30 per cent. Group B was treated with topical chemotherapy. The origins of the curves from the ordinates indicate values considered to be normal for the technic used.

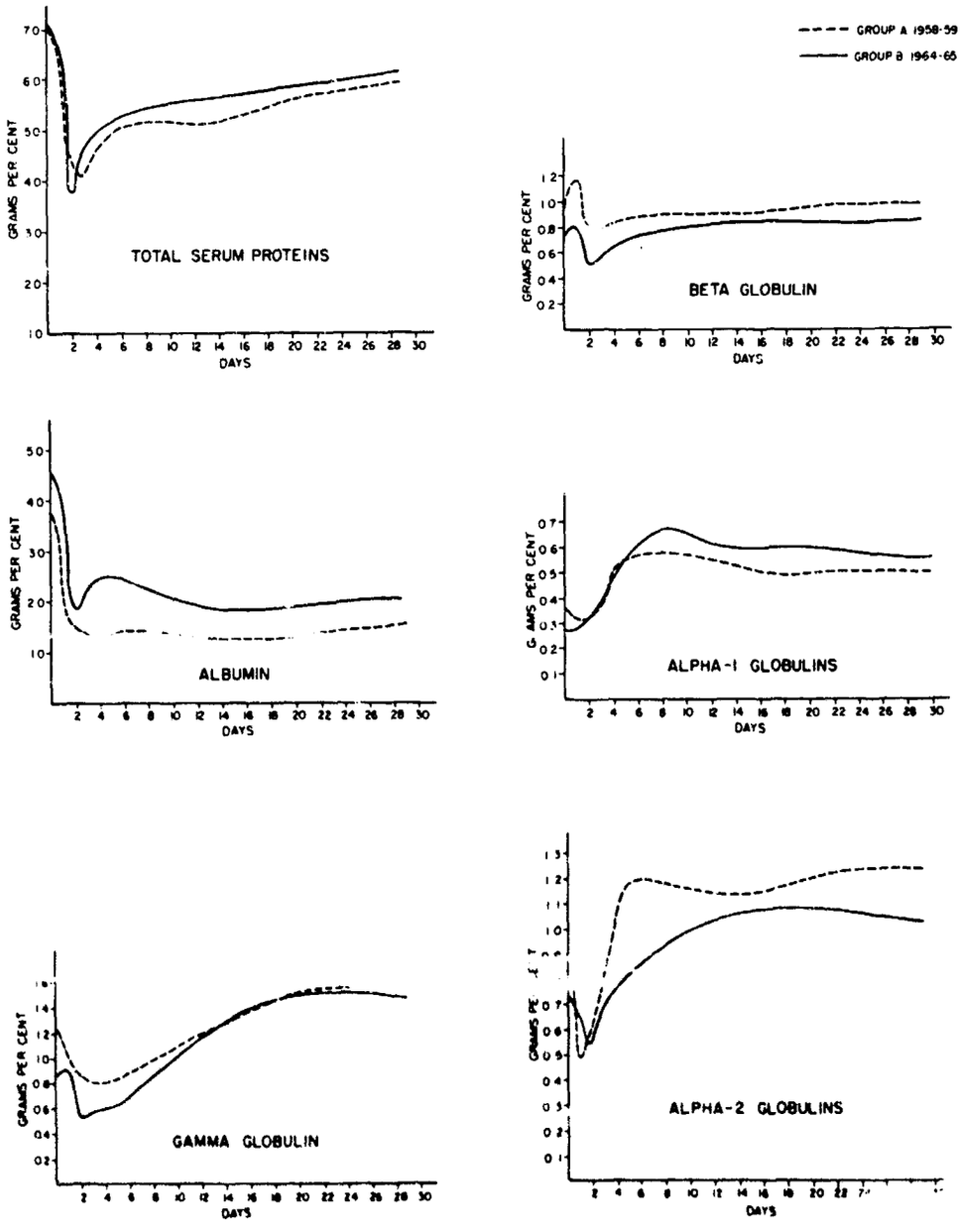
GROUP A 1958-59
GROUP B 1964-65
TOTAL SERUM PROTEINS
BETA GLOBULIN
ALBUMIN
ALPHA-1 GLOBULINS
GAMMA GLOBULIN
ALPHA-2 GLOBULINS
GRAMS PER CENT
DAYS

Figure 1

Table 1. Electrophoretic Values on a 39 Year-Old Patient with Staphylococcal Septicemia
Total Burn = 55% with 15% Third Degree

Postburn Day	Total Serum Proteir	Albumin	Gamma Globuiin	Beta Globulin	Alpha-one Globulin	Alpha-two Globulin
3	3.60	1.10	0.51	0.82	0.37	0.80
4	4.09	1.40	0.67	0.75	0.46	0.81
10*	4.79	0.92	1.23	0.81	0.60	1.23
11*	5.03	0.82	1.53	0.98	0.65	1.05
14	4.50	0.59	1.65	0.71	0.65	0.90
16*	4.92	0.60	1.58	1.00	0.63	1.11
18*	4.57	0.32	1.64	0.75	0.71	1.15
19*	(Died - Staphylococcal septicemia)					

* Positive blood culture.

shown graphically in Figure 2 for Groups A - D. Six animals died in both Groups C and D, resulting in no determination for the 12th postburn day in these two groups.

After a 20 per cent burn without infection (Group B), there was slight drop in the total serum protein, albumin, and globulin, with essentially no change in the beta globulin and alpha-one globulin, but with a rise in the alpha-two globulin consistent with the "pattern of injury" described elsewhere.[4,14]

If animals with the same magnitude of burn injury received bacterial contamination so that a severe and ultimately fatal infection resulted (Group C), there was a much more striking depression of the total serum protein with a profound depression of the serum albumin. In many animals, on the 8th and 10th postburn day, the values for albumin were less than 0.5 gm per cent, a seemingly progressive decrease until the time of death. The gamma globulin levels were only slightly lower than those seen in animals with burn injury alone. Beta globulin levels, however, were higher, reached a peak on the sixth postburn day, and decreased slightly after that time. There was an immediate rise in the alpha-two globulins, which continued upward to two and one-half times normal values by the time of the animal's death. The alpha-one globulins rose more slowly, reached a peak on the sixth postburn day, and dropped toward normal values on the 8th and 10th postburn days.

In Group D, where a subcutaneous abscess was produced, the animals developed a very large swelling in the region of the abscess which resulted in skin necrosis in many animals with rupture by the 8th to 10th day. The return toward normal values at this particular time was felt to represent, in part, spontaneous drainage of the abscess with partial recovery in the remaining animals. The total serum protein levels dropped approximately 1 gm per cent and stayed low throughout the period of observation. Serum albumin levels fell to one gram per cent on the second day after creation of the abscess and decreased still further on the fourth and sixth days. The gamma globulin followed a pattern similar to that seen in the burn-infected animal with higher values noted on the 8th and 10th day. The changes in the alpha-one, alpha-two, and beta globulins were striking, with an immediate rise in all three. The rise occurred sooner than in the burn-infected animal, and the beta globulins and alpha-two globulins reached a higher level although all of these tended to return toward normal in those

Legend for Figure 2.

Electrophoretic determinations in the experimental animals. Each point on the charts represents an average value obtained from six animals. The injury and/or infection was inflicted on Day 0.

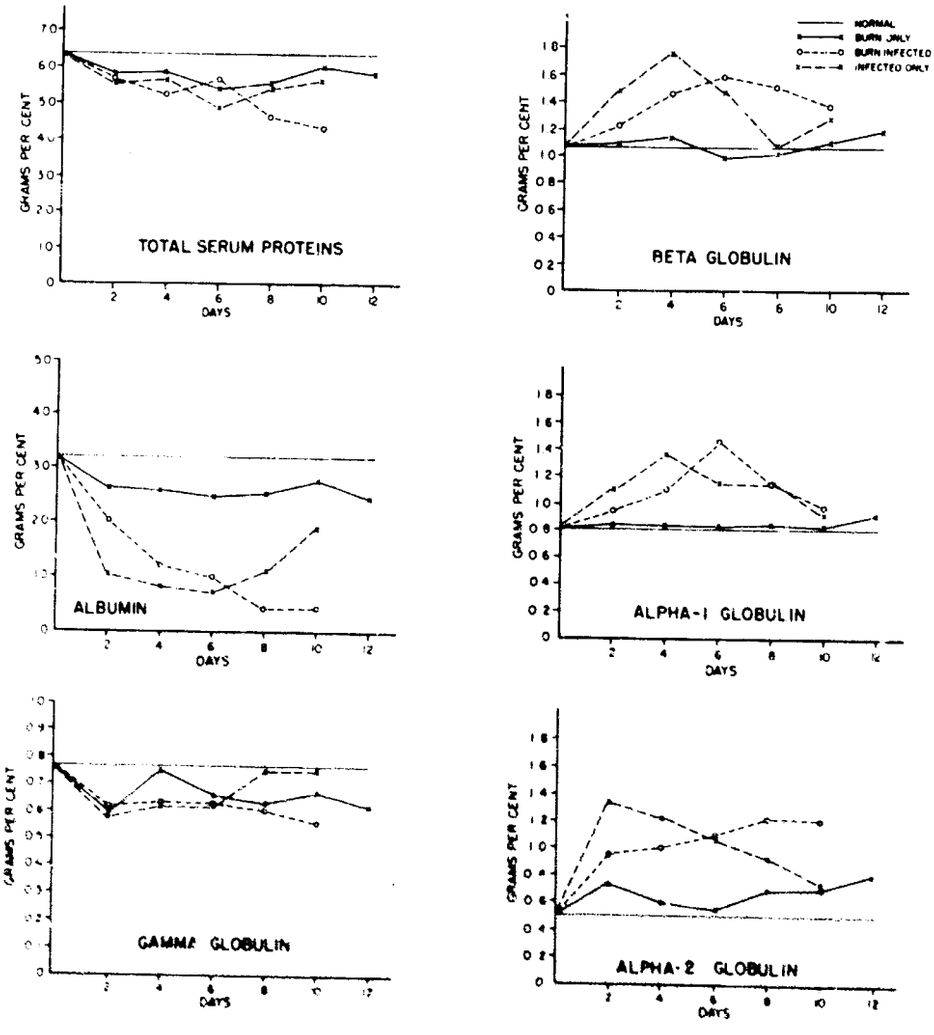
NORMAL
BURN ONLY
BURN INFECTED
INFECTED ONLY
GRAMS PER CENT
DAYS
TOTAL SERUM PROTEINS
BETA GLOBULIN
ALBUMIN
ALPHA-1 GLOBULIN
GAMMA GLOBULIN
ALPHA-2 GLOBULIN

Figure 2

animals in whom spontaneous rupture of an abscess had occurred. An average value of 1.34 gm per cent alpha-two globulin on the second day after production of the abscess as compared to 0.5 gm per cent of the normal value represents a striking increase.

It is significant that the injection of a Pseudomonas extract without viable bacteria (Group E) created changes similar to those seen with burn-infection or infection alone with the exception of alpha-one globulin (Table 2). It would seem from these studies that bacterial products themselves contribute significantly to the serum protein changes following thermal injury. Apparently, it is necessary to have both tissue injury and infection to produce a marked rise in the alpha-one globulins since neither injection of the bacterial extract nor a burn in itself produced the characteristic changes which followed severe thermal injury with surface infection.

Discussion

A number of investigators have described the alterations in the serum protein electrophoretic patterns of burned patients since the first systematic study by Prendergast, Fenichel, and Daly in 1952.[17] The studies of Birke, et al.,[3] and Lanchantin and Deadrick [11] are particularly noteworthy. The major alterations found in our patients with severe thermal injuries do not differ significantly from those previously described in the works of the aforementioned authors. In general, the changes found early in the course following a burn injury have been felt to be a result of loss of protein into the burn wound itself, and the later alterations have been felt to result from tissue injury and repair or possibly from infection in some instances.

The reviews by Pederman[16] and Wall [19] indicated that depression of the serum albumin levels and elevation of alpha-one and alpha-two globulin occurred in several disease entities, but when profound changes occurred in these three regions, associated with an elevated gamma globulin, they were almost always associated with acute infection. Dunn and Pearce [7] recorded similar results in a study of clinical patients having various disease entities in a general hospital. In a study of 83 patients, seriously ill with acute infections, Graham, Dobson, and Yow [8] also described similar depressions of the albumin, with elevations of alpha-one, alpha-two, and also beta globulin and gamma globulin. The increase in the

Table 2. Electrophoretic Values on Rat Sera Following the Injection of Pseudomonas Extract Intraperitoneally (Group E)
(Sera Drawn One Day After Last Injection)

Amt. Extract (cc)	No. Mice	No. Doses	Total Serum Protein	Albumin	Gamma Globulin	Beta Globulin	Alpha-one Globulin	Alpha-two Globulin
0.5	3	2	5.55	2.43	0.58	1.05	0.60	0.91
0.5	2	4	5.40	1.93	0.66	1.24	0.72	0.88
1.0	2	2	5.55	2.16	0.53	1.08	0.77	1.10
1.0	2	4	4.50	1.42	0.44	1.05	0.74	0.86
2.0	2	1	5.20	1.55	0.58	1.33	0.88	0.88
Normal Values			6.40	3.20	0.73	1.06	0.81	0.51

beta globulin was seen primarily with infections caused by gram-positive cocci. It is characteristic of bacterial infections in man that the gamma globulin is elevated, particularly in chronic infections.

This investigation was undertaken to determine the role of infection in producing the serum protein alterations following severe thermal injury. For this purpose, two types of studies were employed. The first of these compared the serum protein alterations in two groups of burned patients, comparable with the exceptions that patients in Group A had slightly more severe burns and were not given as much early albumin replacement, and that fewer fatal septic complications were seen in the more recent group (B) which had been treated with topical chemotherapy (Sulfamylon). This is not to say that surface infections did not occur in the latter group, for they were invariably present although less severe. Bacteria in large numbers have been found in the burn wounds of almost every patient studied who has been treated with Sulfamylon even though invasive infection with septicemia has been less frequent.[13] The serum protein alterations in both groups were quite similar, with only slight differences which could be attributed to a slight variance in the technic and the relative lack of albumin replacement in Group A. However, within the group where sepsis was common (Group A), the serum albumin levels were somewhat lower and the serum alpha-one and alpha-two globulins higher in those patients where infection was fatal. Occasional individual electrophoretic patterns in patients dying with Pseudomonas or Staphylococcal septicemia showed striking changes characterized by progressive depression of the serum albumin, no change or slight elevation in the beta and gamma globulins, and an elevated level of alpha-one globulin. The alpha-two globulin changes were often inconstant although there tended to be an early rise with a subsequent depression shortly before death with Pseudomonas septicemia, possibly a result of hemolysis with removal of haptoglobin by free hemoglobin. The alterations seen in individuals with burn injury ranging from 30 per cent to 50 per cent when compared to individuals with burn injury ranging from 50 per cent to 90 per cent were slightly but significantly less pronounced and tended to return to normal at an earlier time.

The second type of study involved investigations performed in experimental animals in which burn injury with and

without infection was produced and in which a severe abscess produced by Pseudomonas and the injection of bacterial products were used to determine the influence of infection upon the protein changes. The results show that infection itself plays a major role in many of the protein changes, especially those in the albumin, alpha-one, and alpha-two ranges as determined by paper electrophoresis. Alpha-two globulins increased significantly after a 20 per cent third degree burn without bacterial contamination, but the increase was much greater when infection of the burn wounds occurred. An increase was also seen after the intraperitoneal injection of a Pseudomonas extract or in those animals with Pseudomonas abscess. These findings indicate that infection can play an important role in the changes of alpha-two globulin. By contrast, neither the injection of Pseudomonas extract nor burn injury itself significantly altered the alpha-one globulin levels, but active bacterial infection did cause a striking increase in the alpha-one globulin whether or not burn injury was present. The serum albumin was somewhat depressed by burn injury alone, but markedly depressed by the complication of infection, whether or not the infection was associated with burn injury. Likewise, injection of a sterile Pseudomonas extract caused a rather marked depression of serum albumin levels which seemed to be directly proportional to the amount of extract given. In contrast to humans, beta globulins were moderately elevated by infection, and gamma globulins slightly depressed. Burn injury in itself caused a slight depression of gamma globulin, but essentially no change in the beta globulin levels.

The relative lack of difference between patients in Group A and Group B would indicate either that burn injury is more important than infection in producing the characteristic protein changes or that the burn wounds in both groups are infected. Since all burn wounds, even in the second group, invariably had bacterial contamination and growth, and since injury without infection can produce similar protein changes of a lesser magnitude, it is impossible to separate these two possibilities by our study. This lack of separation makes serum electrophoresis an ineffective tool in the clinical management of burn injuries. Nevertheless, it is evident from the animal experiments and from the observations on several of the patients that infection can and probably does contribute greatly to the serum protein alterations which are seen following severe thermal injury.

Summary and Conclusions

A parallel study comparing the electrophoretic patterns in two groups of comparable severely burned patients, where fatal septic complications were seen frequently in one group and much less frequently in the other, was made to determine what influence infection had upon the electrophoretic patterns following severe thermal injury. A second study in experimental animals compared the serum protein changes with burn, burn plus infection, infection associated with abscess formation, and the injection of bacterial extracts with neither burn injury nor infection.

Those patients dying with septicemia caused by Pseudomonas aeruginosa or Staphylococcus aureus definitely tended to have a marked terminal depression of albumin with elevated gamma globulin and alpha-one globulin levels. With both moderate and severe infections, the alpha-two globulin levels tended to be high, but these were usually depressed terminally with fatal infections, possibly because of hemolysis with haptoglobin combining with free hemoglobin. Generally, however, the electrophoretic patterns for both groups were quite similar except at the time of actual septicemia.

Infection of the burn wound in the experimental animal caused a characteristic increase in the alpha-one and alpha-two globulin and beta globulin, a mild depression of the gamma globulin, and severe depression of the albumin, with exceedingly low values found terminally. The induction of an abscess in itself caused similar changes which were even more marked at an earlier time. The changes in the electrophoretic patterns of severely burned patients in both groups tended to more closely resemble those of experimental animals with both burn injury and infection rather than those with burn injury without infection.

It is concluded that the effects of infection may contribute significantly in producing alterations of the serum protein fractions following severe thermal injury. However, the determination of serum protein patterns by electrophoresis has not been found to be a useful tool in clinical management.

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Presentations: None.

Publications

Alexander, J.W., Brown, W., Mason, A.D., Jr., and Moncrief, J.A.: The influence of infection upon serum protein changes in severe burns. J. Trauma (In press).

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(U) Incidence of Positive Antiglobulin Test Following Thermal Injury (44)

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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Thermal injury, Coombs' test

24.
(U) Tech Objective - To determine if Coombs' test becomes positive following thermal injury.

(U) Approach - Direct and indirect Coombs' tests were done on 15 patients admitted to the Burn Study Branch of the Surgical Research Unit. Correlation with clinical hemolysis was attempted.

(U) Progress (Aug 65-Jun 66) - In 11 of the 15 patients included in this pilot study, the direct and indirect Coombs' test remained negative throughout the period of observation. Two patients had a transiently positive direct Coombs' test during the immediate postburn period, while the remaining two patients had transiently positive indirect Coombs' test. In no instance was a positive Coombs' test associated with a clinically recognizable hemolytic episode.

26.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
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31. MISSION OBJECTIVE	32. PARTICIPATION
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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: INCIDENCE OF POSITIVE ANTIGLOBULIN TEST FOLLOWING THERMAL INJURY

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

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William T. Summerlin, Captain, MC
James A. O'Neill, Jr., Captain, MC
Basil A. Pruitt, Jr., Major,MC
Neil Lempert, Captain, MC
Walter E. Switzer, Lt Colonel, MC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: INCIDENCE OF POSITIVE ANTIGLOBULIN TEST FOLLOWING THERMAL INJURY

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert M. Vertel, Captain, MC
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Walter E. Switzer, Lt Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

A high incidence of positive direct Coombs' reactions occurs in burned rats. Rats with negative direct Coombs' develop positive reaction when injected with viable lymph node cells from animals with a positive direct Coombs' This suggests that an autoimmune mechanism is responsible for the positive direct Coombs' test following burns in rats.

Autoimmune hemolytic anemia has been reported in a number of cases characterized by destruction of erythrocytes through mechanical means. Heat induced alterations in the erythrocyte membrane may produce antigenic changes similar to those ascribed to mechanical injury.

Coombs' studies were performed on 15 patients admitted to the Burn Study Branch. The direct and indirect Coombs' test remained negative in 11 patients. Two patients developed a transiently positive direct Coombs' test while the two remaining patients had a transiently positive indirect Coombs' reaction. A positive Coombs' test was not correlated with clinically recognizable hemolysis.

To date there are insufficient data to suggest that an autoimmune mechanism is responsible for the positive Coombs reactions seen in these burned patients.

(1) Thermal injury
(2) Coombs' Test

INCIDENCE OF POSITIVE ANTIGLOBULIN TEST FOLLOWING THERMAL INJURY

Autoimmune hemolytic anemia has been reported in a number of cases characterized by destruction of erythrocytes through mechanical means.[1] These include open heart surgical procedures, implantation of Starr prosthetic heart valves, severe aortic stenosis, and a case involving severe mitral stenosis with turbulent flow. In these cases, it would seem that mechanical damage to the erythrocyte leads to changes in the cell membrane which modifies surface antigens. These modified antigens may appear foreign to the host and initiate antibody formation. Heat causes increased spheroidicity, osmotic and mechanical fragility, and hemolysis of erythrocytes.[2] These heat induced changes in the erythrocyte membrane may produce antigenic changes similar to those ascribed to mechanical injury.

In 1960, McCarthy reported a high incidence of positive direct Coombs' reactions in severely burned Wistar rats.[3] Fifty-one of 56 rats developed positive reactions four days after 37 per cent body surface burns. In these rats, a persistent positive reaction correlated with death and a negative reaction with survival. There is also a correlation with the size of the burn, large burns being more likely to produce positive reactions.[4] An eluate from Coombs' positive cells was capable of giving a positive indirect reaction; however, attempts to demonstrate a circulating red cell specific factor by indirect Coombs' were uniformly unsuccessful.[5] When viable cell suspensions prepared from the lymph node and spleens of those animals with a positive direct Coombs' were injected intraperitoneally into rats with negative direct Coombs', these recipients developed positive reactions and died. Controls injected with cells from Coombs' negative rats remained Coombs' negative and survived. This suggests that an autoimmune mechanism is responsible for the positive direct Coombs' test following thermal injury in rats.[3]

Methods

Fifteen patients admitted to the Burn Study Branch of the Surgical Research Unit have been included in this pilot study. Direct and indirect Coombs' tests have been done on admission and repeated on hospital days 3, 7, and 14.

Hemolysis has been gauged by serial CBC, reticulocyte count, direct and indirect bilirubin, and urinary urobilinogen. This study has been designed to determine the incidence of positive Coombs' test following thermal injury and to correlate this phenomenon with the degree of hemolysis, size of burns, and prognosis.

Results

In 11 of the 15 patients included in this pilot study, the direct and indirect Coombs' test remained negative throughout the period of observation. Two patients had a transiently positive direct Coombs' test during the immediate postburn period, while the two remaining patients had transiently positive indirect Coombs' test. In no instance was a positive Coombs' test associated with a clinically recognizable hemolytic episode.

The Coombs' antiglobulin test has gained wide acceptance in the demonstration of incomplete antibodies. A positive reaction has usually been interpreted to indicate the presence of isoimmune or autoimmune globulins. However, false positive Coombs' tests have been attributed to a number of divergent factors.

Clinical dextran is commonly employed during the immediate postburn resuscitation. This macromolecular plasma volume expander also interferes with the blood clotting mechanism. This antithrombotic effect may result from adherence of dextran to platelets and also to intimal surfaces of arteries, especially at sites of recent injury. Coating of circulating red blood cells has also been demonstrated.[6] Since dextran is known to be antigenic in man, it is possible that anti-dextran antibodies could cause agglutination of dextran-coated red blood cells. Adequate washing of red blood cells prior to performing a direct Coombs' agglutination should prevent a false positive reaction, since erythrocytes which have been exposed to dextran can be readily freed from it by washing. The presence of dextran in a patient's plasma may interfere with indirect Coombs' test by causing heavy rouleaux formation of uncoated red blood cells.[7] We are currently engaged in a prospective study to determine the incidence of positive Coombs' reactions in patients receiving clinical dextran in the treatment of thrombophlebitis.

Identification of reticulocytosis as a cause of a false positive direct Coombs' test was first reported by Southerland, Isentrout, and McCall.[8] Their study revealed that many reticulocytes have a surface coated with globulin.

The role of bacterial contamination in producing false positive agglutination during blood grouping procedures has been well documented. Beck, et al., demonstrated that blood incubated with Pseudomonas effusa gave positive direct and indirect antihuman globulin reactions.[9] The sterile control blood and blood containing other organisms gave negative results with the Coombs' test. This study may have special significance in view of the frequency of Pseudomonas infections in burned patients.

At present we have insufficient clinical data to suggest that an autoimmune mechanism is responsible for the positive Coombs' test which we have found following thermal injury.

References

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Presentations and/or Publications

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6390	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	6. SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A[illegible]4501B71R 01 181	NONE

11. TITLE: (U) The Effects of Administration of Dextran Solutions on Bleeding in Surgical Patients (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	11 64	NA	Other DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	1	2
		CURRENT FY 66	2	4

19. GOVT LAB/INSTALLATION/ACTIVITY

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Washington, DC 20315

RESP. INDIV.: Kovaric, J.J., Lt Col
TEL.: 8 22 63737

20. PERFORMING ORGANIZATION

NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas

INVESTIGATORS PRINCIPAL ASSOCIATE: Foster, L.H., Jr, Maj, MC
Moncrief, J.A., Col, MC

TEL.: 512 - CA1 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS: Dextran, bleeding, donor sites, hemorrhage

24. (U) Tech Objective - The objective is to document or disprove the assertion that the standard dextran solution causes a clinically significant defect in the hemostatic mechanism in traumatized patients.

25. (U) Approach - Donor site blood loss in cubic centimeters per square inch will be determined twice in each of 50 patients undergoing autograft procedures, once after Plasmanate is given preoperatively and once after dextran. The rate of completion of this project is governed by the rate of admission of burn patients requiring two or more autograft procedures.

26. (U) Progress (Nov 64 - Jun66) - Ten pairs of results have been accumulated. Average blood loss after Plasmanate is 0.95 cc/sq in, and after dextran it is 1.54 cc/sq in. This difference is not yet statistically significant.

27.

27. COMMUNICATIONS SECURITY	28.	29. DDC CODE	30. SUBJECT CODE
☐ a. CLASSIFIED OR CONTROL REQUIRED ☒ b. NOT REQUIRED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. GOV. FUNDS (In thousands)	36.

DD FORM 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECTS OF ADMINISTRATION OF DEXTRAN SOLUTIONS ON BLEEDING IN SURGICAL PATIENTS *

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Lawrence H. Foster, Jr., Major, MC
John A. Moncrief, Colonel, MC
Charles R. Ritchey, Captain, MSC

* Joint study in cooperation with the Ad Hoc Committee on Effects of Dextran on Bleeding, National Research Council.

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: THE EFFECTS OF ADMINISTRATION OF DEXTRAN SOLUTIONS ON BLEEDING IN SURGICAL PATIENTS *

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Lawrence H. Foster, Jr., Major, MC
John A. Moncrief, Colonel, MC
Charles R. Ritchey, Captain, MSC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Either dextran or Plasmanate is given preoperatively to autograft patients. Blood loss is measured from the donor site sponges. The 10 pairs of results already accumulated do not yet show a statistically significant difference. Results from 40 more patients will be accumulated.

(1) Dextran
(2) Bleeding
(3) Hemorrhage
(4) Donor sites

* Joint study in cooperation with the Ad Hoc Committee on Effects of Dextran on Bleeding, National Research Council.

THE EFFECTS OF ADMINISTRATION OF DEXTRAN SOLUTIONS ON BLEEDI'G IN SURGICAL PATIENTS

Since 1953, dextran solution has been sold commercially throughout the world and has been infused into many patients. To date, however, no adequately documented reports of controlled studies have become available that would clearly implicate the infusion of dextran as the cause of abnormal bleeding in a patient. On the other hand, many physicians have heard that the administration of dextran is associated with a "bleeding tendency" and that giving it to a patient, even in limited amounts, incurs a serious risk of hemorrhage. There is no evidence that concerted efforts have ever been made to acquire adequate data from controlled studies on the influence of dextran on bleeding in surgical patients.

In view of this situation, a special panel of the Division of Medical Sciences, NAS-NRC, in its Statement Concerning Studies on the Physical, Chemical, and Physiological properties of Certain Artificial Macromolecular Agents, dated 26 April 1963, noted that, "It should be possible within a relatively short time and at comparatively little cost to complete the studies required to document or disprove the assertion that the standard dextran solution causes a clinically significant defect in the hemostatic mechanism in traumatized patients."

Several military hospitals are cooperating on accumulating blood loss figures from an experimental group of 250 patients, 80 of whom will undergo herniorrhaphy after either Plasmanate* or dextran administration, 60 colecystectomy and 60 radical mastectomy, plus 50 to be accumulated at this institution who will undergo skin grafting.

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* Cutter Laboratories, Berkeley, California.

Methods

Patients expected to undergo two or more autograft procedures are chosen at random to receive either Plasmanate or dextran (10 cc/kg) immediately prior to each skin grafting. The surgical sponges applied to the donor sites are collected, washed in a measured amount of distilled water, and an aliquot of this is used to determine the hemoglobin concentration by the cyanmethemoglobin method. Additional blood loss from the donor sites accumulating in the fine-mesh gauze dressing during the first 24 hours postoperatively is estimated and added to the measured amount of surgical loss. Donor sites are then carefully measured and the total square inches of donor site area calculated, dividing the total blood loss in cubic centimeters by the total donor site area in square inches to give two figures for each patient: (1) blood loss in cubic centimeters per square inch after having had Plasmanate, and (2) blood loss in cubic centimeters per square inch of donor site after having had dextran.

Results

At this writing, 10 matched pairs of figures have been accumulated, as follows:

Blood Loss in Cubic Centimeters Per Square Inch of Donor Site

Patient	Plasmanate	Dextran
1	0.70	0.64
2	0.47	1.72
3	0.84	1.62
4	1.25	0.73
5	2.17	1.24
6	1.25	0.73
7	0.50	2.84
8	0.18	2.06
9	1.10	2.10
10	1.06	1.78

Average blood loss for Plasmanate is 0.95, and for dextran 1.54. This difference is not yet statistically significant.

Forty more matched pairs will be accumulated.

Summary

Either dextran or Plasmanate is given preoperatively to autograft patients. Blood loss is measured from the donor site sponges. The 10 pairs of results already accumulated do not yet show a statistically significant difference. Results from 40 more patients will be accumulated.

References

1. Hainline, A., Jr.: Hemoglobin, in Standard Methods of Clinical Chemistry, American Association of Clinical Chemists, ed. D. Seligson, Academic Press, Inc., New York, 1958, vol. 2, p. 49.

Publications and/or Presentations

None.

GPO 710004

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 0A 6960	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A0145018 71R 01 201	NONE

11. TITLE: (U) A Study of White Phosphorus Burns in Humans Regarding the [illegible]tion of Resulting Systemic Phosphorus Poisoning (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	03 66	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	4

19. GOV'T LAB/INSTALLATION/ACTIVITY

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20. PERFORMING ORGANIZATION

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ADDRESS Fort Sam Houston, Texas

INVESTIGATORS PRINCIPAL ASSOCIATE Summerlin, W.T., Capt, MC
Walder, A.I., Capt, MC
TEL. 512 - CA1 2720 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS

White phosphorus burns, systemic phosphorus poisoning

24. (U) Tech Objective - To better define the characteristics of and etiology of the clinical picture seen with systemic phosphorus poisoning due to white phosphorus burns.

25. (U) Approach - To regularly measure and test: urine, Coombs, serum phosphorus and calcium, blood sugar, total proteins and A/G ratio, thymol turbidity, prothrombin time, bilirubin and where indicated - plasma hemoglobin, serum haptoglobin, and urine hemoglobin.

(U) Progress (Mar 66 - Jun 66) - Seven patients studied so far; (2 with systemic poisoning - entirely new clinical syndrome with their white phosphorus burn - hyperphosphatemia, intermittent hyperphosphaturia, hypocalcemia, bilirubinemia, hemoglobinemia, hemoglobinuria along with hypoproteinemia, hematuria and oliguria).

26.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
[ ] a. CONNEC OR COMSEC RELATED [X] b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. [illegible] FUNDS (In thousands)	36.
FY [illegible]	

DD FORM 1498 (Items 1 to 36 identical to AMXRA Form 1722) SUPERSEDES PREVIOUS EDITIONS OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: A STUDY OF WHITE PHOSPHORUS BURNS IN HUMANS REGARDING THE QUESTION OF RESULTING SYSTEMIC PHOSPHORUS POISONING

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

William T. Summerlin, Captain, MC
Arnold I. Walder, Captain, MC
Ami Giladi, Lt Colonel, MC (Israeli Army)
Walter E. Switzer, Lt Colonel, MC
John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: A STUDY OF WHITE PHOSPHORUS BURNS IN HUMANS REGARDING THE QUESTION OF RESULTING SYSTEMIC PHOSPHORUS POISONING

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: William T. Summerlin, Captain, MC
Arnold I. Walder, Captain, MC
Ami Giladi, Lt Colonel (Israeli Army)
Walter E. Switzer, Lt Colonel, MC
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

Hypoglycemia, hypoproteinemia, hematuria, oliguria, generalized petechiae, icterus, yellow atrophy of the liver, seizures, impaired glycogenolysis, and ischemic-like ECG changes have been reported as toxic manifestations of phosphorus ingestion and/or inhalation. Nowhere does the literature document systemic toxicity secondary to absorption of phosphorus by way of a burn wound. It has been stated, however, that toxic quantities of yellow phosphorus may be absorbed through the skin.

In the past four months, the Surgical Research Unit has admitted two patients with white phosphorus burns who have manifested some of the classically cited systemic stigmata of white phosphorus intoxication (hypoproteinemia, hematuria, and oliguria). One of these patients became anuric and required hemodialysis. Although the literature cites hepatic damage as the most frequent sign of systemic poisoning, liver function studies remained normal in our patients. These two patients manifested what appears to be an entirely new and different clinical picture with their phosphorus injury: hyperphosphatemia and intermittent hyperphosphaturia, hypocalcemia, bilirubinemia, hemoglobinemia, and hemoglobinuria (all documented quantitatively). The urine has been dark

to red-black, with 4+ albumin, 4+ occult blood, red blood cells to numerous to count, and hemoglobin casts. In these phosphorus burned patients, the red blood cell appears to bear the burden of toxicity, not the liver, and with the massive hemolysis that occurs kidney function is jeopardized.

Certain measurable clinical parameters have been examined in all phosphorus burned patients admitted to the Surgical Research Unit in an effort to quantitatively document and better characterize what appears to be a heretofore undescribed clinical syndrome. Seven patients have been studied to date, and present data are insufficient to make any well defined conclusions.

(1) White phosphorus burn injury in humans

A STUDY OF WHITE PHOSPHORUS BURNS IN HUMANS REGARDING THE QUESTION OF RESULTING SYSTEMIC PHOSPHORUS POISONING

This project is designed to study all patients burned with white phosphorus to determine if there is any evidence of systemic toxicity following such injury.

Hypoglycemia, hypoproteinemia, hematuria, oliguria, generalized petechiae, icterus, acute yellow atrophy of the liver, seizures, impaired glycogenolysis, and ischemic-like ECG changes have been reported as toxic manifestations of phosphorus ingestion and/or inhalation.[1-7] Nowhere does the literature document systemic toxicity secondary to absorption of phosphorus by way of a burn wound. Toxic serum levels of inorganic phosphorus have not been established nor have serum levels of inorganic phosphorus been measured in patients with phosphorus burn wounds. Absorption of gaseous phosphorus preparations via the intact skin has been demonstrated as long ago as 1950,[7] and in 1949 it was found that toxic quantities of yellow phosphorus may be absorbed through the skin and by inhalation of phosphorus vapors.[8] It was also noted at this time that the oxidation of phosphorus in the blood requires days.[8] The smallest lethal dose of phosphorus in man has been noted to be 50 mg whereas in one instance a patient was known to survive although he had ingested 800 mg.[5]

Methods

In an effort to better characterize this intriguing problem, the following determinations are being made on each white phosphorus burned patient admitted to the Surgical Research Unit: (Items 3-9 are being investigated on admission and on the 4th, 9th and 14th postburn days.)

1. Daily urinalysis.
2. Daily qualitative urinary urobilinogen (quantitative if positive).
3. Coombs' test, both direct and indirect.
4. Serum phosphorus and calcium and urinary calcium and phosphorus.
5. Blood sugar.
6. Total proteins and AG ratio.
7. Thymol turbidity.
8. Prothrombin time.
9. Bilirubin.

10. Electrocardiogram daily, initially, to determine if there are any ischemic changes.

11. Portable electroencephalogram for any patient showing signs of central nervous system toxicity and/or papilledema.

12. CSF glucose where feasible and advisable (It has been shown that the absence of sugar in otherwise normal cerebrospinal fluid is pathognomonic of phosphorus poisoning.[1]).

13. Where indicated, G6PD deficiency and hemoglobinopathies will be investigated.

14. Where indicated, plasma hemoglobin, serum haptoglobin, and urine hemoglobin.

Results

Seven patients have thus far been studied, three of these thoroughly. Coombs' studies have been entirely negative throughout except for one 3+ positive indirect reaction seen on the fourth postburn day in a 17 per cent burn (four per cent third degree). Urine urobilinogen has been consistently negative qualitatively or within normal limit quantitatively. There has been a decrease seen in the albumin fraction of serum proteins during the first week postburn. Both total and direct bilirubin was increased up to two weeks after injury in two of the patients. In two of the patients, serum phosphorus and calcium were increased and decreased, respectively, for seven days in one case and 21 days in the other.

In the past four months this Unit has admitted two patients with white phosphorus burns who manifested some of the classically cited systemic stigmata of white phosphorus intoxication--i.e., hypoproteinemia, hematuria, and oliguria. One of these patients became anuric and required hemodialysis. Although the literature cites hepatic damage as the most frequent sign of systemic poisoning, liver function studies have remained within normal limits in the Surgical Research Unit patients. These two patients have manifested what appears to be an entirely new clinical syndrome with their phosphorus injury: hyperphosphatemia, intermittent hyperphosphaturia, hypocalcemia, bilirubinemia, hemoglobinemia, and hemoglobinuria. (All have been documented quantitatively.) The urine has been dark to red-black with 4+ albumin, 4+ occult blood, and red blood cells too numerous to count with hemoglobin casts present. In these patients, the red blood cell appears to bear the burden of toxicity, not the liver, and with the massive hemolysis that occurs, kidney function is jeopardized.

Although there are insufficient data to date to make any conclusions, this study will continue in order to better characterize what appears to be a new and heretofore undescribed clinical syndrome.

References

1. Brescia, M.A., and Dobbins, J.M.: Acute phosphorus poisoning. J. Pediat. 21:378-381, 1942.

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4. Brown, C.A., and Halpert, B.: Case report of patient with phosphorus poisoning. Southern Med. J. 50:740-742, 1957.

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6. Dathe, R.A.: ECG changes resulting from phosphorus poisoning. Amer. Heart J. 31:98-102, 1946.

7. Diaz, R.: ECG changes in acute phosphorus poisoning in man. Amer. J. Med. Sci. 241:758-765, 1961.

8. Hunter, D.: Protection of the worker. Brit. Med. J. 1: 506-513, 1950.

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Presentations and/or Publications

None.

GPO 770063

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6983	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A, NEW"	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 168

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) Clinical Operation, Trauma Study Branch (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	02 62	NA	Other DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	10	25

19. GOV'T LAB/INSTALLATION/ACTIVITY
NAME: Headquarters
ADDRESS: US Army Med Res & Dev Command
Washington, DC 20315
RESP. INDIV.: Kovaric, J.J., Lt Col
TEL.: 8 22 63737

20. PERFORMING ORGANIZATION
NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas
INVESTIGATORS PRINCIPAL ASSOCIATE: Alexander, J.W., Capt, MC
Kaye, M.P., Capt, MC
TEL.: 512 - CA1 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Trauma, thrombophlebitis, crush injury

24. (U) Tech Objective - Care and study of acutely traumatized patients. Principal objective is to study the physiological changes that occur in patients with severe traumatic injury in order to improve care of such patients. Subordinate objectives have been the study of crush injury and of thrombophlebitis.

25. (U) Approach - Planned study of patients with acute traumatic injury. Progress has been hampered by lack of clinically suitable patients. Selected patients with thrombophlebitis admitted for study. Data of possible significance recorded with careful followup employed in order to improve technics and procedures used in treatment of patients with traumatic injuries and thrombophlebitis.

26. (U) Progress (Jan-Dec 65) - During this period, 16 patients were admitted to the Trauma Study Branch. Eleven patients with thrombophlebitis were treated. All were given clinical Dextran therapy, and two were treated surgically in addition. Study in crush injury was continued. (See "Crush Injury" elsewhere in this report.) Surgical consultation for the Renal Branch was provided as was outpatient and inpatient consultation service for patients with thrombophlebitis seen by other services of Brooke General Hospital.

27. COMMUNICATIONS SECURITY	28.	29. DOD CODE	30. BUDGET CODE
a. CONSEC OR CONSEC RELATED ☐ b. NOT RELATED ☒		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST FUNDS (In thousands)	36.

DD FORM 1498 (Items 1 thru 36 identical to NAVY Form 1222) SUPERSEDES DD FORM 1498 WHICH WAS OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CLINICAL OPERATION, TRAUMA STUDY BRANCH

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 January to 31 December 1965

Investigators:

J. Wesley Alexander, Captain, MC
Michael P. Kaye, Captain, MC
Charles T. Fitts, Captain, MC
John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: CLINICAL OPERATION, TRAUMA STUDY BRANCH

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 January to 31 December 1965

5. Investigators: J. Wesley Alexander, Captain, MC
Michael P. Kaye, Captain, MC
Charles T. Fitts, Captain, MC
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

The primary responsibility of the Trauma Study Branch of the US Army Surgical Research Unit is to perform clinical investigations on patients with severe physical trauma. Since only a few patients have been available for clinical study, the investigators' activities, for the most part, have been diverted elsewhere, primarily to laboratory investigations and to the treatment and study of thrombophlebitis.

(1) Trauma
(2) Thrombophlebitis
(3) Crush injury

CLINICAL OPERATION, TRAUMA STUDY BRANCH

It is the purpose of the Trauma Study Branch to study the physiological changes that occur in patients with severe traumatic injury. Subordinate objectives have been the study of crush injury and of thrombophlebitis.

Because of a lack of trauma patients at this institution, the investigators have attempted to establish a working relationship with the Robert B. Green Hospital to obtain patients suitable for the study of traumatic injuries. In doing so, they have attended weekly conferences at the Robert B. Green Hospital, including Surgical Grand Rounds, and also have held a course in Surgical Physiology for the surgical residents during the first half of the year. Despite an agreement to initiate a study program of trauma patients with the Robert B. Green Hospital, such was not possible because of a limited resident staff at the Robert B. Green during the period of this report. A few cardiac output studies, blood volume studies, and blood gas studies have been done on patients from Brooke General Hospital Surgical Service, but suitable patients have been infrequent. Efforts are being continued to obtain suitable material.

During the period covered by this report, 16 patients have been admitted to the Trauma Study Branch, Ward 13B. These include 11 patients with thrombophlebitis, and one patient each for crush injuries plus renal shutdown, muscle necrosis and renal shutdown, a retained foreign body, a stab wound, and one patient was admitted as a skin donor. Five patients received operations including two ileofemoral venous thrombectomies, removal of foreign body, a fasciotomy, and a multiple-staged procedure for excision of necrotic muscle in a patient with crush injury.

There have been two publications during the period covered by this report relevant to clinical patients, one involving crush injury and the other thrombophlebitis. Both of these are included as part of this report.

In addition to the above activities, the investigators

have been actively engaged in research projects which are reported elsewhere in this volume. They have also provided surgical consultation for the Renal Branch, including the performance of arteriovenous shunts, and have provided outpatient and inpatient consultation service for patients with thrombophlebitis seen by other services.

Publications

Fitts, C.T., Easterling,R.E., Switzer, W.E., and Moncrief, J.A.: Crush injury. J. Trauma, in press.

Sawyer, R.B., Moncrief, J.A., and Canizaro, P.C.: Dextran therapy in thrombophlebitis, J. Amer. Med. Assoc. 191, 740-742, 1965.

Presentations

Alexander, J.W., Kaye, M.P., and Fitts, C.T.: Shock; Pulmonary Physiology, and Cardiac Physiology. Weekly Surgical Physiology Conference, Robert B. Green Hosp., San Antonio, Texas, from January 1965 to June 1965.

Fitts, C.T.: Crush Injury. Amer. Assoc. for Surgery of Trauma, 14-16 Oct. 1965, Philadelphia, Penna.

Alexander, J.W.: Enhancement of Tumor Growth by Growth-Promoting Factor from Injured Tissues. Surgical Forum, American College of Surgeons, 18-22 Oct. 1965, Atlantic City, New Jersey.

Alexander, J.W.: Alterations of the Immune Response in Thermal Injury. Surgical Seminar, Dept. of Surgery, Univ. of Cincinnati, Cincinnati, Ohio, 3 Nov. 1965.

Alexander, J.W.: Studies on Pseudomonas Immunology. Surgical Seminar, Dept. of Surgery, Univ.of Cincinnati, Cincinnati, Ohio, 3 Nov. 1965.

Alexander, J.W.: Recent Advances in the Treatment of Burns. Ex-Interns and Residents Association of Brackenridge Hosp., Austin, Texas, 6 Nov. 1965.

FINAL REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CRUSH INJURY

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Charles T. Fitts, Captain, MC
Ronald E. Easterling, Major, MC
Walter E. Switzer, Lt. Colonel, MC
John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288 (R1)

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Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: CRUSH INJURY

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Charles T. Fitts, Captain, MC
Ronald E. Easterling, Major, MC
Walter E. Switzer, Lt. Colonel, MC
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

The clinical picture of crush syndrome is characteristic and was carefully observed and recorded during World War II. The intervening years, however, during which such injuries have occurred rarely have dimmed the general appreciation of this problem among physicians. Because of the advent of the artificial kidney even severe examples of this injury can survive with treatment and will probably present the surgeon with the problems of massive debridement, in which the time of debridement, the question of staged versus total excision, and the difficulty of large fluid loss and secondary hemorrhage will present themselves. The experimental work engendered by World War II has continued in the interim and is still going on at the present time, but the lack of large numbers of patients since that time has resulted in few, if any, clinical trials of methods which have shown some promise in the laboratory. It is felt that at the present time sufficient evidence is available to warrant the following program of management in an attempt to prevent renal injury secondary to crush syndrome:

1. Every effort should be made to have a physician present at the site of the accident before the patient is extricated.

2. Intravenous fluid therapy should be started before the crushed limbs are released.

3. Following release of the limb it should be dressed with a compression bandage to inhibit sudden release of a flood of myohemoglobin into the circulation.

4. Serious consideration must be given to immediate administration of a renal vasodilator or adrenergic blocking agent.

5. The use of alkalizing agents and osmotic diuretics have some experimental evidence to support their use and should also be considered.

6. The optimum time for initial surgical debridement has not been determined; this must be determined by the surgeon on the basis of his judgment of the facts at hand as they apply to his particular patient.

(1) Crush Injury
(2) Renal Failure

CRUSH INJURY

Although crush injury has probably been observed and treated by physicians as long as there have been people and physicians, the recognition of the syndrome and most of its ramifications did not occur until World War II when relatively great numbers of patients were crushed, especially in England during the air blitz. At this time, Bywaters [3-9] in London directed his attention toward a group of patients characterized by a history of having been buried beneath debris for several hours, often with compression of a limb by fallen masonry. His description of the clinical finding is lucid, concise, and complete; and one reading of his work will more than likely impart a lifetime ability to recognize the disease entity when confronted with it. The salient features include gradual loss of sensation and paralysis of the pinned extremity, tense swelling shortly after release from the crushing force, and either no skin changes or patchy erythematous areas occasionally progressing to blister formation. The blood pressure is satisfactory initially, but shock may supervene over a period of hours and is almost invariably responsive to fluid therapy. At this time hemoglobin values may climb to as high as 22 gm/100 ml. The first urine passed is colored. The urine volume progressively decreases and nitrogen retention occurs in severe cases.

Approximately two-thirds of the patients in Bywaters' series died toward the end of the first week. He noted that death usually occurred suddenly and was occasionally preceded by cardiac irregularities. At autopsy, the muscles involved in the crushed area were noted to be remarkably pale and the renal tubules were filled with brownish pigment. These two facts were put together by Bywaters and coworkers and an attempt was made to identify the pigment in the urine. Using a Hartridge reversion spectroscope, they were able to satisfy themselves that the pigment was myohemoglobin. Analysis of undamaged muscle and damaged muscle from the same patient revealed that the damaged muscle had lost 75 per cent

of its pigment and 66 per cent of its potassium. The problem was then carried to the laboratory. Using rabbits, whose muscles contain no myohemoglobin, it was found that crush injury to the muscles, produced by tight winding of rubber tubing, resulted in all the characteristics of crush syndrome in man except myonemoglobinuria and renal failure. Renal failure could be produced in the rabbit, however, if human myohemoglobin was injected and the urine made acid.

The following is a case history of classical crush syndrome incurred in the Vietnamese war. On 10 February 1965, the barracks at Qui Nhon Airfield, Viet Nam, was blown up by the Viet Cong. Among the injured was a 20-year-old American soldier who was buried in the rubble for 20 hours before he could be extricated. Initial evaluation revealed that the left leg, buttocks and flank had been pinned beneath masonry resulting in moderate swelling and paralysis of the left leg. On arrival at the Eighth Field Hospital, he was evaluated and over the next 24 hours given 5,000 ml of fluid. At no time was he observed to be in shock.

Several hours following admission, 300 ml of dark brown urine was obtained by catheterization. In the next 24 hours, urine output was 167 ml and the diagnosis of renal shutdown was made. He was air evacuated to Tachikawa AFB Hospital where a Quinton Silastic shunt system was put in place, and hemodialysis was performed five times in the first eight postinjury days. Despite this, on four of these eight days potassium levels of 7.0 mEq/L or higher were recorded. During this period of time, the paralysis of his left leg continued, and a brawny swelling of the left buttocks and flank with bruises was evident. He was then transferred to the US Army Surgical Research Unit, Fort Sam Houston, Texas, arriving on his eleventh postinjury day, having had hemodialysis performed at Travis AFB en route. On admission, the patient was somnolent, and had a low-grade fever which had been present since his sixth postinjury day. The left leg was completely paralyzed and the buttocks and flank were edematous. Patchy, erythematous areas intermixed with

bruises of the skin were present. He was hemodialyzed on the eleventh postinjury day which dropped his non-protein nitrogen from 159 mg to 56 mg per 100 ml. Serum potassium level decreased from 5.5 mEq/L to 4.8 mEq/L. His clinical course appeared relatively benign during this period of time; however on the fourteenth postinjury day blood cultures were reported positive for beta hemolytic streptococci and staphylococci. At this time Prostaphlin was added to the penicillin and streptomycin the patient was already on. Examination of the left buttocks on the fifteenth postinjury day revealed an area of possible fluctuance which yielded purulent material upon aspiration. Smear revealed gram-positive cocci. On that day the patient was taken to the operating room, and under general anesthesia the fluctuant area was incised. An abscess pocket containing approximately 200 ml of pus was drained without difficulty; however exploration revealed that the muscle in the gluteal area was pale yellow and necrotic wherever it could be seen. The incision was extended superiorly onto the flank and inferiorly to the inferior gluteal fold. All muscle visualized was necrotic. Large portions of the gluteus maximus muscle were debrided and the wound was left open. Following this procedure, multiple blood cultures became negative and remained so. Hemodialysis was performed that night using regional heparinization. The following day the dressing was soaked with blood necessitating clamping of several bleeders and the administration of 500 ml whole blood. On the seventeenth postinjury day, he was dialyzed for the last time. Urine output was 600 ml on the eighteenth postinjury day, and he continued on through an uneventful diuretic phase.

The wound was debrided under general anesthesia on the nineteenth and twenty-first postinjury days with large amounts of necrotic gluteal and paraspinal musculature being removed. (Fig. 1.) The wound was dressed open and dressing changes and debridement were done on the ward from that time onward. On the twenty-third and twenty-fifth postinjury days, the patient bled suddenly and briskly from a branch of the gluteal artery. Both episodes were associated with drops in blood pressure and

Figure 1. Appearance of wound after incision and prior to debridement on nineteenth postinjury day.

required 500 and 2,000 ml of blood respectively. The large open wound with irregular surfaces, exposed iliac fossa, and deep pockets was debrided daily and treated with wet soaks on the ward. (Fig. 2.) Fluid loss from the wound was estimated and replaced at as high as 3,000 ml daily before homografting was started. On the fortieth post-injury day, the first of a series of homografting procedures was performed after approximating the skin flaps with butterfly bandages. (Fig. 3.) On his sixty-ninth postinjury day, the remaining area was autografted with a successful take. The function of the femoral nerve had largely returned and function of the sciatic nerve was showing signs of very early positive return. The patient was ambulatory with crutches and because of proximity to his home, was transferred to Walter Reed Army Hospital for further rehabilitation. (Fig. 4).

Discussion

Several cardinal manifestations of the crush syndrome were present in this case. As Bywaters[3] pointed out, large areas of crush involving the trunk frequently go relatively unnoticed because of the absence or mildness of the overlying skin changes. Paralysis of the involved extremity was characteristic in the series of Bywaters[3] and others[2] and was present in this case. The first urine obtained was reddish brown in color as would be expected. It has been pointed out[15] that as a result of crushed tissue, the NPN may rise at four times the rate and potassium at twice the rate one might expect in renal failure unassociated with this type of trauma. It is felt that the frequent necessity for dialysis early in this patient's course was due in part to this factor. In addition, this patient presented a problem rarely encountered in the World War II experience, that is, the necessity for massive debridement of necrotic muscle. Without the benefit of the artificial kidney, approximately two-thirds of the patients with crush syndrome succumbed within seven to ten days. The large amounts of necrotic muscle did not give rise to any apparent complication during that period of time and usually there was no operative debridement. (In the present case,

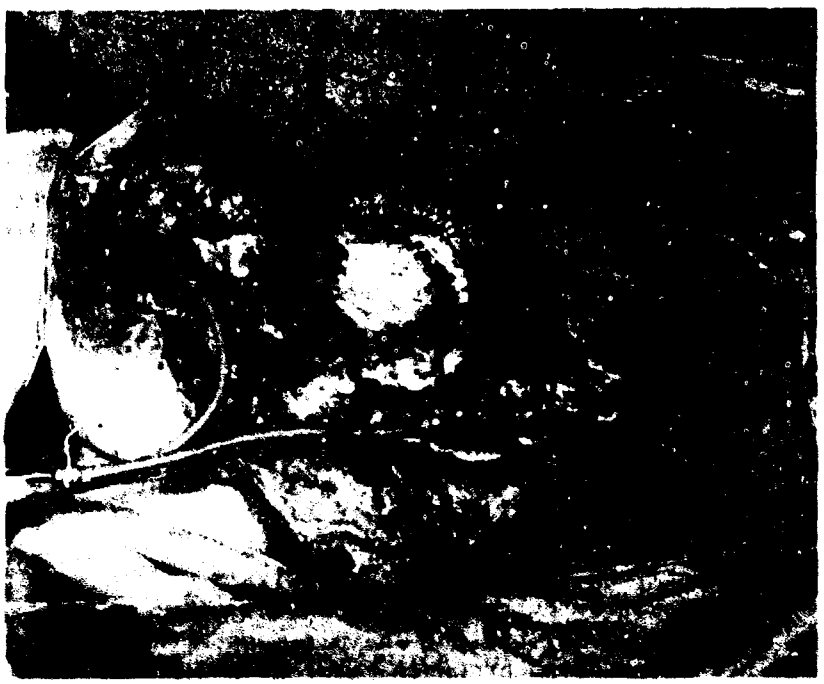

Figure 2. Appearance of wound after several operations on the thirty-seventh postinjury day.

Figure 3. Wound edges pulled together and open areas covered with homografts on fortieth day.

Figure 4. Photograph of healed wound and patient on crutches immediately prior to discharge.

sepsis did not become evident until approximately the fourteenth postinjury day.) Those who survived apparently had smaller amounts of necrotic muscle because it is described as eventually calcifying and leading to little if any difficulty.

This patient was handled with staged debridement. Once the abscess was drained, the patient's condition improved and he tolerated subsequent excisions. It was felt that an attempt to excise the entire amount of necrotic muscle at one time would have been too much operative trauma although this opinion was not unanimous. The possibility of early excision of necrotic muscle, within hours of the injury, apparently has not been evaluated although Bywaters[5] mentions a case where amputation of the crushed extremity at 36 hours was of no avail.

The investigative work in the field of crush injury has followed the classical line of first attempting to outline the pathophysiology and then apply therapeutic methods according to this knowledge. Bywaters[9] felt that the crushed muscle liberated myohemoglobin which was then fed in large amounts into the circulation upon release of the crushing force. The myohemoglobin was then felt to be filtered into the urine, concentrated and precipitated in the distal tubules as casts which caused obstruction and tubular degeneration. Baker and Dodds[1] had pointed out as early as 1925 that in acid urine the myohemoglobin was precipitated as acid hematin. Bywaters[9] felt that the primary damage caused by the pigment was probably due to a direct toxic effect more then an effect secondary to obstruction. Corcoran and Page in 1945[10] studied crush syndrome experimentally and felt that in addition to pigment in the tubules, renal vasoconstriction was necessary to get lower nephron nephrosis. In an extensive study, they noted that dehydration, large doses of diuretics, and alkalizing agents such as sodium bicarbonate or sodium citrate were harmful or of no help. Moderate amelioration of the syndrome was noted with the use of small doses of diuretics such as mannitol, infusions of gelatin and

compression of the crushed leg after removal of the crushing force Patey and Robertson[14] had reported excellent results with compression treatment of crush injuries of limbs in 1941. In 1958, Powers, et al.,[16] confirmed the decrease in renal blood flow that accompanies crush type injuries and reported amelioration of the syndrome with surgical denervation, vasodilators and later[11] mannitol. Thompson and Campbell[17] in 1959 found that perfusion of an ischemic limb with dextran before re-establishing circulation resulted in decreased levels of hemoglobin and myohemoglobin in blood and urine. Litwin, et al.,[12] in 1960 found that the administration of acid hematin alone did not produce renal failure but that acid hematin given in the presence of dehydration, renal vasoconstriction or renal ischemia produced acute renal failure in dogs. Mehl, et al.,[13] in 1964 found that they could prevent shock, prevent renal changes, and lower mortality following five-hour crush injuries to dogs by administering THAM.

Conclusions

The clinical picture of crush syndrome is characteristic and was carefully observed and recorded during World War II. The intervening years, however, during which such injuries have occurred rarely have dimmed the general appreciation of this problem among physicians. Because of the advent of the artificial kidney even severe examples of this injury can survive with treatment and will probably present the surgeon with the problems of massive debridement, in which the time of debridement, the question of staged versus total excision, and the difficulty of large fluid loss and secondary hemorrhage will present themselves. The experimental work engendered by World War II has continued in the interim and is still going on at the present time, but the lack of large numbers of patients since that time has resulted in few, if any, clinical trials of methods which have shown some promise in the laboratory. It is felt that at the present time sufficient evidence is available to warrant the following program of management in an attempt to prevent renal injury secondary to crush syndrome:

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Publications

Fitts, C.T., Easterling, R.E., Switzer, W.E., and Moncrief, J.A.: Crush injury. J. Trauma, in press.

Presentations

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AG INCV ACCESSION	REPORT CONTROL SYMBOL
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11. TITLE:
(U) Clinical Operation, Renal Center (44)

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19. GOVT LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Renal failure, hemodialysis, peritoneal dialysis

24. (U) Tech Objective - Care for acute post-traumatic renal failure, evaluate and test new techniques and develop new concepts.

(U) Approach - Chronic cannulation, peritoneal dialysis, etc. Evaluated on basis of clearance and clinical course of the patient.

25. (U) Progress (Jan 65-Dec 65) - Thirteen patients were treated for renal failure. This represented 1548 patient days of hospitalization, the largest total in the past 10 years of operation of the Renal Branch. During 196, 217 hemodialyses were performed, the largest yearly total in the past 10 years; 190 of the 217 hemodialyses (87.5%) were performed in the three patients with chronic renal failure. Nine patients with acute renal failure were treated during the year with a 67% survival rate. In this group, 27 hemodialyses and 371 peritoneal dialyses were performed. The mainstay of therapy in the severely ill hypercatabolic patient remains the 26. Travenol Twin-Coil Artificial Kidney. Several of the patients with smaller increments of nitrogen accumulation were managed successfully with peritoneal dialysis alone. The prophylactic dialysis protocol has been adhered to in all cases.

27. COMMUNICATIONS SECURITY	28.	29. DDC CODE	30. SUBJECT CODE
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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CLINICAL OPERATION, RENAL CENTER

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 January to 31 December 1965

Investigators:

Robert M. Vertel, Captain, MC
Ronald E. Easteriing, Major, MC
Richard M. Freeman, Captain, MC
Myers W. Henry, Sp7
James P. Knochel, Major, MC

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: CLINICAL OPERATION, RENAL CENTER

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 January to 31 December 1965

5. Investigators: Robert M. Vertel, Captain, MC
Ronald E. Easterling, Major, MC
Richard M. Freeman, Captain, MC
Myers W. Henry, Sp7
James P. Knochel, Major, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

Thirteen patients with renal failure were treated in the Renal Branch in 1965. Twenty-seven hemodialyses and 371 peritoneal dialyses were performed on nine patients with acute renal failure. Six of these patients survived. One hundred ninety hemodialyses were performed on three patients with chronic renal failure. One patient has had 98 hemodialyses in 1965, and a total of 119 hemodialyses and 457 peritoneal dialyses during 604 days of oliguria. A second patient has had 66 hemodialyses in 1965; he has had 74 hemodialyses during 437 days of oliguria. A third patient died on the 200th day of oliguria after 26 hemodialyses.

One hundred seventy-seven hemodialyses were performed with the Travenol Twin-Coil Artificial Kidney; 40 hemodialyses were done with the Two-Layer Kill Hemodialyzer. The failure rate of the Travenol Twin-Coils was six per cent compared to a 44 per cent failure rate with the Kill hemodialyzer.

The Quinton Silastic-Teflon Cannula System was used exclusively. The two surviving patients with chronic renal

failure have had successfully functioning arterial cannulae in place for 424 and 367 days, respectively. The current venous cannulae have been functional for 244 days and 109 days, respectively. Quinton cannulae have been successfully employed in four patients with acute renal failure.

(1) Renal failure
(2) Hemodialysis

CLINICAL OPERATION, RENAL CENTER

Thirteen patients were treated for renal failure at the Renal Branch during 1965. This report reviews the course of these patients and the clinical results with the dialysis procedures performed during the report period.

Case Reports

Acute Renal Failure

Patient 1. A 19-year-old Negro female with sickle cell anemia developed acute renal failure following postpartum shock. She subsequently developed progressive nephrocalcinosis and shrinkage of the kidneys, suggesting the diagnosis of bilateral renal cortical necrosis. This patient is discussed in the report entitled, "Postpartum Acute Renal Failure (Bilateral Renal Cortical Necrosis) Associated with Sickle Cell Anemia", elsewhere in this volume.

Patient 2. This 50-year-old Negro female had a history of hypertension first noted in February 1965. At that time, the BUN was 26 mg% and serum creatinine 1.4 mg%. Blood pressure was 170/110 mmHg. The patient was admitted to the hospital in May 1965 because of rapidly progressive azotemia. At the time of this admission, BUN was 152%, serum creatinine 14.8 mg%. A left renal biopsy was attempted on 27 May 1965, which yielded no tissue. Following the biopsy attempt, the patient had an episode of bleeding, most of which was confined to Gerota's space. Her subsequent course was one of rapidly progressive oliguric renal insufficiency. Total anuria was present for the final 28 days of life. During the hospital course, 124 instillations of peritoneal dialysate were required. The patient expired on the 72nd post-onset day. Autopsy revealed renal changes compatible with the diagnosis of acute glomerulonephritis. There was also evidence of advanced arteriolar nephrosclerosis.

Patient 3. A 20-year-old white male Sp4 was injured in a bomb explosion in Viet Nam on 10 February 1965. He

was trapped under debris for 20 hours. During the ensuing 21-day period of oliguria, the patient was hemodialyzed 10 times with the Travenol Twin-Coil Kidney.

Patient 4. This 34-year-old Latin American female developed acute renal failure following postpartum hemorrhage and shock. The patient had a very mild course with day of diuresis on Day 8. No dialyses were required.

Patient 5. A 49-year-old white male, retired Navy corpsman was treated initially at Wright Patterson AFB Hospital for seven days prior to admission. On 11 April 1965, the patient developed fever, rigors, nausea, vomiting, diarrhea, and lower abdominal pain. In the Emergency Room, the patient was noted to have right lower quadrant and suprapubic pain. Temperature was 101°, and urinalysis revealed 3+ albumin, 3-6 white blood cells, and red blood cells too numerous to count. He was begun on Gantrisin and Pyridium and sent home. Urine culture subsequently grew E. coli. His symptoms persisted in spite of this therapy, and the patient returned to the Emergency Room at Wright Patterson AFB Hospital on 16 April 1965, where he was found to be acutely ill. Later that evening, the patient underwent an exploratory laparotomy. A ruptured appendix with subphrenic and subhepatic abscesses was found and appropriate drainage established. The patient subsequently became severely oliguric and was transferred to the Renal Branch of the Surgical Research Unit. During the next 11 days of life, the patient underwent seven hemodialyses. However, he succumbed to his overwhelming infection. At autopsy, purulent peritonitis, subdiaphragmatic abscess, and bronchopneumonia were found.

Patient 6. This 60-year-old white female had a long history of chronic bronchiectasis and several hemoptysis in the past. In May 1965, the patient had a massive hemoptysis associated with marked tachycardia, respiratory distress, and hypotension. Following tracheotomy and blood replacement, the patient's condition stabilized. However, oliguria ensued and the patient was transferred from Fort Hood Army Hospital to the Surgical Research Unit. During the following 16 days of oliguria, the patient underwent four hemodialyses

with the Twin-Coil Kidney and had an uneventful recovery. The patient was transferred back to her referring hospital for further evaluation and treatment of her pulmonary disease.

Patient 7. A 40-year-old white male sustained a 50 per cent total body burn (30.5 per cent third degree) on 30 July 1965 in a plane crash. The patient was without aid of any sort for 36 hours postinjury. When initially seen, in Anchorage, Alaska, the left foot was considered nonviable and disarticulation at the left knee was performed on 1 August 1965. Because of oliguria and hyperkalemia, the patient was transferred to the Surgical Research Unit. During the remaining six days of life, the patient received 63 peritoneal exchanges. However, the burn wound on his back, buttocks, and hip had obvious bacterial invasion. The stump of the left knee was grossly necrotic. The patient died of overwhelming sepsis.

Patients 8 and 9. Two young Army recruits developed acute renal failure following recovery from heat stroke. These patients are discussed in the report entitled, "Heat Stroke and Acute Renal Failure", elsewhere in this report.

Chronic Renal Failure

Patient 1. This 25-year-old white male with Goodpasture's syndrome was initially described in the USASRU Annual Research Progress Report of 30 June 1965. During the past year, the patient has had a total of 98 hemodialyses (67 Travenol Twin-Coil, 31 Two-Layer Kiil) A detailed discussion of this patient's course is described elsewhere in this report under the title, "Goodpasture's Syndrome--Prolonged Survival with Chronic Hemodialysis."

Patient 2. This 32-year-old Latin American male was also described in the USASRU Annual Research Progress Report of 30 June 1965. During the past year, the patient has had 66 hemodialyses (57 Twin-Coil, 9 Two-Layer Kiil). He has now been oliguric for 437 days and has presented no serious problems of management.

Patient 3. This 23-year-old white male was previously described (Case 12) in the report entitled, "Clinical Operation, Renal Center", in the USASRU Annual Research Progress Report of 30 June 1965. This patient had a diagnosis of chronic glomerulonephritis made seven years befor transfer to the Surgical Research Unit, and was known to have had hypertension and azotemia for one year prior to admission. In 1964, the patient was hemodialyzed 15 times and had 145 peritoneal exchanges. Throughout the first four months of 1965, the patient was hemodialyzed 26 times. Despite adequate dialysis, the patient continued to have severe hypertension which did not respond well to drug therapy. The patient expired on 11 April 1965, following an episode of left lower lobe pneumonia. Autopsy revealed chronic glomerulonephritis, left lower lobe pneumonia, and pericarditis with 775 ml of sanguineous effusion.

Not Elsewhere Classified

This 37-year-old white female, gravida six, para six, was well until two weeks after delivery of her sixth child. She suddenly suffered a fulminating illness manifested by peripheral vascular collapse, hepatic and renal failure, leading to death four days after onset. The patient was admitted to the Surgical Research Unit with the diagnosis of acute renal failure. However, on admission the patient was in profound shock and was deeply jaundiced. She expired 12 hours after admission. Autopsy revealed massive hepatic necrosis involving an estimated 90 per cent of the total liver parenchyma. The etiology of the hepatic necrosis could not be determined with certainty. In the absence of known exposure to hepatotoxins, the patient was presumed to have suffered acute fulminating infectious hepatitis. Since this patient presented with shock, a diagnosis of acute renal failure is not warranted.

Summary

Thirteen patients were treated for renal failure in the Renal Branch, Surgical Research Unit, in 1965 (Tables 1,2).

This represented 1548 patient days of hospitalization, the largest total in the past 10 years of operation of the Renal Branch (Table 3). During 1965, 217 hemodialyses were performed, the largest yearly total in the past 10 years (Table 4); 190 of the 217 hemodialyses (87.5 per cent) were performed in the three patients with chronic renal failure. Nine patients with acute renal failure were treated during the year with a 67 per cent survival rate. In this group, 27 hemodialyses and 371 peritoneal dialyses (each instillation of 1, 2, or 3 liters of solution counted as one dialysis) were performed. The mainstay of therapy in the severely ill hypercatabolic patient remains the Travenol Twin-Coil Artificial Kidney. Several of the patients with smaller increments of nitrogen accumulation were managed successfully with peritoneal dialysis alone. The prophylactic dialysis protocol has been adhered to in all cases.

Dialyzer Performance

During the past year, 177 dialyses have been performed with the Travenol Twin-Coil Artificial Kidney. Coil failure was encountered only 12 times during the year (failure rate six per cent). Six coils leaked or ruptured; 3 coils clotted, and an additional 3 could not be primed.

Forty hemodialyses were performed with the Two-Layer Kiil Hemodialyzer. Cuprophan PT 150 membranes have been used. The dialysate is aspirated through the dialyzer opposite the direction of the blood flow and increased negative pressure for enhanced ultrafiltration is obtained by partially occluding the dialysate inflow. In order to achieve 40 successful Kiil dialyses, a total of 71 dialyzers were assembled (44 per cent failure rate). Leaks were detected in 25 dialyzers by *in vitro* testing. The dialysate compartment is opened to the air while the blood compartment is rapidly inflated to approximately 150 mmHg. If this pressure cannot be easily achieved or if the pressure rapidly falls (over 25 mmHg in 30 seconds) the membrane is faulty and the dialyzer must be rebuilt. If this preliminary test is satisfactory, both dialysate

Table 1. Acute Renal Failure - 1 Jan 1965 to 31 Dec 1965

Case	Diagnosis	Age	Sex	Day of Diuresis	Day of Death	Number of Peritoneal Dialyses	Number of Hemodialyses
1.	Postpartum Hemorrhage, Bilateral Cortical Necrosis	19	F	15		50	0
2.	Acute Glomerulonephritis	50	F		72	124	0
3.	Post Traumatic Hemorrhagic Shock	20	M	21		0	10
4.	Post partum hemorrhage	34	F	8		0	0
5.	Acute appendicitis with perforation and peritonitis.	49	M		11	0	7
6.	Post Hemorrhagic Shock	60	F	16		0	4
7.	50% Total Body Burns	40	M		6	63	0
8.	Heat Stroke	21	M	21		48	6
9.	Heat Stroke	21	M	16		86	6

Table 2. Chronic Renal Failure - 1 Jan 1965 to 31 Dec 1965

Case	Diagnosis	Age	Sex	Days of Oliguria	Day of Death	Number of Peritoneal Dialysis	Number of Hemodialysis
1.	Goodpasture's Syndrome (Pulmonary hemorrhage and glomerulonephritis)	25	M	604		0	98
2.	Chronic Glomerulonephritis	52	M	437		0	66
3.	Chronic Glomerulonephritis	23	M		200	0	26

Table 3

PATIENT LOAD 1956 - 1965

YEAR	CARRY OVER	ADMITTED	TOTAL	TOTAL DAYS HOSPITALIZED	AVERAGE DAYS PER PATIENT
1956	0	13	13	157	12.08
1957	0	8	8	124	15.50
1958	0	15	15	289	19.27
1959	0	19	19	460	24.21
1960	3	24	27	791	29.33
1961	1	18	19	402	21.16
1962	2	12	14	398	28.43
1963	1	15	16	793	49.56
1964	0	22	22	1123	51.05
1965	3	10	13	1548	119.07

Table 4

DIALYSIS DATA 1956 - 1965

YEAR	HEMODIALYSIS	PERITONEAL (each installation of one, two or three liters of solution is counted as one dialysis.)
1956	16	0
1957	2	0
1958	71	0
1959	176	0
1960	135	102
1961	39	241
1962	31	109
1963	98	938
1964	142	695
1965	217	371

and blood compartments are inflated to 200 mmHg and observed for loss of pressure during a three-minute period. The dialysate compartment is then deflated to about 50 mmHg while keeping the blood compartment at 200 mmHg. The dialyzer's ability to maintain this differential pressure during a three-minute observation period is noted. Only five dialyzers that passed these rigid *in vitro* standards subsequently leaked.*

Cannulation Experience

All cannulations were done with the Quinton Silastic-Teflon System. The present arterial cannula in Patient 1 (chronic renal failure) was implanted on 12 November 1964 in the left leg. As of 31 December 1965, this cannula had functioned successfully for 424 days. On 1 May 1965, the venous cannula was moved from the lesser saphenous vein to the greater saphenous. This venous cannula was still functioning well on 31 December, 244 days later. On 12 October 1965, the Silastic-Teflon shunt was converted to a single break system by making the venous Teflon extension long enough to reach and connect with the arterial Teflon extension.

Patient 2 (chronic renal failure) has had his present arterial cannula in his left arm since 29 December 1964 (367 days). His present venous cannula has been functioning well for 109 days. On 27 October 1965, the arterial-venous junction of the Teflon cannulae separated, apparently spontaneously. Although the patient had been provided with a hemostat and had been well rehearsed in the management of such cannula emergencies, he apparently panicked and attempted to control bleeding with tourniquests and a clothespin in the cannula. He arrived at the hospital with his clothing and several towels soaked with blood. Bleeding was immediately controlled. His blood pressure was 80/60, and his skin was moist and cool. The

* During the first three months of 1966, 13 additional Kiil dialyses were performed. Only one dialyzer failed to meet *in vitro* standards. One additional dialyzer leaked (failure rate 13 per cent).

patient required four units of whole blood to reestablish cardiovascular stability.

The Quinton cannulae are routinely placed in patients with acute renal failure when hemodialysis therapy is anticipated. In the four patients who were so cannulated, the cannulae performed successfully throughout the entire course of acute renal failure.

Publications

1. Freeman, R.M. and Pearson, E.: Hypomagnesemia of unknown etiology. Amer. J. Med, in press.

2. Freeman, R.M., Vertel, R.M. and Easterling, R.E.: Goodpasture's syndrome. Prolonged survival with chronic hemodialysis. Arch. Internal Med., in press.

3. Freeman, R.M., Forrester, R.H. and Knochel, J.P.: Postpartum acute renal failure (bilateral renal cortical necrosis) associated with sickle cell anemia. To be published.

Presentations

None.

FINAL REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: POSTPARTUM ACUTE RENAL FAILURE (BILATERAL RENAL CORTICAL NECROSIS) ASSOCIATED WITH SICKLE CELL ANEMIA

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 January to 31 December 1965

Investigators:

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Ralph H. Forrester, Colonel, MC *
James P. Knochel, Major, MC

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Reports Control Symbol: RCS-MEDDH-28 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: POSTPARTUM ACUTE RENAL FAILURE (BILATERAL RENAL CORTICAL NECROSIS) ASSOCIATED WITH SICKLE CELL ANEMIA

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 January to 31 December 1966

5. Investigators: Richard M. Freeman, Captain, MC
Ralph H. Forrester, Colonel, MC*
James P. Knochel, Major, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

An unusual case of acute renal failure following postpartum shock during a sickle cell crisis is reported. The development of progressive renal calcification established the diagnosis of bilateral renal cortical necrosis. Unusual aspects of this case of BRCN were the following: (1) an unusually brief period of oliguria, (2) the association with sickle cell anemia, (3) survival of both mother and infant (4) an unexplained elevation of serum alkaline phosphatase activity.

(1) Sickle Cell Anemia
(2) Acute Renal Failure

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POSTPARTUM ACUTE RENAL FAILURE (BILATERAL RENAL CORTICAL NECROSIS) ASSOCIATED WITH SICKLE CELL ANEMIA

The association of sickle cell anemia and acute renal failure is a surprisingly rare phenomenon. In some recent excellent review articles on the renal manifestations of sickle cell anemia,[1,2,3] acute renal failure is not mentioned. This is the first report of bilateral renal cortical necrosis associated with sickle cell anemia.

Case Report

This 18-year-old Negro housewife was discovered to be anemic at four years of age. The only sign of sickle cell anemia, however, was the presence of scleral icterus until age 12 years, when she was hospitalized for several blood transfusions. She estimated that not more than 20 units of blood had been given to her prior to the present illness. She specifically denied previous bone, joint, abdominal, or chest pain.

In November 1964, she was evaluated elsewhere during the fourth month of her first pregnancy. At that time, she had a hematocrit of 18 volumes %, hemoglobin 6.4 g/100 ml, and a reticulocyte count of 28%. She was given 250 ml of packed red blood cells. During the subsequent antepartum course, the hematocrit ranged from 18 to 21%, and the reticulocyte count from 7.8 to 22.9%.* Urinalyses on 13 different occasions were normal; random specific gravities were 1.010 or less. Total serum bilirubin varied from 5 to 10 mg/100 ml. On 25 January 1965, blood urea nitrogen was 7 mg/100 ml, alkaline phosphatase 11 Bodansky units, and serum transaminase 19 units.

She was admitted to the hospital in early labor on 10 April 1965, and delivered a normal infant, without difficulty, under oxygen and pudendal block anesthesia at 9:30 p.m. At 6:45 a.m. the following morning, she developed sudden dyspnea, weakness, and restlessness; her

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* Sickle cell anemia was confirmed by filter paper electrophoresis and a positive sickle cell preparation.

blood pressure became unobtainable. Thirty minutes later, she was unresponsive and required mouth-to-mouth resuscitation because of shallow, slow, and ineffectual breathing. Two units of packed red blood cells were rapidly given, and by 8:00 a.m., her blood pressure was obtainable and her breathing had improved. By 12:00 a.m., she had received three and one-half units of packed red blood cells and 500 cc of 6% dextran. Urinary output approximated 30 to 40 cc per hour throughout the day, but a catheterized speciment the following morning revealed gross hematuria. At 10:00 a.m., the urinary output had dropped to 10 cc per hour, and there was no response to 12.5 grams of Mannitol. Because of oliguria, she was transferred to the Surgical Research Unit. Her complaints at the time of transfer were those of extreme fatigue, some dizziness upon sitting, and bilateral flank pain which had its onset following the hypotensive episode. The pain was initially dull and aching, but had become progressively severe. She denied dyspnea or chest pain at the time of transfer.

Physical Examination: The blood pressure was 120/58, pulse 116 and regular. She was alert and resting comfortably, lying flat in bed. There was marked scleral icterus and pallor of the mucous membranes. The skin was warm and dry. There was no venous distention or palpable lymphadenopathy. The chest was clear. The heart was enlarged almost to the anterior axillary line. There was a heaving, well-localized, apical impulse, and a Grade II/VI soft blowing systolic murmur, especially prominent in the pulmonic area. There was no cardiac gallop or friction rub. Peripheral pulses were all strong and equal. The abdomen was diffusely tender, distended, and tympanitic; bowel sounds were absent. A tender uterus was palpable to the umbilicus. There was bilateral costovertebral angle tenderness to percussion. There was no sacral edema, cyanosis, clubbing, or evidence of previous or active skin ulcers. Deep tendon reflexes were normal.

Laboratory findings at the time of transfer were as follows: hematocrit 26 volumes %, hemoglobin 7.6 g/100 ml, white blood count 25,900, with 89 per cent neutrophils, 4% bands, 6% lymphocytes, 1% basophils, and 21 nucleated red blood cells per 100 white blood cells. Urine was grossly bloody, with an acid pH, 4+ albumin, negative

sugar, acetone, and bile; sediment was loaded with red blood cells, as well as 30 to 40 white blood cells per high power field. Urine culture showed no growth. Urine chemistries: sodium 73 mEq/L, potassium 17 mEq/L, NPN 99 mg/100 ml, osmolality 191 mOsm/kg. Serum chemistries: sodium 118 mEq/L, potassium 6.1 mEq/L, chloride 106 mEq/L, CO2 10 mEq/L, NPN 70 mg/100 ml, creatinine 5.5 mg/100 ml, calcium 9.3 mg/100 ml, phosphorus 6.6 mg/100 ml, osmolality 261 mOsm/kg, total bilirubin 15 mg/100 ml (9 mg/100 ml direct), total protein 4.5 g/100 ml (albumin 1.3 g/100 ml), thymol turbidity 4 units, alkaline phosphatase 11.2 Bodansky units.

Hospital Course: Following admission, hyperkalemia was treated with Kayexalate and intravenous hypertonic glucose solution containing sodium bicarbonate. A nasogastric tube was passed because of abdominal distention and guaiac-positive material was obtained. No urinary response was obtained with the administration of Mannitol, 12.5 gm.

Abdominal pain became more severe, suggesting an acute surgical abdomen, on 13 April, postonset day number 2.* Liver damage was suggested by a prolonged prothrombin time (18.5 seconds with control 13 seconds), serum transaminase of 1420 units and total bilirubin of 14 mg/100 ml (12 mg/100 ml direct)(Fig. 1). Following AquaMephyton, the prothrombin time became normal. Although there was no clinical evidence of localized infection, penicillin and Streptomycin were administered because of low grade fever.

On POD 3, there was a decrease in abdominal distention and nasogastric drainage, and active peristalsis was noted. The hyponatremia, hyperkalemia, and metabolic acidosis had been corrected by moderate sodium bicarbonate supplements and mild fluid restriction. Transfusions were given in an attempt to maintain hemoglobin at 10 g/100 ml (Fig. 2). In spite of a daily urinary output between 500 and 600 cc, the serum creatinine had risen from 5.5 to

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* For convenience, the day of the hypotensive episode is called Day 0, and each subsequent day is numbered, i.e., POD 2 means the second hospitalization day following the day of shock.

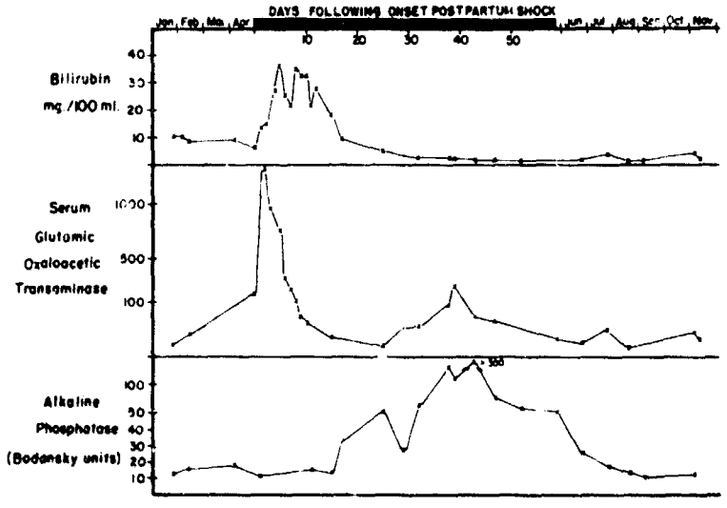

Figure 1. Laboratory data

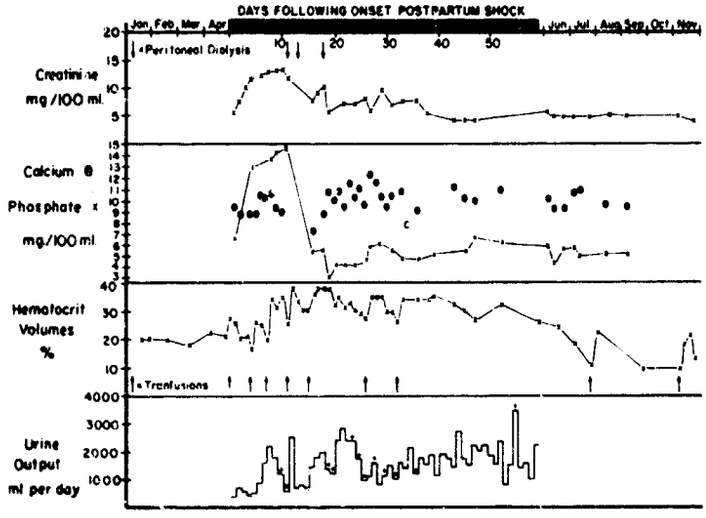

Figure 2. Laboratory data

13.2 mg/100 ml on POD 4 (Fig. 2). Although the urinary output was 1600 cc on the sixth postonset day, azotemia and clinical deterioration were progressive.

On POD 8, she complained of weakness of her arms and legs, and hyperkalemia was confirmed both chemically (serum potassium 7.4 mEq/L) and by striking tenting of the T-waves on electrocardiogram. Ankle and knee reflexes were unobtainable, and sustained clonus was noted on the right side. Kayexalate was given by rectum, and intravenous hypertonic glucose and sodium bicarbonate infusions were administered.

Her mental behavior became progressively bizarre, and on POD 10 she was unresponsive. She had a seizure, characterized by opisthotonic movements of the head, eyes moving to the right, sweating, tachycardia, and a drop of blood pressure to 80 systolic. Chvostek and Trousseau signs were absent, but sustained ankle clonus was noted bilaterally. She became more responsive following the infusion of Plasmanate and treatment with oxygen, phenobarbital, and Dilantin. Colistin was administered because of recurrent fever, pyuria, and the isolation of Pseudomonas (7.8×10^7 organisms per ml) from a catheterized urine specimen. Colistin, 150 mg, was given on POD 10, 75 mg on POD 11, and 150 mg on postonset days 12 and 15.

On POD 11, peritoneal dialysis was initiated because of progressive deterioration, manifested by coma and diffuse bleeding. Intravenous hydrocortisone was started at the same time because of an isolated report suggesting its therapeutic value in a patient with sickle cell anemia and acute renal failure.[4] A lumbar puncture was unremarkable except for a spinal fluid protein of 126 mg/100 ml. On POD 15, she was more alert, although her behavior intermittently was inoppropriate. I.V. infusions were discontinued, and the hydrocortisone dose was gradually decreased. The last of the three 24-hour periods of peritoneal dialysis was performed on POD 18. Postdialysis the serum creatinine value rose slowly, and then fell to a stable level, approximating 4 mg/100 ml.

On POD 19, Pseudomonas was isolated from the peritoneal dialysate for the first time, and she was given a final injection of Colistin, 150 mg. Low grade fever was noted

the same evening and continued intermittently for the next two weeks. On POD 29, a morbilliform rash appeared on the face, arms, and trunk in association with an eosinophil count of 13 per cent. The only medication she was taking at that time, Dilantin, was discontinued. The skin rash cleared within two days, but eosinophilia as high as 30 per cent persisted.

Coincidental with this apparent allergic reaction was a striking rise in the serum alkaline phosphatase, reaching 326 Bodansky units on POD 43 (Fig. 1). At this time she was asymptomatic. X-rays of her bones showed no abnormalities. A liver scan revealed only moderate hepatomegaly. The serum bilirubin was unchanged, but there was a moderate rise in the serum glutamic oxaloacetic transaminase. On POD 53, the creatinine clearance was 7.1 ml/min, the phosphate clearance 4.5 ml/min, and per cent tubular reabsorption of phosphate was 56. Serum calcium and phosphate values appear in Figure 2. On a self-selected diet, urinary calcium was regularly less than 50 mg per 24 hours. Within two months, the serum alkaline phosphatase had returned to "baseline" values for this patient (Fig. 2).

She was discharged from the hospital on June 9, 1965, 59 days following the onset of renal insufficiency. Since discharge, the serum creatinine has fluctuated between 4 and 5 mg/100 ml. Urine cultures have remained sterile and she has been free of uremic symptoms. The hematocrit has dropped to values as low as 10 volumes %, but the patient has vigorously denied associated symptoms or improvement following transfusion.

Absent menses and cold intolerance were responsible for further hospitalization in November, 1965, when the diagnosis of postpartum hypopituitarism was investigated. The 24-hour urinary 17-hydroxycorticosteroids were low. There was a definite rise in plasma cortisol following the administration of ACTH, but no urinary response to Metopirone. The RAI uptake was moderately reduced, and the gonadotropin assay was less than 4 mouse units.

Radiographic Findings (Fig. 3)

During the initial few days of her hospitalization, scout films of the abdomen were taken on three occasions,

and the renal shadows could not be identified definitely on any of them. On May 24, POD 43, the renal shadows were obvious without the use of contrast media (Fig. 3A). The right kidney measured 13 centimeters in length; the indefinite lower pole of the left kidney made accurate measurement of this kidney impossible. Laminograms at that time revealed the so-called "tram-line" calcification of the cortex (Fig. 3B) which has been considered characteristic of bilateral renal cortical necrosis. By June 24, POD 74, the renal calcification had become more prominent; there was a decrease in kidney size bilaterally, both measuring 11.2 cm in length (Fig. 3C). Progressive calcification and decrease in size were noted on July 9, POD 89, when each kidney measured 10.5 cm (Fig. 3D). Both kidneys measured 9.5 cm on August 17, POD 128 (Fig.3E), but no further decrease in kidney size was apparent (Figs. 3F and G). On November 15, POD 218, the rim of cortical calcification was especially prominent in the laminograms (Fig. 3H). The size of the kidneys as measured on the conventional abdominal plain films decreased during the observation period from 13 x 5.3 cm to 9.5 x 4 cm (right kidney).

No roentgenologic evidence of metastatic calcification was noted elsehere.

Discussion

Bilateral renal cortical necrosis (BRCN) is the most reasonable cause for renal insufficiency in this patient. Bilateral flank pain and gross hematuria are characteristic findings in BRCN, and parturition is the most common clinical setting in which this disease develops. A number of patients have survived the acute episode[7-16] and like the present patient, renal function has not returned to normal. An association between postpartum pituitary insufficiency and BRCN has been recognized for many years.[17] Finally, the development of renal calcifications has been a prominent radiographic finding in patients with BRCN who have lived beyond four weeks.[5,6,12,13,14,16,18-20]

There are, however, atypical features of bilateral renal cortical necrosis in the present case. Most postpartum cases of BRCN have been associated with abruptio placenta and stillbirth, both of which were absent in the present patient.

Figures 3A - 3H. Radiographic findings

Figure 3A

Figure 3B

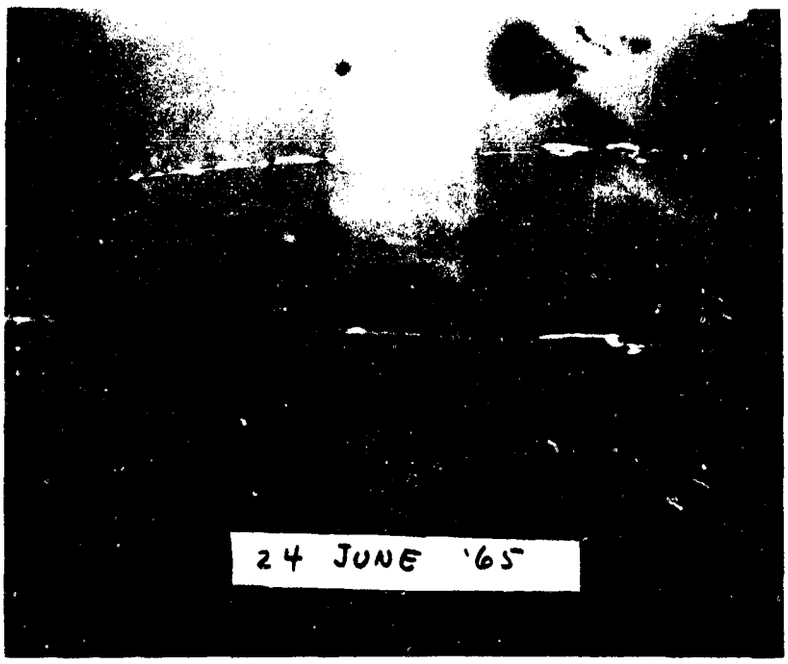

Figure 3C

Figure 3D

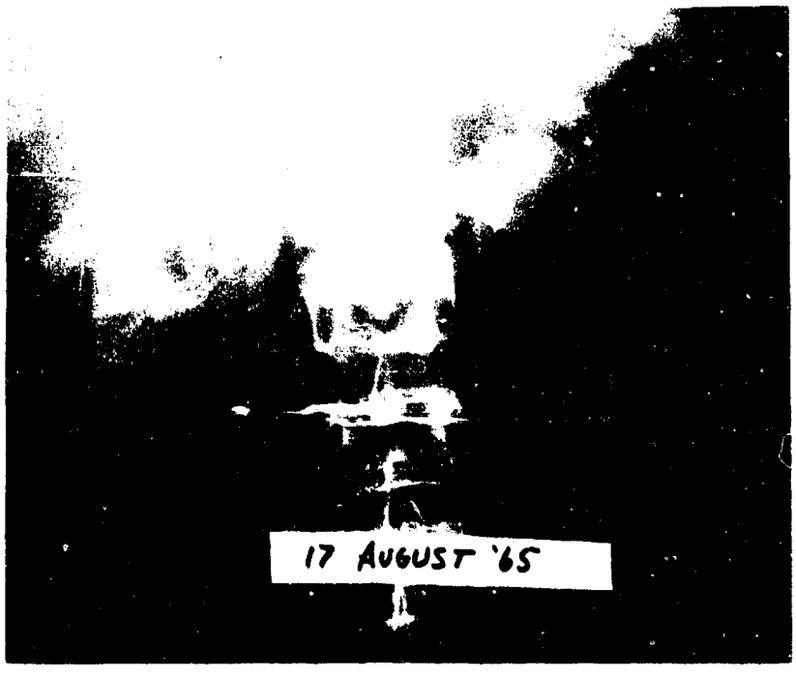

Figure 3E

Figure 3F

Figure 3G

Figure 3H

Anuria is generally so striking that the possibility of bilateral obstruction to the ureters must be considered in the differential diagnosis of patients with BRCN. Although the urinary output in the present patient was transiently as low as 10 ml per hour, the patient was never anuric and the period of oliguria was brief.

A less likely cause of renal insufficiency and calcification in this patient is pyelonephritis. Evidence for acute pyelonephritis was observed on the 10th day following delivery when fever and pyuria were noted, and a significant colony count of Pseudomonas was isolated from the urine. There was no evidence of renal infection before delivery, however, and renal insufficiency was well developed prior to any objective evidence of pyelonephritis. The infection was most likely a complication of the urinary catheter which was in place for two days following delivery. In those few cases in which renal calcification appears to be secondary to chronic pyelonephritis, nephrocalcinosis developed over months rather than days as observed in this patient and is not cortical in distribution. Furthermore, a high incidence of pyelonephritis during pregnancy in both sickle cell anemia and trait has been reported, but renal calcification has not been mentioned.[21-23]

A final factor which may have contributed to the renal damage is the antibiotic, colistin. The potential nephrotoxic effects of colistin in patients with renal insufficiency are now well documented.[24-26] Because of this relationship, the dosage of the drug was reduced. The sterile urine cultures suggest adequacy of this decreased dose of colistin in curing the renal infection. Although renal insufficiency was well established prior to the administration of colistin, we know of no way to rule out superimposed renal damage from this medication.

The value of progressive calcification in the diagnosis of BRCN has only recently been emphasized. At least 10 cases of this association are now recorded.[5-7,12-14,16,18-20] Prior to the clinical use of dialysis procedures, patients with this disorder seldom survived beyond two weeks. Since the earliest demonstration of radiologic renal calcification has been noted at 31 days,[6] it is not surprising that this aspect of the disorder has been rarely mentioned in earlier publications on BRCN. Among the diseases known to produce renal calcification (Table 1), only glomerulonephritis

Table 1. Diseases Associated with Nephrocalcinosis

1.	Primary hyperparathyroidism
2.	Renal tubular acidosis
3.	Chronic glomerulonephritis
4.	Milk-alkali (Burnett's) syndrome
5.	Vitamin D intoxication
6.	Sarcoidosis
7.	Bilateral renal cortical necrosis
8.	Chronic pyelonephritis
9.	Renal tuberculosis
10.	Brucellosis
11.	Idiopathic hypercalcemia of infancy
12.	Hypercalcemia of malignancy
13.	Thyrotoxicosis

produces a similar roentgenographic appearance, and this disease can usually be distinguished from BRCN on clinical grounds. While the development of cortical calcification following acute renal failure is diagnostic of BRCN, its absence does not exclude this diagnosis. A patient with histologically proved BRCN has recently been reported. There was no radiologic renal calcification eight months following the onset of renal insufficiency.[15]

Although BRCN was at one time considered uniformly fatal, a number of survivors have been reported within the past decade.[7-16] Unlike patients with acute tubular necrosis, however, evidence of severely depressed glomerular function has been present in these patients at followup examination. The longest known survivor has lived eight years following the onset of renal insufficiency.[7]

Although we think it likely that the present patient sustained damage to the pituitary gland during the episode of postpartum shock, abnormalities of pituitary and adrenal function are not easily diagnosed in the presence of renal insufficiency. The low urinary corticosteroid values are, of course, consistent with the diagnosis of hypopituitarism; however comparable levels have been demonstrated in patients with chronic renal failure and presumably normal pituitary and adrenal function.[27] These low urinary values have been attributed to decreased clearance by the damaged kidneys. The minimal urinary response to metyrapone also suggests pituitary damage, but the interpretation of this test is also limited by the above considerations. The rise in plasma cortisol two hours following the administration of ACTH eliminates the possibility of adrenal insufficiency damage. The values for urinary gonadotropins and radioactive iodine uptake are also compatible with the pituitary insufficiency of hypopituitarism. While her symptoms of cold intolerance and absent menses suggest hypopituitarism, such findings are frequently present in patients with severe anemia and normal pituitary function. She has none the less been placed on substitution therapy with hydrocortisone.

An association between renal and anterior pituitary necrosis should certainly not be considered rare. Since the original description of this combination by Sheldon and Hertig in 1942,[17] there have been approximately 60 case reports of bilateral renal cortical necrosis associated with pregnancy. Pituitary necrosis has been documented in 12 instances, usually on a histological basis. The incidence may be greater than 20 per cent since in many of the reported cases, the pituitary gland was not specifically studied.

During the past decade, a number of excellent review articles and original papers on the renal aspects of sickle cell anemia have been published.[1-4, 21-23, 28-30] The renal

manifestations of sickle cell anemia are listed in Table 2. An association between BRCN and sickle cell anemia has not been reported, and we are aware of only one other case of acute renal failure complicating sickle cell anemia.[4] This 42-year-old woman went into shock following cholecystectomy for cholelithiasis. Metabolic acidosis developed promptly, and, like the present case, the period of oliguria was relatively short. Seizures which occurred on the 10th postonset day were attributed to acidosis and she recovered without dialysis. Unlike the present patient, recovery of renal function apparently was complete, and clinically she followed the course of patients with acute tubular necrosis.

The abnormal liver function tests in this patient are similar to those observed in five patients with sickle cell anemia in crisis reported by Owen, et al.[39] These patients showed high initial levels of serum transaminase and progressive bilirubinemia. Histologically, plugging of the hepatic sinusoids by sickled red blood cells was prominent. Unlike the present patient, however, there was minimal evidence of renal dysfunction until the terminal onset of oliguria and azotemia. A pathologic picture consistent with "cholemic nephrosis" was noted in the latter cases. Since none of the patients reported by Owen survived, it is tempting to suggest that peritoneal dialysis was of therapeutic value in the treatment of hepatic as well as renal insufficiency in the present patient although information was not collected to document this contention.

During the antepartum course and the seven days following delivery, the serum alkaline phosphatase value varied between 11 and 16 Bodansky units. Elevated alkaline phosphatase activity of this degree is a well documented finding in adults with sickle cell anemia.[40] A value of 34 units was noted on the 17th postonset day, rising to 326 units on postonset day 43, at which time the patient was asymptomatic. We are unaware of such high levels previously being reported in association with acute renal failure. We furthermore have no good explanation for this chemical abnormality. There was no radiologic or clinical evidence of skeletal involvement. Standard clearance tests for parathyroid activity were performed in this patient, but they have little validity in the presence of decreased glomerular filtration. Although there was a coincidental rise in serum glutamic oxaloacetic transaminase, the serum bilirubin was

Table 2. Renal Manifestations of Sickle Cell Disease

Common:
1. Inability to concentrate urine[29,31]
2. Unilateral hematuria[36,38]
3. Pyelonephritis of pregnancy[21,23]
4. Radiographic abnormalities[33,37]
Rare:
1. Nephrotic syndrome[30,32]
2. Chronic renal failure[34]
3. Acute renal failure[4]
4. Acute glomerulonephritis?[35]

stable and other tests of liver function had become normal.

The possibility that alkaline phosphatase originating from renal tissue contributed to the elevated serum level cannot be excluded, since the activity in renal tissue is decreased following experimental kidney damage.[41]

Summary

An unusual case of acute renal failure following postpartum shock during a sickle cell crisis is reported. The development of progressive renal calcification established the diagnosis of bilateral renal cortical necrosis. Unusual aspects of this case of BRCN were the following: (1) an unusually brief period of oliguria, (2) the association with sickle cell anemia, (3) survival of both mother and infant, (4) an unexplained elevation of serum alkaline phosphatase activity.

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Publications and/or Presentations

None.

FINAL REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: GOODPASTURE'S SYNDROME. PROLONGED SURVIVAL WITH CHRONIC HEMODIALYSIS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 January to 31 December 1965

Investigators:

Richard M. Freeman, Captain, MC
Robert M. Vertel, Captain, MC
Ronald E. Easterling, Major, MC

Reports Control Symbol: RCS-MEDDH-288 (R1)

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ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: GOODPASTURE'S SYNDROME. PROLONGED SURVIVAL WITH CHRONIC HEMODIALYSIS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 January to 31 December 1965

5. Investigators: Richard M. Freeman, Captain, MC
Robert M. Vertel, Captain, MC
Ronald E. Easterling, Major, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

A patient with Goodpasture's syndrome maintained by chronic hemodialysis is presented. The total course has exceeded 22 months with anuria existing for the past 16 months. Pulmonary remission has persisted for one year since prednisone was discontinued. The diagnosis of Goodpasture's syndrome need not preclude consideration for chronic dialysis or renal homotransplantation.

(1) Chronic Hemodialysis
(2) Goodpasture's Syndrome

GOODPASTURE'S SYNDROME
PROLONGED SURVIVAL WITH CHRONIC HEMODIALYSIS

The publication of many case reports in the past several years suggests that Goodpasture's syndrome is not as rare as previously emphasized. In the past, this disease has been uniformly fatal when associated with a severe underlying renal disease. Since many of the clinical manifestations of anuric renal disease can now be controlled by modern dialysis techniques, the natural history of the associated pulmonary disease in this syndrome is of particular interest. The patient to be described has been maintained by artificial dialysis procedures for over 22 months. We wish especially to emphasize the absence of recurrent pulmonary hemorrhage during this period of observation.

Case Report

This 24-year-old Caucasian male was in good health until mid-March 1964, when while stationed in Germany he noted the onset of nasal congestion, sore throat, cough, greenish sputum, malaise, and fatigue. The acute symptoms disappeared after several days, but fatigue continued. In early April, he was noted to be pale by his associates, and from April 9 to 14, he produced blood-streaked sputum daily. There was associated right pleuritic chest pain.

On April 14, 1964, he was admitted to the US Army Hospital at Bremerhaven, Germany. On admission, crepitant rales were heard over the lower lung fields and a chest x-ray revealed bilateral pulmonary infiltrates radiating from the hilus with apical sparring (Fig. 1A). The hematocrit was 11 volumes %, hemoglobin 2.9 grams %. Urinalysis revealed a specific gravity of 1.018, acid reaction, 2+ albumin, negative sugar, and a sediment containing numerous red blood cells and casts. The patient was treated with transfusions and penicillin. For two days, his temperature ranged from 102 to 103°F. The admission chest findings and chest x-ray gradually improved (Fig. 1B), but on the third hospital day the patient noted dark urine, and numerous red blood cells were found in the urinary sediment. Within a week, the blood urea nitrogen value was found to be elevated.

On April 22, 1964, he was transferred to the US Army Hospital at Landstuhl, Germany. The azotemia was confirmed

and urinalyses continued to show albuminuria and hematuria. On April 30, 1964, he developed marked swelling of the right upper extremity which was attributed to a thrombophlebitis, secondary to a sulfobromphthalein sodium injection. The following day he developed right chest pains and two days later recurrent hemoptysis. Chest films demonstrated infiltration in both lower lobes and the x-ray findings gradually progressed to a picture similar to that seen on admission at Bremerhaven.

In the process of being evacuated to the United States, he became acutely dyspneic and crepitant rales were heard throughout both lung fields. Chest x-ray revealed extensive bilateral infiltrates (Fig. 1C). Chemical findings demonstrated hyponatremia, hyperkalemia, and increasing azotomia (creatinine 11.7 mg/100 ml). Two units of whole blood were administered and prednisone therapy was instituted (100 mg daily). Within 12-18 hours following the institution of corticosteroid therapy, the patient's dyspnea markedly improved and the rales heard on the initial examination cleared. However, oliguria was noted for the first time, and the patient was transferred to the Surgical Research Unit. Oliguria persisted in flight and did not respond to the administration of 25 gm of Mannitol.

Past medical history was not remarkable except for an episode of pneumonia in 1962. A physical examination in February 1964, one month prior to the onset of his present illness, was normal.

Physical examination on transfer revealed a pale, chronically ill, white male, with blood pressure 130/80, pulse 60, and temperature 96.7°F. Auscultation of the chest revealed only occasional respiratory wheezes at the left anterior lower lung field. There was a sinus arrhythmia but no evidence of cardiomegaly, murmurs, or gallops was noted. There was mild pedal edema. The right arm was tender, and a firm cord-like structure could be palpated in the vicinity of the antecubital vein. The remainder of the physical examination was within normal limits.

Laboratory findings on admission revealed the following: Hematocrit 29 volumes %, hemoglobin 9.2 gm%, WBC 23,700, with 84% neutrophils, 1% bands, 7% lymphocytes,

and 8% monocytes; serum sodium 120 mEq/L, potassium 8.4 mEq/L, chloride 90 mEq/L, CO_2 12 mEq/L, NPN 156 mg%, creatinine 22.8 mg/100 ml, calcium 9.1 mg/100 ml, phosphate 6.6 mg/100 ml. Urinalysis revealed an acid reaction, 4+ albuminuria, 2+ glycosuria, 6-12 white blood cells per high power field, red blood cells too numerous to count, and occasional granular and red blood cell casts. Urine sodium 7 mEq/L, NPN 450 mg%, osmolality 261 mOsm/kg water. Protein electrophoresis revealed total protein of 5.6 gm%, with 51.5% albumin, 7.8% alpha 1 globulin, 14.1% alpha 11 globulin, 13.3% beta globulin, and 13.3% gamma globulin. ASO titer was 166 units.

On the day following admission to the Surgical Research Unit, hemodialysis was instituted because of hyperkalemia and increasing azotemia. Corticosteroid therapy was continued and the chest film cleared promptly (Fig.1D). During initial therapy with hemodialysis, his appetite remained good with a mean caloric intake greater than 2000 daily. A renal biopsy was performed on June 10, 1964, and revealed the following: the vasculature was normal. The tubules were ectopic with focal regions of epithelial degeneration and small patchy areas of interstitial fibrosis. The primary change was localized to the glomeruli. All showed varying degrees of proliferative and fibrous obliteration which was severe in most instances. Because of the chronicity of the renal histology, the prednisone was gradually tapered over the succeeding weeks (Fig. 2).

Although the patient tolerated hemodialysis well, recurrent clotting of the arteriovenous shunts prompted institution of peritoneal dialysis on June 15, 1964, using a silastic conduit which was inserted surgically. From June 15 to October 12, 1964, he was primarily maintained by peritoneal dialysis. He lost over 25 kg of weight during this time, appetite and caloric intake decreased markedly, and daily intermittent vomiting became a clinical problem. Hemodialysis was reinstituted on October 14, 1964, at approximately weekly intervals. His appetite remained poor and vomiting persisted. His lowest weight of 43.4 kg occurred on February 15, 1965. Coincident with increased frequency of hemodialysis, his appetite improved and he has subsequently gained over 16 kg.

In addition to severe nutritional problems prior to February 1965, the following major clinical disturbances

Figures 1A - 1F. Serial roentgenograms of the chest in a patient with Goodpasture's syndrome

Figure 1A. Diffuse pulmonary infiltration on admission to the hospital (14 April 1964).

Figure 18. Spontaneous improvement noted six days later (20 April 1964).

Figure 1C. Appearance of the chest during an episode of recurrent pulmonary hemorrhage (7 May 1964).

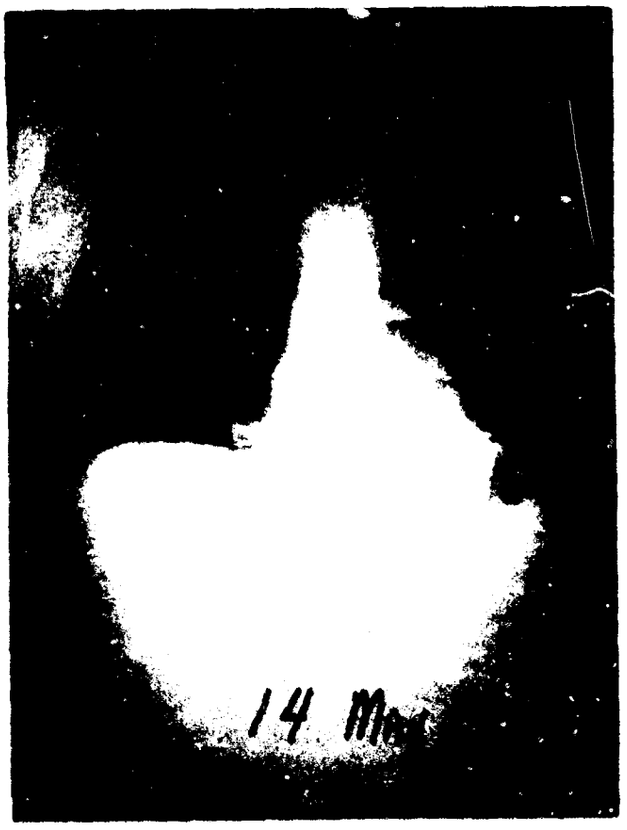

Figure 10. Marked clearing of pulmonary infiltration seven days following the institution of prednisone (14 May 1964).

Figure 1E. Appearance of chest on 3 August 1964.

Figure 1F. Normal chest x-ray taken on 16 February 1965.

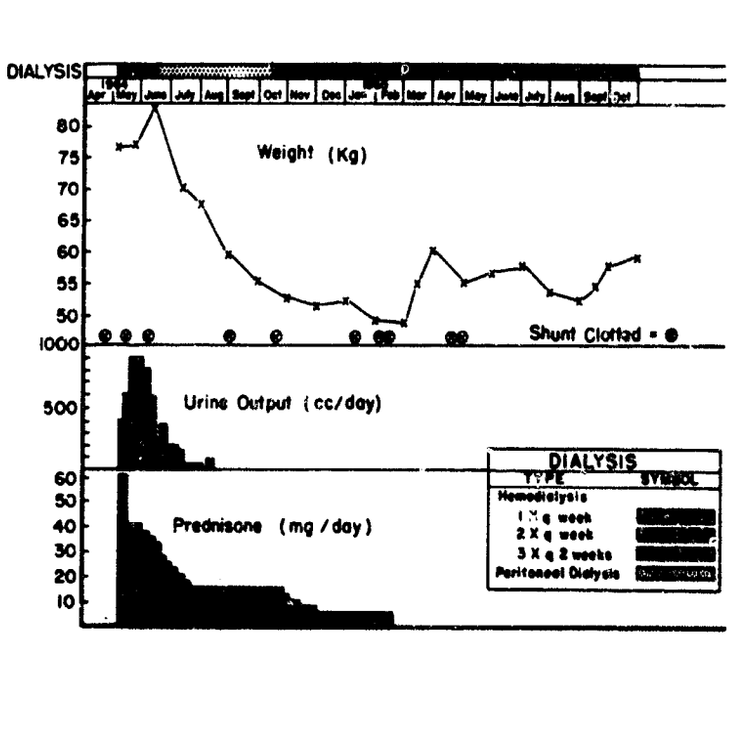

Figure 2. Urinary output, prednisone dosage, and frequency of dialysis during the course of Goodpasture's syndrome.

were encountered:

(1) Three grand mal seizures on August 13, 1964, presumably secondary to hypertension and fluid excess.

(2) Diarrhea followed by hypotension and tachycardia on March 29, 1965. Hyperkalemia (8.5 mEq/L) was associated with right bundle branch block on electrocardiogram. The clinical findings and electrocardiographic abnormality disappeared within one hour following institution of hemodialysis.

(3) Sudden severe pain due to a hematoma in the left calf on April 19, 1965. Presumably secondary to heparin given in an attempt to prevent clotting of the arteriovenous shunts, this hematoma was of clinical significance because it restricted his physical activity.

(4) The onset of arthralgias, involving the hands, shoulders, elbows, and feet, on May 12, 1965. Beneficial response to colchicine has been questionable, but the arthralgias have in general been responsive to mild analgesics.

(5) Two grand mal seizures on July 21, 1965, for unexplained reasons. He was subsequently placed on Dilantin and phenobarbital without recurrence.

At the present time, he is hemodialyzed twice weekly on the Kiil dialyzer for 12 to 15 hours on each occasion. He spends the remainder of his time at home. Physical strength has greatly increased over the past three months, and the physical inactivity associated with previous immobilization has responded well to therapy. His appetite is now excellent with a daily intake of greater than 2000 calories. There has been no recurrence of pulmonary hemorrhage. He has been totally anuric since August 1964.

Discussion

The clinical characteristics of Goodpasture's syndrome are very striking. Pulmonary hemorrhage is the most frequent finding, hemoptysis occuring in a majority of cases. There is evidence of diffuse pulmonary infiltration radiographically. Anemia is invariably present and is often of severe degree. Proteinuria, hematuria, and cylindruria may be present during the first examination, but chemical

evidence of severe renal damage, i.e., marked azotemia, follows rather than precedes pulmonary alveolar bleeding. The disease typically afflicts young adult males.

No attempt will be made to re-examine the clinical and pathological aspects of Goodpasture's syndrome. A number of excellent reviews have been recently published on this subject. We have restricted our discussion to those factors which appear to influence survival in this disease. The use of corticosteroids in the management of Goodpasture's syndrome is also briefly reviewed.

Numerous reports have emphasized the fulminant nature of Goodpasture's syndrome.[1-4] Of the 52 cases reviewed by Benoit, et al.,[3] only two survived. The median duration of illness for the 50 patients who died was 15 weeks. In the 27 patients dying from pulmonary hemorrhage and asphyxia, the median duration of illness was 10 weeks. The remaining 23 patients who died of uremia lived somewhat longer (24 weeks).

During the past two years, many additional cases of this syndrome have been described.[2-3,5-9] As more cases are being recognized, it is becoming clear that all cases do not run a rapidly fatal course. In the 100 cases collected by Duncan,[2] there were 11 survivors. We have found four additional surviving patients in the literature and our own case brings this total to 16.[5,8,9,10] In addition, six reported patients died of their disease after a duration of illness exceeding 18 months.[3,6,10-12]

Although the natural history of this disease is variable, the majority of fatalities occur in the first six months of illness. It appears, however, that if the patient survives the initial phase of his disease, his prognosis for an extended remission may be quite good. This point can be illustrated from Saltzman's review of 39 cases.[1] In the 26 patients who died within the first six months, the mean duration of illness was 2.3 months. In those patients who survived at least six months (13 patients), the mean survival was 16.9 months.

The vast majority of surviving patients have not had advanced renal involvement. To our knowledge, there are only two examples of severe renal failure in Goodpasture's syndrome which proved to be reversible. Pasternack's patient

had an episode of severe azotemia with a spontaneous reversal of nitrogen retention. This patient was well with normal renal function six months later.[5] Bloom, et al., have recently reported a case in which renal function improved following high doses of prednisone.[9] This must be considered an atypical case of Goodpasture's syndrome since hematuria and azotemia were present prior to any evidence of hemoptysis and lung involvement. There was chemical evidence of renal involvement 21 months following the onset of illness although the patient was clinically well. There are, in addition, several reported instances of prolonged survival following onset of end stage renal involvement.[3,4,8] One patient was kept alive for 61 days following the onset of anuria.[3] This patient died following a dialysis schedule which we consider to be inadequate by current chronic dialysis standards. Canfield[4] described a case in which severe oliguria occurred four months after the onset of the disease. Hemodialysis was performed 17 times during the following month; for the next three months intermittent peritoneal dialysis was carried out. Despite these measures, pericarditis and recurrent pulmonary hemorrhage occurred and the patient died four months after the initiation of dialysis. Cleveland, et al.,[8] accepted a patient with Goodpasture's disease for renal homotransplantation. This patient received a cadaver kidney, and at last report was still alive 16 months later. Dialysis has been employed in several other cases.[6,2] However, in most instances, no attempt was made to maintain life once it became clear that the renal disease was irreversible.

The efficacy of steroid therapy in this disease is not clear. In Benoit's series,[3] the steroid-treated group of 25 patients had a median survival of 24 weeks, not impressively different from the total group survival of 15 weeks. However, fewer patients treated with steroids had pulmonary deaths; of the 24 deaths in this group, only eight were attributed to pulmonary hemorrhage. That corticosteroid therapy may have a pulmonary sparing effect is further suggested by several case reports in which arrest of pulmonary bleeding and roentgenographic clearing of the pulmonary infiltrate were temporally related to steroid therapy.[2,9,13,14] In addition, Fairley[13] has described two patients who had immediate recurrence of hemoptysis and hematuria following a sudden large reduction in dosage of prednisone. Unfortunately, there is no way to distinguish a spontaneous remission from treatment effect. The present

patient is an excellent illustration of this problem. The pulmonary infiltrate observed on April 14, 1964 (Fig. 1A), had cleared significantly within six days (Fig. 1B) without specific therapy. The second episode of pulmonary hemorrhage (Fig. 1C) was treated with prednisone and again prompt improvement was noted (Fig. 1D). These observations do not vitiate the efficacy of corticosteroid therapy in Goodpasture's syndrome; it is conceivable, for example, that the administration of prednisone during the first episode of pulmonary hemorrhage would have prevented its recurrence. The frequency of spontaneous remissions of pulmonary hemorrhage, however, makes it impossible to conclusively establish the efficacy of corticosteroids in the treatment of this disease.

A beneficial effect of corticosteroid therapy on the nephritis of Goodpasture's syndrome has also not been clearly established. In certain cases, reversal of renal involvement has followed early therapy.[2,7,9,10,13,14] Other cases have demonstrated relentless progression of nephritis despite the initiation of steroid therapy before signs of advanced renal insufficiency were present.[9,10,15]

Corticosteroids initiated in the presence of advanced azotemia have never, to our knowledge, produced an arrest or reversal of the nephritis. A possible exception is the atypical case of Goodpasture's syndrome reported by Bloom and mentioned previously.[9] Although corticosteroids in the present patient may have been of value in treating the second episode of pulmonary hemorrhage, severe renal involvement was already apparent at this time. The absence of improvement in renal function was therefore not surprising.

The successful maintenance of this patient demonstrates that the diagnosis of Goodpasture's syndrome need not preclude consideration for chronic dialysis or renal homotransplantation.

Summary

A patient with Goodpasture's syndrome maintained by chronic hemodialysis is presented. The total course has exceeded 22 months with anuria existing for the past 16 months. Pulmonary remission has persisted for one year since prednisone was discontinued. The diagnosis of Goodpasture's syndrome need not preclude consideration for chronic dialysis or renal homotransplantation.

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Publications

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Presentations

None.

FINAL REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: HYPOMAGNESEMIA OF UNKNOWN ETIOLOGY

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 January to 31 December 1965

Investigators:

Richard M. Freeman, Captain, MC
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Reports Control Symbol: RCS-MEDDH-28 (R1)

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6. Reports Control Symbol: RCS-MEDDH-288 (R1)

A 38-year-old Caucasian housewife had repeatedly demonstrated hypomagnesemia, symptoms of intermittent weakness and abnormal personality behavior, and a history of dermatographism and bronchial asthma. During a period of supplemental oral magnesium, there was a positive balance of magnesium and a rise in the serum magnesium level. The hypomagnesemia is most likely secondary to urinary losses of this cation, although no evidence for associated renal potassium wasting could be established. The demonstration of hypomagnesemia in one of her children suggests a possible familial tendency for this chemical abnormality. Data were not collected which will allow us to assess the pathological significance of hypomagnesemia in this patient.

(1) Hypomagnesemia
(2) Magnesium Metabolism

HYPOMAGNESEMIA OF UNKNOWN ETIOLOGY

Although detailed knowledge regarding human magnesium metabolism is still lacking, hypomagnesemia has now been described in association with many disease states. Hypomagnesemia in the present patient was unassociated with any of these diseases. However, the discovery of hypomagnesemia in one of the patient's children has suggested a possible familial tendency for this chemical abnormality.

Case Report

This 38-year-old Caucasian housewife was first seen in June 1964 when she came to the Allergy Clinic to continue desensitization for bronchial asthma. Because of psychiatric symptoms, the diagnosis of primary hyperparathyroidism was considered. Although serum calcium and phosphate values were normal, the serum magnesium determinations were repeatedly less than 1 mEq/L (normal mean 1.7 mEq/L with range from 1.4 to 2.0 mEq/L.)

Because of her psychiatric difficulties, it was difficult to pinpoint major complaints. For several years, however, she had noticed a "weak feeling" which seemed to occur with progressive frequency. This weakness appeared abruptly, lasted a few minutes, and was occasionally associated with numbness and tingling on the left side of her body. These symptoms occurred at no particular time and were not related to exertion.

She had episodes of shortness of breath which were not asthmatic in origin. The above symptoms were considered typical of the hyperventilation syndrome. She complained of intermittent headaches of progressive severity. These usually began at the occiput and radiated to the face; they lasted from a few minutes to many hours. Menstrual periods had been irregular throughout her life, usually associated with premenstrual cramps. Although a large variety of medications had been taken prior to June 1964, she denied the regular use of any medications subsequently except for an infrequent Quadrinal tablet for asthma.

The mother furnished information about the patient's infancy and childhood. She weighed six pounds at birth and developed normally until nine months when she refused

cow's milk after being weaned from the breast. Her appetite was poor and "enormous stools" were noted. At one year, she weighed 12 pounds and was said to be suffering from "fat indigestion." She was given a special diet, and by 18 months of age had reached normal weight.

At 12 years, she developed an acute episode of wheezing. The diagnosis of bronchial asthma was made subsequently and she has had intermittent asthmatic attacks since that time. There was a documented history of dermatographism. In June 1960, she was hospitalized because of chills, fever, dysuria, and diarrhea. The initial impression of urinary tract infection was not confirmed by cultures. Low grade fever continued for 11 days, but finally disappeared without specific therapy.

In April 1961, she was admitted to a psychiatric service because of abnormal mental behavior. Schizophrenic reaction was diagnosed, and she left the hospital against the advice of her doctor 10 days later. A second psychiatric admission occurred in July 1961, during the third month of her fourth pregnancy, again for schizophrenic behavior. Mental improvement followed large doses of Thorazine.

Her father died at 56 years from carcinoma of the pancreas and rheumatic heart disease. Her 60-year-old mother is living and well. Her 36-year-old sister is nervous but otherwise healthy.

Physical examination revealed a thin but normally developed white woman with height 160 centimeters (63 inches), weight 45 kilograms (99 pounds), blood pressure 90/60 and pulse 60. There was mild facial flushing and evidence of healed furunculosis over the back. Dermatographism could not be demonstrated. Examination of the eyes, ears, nose, throat and breasts was normal. There was no palpable thyroid or lymphadenopathy. Auscultation of the lungs was normal. The heart was not enlarged; heart sounds were normal and no murmurs were heard. There were no palpable abdominal organs. Pelvic examination revealed a marked rectocele. Mild varicosities were noted on the lateral aspects of both hips. Peripheral pulses were all strong. Both Chvostek's and Trousseau's signs were negative, and deep tendon reflexes were hyperactive bilaterally. No fasciculations were noted, and the sensory examination was normal.

From February to June 1965, she was seen as an outpatient on numerous occasions with multiple, nonspecific complaints. Although hypomagnesemia was confirmed repeatedly, none of her symptoms could be ascribed with certainty to magnesium deficiency. The other serum electrolytes were within normal limits. Eosinophilia was demonstrated on peripheral blood smear and an intermittent tendency toward lymphocytosis was noted. She excreted between 5 and 15 mEq of magnesium in the urine daily on a self-selected diet. The following X-rays were normal: chest, bone survey, intravenous pyelogram. An electroencephalogram revealed a borderline, nonfocal, slow record with intermittent fast activity. Miscellaneous laboratory data appear in Table 1.

Since the hypomagnesemia in this patient did not appear to be associated with any of its recognized causes (Table 2), a description of the case was sent to Dr. Louis G. Welt of the University of North Carolina School of Medicine. Dr. Welt was in the process of evaluating two separate families with hypomagnesemia and suggested that the family members of the present patient be checked for this chemical abnormality. Table 3 shows the results of this undertaking. Of the seven family members tested, only the three-year-old son had a definitely low serum magnesium level on two separate occasions. Unfortunately, the family moved to a different area so that further evaluation of hypomagnesemia in this child could not be undertaken.

On June 30, 1965, the patient was admitted to the Surgical Research Unit for metabolic studies.

Methods

The 17-day study was divided into four periods with specific treatment as follows:

Period One: Five days on constant diet to establish a baseline.

Period Two: Three days with the same constant diet. On the first day of this period, 80 mEq Mg SO4 were infused over a six-hour period.

Period Three: Five days of constant diet continued, with 40 mEq magnesium supplement given as oxide.

Table 1. Miscellaneous Laboratory Data

		Hematocrit [illegible]/100 ml	WBC/cu mm	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
March	1963	44	5600	34	51	12	2	1
December	1964		7900	54	37	1	8	
March	1965	41	7800	35	52	4	7	2
June	1965	42	7600	31	55	3	10	
July	1965	38	7600	51	42	2	5	

Urinalyses: pH 6.5 or less on most determinations; sugar, protein, and sediment negative on numerous determinations

Urine cultures: no growth on three occasions.

Total protein 7.1 gm/100 ml; albumin 66.5%; alpha-1-globulin 3.5%; alpha-2-globulin 9.7%; beta globulin 10.6%; gamma globulin 9.7%.

Serum bilirubin 0.5 mg/100 ml; thymol turbidity 4 units; serum alkaline phosphatase 3.4 Bodansky units; serum glutamic oxaloacetic transaminase 14 units; serum cholesterol 142 mg/100 ml; serologic test for syphilis nonreactive.

Plasma cortisol 20 mcg/100 ml.

Blood volume (RISA method) 3600 ml (76 ml/kg).

% absorption of radioactive triolein: 2 hours - 10.3%; 3 hours - 10.3%; 4 hours - 10.8%; 5 hours - 9.7%; 6 hours - 8.7%.

Urinary porphobilinogens none; urinary coproporphyrins 24.9 mcg/24 hr; urinary uroporphyrins none.

Urinary 17-hydroxycorticosteroids 2.2 mg/24 hr; urinary ketosteroids 4.8 mg/24 hr; urinary uric acid 352 mg/24 hr.

Radioactive iodine uptake 15%/24 hr

Parotid fluid flow rate 1.37 ml/min; sodium 76.5 mEq/L; potassium 19.6 mEq/L.

Table 2. Diseases Associated with Hypomagnesemia

Disease	Reasons for Exclusion
Primary aldosteronism[1,2,3]	1. Absence of hypertension 2. Normal serum K, CO_2 3. Normal Na/K of saliva, urine 4. Normal blood volume
Primary hyperparathyroidism, esp. following removal of tumor[4,9]	1. Normal serum Ca, pO_4, alkaline phosphatase 2. Absence of kidney stones, bone lesions 3. Absence of hypercalciuria
Malabsorption syndrome[10-18]	1. Normal urinary Mg 2. Absence of symptoms 3. Normal radioactive triolein absorption
Hyperthyroidism[19]	Normal RAI uptake
Renal tubular acidosis[20]	1. Normal serum Na, K, Cl, CO_2 2. Acid urine
Inappropriate secretion of ADH associated with acute intermittent porphyria[21,22]	1. Normal serum Na and osmolality 2. Absence of urinary porphobilinogens
Bronchial asthma[23]	Hypomagnesemia of this degree not previously observed (see discussion)

Diseases Not Pertinent to Present Patient

Excessive lactation[24]	During treatment of diabetic acidosis[31]
Hypercalcemia of malignancy[9,25]	Chronic alcoholism, delirium tremens, cirrhosis[32-38]
Diuretic phase of acute tubular necrosis	Prolonged parenteral fluids[39,40]
Diuretic therapy[26-30]; Chlorothiazides, mercurials, NH_4Cl	Acute pancreatitis[41]
Excessive use of purgatives	Malnutrition[42]

Table 3. Serum Magnesium on Family Members

Relative	Serum Magnesium (mEq/L)
Mother	1.70
Sister	1.47
Thirteen-year-old son	1.50
Seven-year-old son	1.70
Five-year-oid daughter	1.68; 1.69
Three-year-old son	1.12; 1.20
Maternal cousin	1.51
Normal value	1.70
Range	1.40 - 2.00

Period Four: Low potassium formula given plus Kayexalate, 10 gm t.i.d. A 78 kilogram male was on the same dietary intake and dose of Kayexalate during this period.

The composition of the diets is presented in Table 4. Heparinized blood samples were obtained intermittently throughout the study; 24-hour urines were collected daily. Carmine stool markers were administered at the beginning of each period and at the end of Period Four. Inadequate stool collections, however, vitiated the data on the first eight days, and values are given only for Periods Three and Four. Stools were ashed and dissolved in hydrochloric acid prior to chemical determination.

The following chemical methods were used: magnesium - automated spectrophotometric method with Eriochrome Black T[43]; calcium - modification of method of Kessler and Wolfman[44]; phosphate - Fiske-Subbarow method adapted to the Auto Analyzer; sodium - Baird flame photometer; potassium - Baird flame photometer; chloride - Schales and Schales; total CO_2 - Van Slyke manometric method; creatinine - Folin-Wu.

Results

Period One: During the first five days, the serum magnesium varied between 0.90 and 1.01 mEq/L (see Table 5 and Fig. 1). The other serum electrolytes were normal. The daily urinary excretion of magnesium averaged 7.6 mEq or approximately 43% of the analyzed intake.

Period Two: On July 6, 80 mEq of magnesium sulfate were administered intravenously over a six-hour period. The serum magnesium was 1.01 mEq/L prior to the infusion and 3.87 mEq/L at the end of six hours. The serum calcium dropped from 4.69 to 4.32 mEq/L; the serum phosphate decreased from 3.33 to 2.64 mg/100 ml. During the six hours of magnesium infusion, no clinical or electrocardiographic changes were noted. An estimate of the urinary excretion of the infused magnesium was made by subtracting twice the average daily excretion of the baseline period from the amount excreted during the 48 hours following the infusion. The remaining amount was 63% of the parenterally administered

Table 4. Daily Dietary Intake for Balance Periods

Element	Period	
	I, II, III	IV
Magnesium, mEq	17.8*	10.5*
Calcium, mEq	64.1*	10.8*
Phosphorus, mg	1613	608
Sodium, mEq	78*	285*#
Potassium, mEq	72*	22*
Protein, gm	98.5	39.4
Calories	1832	1831

*By chemical analysis

#Includes additional 84 mEq/day in Kayexalate

Table 5. Metabolic Data

	Plasma*									Urine#							Feces#			
	Wt Kg	Mg	Ca	PO_4	Na	K	Cl	CO_2	Creat.	Volume ml	Mg mEq	Ca mEq	PO_4 mg	Na mEq	K mEq	Creat. mg	Mg mEq	Ca mEq	Na mEq	K mEq
Period I																				
Jul 1	43.9	0.95	4.73	3.86						2220	9.0	5.3	689	111	54	835				
Jul 2	43.7	0.96	5.10	4.00	143	4.1	108	28	1.10	1880	6.4	4.2	705	38	51	9[illegible]				
Jul 3	43.9	0.90	4.79	3.94	139	4.0	104	29	1.20	2930	8.1	7.0	1116	70	67	1084				
Jul 4	43.5									2550	8.2	5.2	908	43	61	1046				
Jul 5	43.8	0.97	4.65	3.25						2610	6.2	3.7	647	31	65	1096				
Period II																				
Jul 6	44.0	1.01	4.69	3.33						4100	50.8	7.0	1168	41	74	1394				
Jul 7	44.5	1.45	4.20	2.64	140	3.7	104	28		2340	14.3	1.8	936	33	58	1100				
Jul 8	44.1	1.10	4.60	3.12						2570	10.8	3.4	892	49	56	1208				
Period III																				
Jul 9	44.6	0.99	4.70	3.35						2610	12.3	4.2	744	59	65	966	35.4	78.3	2.1	3.0
Jul 10	44.1									2760	15.3	3.6	831	55	66	883	35.4	78.3	2.1	3.0
Jul 11	44.1									2530	12.4	3.4	706	61	63	860	35.4	78.3	2.1	3.0
Jul 12		1.25	4.72	3.65						3030	13.8	4.2	836	97	52	818	35.4	78.3	2.1	3.0
Jul 13	44.1	1.23	4.64	3.60						3175	15.1	2.6	1050	44	76	1207	35.4	78.3	2.1	3.0
Period IV																				
Jul 14	43.9	1.31	4.90	3.92	135	4.3	104	29	1.30	2400	8.1	1.2	886	106	41	1032	20.4	18.4	68.9	24.0
Jul 15	44.6				139	4.0	109	27	1.05	2640	4.2	0.5	544	103	29	898	20.4	18.4	68.9	24.0
Jul 16	45.1				140	3.9	106	25	1.00	2850	4.4	1.2	439	199	31	912	20.4	18.4	68.9	24.0
Jul 17	45.5				141	2.8	108	30	1.00	1820	2.5	0.6	402	126	20	673	20.4	18.4	68.9	24.0
Jul 18	45.4	0.76	3.88	2.88	140	2.6	108	28	0.93											

* Plasma Mg, Ca, Na, K, Cl and CO_2 expressed in mEq/L; plasma phosphate and creatinine expressed in mg/100 ml.

Expressed as total output per 24 hours.

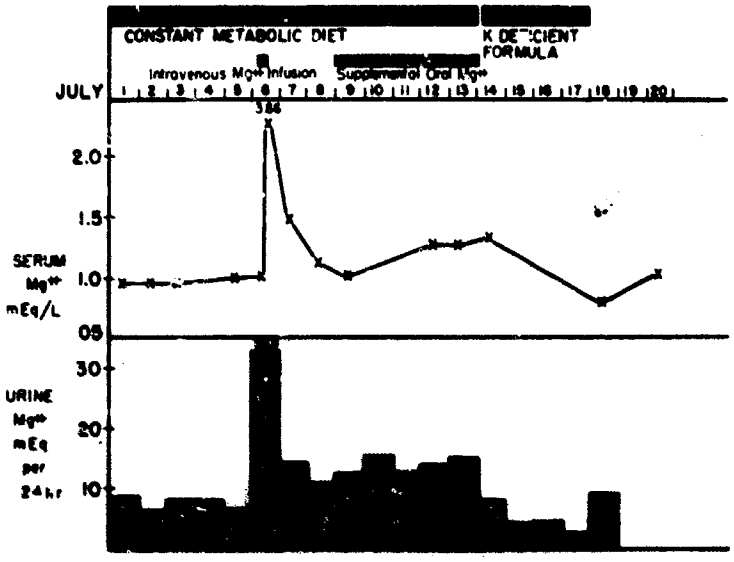

Figure 1. Protocol of metabolic study reflecting changes in serum and urinary magnesium.

magnesium. Three days following the infusion, the serum magnesium had returned to baseline levels.

Period Three: During the five-day period of supplemental magnesium oxide, the serum magnesium rose from 1.0 to 1.3 mEq/L. A positive balance of 43 mEq of magnesium was achieved in spite of an associated increased urinary output of magnesium. Thirty-nine per cent of the oral magnesium was absorbed, and 62 per cent of the absorbed magnesium appeared in the urine. No changes in urinary calcium and phosphate excretion were observed during this period of supplemental magnesium intake.

Period Four: During the four-day period of potassium restriction, negative potassium balances of 129 mEq and 215 mEq were observed for the patient and control, respectively. This loss was 2.9 mEq/kg body weight in the patient and 2.8 mEq/kg body weight in the control. In the patient, the serum potassium dropped from 4.3 to 2.6 mEq/L with no change in total CO_2 content during this interval. This drop of 1.7 mEq/L was comparable to the decrease in serum potassium noted in the control subject (Table 6). The reduction in urinary potassium in the patient was prompt with excretion of 20 mEq on the fourth day of restriction. Both patient and control demonstrated irritability and lessened ability to concentrate during this period.

Although the patient's weight was stable during the first 14 days of study, an increase of 1.45 kilograms occurred during the four days of potassium restriction. This gain corresponds well with the positive sodium balance of 330 mEq observed during the same time.

Fortuitously, the potassium deficient formula was also moderately restricted in magnesium (10 mEq/day). A negative magnesium balance of 54 mEq was measured, and the serum magnesium dropped from 1.30 to 0.76 mEq/L. Stool losses accounted for most of the magnesium deficit, and some renal conservation of magnesium was apparent on the fourth day.

Discussion

In spite of the renewed interest in magnesium metabolism during the past decade, detailed knowledge regarding human magnesium deficiency is still lacking. Typical characteristics have been difficult to define because other electrolyte abnormalities are generally associated with magnesium

Table 6. Influence of Potassium Restriction on Plasma and Urine Potassium in a Hypomagnesemic Patient

	Plasma Potassium (mEq/L)		Urine Potassium (mEq/Day)	
	Patient	Control	Patient	Control
14 July	4.3	5.0	41	51
15 July	4.0	4.7	29	43
16 July	3.9	4.3	31	44
17 July	2.8	3.4	20	25
18 July	2.6	3.4	18	--

depletion. The signs and symptoms most frequently associated with this condition are listed in Table 7. Some are controversial. For example, is tetany in its classical form a manifestation of magnesium deficiency? Vallee and his coworkers have described carpopedal spasms associated with low serum magnesium values in a number of disease states.[45] MacIntyre, on the other hand, has stressed that calcium abnormalities may have been responsible for the tetany observed, and that the latter symptom is not part of the magnesium deficiency syndrome.[46] Although we are unable to resolve this controversy, it is clear that marked hypomagnesemia may be present in a patient without manifestations of overt or latent tetany. Certainly, the nonspecificity of the signs and symptoms of magnesium deficiency emphasizes the need for confirmatory evidence of this disorder.

The following methods have been utilized by various investigators in the diagnosis of magnesium deficiency: serum magnesium, erythrocyte magnesium; muscle magnesium; measurement of "exchangeable" magnesium; metabolic balance studies of magnesium; and the renal response to parenterally administered magnesium. Since there are technical and/or theoretical objections to each of these procedures, magnesium deficiency remains difficult to prove clinically. These methods and some of their limitations will be considered briefly.

It has been established that the range of serum magnesium values in healthy humans is quite narrow since reliable and precise methods for the measurement of magnesium have become available.[43,47-52] The wide ranges in normal values quoted from the earlier literature are probably due to the imprecision of the older methods. Although most cases of magnesium deficiency have had demonstrable hypomagnesemia, documented instances of magnesium depletion with normal serum values have been reported.[17,65] Clinical investigations are still too limited to state with certainty that hypomagnesemia is always indicative of magnesium depletion, although such a relationship has been true in most reports. In animal studies, however, hypomagnesemia does not necessarily imply magnesium deficiency. It has been demonstrated in rats that hypomagnesemia in the absence of tissue depletion of magnesium follows the administration of Vitamin D.[53]

Table 7. Symptoms of Magnesium Deficiency

(From Welt and Gitelman: Disorders of Magnesium Metabolism)

1. Neuromuscular disorders
   - Weakness
   - Muscle fasciculation
   - Gross physical tremors
   - Positive Chvostek's sign (occasionally)
   - Classical tetany (controversial)

2. Central nervous system changes
   - Personality changes
     - Anxiety, agitation, delirium, psychosis
   - Convulsions
   - Choreo-athetoid movements

been used clinically to confirm the diagnosis of magnesium deficiency. Smith, et al., for example, found a higher incidence of decreased erythrocyte magnesium than hypomagnesemia in patients with delirium tremens.[33] They suggested that the former measurement was a better estimate of the magnesium deficient state than the serum magnesium level. Erythrocyte magnesium has been measured in a variety of clinical states with conflicting results.[19,54-62] A pertinent limitation to this method may be related to the age of the red blood cells which are analyzed. Ginsberg, et al., induced reticulocytosis in rabbits with the administration of phenylhydrazine.[63] These reticulocytes had a higher magnesium concentration than older red blood cells. They noted also a positive relationship between the reticulocyte count and erythrocyte magnesium concentration in man. These findings have been confirmed more recently by Erlandson, et al., who demonstrated increased erythrocyte magnesium levels in patients with thalassemia and sickle cell anemia.[64] The above considerations make it unlikely that the magnesium concentration of red blood cells will be a useful confirmatory test for magnesium deficiency.

MacIntyre and his colleagues have utilized the measurement of the magnesium concentration in biopsied muscle to aid in diagnosing clinical magnesium deficiency.[65] The positive relationship between depressed muscle magnesium and magnesium deficiency in rats provides a firm foundation for the utilization of this test in man.[66,67] However, data from normal humans are still scanty at this time. In the few patients in which this test has been performed, the relationship between muscle magnesium and the apparent deficit of magnesium was good. In one patient, decreased muscle magnesium was considered diagnostic of magnesium deficiency in the presence of normal serum magnesium.[65] Unfortunately, this technique requires a generous amount of tissue, and the preparation for analysis is time consuming.

Isotope studies of "exchangeable" magnesium have been performed by a number of investigators, especially in animal experiments.[65,68-72] However, such studies in health and disease are few, and attempts to correlate other tests of magnesium deficiency with exchangeable magnesium values are limited. The values which have been obtained suggest that a relatively small proportion of the total body magnesium

is available to replace acute losses of magnesium from the extracellular fluid.

The demonstration of a positive magnesium balance during a period of prolonged metabolic observation is also evidence of a magnesium depleted state.[73] (Table 8)

In 1958, Fitzgerald and Fourman studied experimentally-induced magnesium deficiency in two healthy males.[74] At the end of the period of magnesium depletion, the calculated magnesium deficits of each were administered by vein. Unlike normals, who usually excrete intravenously administered magnesium quantitatively in the urine within 48 hours, these subjects showed significant retention of the administered cation. On the basis of this observation, Fourman, et al., subsequently suggested that retention of intravenously administered magnesium is consistent with magnesium deficiency even when serum levels are normal.[17] A prerequisite for this test is normal renal mechanisms for conserving magnesium. This test would therefore be of no diagnostic value if the magnesium deficient state were secondary to faulty renal mechanisms. For example, increased renal clearances of magnesium, as well as hypomagnesemia, have been demonstrated in patients with primary aldosteronism.[1-3] In such patients, the absence of retention of parenterally injected magnesium could not be used as evidence against underlying magnesium deficiency. Conversely, patients with severe renal disease and reduced glomerular filtration rates excrete magnesium slowly.[75,76] Retention of magnesium in such patients does not necessarily imply associated magnesium depleted state.

Three of the tests summarized above were performed in the present patient. The serum magnesium was markedly depressed on numerous occasions. The hypomagnesemia was confirmed by analysis in two separate laboratories using different methods of magnesium determination. This should eliminate any possible artefactual depression of serum magnesium due to methodology.

During five days of supplemental oral magnesium (Period III), a positive magnesium balance of 43 mEq was noted as well as a rise in serum magnesium from 1 to 1.3 mEq/L. Further suggestive evidence of magnesium depletion appeared during Period IV when a drop in serum magnesium from 1.3 to

Table 8. Laboratory Methods Used to Establish Diagnosis of Magnesium Deficiency

(From Welt and Gitelman: Disorders of Magnesium Metabolism)

1. Magnesium balance
2. Muscle analysis
3. Retention of magnesium following acute parenteral administration
4. Isotope studies
5. Serum magnesium
6. Erythrocyte magnesium

0.76 mEq/L was demonstrated and a negative magnesium balance of 53 mEq was calculated. Although longer periods of metabolic balance would have been desirable, the results in Periods III and IV are consistent with a magnesium depleted state (Fig. 2).

The interpretation of the response of this patient to the intravenously administered magnesium is equivocal. Approximately 63 per cent of the administered magnesium was excreted in the urine within a 48-hour period. This figure is somewhat less than "normal" but higher than that demonstrated in patients with malabsorption and presumed magnesium deficiency.[17] As mentioned above, a prerequisite for this test is normal renal mechanisms for conserving magnesium. The patients of Fourman, et al., with malabsorption had negligible amounts of magnesium in the urine prior to the test, suggesting excellent renal conservation. Low urinary magnesium excretion was accomplished in spite of normal serum levels of magnesium and therefore presumably normal filtered loads of this cation.

In the present patient, however, the quantity of magnesium excreted in the urine in the face of marked hypomagnesemia was inappropriately high. This suggests to us that the inability of her kidneys to conserve magnesium may have been responsible for the hypomagnesemia observed. The protocol was not specifically designed to test the ability of this patient's kidneys to conserve magnesium. During potassium restriction (Period IV), however, the intake of magnesium was moderately reduced to 10 mEq per day, a negative magnesium balance was demonstrated, and the serum magnesium decreased from 1.3 to 0.76 mEq/L. Some renal conservation of magnesium was apparent since the urinary magnesium excretion was down to 2.5 mEq on the fourth day of this period. In those few studies available on experimentally induced magnesium deficiency in normals, however, the urinary magnesium has been 1 mEq per day or less by the time a negative magnesium balance of 50 mEq has been achieved.[74-81] Unfortunately, we are unable to estimate the total magnesium deficit in this patient when her serum magnesium was 0.76 mEq/L.

Aberrations in potassium metabolism have been present in many diseases in which magnesium deficiency has been discovered.[1-3,26,27,31,41] The patients with familial

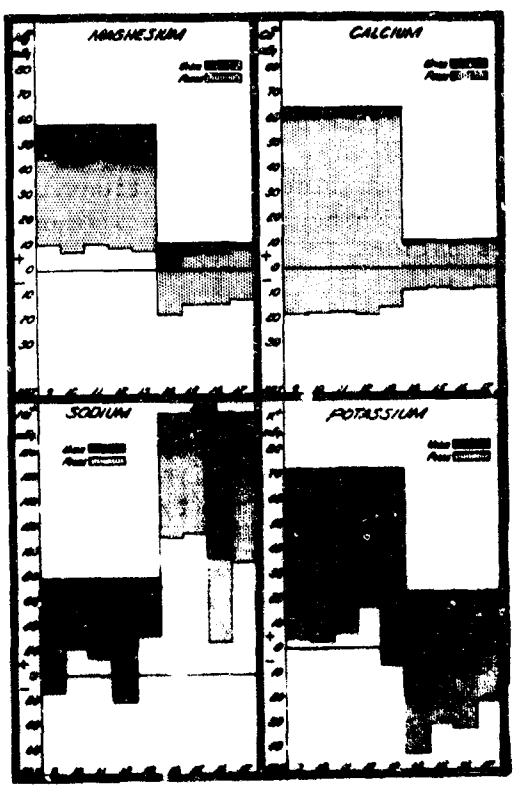

Figure 2. Metabolic balance during Periods III and IV.

hypomagnesemia under investigation by Dr. Louis G. Welt had evidence of associated potassium deficiency. It was for this reason that she was placed on a potassium deficient formula. While on this diet, the decrease in serum potassium of the patient was comparable to that of the control, and similar losses of potassium were noted in each when expressed per kilogram of body weight (Table 6). The patient excreted 20 mEq of potassium on the fourth day of restriction. This excretion compares favorably to the response of normals to potassium restricted diets as noted in the literature,[80] although there are admittedly limitations to such comparisons. We therefore have nothing to confirm a renal potassium wasting defect in this patient.

The patient, therefore, had repeatedly demonstrated hypomagnesemia without other evidence of electrolyte abnormalities. Documentation of hypomagnesemia in one of her children suggests a familial tendency. Since we are unable to rule out recognized causes of hypomagnesemia in this child, however, further speculation regarding a familial origin of this chemical abnormality seems inadvisable at this time.

Of the various signs and symptoms associated with magnesium deficiency (Table 7), only weakness and personality changes were present in this patient. Weakness was a subjective complaint; no evidence of decreased strength was apparent on physical examination. She had twice been hospitalized for psychotic behavior, and she showed evidence of agitated depression while under our observation.

Of the many diseases which have been associated with hypomagnesemia (Table 2), only two deserve more than passing reference in this patient.

The most frequent and best documented case reports of magnesium deficiency have occurred in patients with malabsorption.[10-18] The present patient had a clinical history suggestive of celiac disease in infancy although no symptoms of malabsorption were present during her adult life. The best evidence against malabsorption in this patient is the significant amount of magnesium appearing in her urine. In patients with magnesium deficiency secondary to malabsorption, urinary magnesium excretion has been extremely low, usually less than 1 mEq per day.[13-15,17] Hypocalcemia and hypoalbuminemia have been present in many of these cases,

and clinical evidence of gastrointestinal disturbance has been obvious.

Because this patient has a history of asthma, the association of hypomagnesemia and bronchial asthma reported Haury also warrants discussion.[23] In 1940, he reported that 13 of 26 patients were hypomagnesemic during acute attacks of bronchial asthma; 37 out of 40 asymptomatic asthmatic individuals, however, had normal serum magnesium values. We are unaware of any followup study which has confirmed Haury's findings, although magnesium is occasionally recommended in the treatment of bronchial asthma.[82] During observation of the present patient, asthma was not a clinical problem.

Although the association of bronchial asthma with hypomagnesemia may be fortuitous in this patient, certain aspects of magnesium deficiency in animals have suggested a relationship between allergic states and magnesium metabolism. Peripheral eosinophilia of the blood is a recognized part of the magnesium deficiency syndrome in rats.[83] Eosinophilia of various organs in association with decreased mast cells has also been described, and more recently increased plasma and urinary levels of histamine have been measured in these animals.[84-86] The eosinophilia can be blocked by pretreatment with an antihistamine, Pyribenzamine. The peripheral vasodilatation of the skin characteristic of magnesium depleted rats is also presumably related to endogenous histamine release. Both the eosinophilia and peripheral vasodilatation are especially prominent during the acute phase of magnesium depletion. Urticarial reactions have also been noted in dogs acutely depleted of magnesium by hemodialysis.[87] Finally, magnesium has been said to exert a protective influence against acute anaphylactic shock in guinea pigs.[88]

Although dermatographism was not present while the patient was under our observation, it had at one time been striking. During that interval, her case had been utilized for teaching purposes by the Dermatology Service. Because of the similarity between human dermatographism and peripheral vasodilatation in rats, one cannot resist the speculation that dermatographism in this patient may have been a manifestation of an acute phase of magnesium deficiency. We are unaware, however, of a previous clinical association between hypomagnesemia and dermatographism.

In summary, this patient had repeatedly demonstrated hypomagnesemia, symptoms of intermittent weakness and abnormal personality behavior, and a history of dermatographism and bronchial asthma. During a period of supplemental oral magnesium, there was a positive balance of magnesium and a rise in the serum magnesium level. The hypomagnesemia is most likely secondary to urinary losses of this cation, although no evidence for associated renal potassium wasting could be established. The demonstration of hypomagnesemia in one of her children suggests a possible familial tendency for this chemical abnormality. Data were not collected which will allow us to assess the pathological significance of hypomagnesemia in this patient.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501871R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: HEAT STROKE AND ACUTE RENAL FAILURE

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 January to 31 December 1965

Investigators:

Robert M. Vertel, Captain, MC
James P. Knochel, Major, MC

Reports Control Symbol: RCS-MEDDH-288(R1)

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Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: HEAT STROKE AND ACUTE RENAL FAILURE

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 January to 31 December 1965

5. Investigators: Robert M. Vertel, Captain, MC
James P. Knochel, Major, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

During the past year, the Renal Branch has seen two cases of acute renal failure complicating heat stroke which occurred in healthy young Army recruits. Both patients were dialyzed frequently according to the Surgical Research Unit prophylactic dialysis protocol and had uneventful recovery with complete return of normal renal function. These cases stress the importance of prompt, rapid cooling, and awareness of the significance of persistent oliguria following reversal of hyperpyrexia and hypotension.

(1) Sickle cell
(2) Goodpasture syndrome
(3) Heat stroke
(4) Hypomagnesemia

HEAT STROKE AND ACUTE RENAL FAILURE

Heat stroke is a syndrome which follows exposure to high environmental temperatures characterized by severe hyperpyrexia, failure of the sweating mechanism, and central nervous system signs. Without treatment, this condition is uniformly fatal.[1] With treatment, the mortality varies from 20-50 per cent. Complications, such as prolonged coma, shock, acute renal failure, jaundice, and hyperkalemia, raise the death rate to 75 per cent or more.[1] The cornerstone of treatment is rapid cooling, followed by rapid reconstitution of the circulating blood volume. Acute renal failure complicates heat stroke in 2-9 per cent of cases,[2] however, the significance of this association needs clarification. Most of the well-documented cases described in the literature have been further complicated by persistent shock, severe hepatic necrosis, irreversible central nervous system damage, persistent hyperpyrexia due to delayed inadequate cooling, or hemorrhagic diatheses secondary to heat-induced thrombocytopienia and/or fibrinolysis. It is not surprising, therefore, that these cases have often had a fatal outcome, despite modern dialysis therapy. During the past year, the Renal Branch has seen two cases of acute renal failure complicating heat stroke which occurred in healthy young Army recruits despite prompt and adequate cooling. Both patients were dialyzed frequently according to the Surgical Research Unit prophylactic dialysis protocol and had uneventful recovery with complete return of normal renal function. These cases stress the importance of prompt, rapid cooling, and awareness of the significance of persistent oliguria following reversal of hyperpyrexia and hypotension.

Case Report Number 1

A 21 year old white male Army Private collapsed during a forced march on 2 June 1965, during his first month of basic training at Fort Polk, Louisiana. On his arrival at the heat ward the patient was comatose, rectal temperature 107° F, blood pressure 90/50, pulse 150 beats per minute, skin hot and dry. The patient was rapidly cooled in an iced tub and his temperature dropped within minutes to 104° F. Admission laboratory data: Hematocrit 44%, hemoglobin 14.3 gm%, white count 19,200, with 77 neutrophils, 13 lymphocytes, 10 monocytes. Serum sodium 140 mEq/L, chloride 114 mEq/L, CO_2 16 mEq/L, BUN 29 mg%, calcium 10.2 mg%, glucose 66 mg%. Within five hours his temperature had fallen to 101° F and his

sensorium cleared. During the first 24 hours the patient was given 6,500 cc of intravenous fluid, 4,000 cc of this as normal saline. With the rapid cooling and fluid administration the blood pressure rose to 125/75. Despite this large fluid replacement, the patient voided only 350 cc of urine during the first 24 hour period. On the morning after admission, examination revealed a swollen left leg. This was felt to be due to the presence of an extremely tight elastic bandage on the left knee which the patient had in place on arrival at the hospital. During the following 48 hour period the patient passed only 40 cc of urine. The BUN had risen to 95 mg per cent and the serum ptoassium was 6.5 mEq/L. Sixty hours after admission the patient was transferred to the Surgical Research Unit at Brooke Army Medical Center. On admission to the Surgical Research Unit a test dose of Mannitol was given with no urinary response. The following day the serum potassium reached a peak of 7.8 mEq/L with a BUN of 102 mg per cent. The patient was treated with oral Kayexalate and sodium bicarbonate intravenously. On this therapy, the potassium rapidly fell to 5.6 mEq/L. On 5 June a fasciotomy of the anterior and posterior compartments of the left leg was performed; both compartments were noted to be under considerable tension. Necrotic muscle was removed from the anterior compartment. On 6 June peritoneal dialysis was begun. During the following 72 hours a total of 48 two-liter exchanges were performed. During this time there was considerable improvement in the patient's mental status. However, it soon became clear that persistent hyperkalemia, and azotemia could not be adequately controlled with peritoneal dialysis. Therefore, on 10 June, a Silastic-Teflon arterio-venous shunt was placed in the left forearm. Six hemodialyses were performed during the remaining 13 days of the oliguric phase. Each dialysis was performed for a duration of six hours, with the Travenol Twin-Coil Kidney, regional heparinization being employed. Two units of whole blood were used to prime the coils for the initial hemodialysis, priming thereafter was performed with autotransfusion. On 11 June, fever was noted, and a chest x-ray revealed a right lower lobe pneumonia. The patient was treated with penicillin; subsequent chest x-rays showed progressive clearing of the pneumonic infiltrate. On 23 June, the 21st day post heat stroke, the patient's urinary output exceeded one liter; a brisk diuresis ensued associated with a rapid fall in the patient's weight and improvement in the azotemia and electrolyte balance. On 28 June a secondary

closure of the fasciotomy was performed and the skin defect subsequently homografted. The patient has had complete return of normal renal function, as judged by concentrating ability and creatinine clearance. During his acute illness there was evidence of hepatic injury, reflected in a transaminase level of 1200 units, total bilirubin of 7.0 mg per cent, with 6.0 mg per cent direct bilirubin, alkaline phosphatase was 10.2 Bodansky units. The serum bilirubin reached a peak of 13 mg per cent total with 12.0 mg per cent direct bilirubin on 8 June. Following this, there was steady improvement of liver function with complete return to normal of all parameters during the third week of convalescence. The patient has no residual central nervous system sequelae.

Case Report Number 2

A 21 year old white male Army Private collapsed while on manuevers in fatigues and in full field pack on 19 April 1965, during the second week of basic training at Fort Polk, Louisiana. When initially seen the patient was unresponsive, axillary temperature 106° and had intermittent tetanic contractions of all muscle groups. A lumbar puncture done at that time was normal. The patient was cooled rapidly in an iced tub and during the initial 12 hours was given a total of 3,000 cc of intravenous fluid. Admission laboratory data: Hematocrit 49%, hemoglobin 16.4 gm%, white count 35,600, 83% neutrophils, 14 lymphocytes, 3 monocytes, sodium 130 mEq/L, potassium 3.9 mEq/L, chloride 108 mEq/L, CO_2 19 mEq/L, BUN 22 mg%, calcium 10.0 mg%, glucose 100 mg%. During the initial 12-hour observation, the patient voided 1100 cc. Total fluid intake during the next 24 hours was 7,900 cc. Despite this massive fluid intake, the patient voided only 365 cc of urine. His BUN rose to 51 mg%. The following day oliguria persisted and the patient was transferred to the Surgical Research Unit. On admission to the Surgical Research Unit on 22 April 1965, the patient had already recovered from the heat stroke, however, it was apparent that he was suffering from acute renal failure. Shortly after admission, two test doses of Mannitol, each containing 12.5 gm were given in an attempt to verify this diagnosis There was no response to Mannitol. In keeping with the prophylactic dialysis protocol, early dialysis was instituted in an effort to present the uremic syndrome. On 23 April a peritoneal catheter was inserted and peritoneal dialysis was begun. During the next 10 days, eight courses of peritoneal dialysis were performed with a total of 86 liters of exchange fluid. During this period or

dialysis the BUN was maintained at levels below 120 mg%. On 28 April Pseudomonas was cultured from the dialysate. This organism was found to be resistant to all antibiotics except Coly-Mycin and Polymyxin. Fifteen mg of Coly-Mycin was added to one bottle of each pair of dialysate solution prior to instillation into the abdomen. On this therapy, cultures of Pseudomonas disappeared from the dialysate. However, on 1 May, it was again noted that Pseudomonas was present in the dialysate fluid and in addition, the implantation site of the peritoneal cannula was painful. On 4 May because of continual leakage and partial obstruction of the cannula, peritoneal dialysis was discontinued and the cannula was removed. No further dialysis was required, as diuresis began on 5 May and the patient had an uneventful convalescence from his renal failure. The abdominal wound healed well following removal of the cannula. On 8 May, chest x-ray revealed a right lower lobe infiltrate. The patient had an uneventful recovery from this brief episode of pneumonia, following appropriate antibiotic therapy. By the third week of May the BUN and creatinine had returned to normal values and at present urinary concentrating ability is normal. This patient had evidence of mild hepatic necrosis, as evidenced by rise in transaminase values to 780 units; however, serum bilirubin and alkaline phosphatase remained normal. Transaminase values steadily fell, reaching normal values on the 11th post onset day. This patient shows no signs of any permanent neurologic deficit attributable to his heat stroke.

Discussion

Acute renal failure is not an uncommon complication of heat stroke. Although the incidence of renal failure in several large series of reported cases of heat stroke varies from 2 to 9 per cent, it is apparent that evidence of some degree of renal damage occurs in most heat stroke cases. Thus, Barry found an incidence of marked proteinuria in 85 per cent of 74 cases,[3] while Austin and Berry noted albuminuria in 60 per cent and an elevation of BUN in 42 per cent of cases.[4] A high incidence of granular casts and hematuria was also found.

Anoxia of the renal tubules is thought to be the common denominator in the production of acute tubular necrosis. The kidneys normally exist under a high oxygen tension, receiving about one-fourth of the total cardiac output. The amount of

oxygen released by each ml of arterial blood as it traverses the kidney is small in comparison to some other organs, but because of the tremendous blood flow to the kidneys their total oxygen extraction is actually greater than any other organ in the body.[5] Renal anoxia may occur in heat strokes via several mechanisms. Smith, et al., observed a 19 per cent decrease in glomerular filtration rate in men while exercising during heat stress. When the men were dehydrated, the rates fell 51 per cent.[6] Acute redistribution of blood accompanying peripheral vasodilatation causes shunting of blood away from the kidney and splanchnic bed to the skin. During exercise, when muscle blood flow is increased, this phenomenon is even more pronounced. Hartman demonstrated experimentally that severe anoxia was produced constantly in animals with induced fever.[7] It is estimated that a body temperature of 106° increases the metabolic rate by 50 per cent and oxygen utilization proportionately.[7] Also, at increased temperatures the oxygen combining capacity of hemoglobin is diminished.[7] Enzymatic reactions are characteristically sensitive to alterations of temperature and pH. Hyperpyrexia certainly may be expected to have some influence on these systems. If sufficient damage is sustained cellular metabolism will no longer be possible and necrosis will occur. The pathology seen in therapeutic hyperpyrexia illustrates this finding. Of 17 cases of fatal iatrogenic pyrexia reviewed at the Army Institute of Pathology in 1949, nine showed some degree of tubular degeneration of the kidney.[8] The proximal and distal convoluted tubules showed progressive disintegration and detachment of the lining of the epithelium. Five patients had "lower nephron nephrosis". Hartman and Major in 1935 described two deaths from hyperpyrexia; both showed acute parenchymatous degeneration of the liver and kidneys.[9] Bragdon, in 1947, described a fatal case of iatrogenic hyperthermia terminating in severe jaundice and fatal acute renal failure. At autopsy, the kidneys showed evidence of necrosis and degeneration of the distal tubules.[10]

The potential volume of the vascular tree in heat stroke is greatly increased by vasodilatation of the skin and muscle capillary bed. Therefore, the arterial blood pressure may be low, even though dehydration and salt depletion are not marked. The quantities of salt and water needed to return the circulating blood volume to normal can be greatly reduced by rapidly returning the body temperature to normal. Indeed, if large quantities of salt and water are given prior to reducing body temperature, there is a high risk of subsequent

pulmonary edema when peripheral vasoconstriction ultimately ensues, thus returning a large volume of fluid to the already repleted vascular space. Several fatalities in Daily and Harrison's series may have resulted from the overzealous fluid replacement without adequate cooling.[11]

Case No. 1 is an example of hypercatabolic acute renal failure, with increments of BUN rise greater than 30 mg% per day and life-threatening hyperkalemia. In contrast, Case No.2 showed only modest daily increments of BUN and potassium rise. These two cases demonstrate some correlation between the severity of renal failure and the severity of heat stroke. Case No. 1 had a fever of 107°, associated with hypotension, coma, and chemical evidence of hepatic necrosis. He had a longer oliguric phase and required considerably more dialysis than the second case who presented with a temperature of 106°, normotensive and with minimal evidence of hepatic damage. The augmented catabolism in Case No. 1 may certainly be due in part to the necrotic muscle tissue in the anterior compartment of the left leg. However, there is evidence that the severe hyperkalemia often seen in heat stroke may be partly independent of tissue breakdown. Bass, et al., in their studies of the mechanisms of acclimatization to heat in man found that potassium left cells in excess of that made available from tissue breakdown during heat stress.[12] During recovery, potassium returned to cells in excess of that required for protein anabolism. Urinary excretion of potassium increased three fold, while the potassium concentration of sweat was extremely high, often accounting for 50 per cent of the total potassium intake. Hence, the exposure to heat may accelerate the movement of potassium out of the cells and the serum concentration will depend largely on the integrity of the potassium excretory mechanism of kidneys and sweat glands. Also contributing to hyperkalemia in heat stroke is the extracellular movement of potassium in response to metabolic acidosis which may result from the hyperpyrexia induced tissue anoxia. Case No. 1 was indeed acidotic when first seen. The destruction of sequestered heat damaged red blood cells is yet another source of serum potassium.

Both of these cases were treated according to the o-phylactic dialysis concept of the management of acute renal failure originally described by Teschan, et al. in 1960.[13]

This regimen is based on the hypothesis that prevention of uremia reduces the likelihood of potentially lethal complications. Maintenance of the BUN below 120 mg% was arbitrarily selected as a dialysis frequency likely to prevent the uremic syndrome. Because of severe hyperkalemia and a steep increment of BUN rise, peritoneal dialysis was inadequate in Case No. 1 and hemodialysis was instituted early in the oliguric phase. In contrast, Case No. 2 was easily controlled with peritoneal dialysis alone. Maintenance of an adequate state of nutrition is simplified using the prophylactic dialysis method because more frequent dialysis permits a liberalized protein intake. Thus, the first patient, once past the hypercatabolic phase, was given a diet containing up to 25 gm of protein and 1400 calories. The second case was permitted up to 40 gm of protein and 2000 calories while oliguric.

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Presentations and/or Publications

None.

GPO 770984

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 0B 6959	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 01 200	None

11. TITLE: (U) Blood Levels of Colistin Following Its Peritoneal Installation in Patients with Acute and Chronic Renal Failure (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

16. PROCUREMENT METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	1

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS

Intraperitoneal, colistin, peritoneal dialysis

24. (U) Tech Objective - To determine the blood levels of colistin which would be achieved following its administration in the peritoneal dialysate in patients with acute and chronic renal failure.

25. (U) Approach - 15 mg of colistin was added to each peritoneal washing. Dialysate and blood samples were collected at regular intervals throughout the peritoneal dialysis for colistin bioassay.

(U) Progress (Jul 65 - Jun 66) - In four patients with oliguric renal disease, the blood levels obtained following administration via the peritone were low and less than to be anticipated following a standard i.m. dose of colistin.

26.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
[ ] a. COMSEC or COMSEC RELATED [X] b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST FUNDS (In thousands)	36.
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(Items 1, 2, 3b Identical to AMXRA Form 1127) REPLACES DA FORM 611 6 611C WHICH ARE OBSOLETE.

FINAL REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: BLOOD LEVELS OF COLISTIN FOLLOWING ITS PERITONEAL INSTILLATION IN PATIENTS WITH ACUTE AND CHRONIC RENAL FAILURE

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 January to 31 December 1965

Investigators:

Richard M. Freeman, Captain, MC
Robert M. Vertel, Captain, MC
Ronald E. Easterling, Major, MC
Robert B. Lindberg, Colonel, MSC
Glen K. Arney, Colonel, MC *

* From the Department of Medicine, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas

Reports Control Symbol: RCS-MEDDH-28 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: BLOOD LEVELS OF COLISTIN FOLLOWING ITS PERITONEAL INSTILLATION IN PATIENTS WITH ACUTE AND CHRONIC RENAL FAILURE

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 January to 31 December 1965

5. Investigators: Richard M. Freeman, Captain, MC
Robert M. Vertel, Captain, MC
Ronald E. Easterling, Major, MC
Robert B. Lindberg, Colonel, MSC
Glen K. Arney, Colonel, MC*

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

In four patients with oliguric renal disease, a potentially nephrotoxic antibiotic was administered via the peritoneum in small doses. The blood levels obtained were low and less than to be anticipated following the single intramuscular injection of a standard dose of colistin. In one instance, a peritoneal infection with Pseudomonas was controlled. It appears likely that the intraperitoneal administration of colistin can cure a localized peritoneal infection with Pseudomonas and obviate high and potentially nephrotoxic blood levels of this antibiotic.

(1) Colistin
(2) Renal failure

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BLOOD LEVELS OF COLISTIN FOLLOWING ITS PERITONEAL INSTILLATION IN PATIENTS WITH ACUTE AND CHRONIC RENAL FAILURE

Colistin has recently been found to be one of the most effective antibiotics for the treatment of infections by Pseudomonas organisms. The nephrotoxicity of colistin, however, has limited its usefulness in patients with acute or chronic renal failure. Peritonitis with Pseudomonas is therefore an especially hazardous complication in patients with renal disease.

Recently we treated a patient with acute renal failure who developed Pseudomonas peritonitis following peritoneal dialysis. In this patient, small doses of colistin added to the peritoneal dialysate were effective in eradicating the peritoneal fluid of Pseudomonas. An attempt was therefore made to determine the blood levels of colistin which would be achieved following its administration in the peritoneal dialysate.

Methods

Patients requiring peritoneal dialysis for the treatment of acute or chronic renal failure were studied. In order to facilitate the bioassay procedure, only patients who had not been receiving antibiotics were studied; all were severely oliguric at the time of study.

Prior to the initiation of peritoneal dialysis, sterile heparinized blood samples were obtained. Following centrifugation, the plasma was transferred to sterile screw-topped tubes and frozen for later bioassay of colistin. Sterile blood samples were collected at regular intervals throughout the peritoneal dialysis procedure. When feasible, urine samples were obtained prior to and during peritoneal dialysis. The urine was passed through millipore filters, transferred to sterile tubes, and frozen for later bioassay. Dialysate was saved from the first peritoneal wash to which no antibiotic was added. One vial of 150 mg colistin was diluted to 10 cc and 1 cc (15 mg colistin) was added to each subsequent peritoneal washing. Dialysate samples were collected every fourth wash for culture and bioassay. All dialysate samples for bioassay were passed through millipore filters and frozen for later analysis.

Results

At the present time, this procedure has been performed on four different patients.

Case #1. This 21-year-old white male was transferred to the Surgical Research Unit on 22 April 1965, three days following the onset of heat stroke and acute renal failure. On 23 April, a peritoneal catheter was inserted, and peritoneal dialysis was initiated. Peritoneal dialysis was continued for two 24-hour periods, starting at 1300 on 23 April and 1500 on 25 April. The peritoneal dialysis was well tolerated until the end of the second 24-hour period when the dialysate was noted to be blooo, and Pseudomonas was isolated on culture. This organism was resistant to Erythromycin, chloramphenicol, tetracycline and Kantrex, but was responsive to colistin at 3.1 mcg/ml. Because of continued isolation of Pseudomonas from the peritoneal dialysis fluid, 15 mg colistin were added to each peritoneal wash starting at 1545 on 28 April. The results of the administration of colistin may be seen in Table 1.

There was an immediate decrease in the colony count, and Pseudomonas had disappeared from the peritoneal dialysate within two hours following the addition of colistin. Bioassay of colistin from the blood stream was negative in all samples obtained. An uneventful diuresis began on 5 May, 16 days following the onset of acute renal failure.

Summary of Case #1. In this patient the addition of colistin was effective in eradicating the Pseudomonas from the peritoneal dialysate and measurable levels of colistin were not assayed from the blood stream. The quantity of colistin in the dialysate was only slightly less than anticipated from the addition of 15 mg colistin to two liters of fluid (7.5 mcg/ml).

Case #2. This 24-year-old white male was transferred to the Surgical Research Unit two days following the onset of heat stroke and acute renal failure. A 24-hour period of peritoneal dialysis was initiated at 1200 hours on 6 June 1965 without colistin. On 8 June 1965, colistin was added to the peritoneal dialysate. There was no clinical or bacteriologic evidence of peritoneal infection at the time

Table 1. Results of Administration of Colistin, Case #1

Date	Time	Peritoneal Dialysate Wash #	Organism	Colony Ct*	Assay #	Blood Assay #
23 Apr	1730	4	Pseud.	1.0×10^1		
	2330	8	No growth			
24 Apr	0615	12	No growth			
	1240	16	No growth			
26 Apr	0235	24	Pseud. Prov.	1.6×10^3		
	0834	28	Pseud. Prov.	4.5×10^3		
	1505	32	Pseud. Prov.	Not done		
27 Apr	1925	33	Pseud.	3.0×10^2		
28 Apr	0210	37	Prov.	2.1×10^6		
	0915	41	Pseud. Prov.	5.2×10^5		
	1420					Neg.
	1430	44	Pseud. Prov.	7.9×10^5	Neg.	
	1545	45 §	Pseud. Prov.	6.1×10^3	Neg.	Neg.
	1745	46	Prov.	4.1×10^1	6.24	Neg.
	1900	47	Prov.	2.0×10^1	6.24	
	2030	48	Pseud. Prov.	1.1×10^2	3.12	
	2045					Neg.
	2200	49	Pseud. Prov.	8.0×10^2	6.24	
	2328	50	No growth		3.12	
29 Apr	0100	51	Prov.	1.4×10^1	3.12	
	0240	52	No growth		3.12	
	0410	53	No growth		6.24	
	0545	54	Prov.	1.0×10^1	3.12	
	0715	55	Prov.	5.0×10^1	3.12	
	1445					Neg.
30 Apr	0245					Neg.

* Number of organisms per ml. of dialysate.
\# Expressed as microgram per ml.
§ Beginning with Wash # 45, 15 mg colistin added to each dialysate; a total of 150 mg administered via peritoneum.

the investigation was started. Results are in Table 2.

The predialysis blood sample showed colistin activity of 6.2 mcg/ml. A blood sample 28 hours following the onset of dialysis showed colistin activity at 12.4 mcg/ml while three other blood samples were all negative. Urine obtained throughout the period of dialysis was negative for colistin. There was no evidence of bacteriologic infection of the peritoneum either before colistin or during the 34 hours in which colistin was added to the peritoneal dialysate. An uneventful diuresis began on 23 June, 21 days following the onset of acute renal failure.

Summary of Case #2. Unlike Case #1, no measurable colistin activity could be found in the peritoneal dialysate, suggesting greater peritoneal absorption of the antibiotic in Case #2. An unexplained finding was the predialysis colistin level of 6.2 mcg/ml. The elevated blood level of colistin at 1900 on June 9 is also questionable since the blood level was negative two hours before and only six hours following this determination.

Case #3. This 50-year-old negro female suffered from malignant hypertension and progressive azotemia. On 27 May 1965, a left renal biopsy was performed, following which she developed flank pain. A large perirenal hematoma was recognized, and she was transferred to the Surgical Research Unit because of subsequent oliguria. Colistin was added to the peritoneal dialysate on two occasions (14 June and 24 June) with the results listed in Table 3. Predialysis bioassays of the blood were negative for colistin on both occasions, but blood colistin activity was measured at 6 and 12 hours during the first and second peritoneal dialyses, respectively. Urine obtained during both dialyses showed no colistin activity. The dialysate on Wash #39 (prior to the addition of colistin) grew E. coli (1.1×10^5 organisms/ml) but subsequently cultures of the dialysate were sterile. Urinary output averaged less than 100 ml daily throughout her course, and she died on 7 August, 72 days following the the onset of oliguria. Postmortem examination revealed advanced arteriolar nephrosclerosis as well as an organizing hematoma around the left kidney.

Summary of Case #3. In this patient therapeutic blood levels of colistin were obtained within 6 to 12 hours

Table 2. Results of Administration of Colistin, Case #2

		Peritoneal Dialysate				
Date	Time	Wash #	Organism	Assay	Blood Assay	Urine Assay
8 Jun	1500				6.2	Neg.
	1655	19	No growth	Neg.		
	2100				Neg.	
		23	No growth	Neg.		
9 Jun		27	No growth			
	1220	31	No growth	Neg.		
	1700				Neg.	
	1735	35	No growth	Neg.		
	1900				12.4	
	2245	39	No growth	Neg.		Neg.
10 Jun	0235	43	No growth	Neg.		
	0300				Neg.	
	0625	47	No growth	Neg.		Neg.

Beginning with Wash # 20, 15 mg colistin added to each dialysate and total of 405 mg colistin administered via peritoneum.

Table 3. Results of Administration of Colistin, Case # 3

Date	Time	Peritoneal Dialysate			Blood Assay	Urine Assay
		Wash #	Organism	Assay		
14 Jun	0910				Neg.	Neg.
	0930	39	E. coli 1.1×10^5	Neg.		
	1515				6.2	
	1620	43	No growth	Neg.		
	21[illegible]				6.2	
	2310	47		Neg.		
15 Jun	0548	51	No growth	Neg.		
	0917	53		Neg.		
	0945				6.2	Neg.
15 mg colistin added to each Wash starting # 40; total of 210 mg colistin administered via peritoneal dialysate within 24 hours.						
24 Jun	1415				Neg.	Neg.
	1910	60		Neg.		
	2015				Neg.	
25 Jun	0125	64		Neg.		
	0[illegible]				6.2	
	0800	68	No growth	Neg.		
	1345	72		Neg.		Neg.
	1415				6.2	
15 mg colistin added to each Wash starting # 57; total of 240 mg colistin administered via peritoneal dialysate during approximately 24 hours.						

following the addition of colistin to the dialysate. There was no evidence, however, of further accumulation of colistin in terms of increasing blood levels of the antibiotic. The negative bioassay of the dialysate suggests significant absorption of colistin via the peritoneum as in Case #2. Evidence of severe and progressive renal deterioration was apparent prior to the administration of colistin, and indeed the performance of the renal biopsy. During the first dialysis, the small doses of colistin effectively eliminated E. coli from the peritoneal fluid.

Case #4. This 44-year-old Latin American male had known chronic renal disease, presumably glomerulonephritis, since August 1964, when he was treated with hemodialysis during a 21-day period of oliguria. The colistin study was performed in September 1965 when he was hospitalized on Ward 13B for treatment of progressive uremia. Results are in Table 4.

Blood samples were negative for colistin at 6 and 24 hours, but a level of 1.5 mcg/ml was measured on the 42-hour sample. Urine was free of measurable colistin activity. Dialysate was negative until approximately 24 hours following the onset of dialysis. Although he became oliguric during the period of peritoneal dialysis, he was later able to leave the hospital with a urinary output greater than 1000ml daily.

Summary of Case #4. The bioassay figures on the dialysate suggest avid peritoneal absorption of colistin during the first 24 hours of dialysis with subsequent decreased absorption. The measurable blood level of 1.5 mcg/ml at 42 hours is low and would not be therapeutic for certain strains of bacteria.

Discussion

On the basis of these preliminary studies and theoretical considerations, it appears unlikely that high blood levels of colistin will result from the administration of this antibiotic in the manner described. Although there is evidence for peritoneal absorption of colistin, in only one instance (Case #2) was the blood level greater than 6.2 mcg/ml. This particular value must be questioned since no activity was found in a blood sample taken two hours previously. Since the colistin was administered in a concentration of 7.5 mcg/ml,

Table 4. Results of Administration of Colistin, Case #4

Date	Time	Peritoneal Dialysate			Blood Assay	Urine Assay
		Wash #	Organism	Assay		
8 Sep	1435				Neg.	Neg.
	1520	1	No growth	Neg.		
	2035				Neg.	
	2225	5	No growth	Neg.		
9 Sep	0125	9	No growth	Neg.		
	0235				Neg.	
	0719	13	No growth	Neg.		
	1430				Neg.	
	1730	17	No growth	3.1		
	2315	21	No growth	1.5		
10 Sep	0941	25	No growth	3.1	1.5	Neg.

15 mg colistin added to each Wash beginning # 2; total of 360 mg colistin administered via peritoneum in approximately 42 hours.

one would anticipate that equilibration with the blood would produce levels no greater than this unless there is active transport of the antibiotic from the peritoneum to the blood stream.

In contrast, MacKay, et al.,[1] have demonstrated that the single intramuscular injection of 150 mg colistin to anuric patients can produce blood levels of 12.5 to 25 mcg/ml for as long as 96 hours. Since a positive relationship between quantitative blood levels of colistin and nephrotoxicity of this agent has been generally presumed, the administration of small doses via the peritoneum may obviate nephrotoxicity from this agent. The small dose of colistin deserves emphasis. Increasing the colistin to 30 or 45 mg per dialysate would increase the dialysate to 15 and 22.5 mcg/ml., respectively. With these latter concentrations, high blood levels might well be achieved although this point was not specifically tested.

Summary

In four patients with oliguric renal disease, a potentially nephrotoxic antibiotic was administered via the peritoneum in small doses. The blood levels obtained were low and less than anticipated following the single intramuscular injection of a standard dose of colistin. In one instance, a peritoneal infection with Pseudomonas was controlled. It appears likely that the intraperitoneal administration of colistin can cure a localized peritoneal infection with Pseudomonas and obviate high and potentially nephrotoxic blood levels of this antibiotic.

References

1. MacKay, D.N., and Kaye, D.: Serum concentrations of colistin in patients with normal and impaired renal function. New Eng. J. Med., 270:394 20 Feb. 1964.

Publications and/or Presentations

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6984	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	6. SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 01 169	NONE

11. TITLE: (U) The Effect of Intradermal Cortisone upon Skin Anergy in Sarcoidosis and other Disease States (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	09 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	0	1

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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	TEL.: CA1 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS

Sarcoid anergy, skin test conversion

24. (U) Tech Objective - Diagnostic test for sarcoidosis.

(U) Approach - Intradermal skin testing was carried out on four patient groups: (1) sarcoid patients, (2) lymphoma patients, (3) patients with miscellaneous disease states, and (4) controls. Antigens used were PPD #2, Monilia, mumps, and Trichophyton. Tests were repeated with 1.25 mg cortisone acetate incorporated in the test mixture. Readings were made in both instances at 24 and 48 hours. Criteria for positivity were those usually applied.

(U) Progress (Sep 65-Jun 66) - Early suggestive evidence of heightened skin reactivity in sarcoid patients, when a cortisone antigen intradermal mixture was injected, was demonstrated. Within the limits of our time intervals, skin test conversion failed to occur on repeated administration of the test antigen to a normal control population. Further study is required before any firm conclusions or statistical analysis can be carried out.

27. COMMUNICATIONS SECURITY	28.	29. DDC CODE	30. BUDGET CODE
a. CRYPTO RELATED b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECT OF INTRADERMAL CORTISONE UPON SKIN ANERGY IN SARCOIDOSIS AND OTHER DISEASE STATES

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Howard J. Schwartz, Captain, MC *
Robert M. Vertel, Captain, MC

* From the Allergy Clinic, Department of Medicine, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas.

Reports Control Symbol: RCS-MEDDH-28 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: THE EFFECT OF INTRADERMAL CORTISONE UPON SKIN ANERGY IN SARCOIDOSIS AND OTHER DISEASE STATES

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Howard J. Schwartz, Captain, MC *
Robert M. Vertel, Captain, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Patients with sarcoidosis have been shown to have depressed delayed hypersensitivity, as do many with lymphomas and uremia. It has been reported that they react paradoxically to cortisone antigen mixtures, however, in that heightened skin reactivity is displayed. In an effort to determine the possible applicability of this observation in the work-up of sarcoid suspects, intradermal skin testing was carried out on four patient groups: (1) sarcoid patients, (2) lymphoma patients, (3) patients with miscellaneous disease states, and (4) controls. The antigens used were P.P.D. #2, Monilia, mumps and Trichophyton. The tests were repeated with 1.25 mg. of cortisone acetate incorporated in the test mixture. Readings were made in both instances at 24 and 48 hours. Criteria for positivity were those usually applied. Early suggestive evidence of heightened skin reactivity in sarcoid patients, when a cortisone antigen intradermal mixture was injected, was demonstrated. Within the limits of our time intervals, skin test conversion failed to occur on repeated administration of the test antigen to a normal control population. Further study is required before any firm conclusions or statistical analysis can be carried out.

(1) Sarcoid
(2) Anergy
(3) Skin Test Conversion

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THE EFFECT OF INTRADERMAL CORTISONE UPON SKIN ANERGY IN SARCOIDOSIS AND OTHER DISEASE STATES

It has previously been well demonstrated that patients with sarcoidosis have depressed delayed hypersensitivity reactions to a number of antigens, including tuberculin, Candida albacans, mumps, and Trichophyton.[1,2,3,4] The defect seems to be limited to the antibody system involving delayed hypersensitivity, since Sones and Israel[3] have shown normal agglutinin development after typhoid and pertussis immunization. Differences from the anergy noted in uremia and lymphomas have been suggested.[5]

Systemically administered cortisone or intradermal cor-cortisone-antigen test mixtures depress the intensity of skin reactivity in non-sarcoid patients, but sarcoid patients react paradoxically in that 50 per cent or more convert to positive reactions.[4,6,7] Aside from supplying further evidence that differences exist between the suppression mechanisms in sarcoid and non-sarcoid disease states, this affords a possible additional test to be used in the work-up of sarcoid suspects.

Methods

Skin testing was carried out in four groups of subjects with P.P.D. #2, Candida albicans antigen, mumps antigen and Trichophyton. Group I consisted of proven sarcoid patients, Group II patients with lymphomas, Group III patients with miscellaneous disease states and Group IV control subjects including some hospitalized patients being evaluated for peptic ulcer and other non-malignant gastrointestinal diseases. Readings were done at 24 and 48 hours. Criteria for positivity were as follows: mumps skin tests were considered to be positive if 10 mm. or more of erythema and/or induration was seen in 24 to 48 hours. P.P.D. #2, Monilia and Trichophyton were made as follows: negative reactions ranged from 0 to 5 mm. induration, 1+ reaction 5 to 10 mm. induration, 2+ reaction 10 to 15 mm., 3+ reaction 15 to 20 mm., and 4+ reaction 20 mm. and above.

Cortisone conversion was judged to have occurred if a test converted from negative to positive or if a 2+ grade change in response was noted. Tests were also carried out with 0.05 ml of cortisone acetate containing 1.25 mg of cortisone with the same criteria used in interpretation.

In order to determine test reliability, serial testing was carried out on a normal population in an attempt to induce a change in skin reactivity as a consequence of repeated testing.

Results (see Tables 1, 2 and 3)

The sarcoid and lymphoma patients tended to show depressed skin reactivity as compared to the control population. Incorporation of cortisone acetate into the testing material converted three Monilia, one P.P.D. and one Trichophyton test from negative to positive in the sarcoid group, while one Trichophyton test was depressed. One control patient developed heightened skin reactivity to the Monilia antigen, but none converted from negative. All other patients remained negative or, in three cases, had depressed reactivity.

Repeated skin testing in a variety of patterns to the above test antigens failed to elicit a conversion reaction within the period of observation. This confirms previously reported work[8] in which conversion due to repeated testing with mumps antigen failed to occur.

The authors feel that early suggestive evidence of heightened skin reactivity in sarcoid patients, when a cortisone antigen intradermal mixture is injected, has been demonstrated. Within the limits of our time intervals, skin test conversion failed to occur on repeated administration of the test antigen. Further study is required before any firm conclusion or statistical analysis can be carried out.

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Table 1. Incidence of Positive Skin Tests on First Evaluation

	P.P.D. #2	Monilia	Mumps	Trichophyton
Controls	6/18	16/18	5/18	5/12
Lymphoproliferative*	1/5	1/5	3/5	0/5
Sarcoid	0/4	0/4	2/4	2/3
Sarcoid (on steroids)	0/1	1/1	1/1	0/1
Other**	0/4	3/4	3/4	2/4

* Lymphomas and multiple myeloma

** Carcinoma suspects, arthritis, idiopathic hypogammaglobulinemia

Table 2. Steroid Induced Changes in Skin Tests

	No.	P.P.D.		Monilia		Mumps		Trichophyton	
		↑	↓	↑	↓	↑	↓	↑	↓
Sarcoid	4	1	0	3	0	0	0	1	1
Lymphoproliferative	3	0	1	0	0	0	0	0	0
Control	5	0	1	1	0	0	1	0	0

Table 3. Incidence of Skin Test Conversions Induced by Repeated Skin Testing in a Control Population

Material	Subjects	Doses	Interval (Days)	Conversions*
P.P.D. #2	1	4	7	0
	1	3	7	0
	1	4	3	0
	2	2	4	0
	4	3	4	0
	1	4	4	0
	3	5	4	0
Mumps	2	2	4	0
	3	3	4	0
	2	4	4	0
	2	5	4	0
Monilia	1	3	4	0
	1	4	4	0
	1	5	4	0
Trichophyton	2	2	3	0
	4	3	4	0
	1	4	4	0
	3	5	4	0

* 2^{+} change in skin test

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3. Jones, M. and Israel, H.L.: Altered immunologic reactions in sarcoidosis. Ann. Int. Med. 40:260, 1954.

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Publications and/or Presentations

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 08 6957	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 C6 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

10A. CURRENT NUMBER/CODE	10B. PRIOR NUMBER/CODE
61145011 3A014501B71R 01 198	NONE

11. TITLE: (U) The Effect of Various Fluid Regimens and Pharmacologic Agents on the Circulatory Hemodynamics of the Immediate Postburn Period (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

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C. In-House	NA	PRIOR FY 65	4	12
		CURRENT FY 66	6	20

19. GOVT LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME: Headquarters	NAME: US Army Surgical Research Unit
ADDRESS: US Army Medical Res & Dev Command, Washington, DC 20315	ADDRESS: Fort Sam Houston, Texas
RESP. INDIV.: Kovaric, J.J., Lt Col	INVESTIGATOR PRINCIPAL ASSOCIATE: Moncrief, J.A., Col, MC; Mason, A.D., Jr, MD
TEL.: 8 22 67337	TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS: Burns, Cardiac output, hemodynamics, vasodilators

24. (U) Tech Objective - To determine the cardiovascular response of the burned animal to combinations of various fluid regimens and pharmacologic agents.

25. (U) Approach - A standard 50% scald burn was produced in the dog. Cardiac output, pressure, pH, chemistries and blood gases monitored. Resistance and O_2 utilization were calculated. Different volumes and rates of administration of colloid and salt solutions plus various vasodilator agents were administered.

26. (U) Progress (Jul 65-Jun 66) - A total of 247 animals with just under 27,000 determinations made. Brooke formula improves cardiac output very little in the first 8-12 hours. Increasing colloid up to 10 ml/Kg/hr appears optimum. Alpha adrenergic blocking agents are greatly inferior to beta adrenergic stimulants in improving dynamics.

27.

27. CLASSIFICATION SECURITY	28.	29. DOD CODE	30. DUPLICATE CODE
☐ a. CLASSIFIED ☒ b. NOT CLASSIFIED		AR	1

31. WORK UNIT NUMBER	32. PERFORMING
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. GOVT. FUNDS (In thousands)	36.

DD FORM 1498

ANNUAL REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECT OF VARIOUS FLUID REGIMENS AND PHARMACOLOGIC AGENTS ON THE CIRCULATORY HEMODYNAMICS OF THE IMMEDIATE POSTBURN PERIOD

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.
Harrel L. Walker, M.S.
Lee R. Newman, D.A.C.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-28(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: THE EFFECT OF VARIOUS FLUID REGIMENS AND PHARMACOLOGIC AGENTS ON THE CIRCULATORY HEMODYNAMICS OF THE IMMEDIATE POSTBURN PERIOD

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.
Harrel L. Walker, M.S.
Lee R. Newman, D.A.C.

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Dogs subjected to a 50 per cent scald burn were studied in an attempt to delineate the response to various fluid regimens and pharmacologic agents in the period 6-36 hours postburn.

Following burning, cardiac output decreased rapidly to 50 to 60 per cent of preburn levels and by one hour was 30 to 40 per cent of initial values. Without therapy, a gradual slight decline continued. With increasing volumes of fluid administration, output returned toward preburn levels. With addition of pharmacologic agents, further improvement was slight with phenoxybenzamine, moderate with Isoproterenol and marked with hydralazine. Steroids showed no beneficial effect. Salt water alone as a resuscitating agent had little effect in the volumes used and was greatly inferior to colloid. Increases in early resuscitation volumes above 10 ml/Kg/hr and more than 5 ml/Kg/hr of colloid offered no significant advantage. This was particularly true when hydralazine was added as 0.5 mgm/Kg q 2h.

Results indicate the myocardium is capable of a good response to a fluid load but inotropic agents increase the response. Peripheral flow is most effectively increased with hydralazine, less so with Isoproterenol and least of all by phenoxylbenzamine. An explanation for this is offered.

(1) Burn Physiology

THE EFFECT OF VARIOUS FLUID REGIMENS AND PHARMACOLOGIC AGENTS ON THE CIRCULATORY HEMODYNAMICS OF THE IMMEDIATE POSTBURN PERIOD

Support of the cardiovascular system in the immediate postburn period is based primarily on the use of intravenous fluids. Various treatment regimens have been devised as a guide to initial resuscitation with response to such therapy being the primary determinant for guiding treatment. Rate of urine formation is considered the main index of adequacy of therapy. Such a simplified course of events obtains in the smaller injuries but accurate monitoring of the circulatory response is often impossible in the larger burns. One of the main problems is that little is known of the circulatory response in the early postburn period, particularly in relation to fluid therapy.

Some investigators have suggested recently that modifications in the fluid resuscitation regimens in both burn and hemorrhagic shock would be of value. The main alterations proposed involve the use of vasoactive substances.[13,28,41,42] to decrease peripheral resistance and/or support the myocardium, large volumes of salt solutions[33] alone to the exclusion of colloid, and the employment of the strong buffer agents,[4,10,20,39] to sustain normal pH. With few exceptions, these suggestions have been based on theoretical considerations with actual measurements of alterations in physiological response lacking. This is particularly true in regard to comparative activity or efficacy of the various agents. This is partly due to the lack of certainty as to what are the proper parameters to observe.

The present study is an attempt to clarify the circulatory response in the immediate postburn period in relation to rate, volume and composition of replacement fluids, and the various pharmacologic adjuvants currently advocated as having a positive effect in support of the circulation as well as comparative buffering effects of Ringer's lactate and Tris (hydroxymethyl) aminomethane. In order to accomplish this objective, study animals were subjected to varying rates and volumes of the fluid administration, as well as changes in the constituent quantities of colloid and salt solutions. In addition, the following pharmacologic agents were administered during the postburn period in an

attempt to determine their effect on the circulatory response: hydralazine, phenoxybenzamine, Isoproterenol, Methylprednisolone and Tris (hydroxymethyl) aminomethane.

Cardiac output was utilized as the principal measure of the changes in the response of the heart to the different loads of fluids and to the administration of the drugs in the postburn period. Circulatory dynamics were determined from calculations of peripheral resistance, venous and mean arterial pressures, and the effectiveness of the peripheral circulation was estimated from measurements of excess lactate[22,23,24] and calculations of oxygen extraction. Changes in the pH were utilized in a similar manner. As a corollary to the investigations, hematocrit changes and splenic weights revealed unsuspected data.

Method

Previously immunized and quarantined mongrel dogs weighing 9.7 to 22 Kg total body weight were utilized as study subjects. Following administration of anesthesia, polyethylene cannulae were placed in the right atrium, inferior vena cava and aortic arch. An esophageal pressure catheter was placed in the midesophagus and this cannula, along with the right atrial cannula, was attached to a differential pressure transducer (Sanborn Model 268B) to measure absolute right atrial pressures. The aortic cannula was attached to a Statham transducer to constantly record systolic and diastolic arterial pressures. The position of each cannula was verified at the time of sacrifice of the animal. In addition, animals were inspected for the presence of heartworms, which were present in scant numbers in only one animal. Arterial and venous blood samples were drawn from the appropriate cannulae with the venous samples being drawn both from the right atrium and from the inferior vena cava. Pressures and heart rate were recorded on a Sanborn (Model 964) 4-channel recorder. An indwelling catheter recorded urinary output at one-hour intervals. Arterial and venous pO_2, pCO_2 and pH were determined by the Instrumentation Laboratory (Model 105) pH and blood gas analyzer. Lactic acid was determined by the Ellis, Cane and Williams[14] modification of the Friedland and Dietrich method,[17] and pyruvic acid by the method of Friedmann and Haugen.[18] Cardiac output was determined by dye dilution technique utilizing Indocyanine green.[32] The dye was injected as a bolus[35] into the right atrium via the indwelling cannula.[21] A Gilford constant flow system (Model

105-S) was used to withdraw blood from the aortic sampling site. Dye dilution curves were recorded with a Waters densitometer (Model X250) and a Sergeant high impedance recorder (Model MR) and immediate calculations of cardiac output in liters per minute determined by a precalibrated Sanborn cardiac output computer (Model 130.[22,40] After induction with sodium pentothal, the animals were maintained with sodium barbital intravenous anesthesia. Following establishment of baseline values, the animals were subjected to a 50 per cent scald burn (11 seconds in water at 99-100°C) and immediately replaced on the surgical table. As soon as possible, instrumentation was reestablished, and previous determinations repeated. Previous experience comparing this preparation with a similar sham burn showed the manipulation to be of no consequence to subsequent determinations.[31] The delay between the burning and accomplishing the determinations varied from three to seven minutes.

The total of 240 animals studied were primarily divided into two groups based upon the period of the study. The first group of 193 animals were each studied for a period of six hours postburn and the second group of 47 animals were each studied for a total of 36 hours postburn. Following infliction of the burn, each baseline determination was repeated immediately upon return to the laboratory table, and at one-hour intervals subsequent to burning in the six-hour study group. In the 36-hour study group, determinations were done immediately after burning, at one hour postburn, at four hours postburn, and subsequently every four hours for a total of 36 hours of observation.

Heparin was not used in any animal.

The following fluid regimens utilized, beginning immediately after the one-hour postburn determinations, are shown in Table 1.

Tris (hydroxymethyl) aminomethane and Ringer's lactate were compared as to their buffering activity. Tris was administered as a 0.3 molar solution equivalent in volume to one-third of the electrolyte requirements and infused over 40 minute periods, at 1, 3 and 5 hours postburn. This amounted to 300 mgm/Kg the first hour and 150 mgm/Kg the third and fifth hours. Ringer's lactate was administered in volumes equal to the volume of dextrose/water used as a

Legend for Table

Fluid replacement therapy was begun immediately after the one-hour postburn determinations. The fluid volumes listed are the amounts given throughout the six-hour study periods except for the 6 + 19 group in which therapy was given for only four hours. This was the 24-hour requirement as calculated by the Brocke formula given in four hours.

In the 36-hour study groups, the original fluid loads were continued for eight hours, reduced by 50 per cent for the next 16 hours, and that volume reduced by 50 per cent for the next 12 hours in accordance with policy advocated by the Brooke formula and generally practiced clinically.

Fluid Therapy				Pharmacologic Adjuvant Dose and Time	Observation Period Hours
Dextran/glucose (ml/kg/hr)	Dextran/saline (ml/kg/hr)	Ringer's (ml/kg/hr)	Ringer's Lactate		
5				THAM - 0.3 M 300 mgm/kg at 1 hr & 150 mgm/kg at 3 & 5 hrs pb	6
5			8 ml/kg at 1 hr pb 4 ml/kg at 3 & 5 hrs pb		6
	* 1.5		* 4.5 ml/kg/hr	Methylprednisolone 4 mgm/kg at 1 hr & 1 mgm/kg at 4 hrs	6
	5.0		4.5 ml/kg/hr	" " "	6
	* 1.5	* 4.5		Hydralazine 0.5 mgm/kg at 1 hr & q 2 hrs pb	6
	5.0	4.5		" " "	6 & 36
	10.0	3.0		" " "	6 & 36
	6.0	19.0		" " "	6 & 36
	1.5	4.5		Isoproterenol 3 mcgm/kg/hr	6
	5.0	4.5		" "	6
	10.0	3.0		" "	6
	6.0	19.0		" "	6
	1.5	4.5		Phenoxybenzamine 1 mgm/kg at 1 hr & q 24 hrs	6 & 36
	5.0	4.5		" " "	6 & 36
	10.0	3.0		" " "	6 & 36
	6.0	19.0		" " "	6
		14 ml/kg/hr for first 8 hrs 7 ml/kg/hr for next 16 hrs 3.5 ml/kg/hr for next 12 hrs			36

* Brooke formula

vehicle for the Tris. Arterial and venous pH determinations were made in each animal preburn, immediately postburn and at one-hour or six-hour intervals postburn depending on the study group.

In the animals studied for 36 hours, the fluid volumes were reduced in accordance with the usual policy in burn therapy of administering one-half the first 24-hour requirement in the first eight hours postburn and the remainder administered evenly over the next 16 hours. Further reduction in fluid load occurred in the period 24-36 hours postburn. The following pattern was used in the 36-hour periods:

Time	0-8 hours	9-24 hours	25-36 hours
	1.5 + 4.5	0.75 + 2.25	0.37 + 1.12
ml/Kg/hr	5.0 + 4.5	2.5 + 2.2	1.25 + 1.12
	* 10.0 + 3.0	5.0 + 1.5	2.50 + 0.75

* A sharp reduction was necessary after eight hours to prevent overloading.

To test myocardial response to load in a different manner a carotid-jugular shunt was formed and preburn and postburn cardiac outputs measured with the shunt open and with it closed.

Results

The unesthetized, preburn determinations are expressed as 100 per cent. Subsequent determinations are expressed as percentages of preburn values. Results are reported as mean values.

1. Cardiac Output (Figs. 1A, 1B). In the burned control animals that received no fluid or drug therapy, cardiac output immediately dropped after burning to 43 per cent of preburn output. This subsequently gradually declined over the period of observation to a low of 20 per cent and remained there for the last two hours of observation. With increasing the administered fluid volume alone, improvement in cardiac output

could be noted, but at no time did it exceed 72 per cent of preburn determination, and this occurred only with large volumes of fluid (Fig. 1B). At these times, the animals' lungs were wet and fluid was dripping from the nose. The smallest dose of resuscitation fluid utilized (1.5 + 4.5), which approximates the Brooke burn resuscitation formula, showed no ability to improve cardiac output following burning but it did tend to minimize the progressive drop in output.

The addition of pharmacologic agents to the smallest volumes of fluid resuscitation therapy resulted in improvement in the group which received hydralazine but not with the other drug additives. As the volume of fluids increased, hydralazine continued to exert the most striking effect as measured by improvement in cardiac output (Fig.2). While improvement with phenoxybenzamine did occur in the smaller fluid volumes, it was much less than that noted with hydralazine, and in the larger fluid volumes fluids alone were as effective. Isoproterenol showed no beneficial benefit as reflected by improvement in cardiac output. It also appeared that marked increases in volume replacement in the immediate postburn period (6 + 19) offered no advantage over moderate increases (10 + 3) whether utilized with or without the pharmacologic adjuvant. Also, an increase in the proportionate ratio of colloid over electrolyte (5 + 4.5 and 10 + 3) was as important as total volume and of greater significance than massive (6 + 19) increases in salt solution. More than a moderate 5 ml/Kg increase in colloid added nothing.

Cardiac outputs for 36-hour Group (Figs. 3 and 4). The animals who received anesthesia alone without burning had a continuous drop in their cardiac output for a period of 12 hours postburn, at which time they had reached 60 per cent of preburn levels. The cardiac output continued to fluctuate around this value the remainder of the 36-hour period of observation. As increased volumes of fluid (without drugs) were administered to the animals following burning, cardiac output continued to improve. Utilizing the smaller amounts of fluid (1.5 + 4.5) which approximates the Brooke fluid formula, there was minimal if any improvement in cardiac output. In the group receiving salt water only without colloid replacement therapy, only slight improvement in cardiac output occurred over the 36-hour period of observation, from a low of 40 per cent of the preburn level to a high of 64 per cent. In the animals receiving the two higher volumes of fluid

Legends for Figures 1A and 1B

Figure 1A. The shaded area represents the difference in myocardial response to the opening of the carotid-jugular shunt both preburn and immediately postburn. While an increase in output occurs with the shunt open in both treated and untreated groups, the response is somewhat less postburn. However, it is still significant, being approximately 50 per cent of the preburn increase with the shunt open. This decrease may represent some degree of myocardial depression.

Figure 1B. Cardiac output was improved the greatest in the hydralazine groups with moderate fluid replacement volumes. However, no advantage was gained by increasing volume above 5 + 4.5.

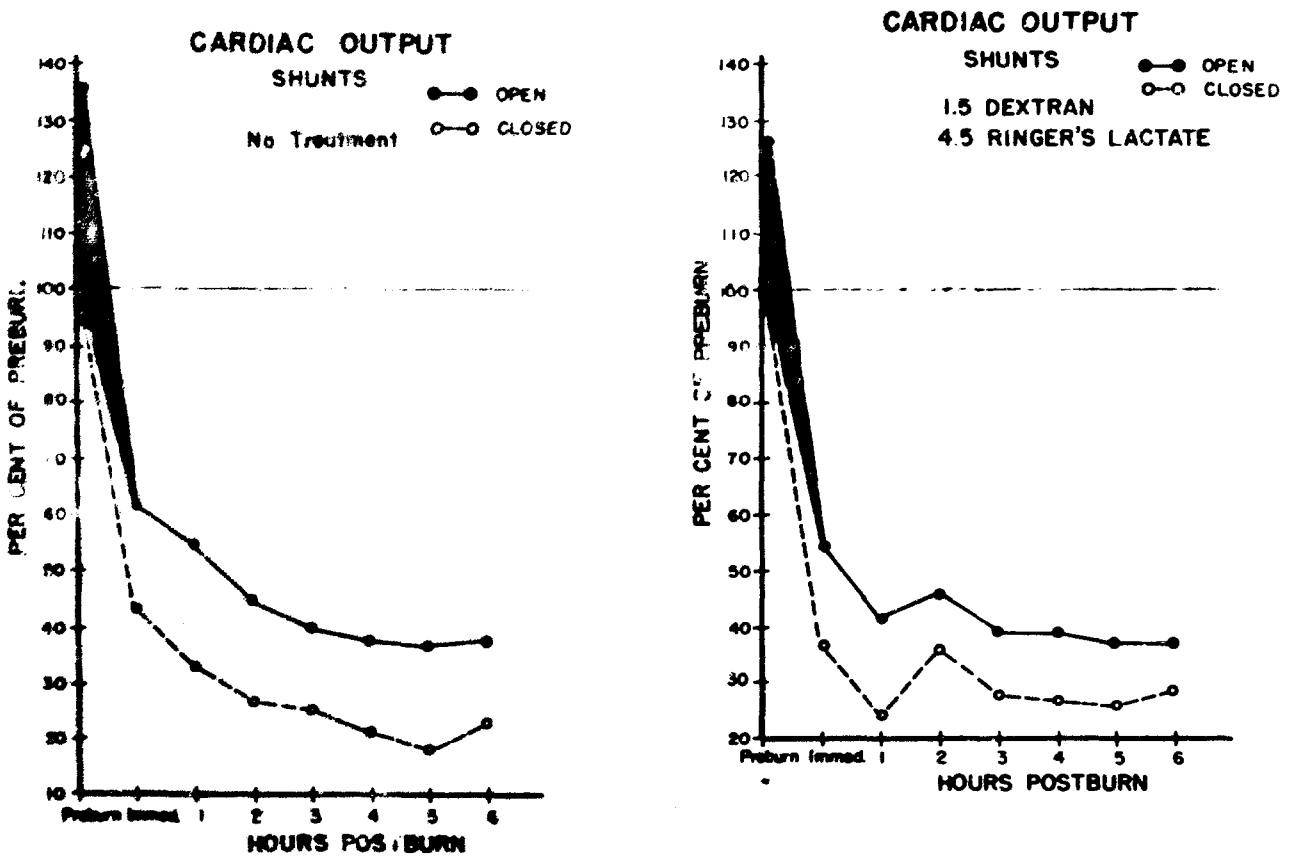

CARDIAC OUTPUT
SHUNTS
No Treatment
OPEN
CLOSED
HOURS POSTBURN
CARDIAC OUTPUT
SHUNTS
1.5 DEXTRAN
4.5 RINGER'S LACTATE
OPEN
CLOSED
HOURS POSTBURN

Figure 1A

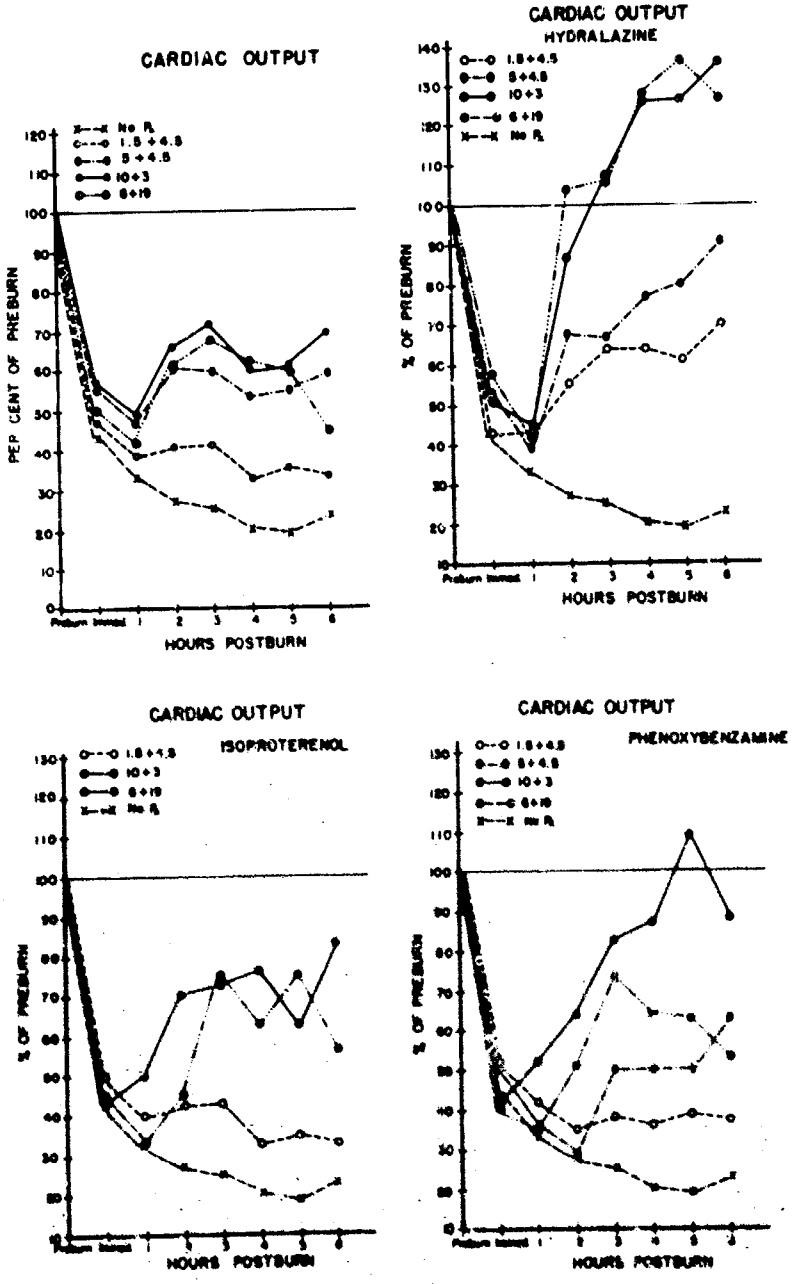

CARDIAC OUTPUT
PER CENT OF PREBURN
HOURS POSTBURN
CARDIAC OUTPUT
HYDRALAZINE
% OF PREBURN
HOURS POSTBURN
CARDIAC OUTPUT
ISOPROTERENOL
% OF PREBURN
HOURS POSTBURN
CARDIAC OUTPUT
PHENOXYBENZAMINE
% OF PREBURN
HOURS POSTBURN

Figure 18

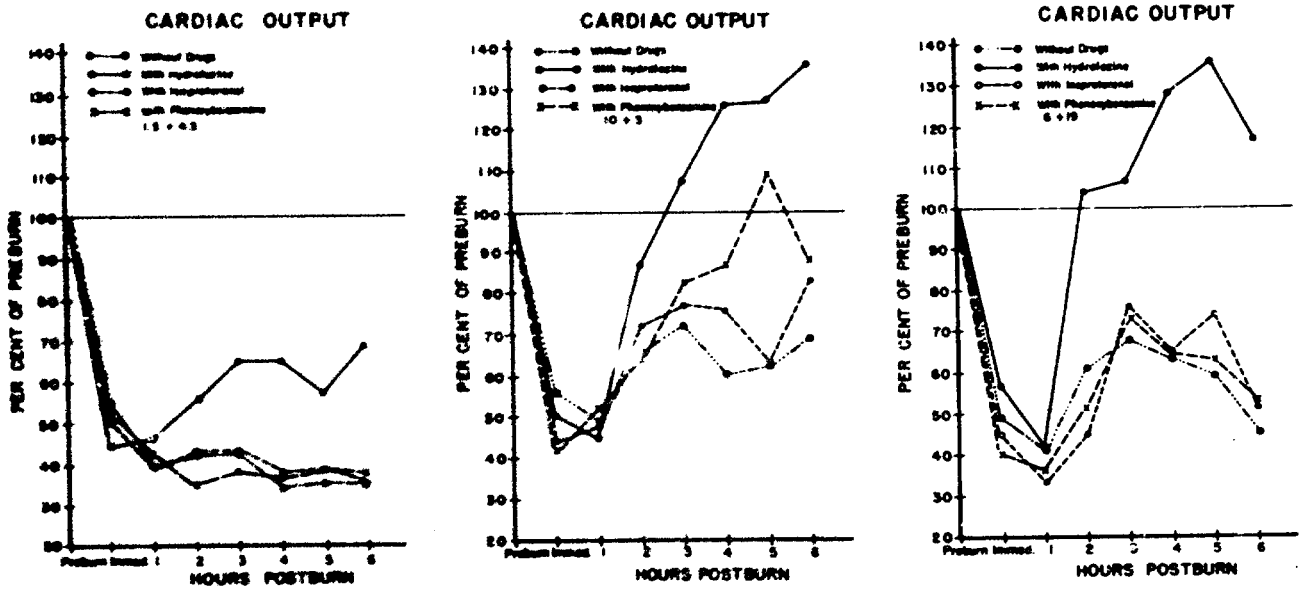
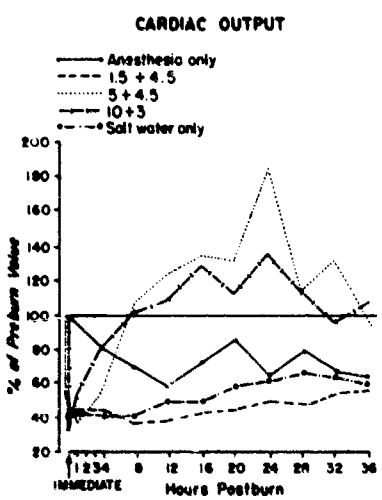
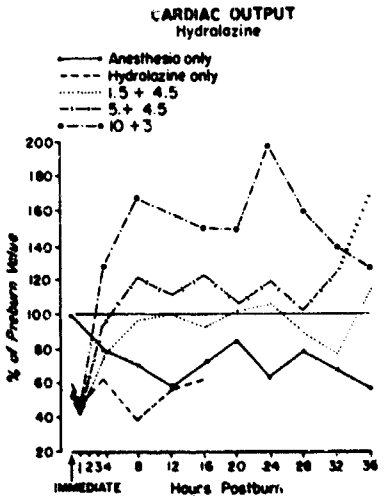


Figure 2. With increasing fluid volumes, cardiac output improved proportionately. This was most marked by far with the hydralazine groups.





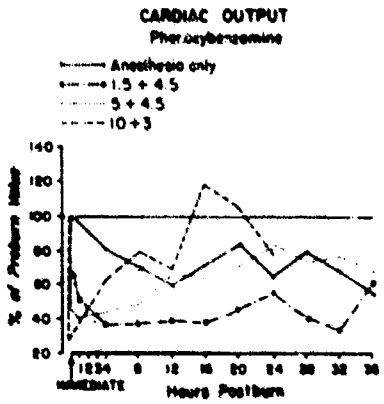


Figure 3. Use of Ringer's only as the resuscitation fluid results in little improvement in output. Hydralazine alone, without fluids, allows survival for 18 hours in spite of low output. Phenoxybenzamine shows minimal improvement even with the larger fluid volumes.

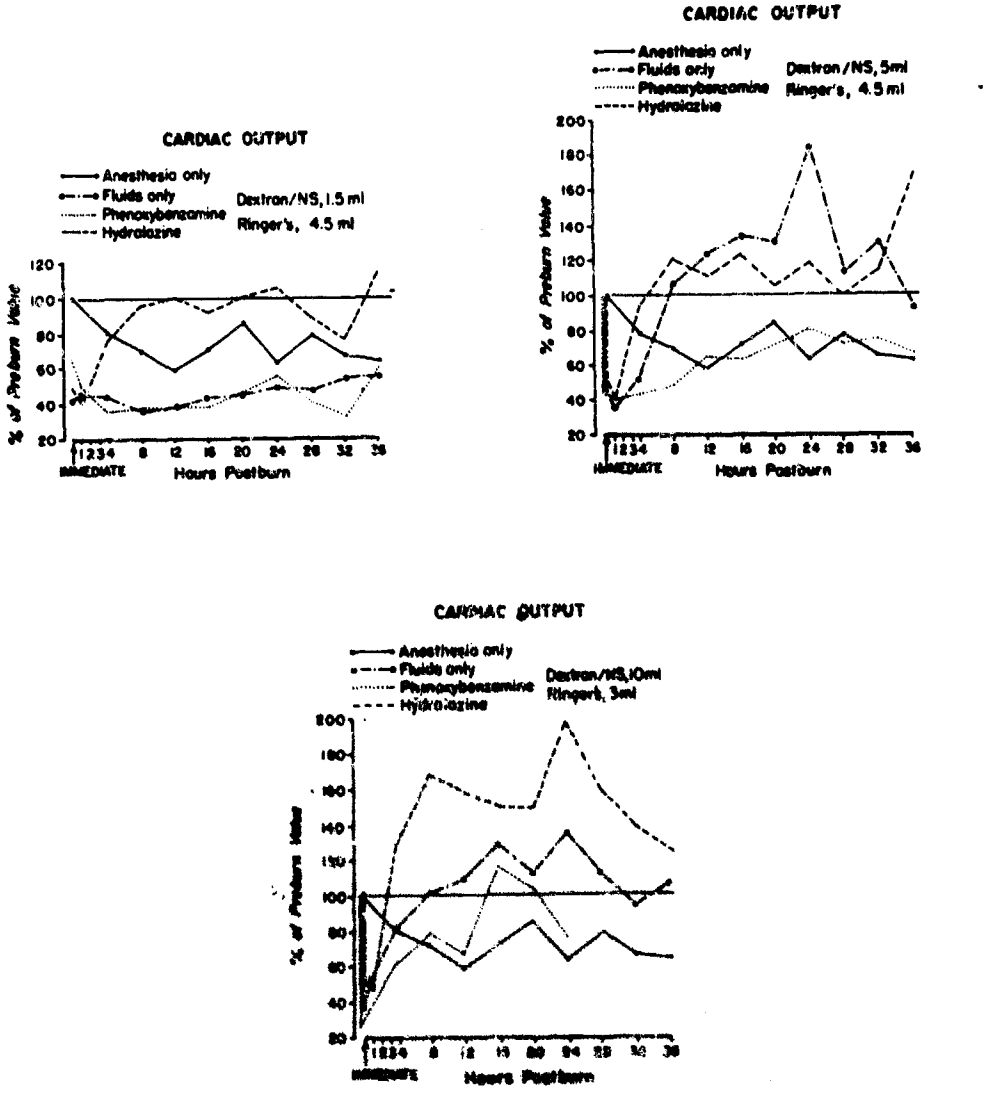


Figure 4. Improvement in cardiac output occurs with increasing fluid volumes and is particularly marked with hydralazine added. The only advantage to increasing volume above 5 + 4.5 is a slightly more rapid rise in output to supernormal levels. Numbers are mean values for three animals. Where line stops, animals died.

(5 + 4.5 and 10 + 3), improvement in cardiac output occurred but was not progressive with increased volume beyond the 5 + 4.5 amount. By eight hours postburn, both had risen to the preburn levels and remained there.

With the addition of hydralazine, a marked improvement in cardiac output followed, even with the smaller fluid volumes (1.5 + 4.5), and it was not until the largest fluid volumes tested (10 + 3) were administered that the phenoxybenzamine showed a return to preburn levels. The improvement in cardiac output appeared most rapid in the group of animals treated with the intermediate fluid dosage (5 + 4.5) and hydrazaline.

In the carotid-jugular shunt preparation, cardiac output increased immediately with opening of the shunt (Fig. 1A).

2. Peripheral Resistance. Peripheral resistance was calculated by considering effective venous pressure as zero, thus deriving peripheral resistance as the quotient of mean arterial pressure divided by cardiac output. Results are expressed as percentages of a preburn, anesthetized value of 100 per cent. In all instances, immediate postburn peripheral resistance showed a marked increase in all instances approaching or exceeding 200 per cent of control values. Within the first hour postburn, prior to therapy, resistances varied, some increasing and others decreasing. With volume replacement alone, there was a sharp drop in peripheral resistance following the administration of the larger volumes. A progressive rise in resistance occurred during the six-hour period of observation with the administration of the smaller volume of fluid.

The addition of pharmacologic agents resulted in precipitous and sustained drops in the peripheral resistance in the animals administered the large volumes of fluid. This was true of hydrazaline, phenoxybenzamine and Isoproterenol. However, in the smaller volumes of fluid, only hydrazaline maintained the decrease in peripheral resistance for the six-hour period of observation (Fig. 5).

3. Arterial and Venous Pressures. Mean arterial pressure showed a sharp rise immediately following burning with a rapid decline to or slightly below preburn value at one-hour postburn. Following administration of fluids with the pharmacologic agents or alone, there was rarely more than

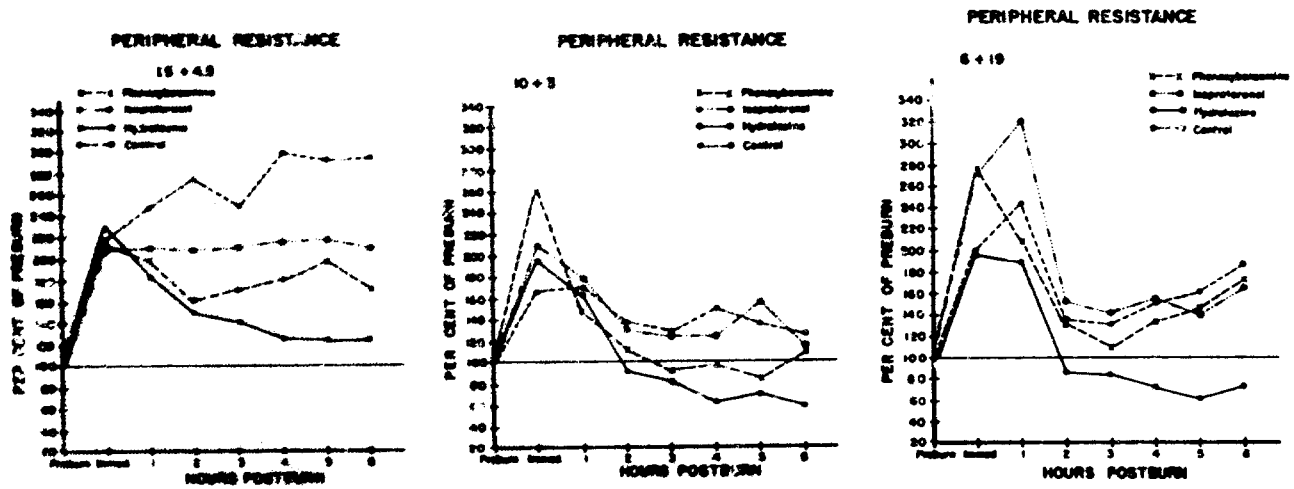


Figure 5. Peripheral resistance showed a sharp rise immediately postburn which was sustained in the control groups until large volumes of fluid were administered. With addition of drugs, resistance decreased. This was most marked with hydralazine and minimally seen with Isoproterenol.

a 10 per cent variation in mean arterial pressure. Venous pressures fluctuated considerably from -1 to +7 mm Hg, and this was associated with two factors: changes in respiratory rate and the obvious pulmonary congestion associated with the very large volumes of fluid. Character of respiration varied so greatly and rapidly throughout the period of observation, however, that it is felt that the validity of the venous pressure observations as a reflection of cardiac dynamics is questionable.

4. Hematocrit. Changes in right atrial hematocrit were directly proportional to volume of fluid administered (Fig. 6) except in the group of animals receiving phenoxybenzamine. In these animals there was a precipitous and sustained drop in hematocrit which, though influenced by volume of fluid administered, was out of proportion to that seen in the other animals (Fig. 7). Observation of the animals at sacrifice revealed that the burned animals without treatment and those treated with fluids and hydralazine showed an almost 50 per cent decrease in mean spleen weights when compared with control animals which were anesthetized but not burned. In contrast, the phenoxybenzamine animals all had an engorged spleen with mean weight of almost twice that of control animals.

This decrease in hematocrit would obviously effect peripheral viscosity and also be a consideration in calculations of oxygen availability or oxygen extraction.

SPLEEN WEIGHTS (MEAN)

	Unburned Anesthetized Controls(11)	Burn Treatment-- None	Burn Treatment-- Fluids, Hydralazine	Burn Treatment-- Fluids, Phenoxybenzamine
Spleen wt.	142 gm	67 gm	69.2 gm	211.4 gm
Spleen wt. / Kg. dog wt.	7.5 gm/Kg	4.23 gm/Kg	4.42 gm/Kg	14.77 gm/Kg

5. Excess Lactate. Arterial samples were drawn from the aorta. Venous samples were obtained from the inferior vena cava. This

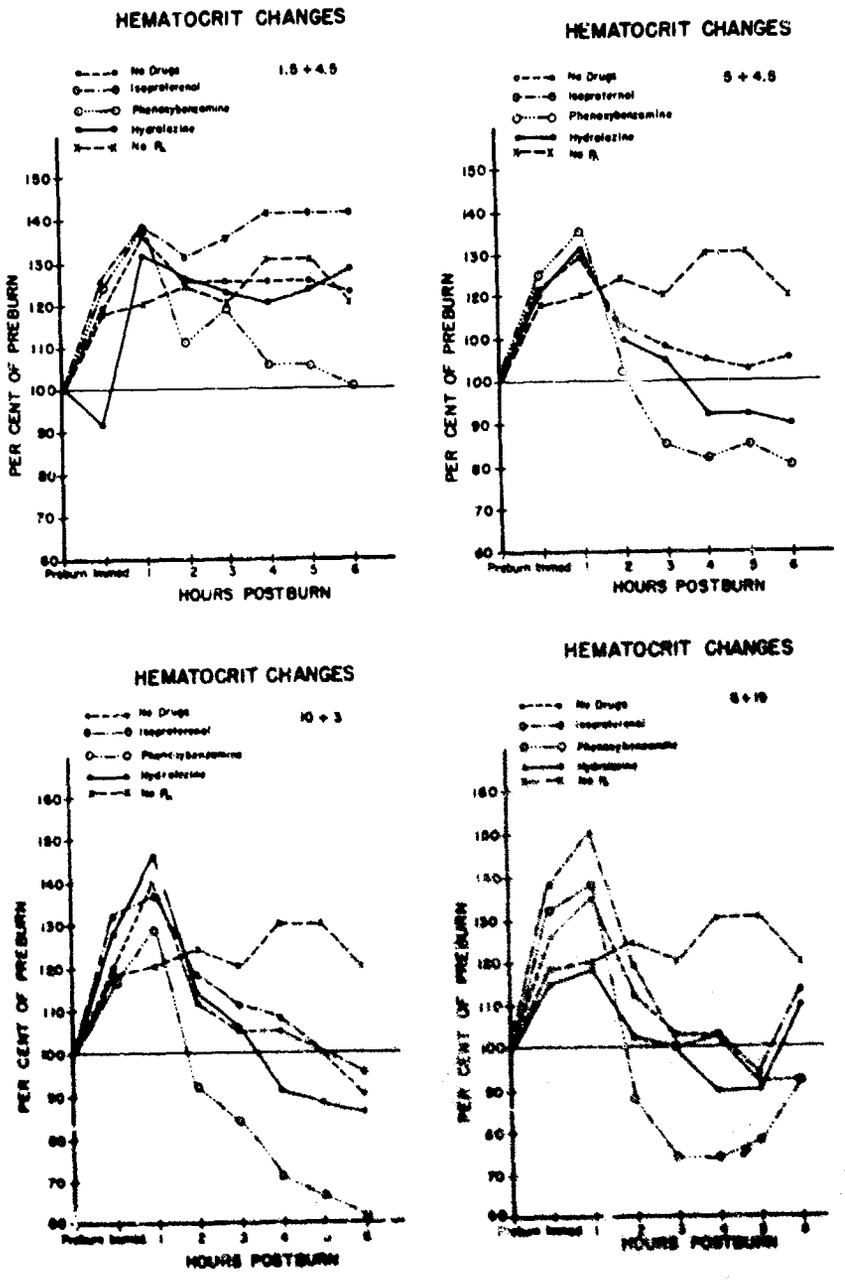


Figure 6. While the anticipated decrease in hematocrit occurred with increasing volumes of fluid replacement, it was most striking when phenoxybenzamine was given also. This is reflected in spleen weights as well.

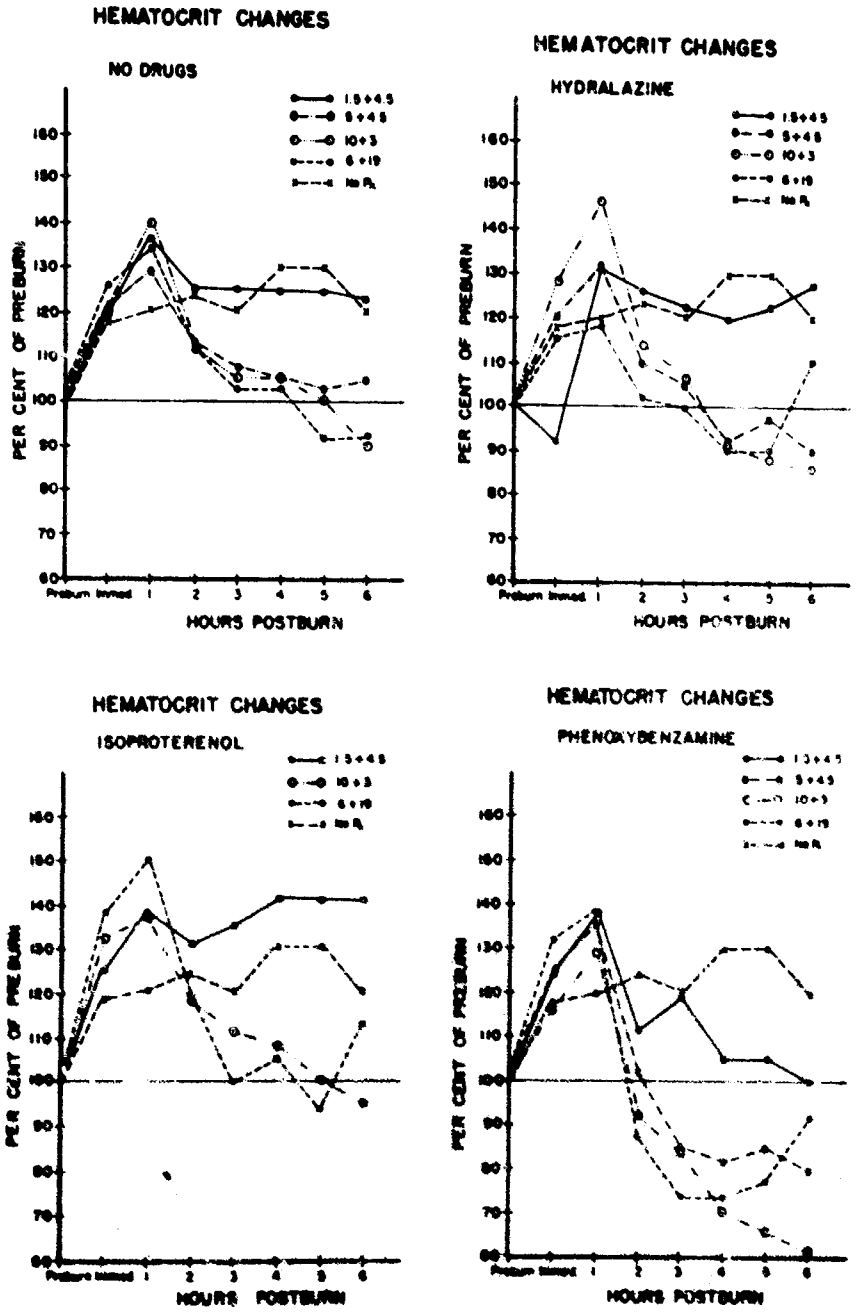


Figure 7. Phanoxybenzamine administration resulted in more marked hematocrit drops in all categories of fluid administration.

source was chosen in an attempt to measure any changes in the circulation of the burn area. In the control animals, both arterial and venous lactate showed a precipitous rise. The arterial lactate (Fig. 8) was maintained for the six-hour observation period at the immediate postburn level, while the venous excess lactate (inferior cava), as one would expect, continued to rise (Fig. 9). Therapy with fluids alone during the six-hour period resulted in a gradual return toward normal levels but during the six-hour period there still remained some excess. During the 36-hour periods of observation, treatment with fluids alone resulted in return to normal baseline values by 8 to 36 hours postburn with some fluctuation around the baseline level, except in the animals receiving the small volumes of fluid (Fig. 10). In those, there was a rather marked rise between 28 and 36 hours postburn when the animals all appeared in terminal failure. While the peak levels in the groups receiving the fluids alone as compared to those receiving fluids and pharmacologic agents varied little, those animals receiving the drugs showed more tendency to return toward the baseline during the six-hour period of observation. When observed for a period of 36 hours, the animals treated with fluids and hydrazaline demonstrated a return of excess lactate values to the normal baseline at 16 hours postburn. Surprisingly, the animals receiving fluids and phenoxybenzamine in the 36-hour group showed a less extreme rise in excess lactate values and a more precipitous and sustained drop to below preburn levels which was difficult to explain.

6. Oxygen Extraction. This was calculated by the following formula:

$$\%\ \text{A-V sat} \times \frac{\text{Hct} \times 0.33\ (\text{ratio hct/hgb}) \times 1.34\ (\text{ml } O_2/\text{gm hgb at saturation}) \times \text{cardiac output} \times 1000}{100}$$

$$= \%\ \text{A-V sat} \times \text{hematocrit} \times 4.46 \times \text{cardiac output}$$

$$= O_2 \text{ extraction}$$

Calculation of oxygen extraction by the above method revealed that in animals receiving fluids only, oxygen extraction improved with increasing volumes of fluid. Only moderate increases (5 + 4.5) in the volume of fluid administered

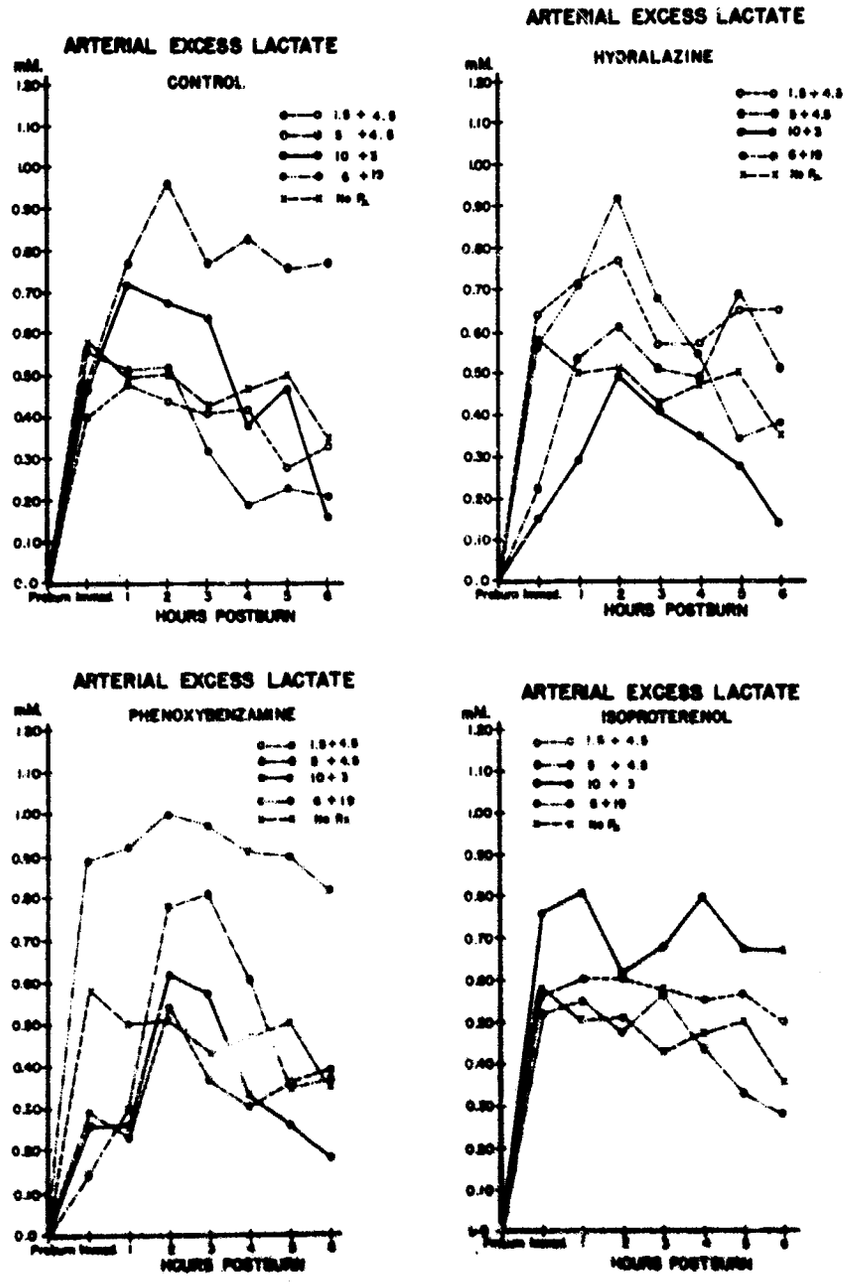


Figure 8. Arterial excess lactate (XSL) displayed an initial sharp rise and a gradual return toward normal in the six-hour period. The decrease was most noticeable in the phenoxybenzamine groups.

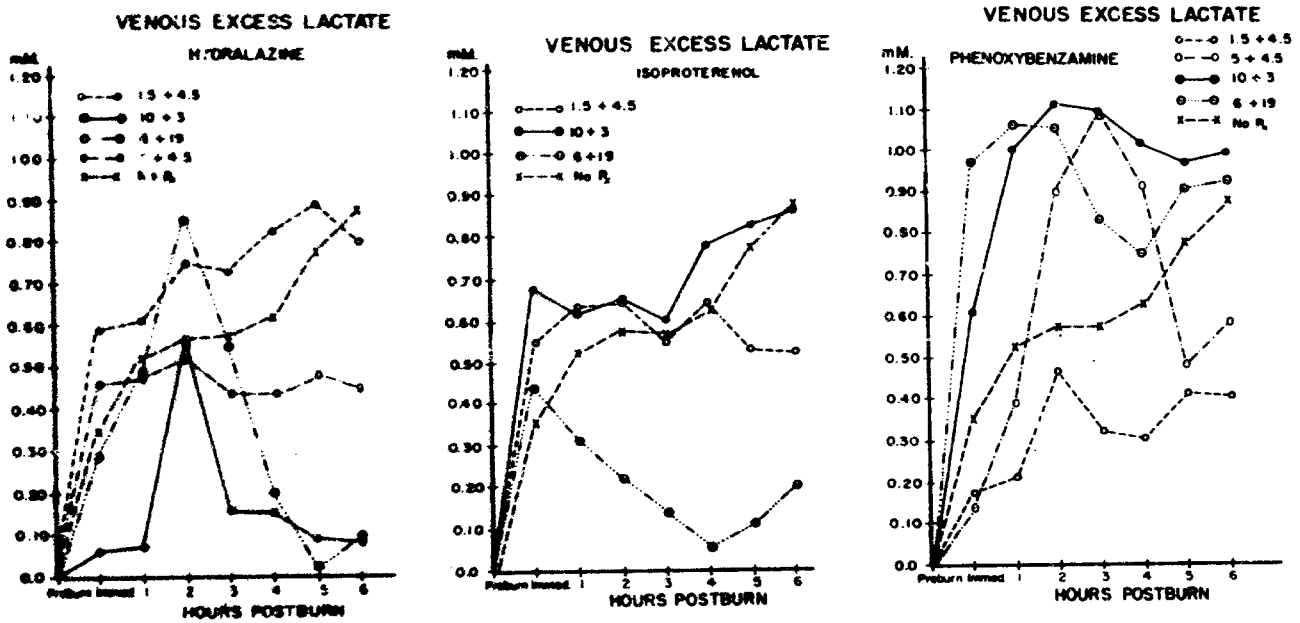


Figure 9. Venous excess lactate values in general were closely related to the arterial except in the phenoxybenzamine groups in particular. In those animals, the venous samples, drawn from the inferior vena cava draining the burned area, showed a sustained level much above the arterial. Why this veno-arterial gradient should exist is difficult to explain.

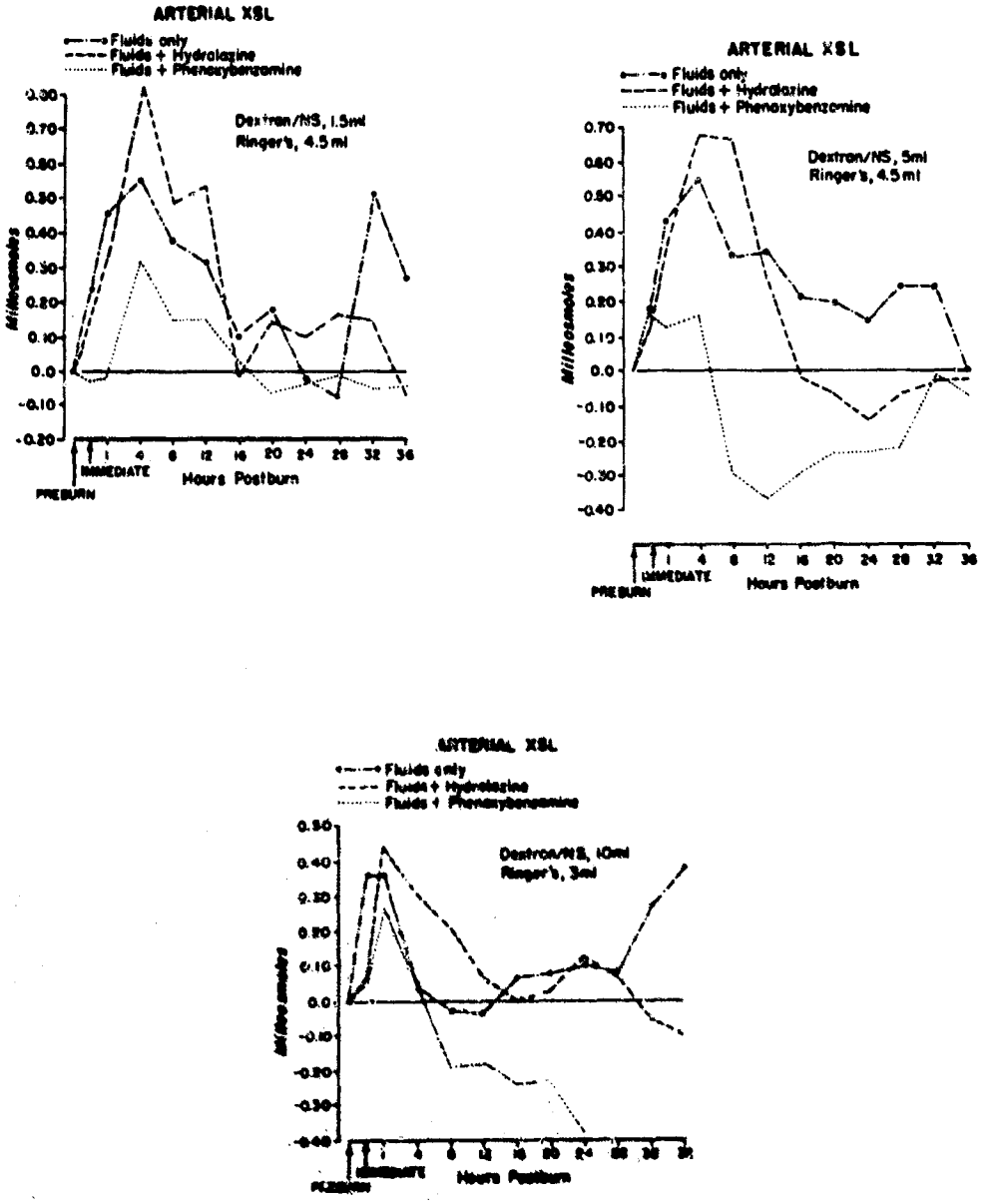


Figure 10. Excess lactate (XSL) gradually declined from peak postburn values. The most striking was the reduction with phenoxy-benzamine. Values beyond 28 hours are difficult to evaluate for many animals are dying with pneumonia and congestion at that time although they survive the 36-hour observation period.

returned oxygen extractions to preburn levels. Increasing fluids above that volume resulted in supernormal oxygen extractions (Fig. 11). With the small volumes of fluid, both hydralazine and Isoproterenol resulted in a return of the oxygen extractions toward the normal baseline during the six-hour period of observation. Phenoxybenzamine therapy showed no tendency towards improvement and in fact resulted in a continuous drop in oxygen extraction. As the volume of fluids was increased, both hydrazaline and Isoproterenol showed rather striking improvements in oxygen extraction, while it was not until extremely large volumes of fluids (6 + 19) were administered that any improvement in oxygen extraction was noted in the animals receiving phenoxybenzamine.

In the animals observed for 36 hours, oxygen extraction values fluctuated widely beyond eight hours both within groups and between groups. This reflected the instability of the study preparation during that period probably due to varying requirements for anesthesia supplements for this prolonged period of observation and frequent findings of pulmonary congestion and/or pneumonia at sacrifice. Even so, by 24 hours postburn all groups had oxygen extraction values at preburn levels or far above.

In general, unless oxygen extraction exceeded preburn values, the excess lactate levels, particularly in venous blood, tended to remain elevated for longer periods of time and the latter often exceeded the values for arterial blood (Fig. 12).

Comparative buffering: Tris (hydroxymethyl) aminomethane demonstrated effective buffering activity but was no more effective than equal volumes of Ringer's lactate (Fig. 13). While urinary output was far greater in the Tris animals, there was no discernable difference in the amount or rate of edema forming in the burn. Nor did cardiac output improve with a buffer added to fluid therapy (Fig. 14).

Steroids: The addition of large doses of steroids to the fluid resuscitation schedules resulted in no improvement in cardiac output.

Discussion

The use of the various resuscitation formulae for the initiation of fluid replacement therapy in the acutely burned

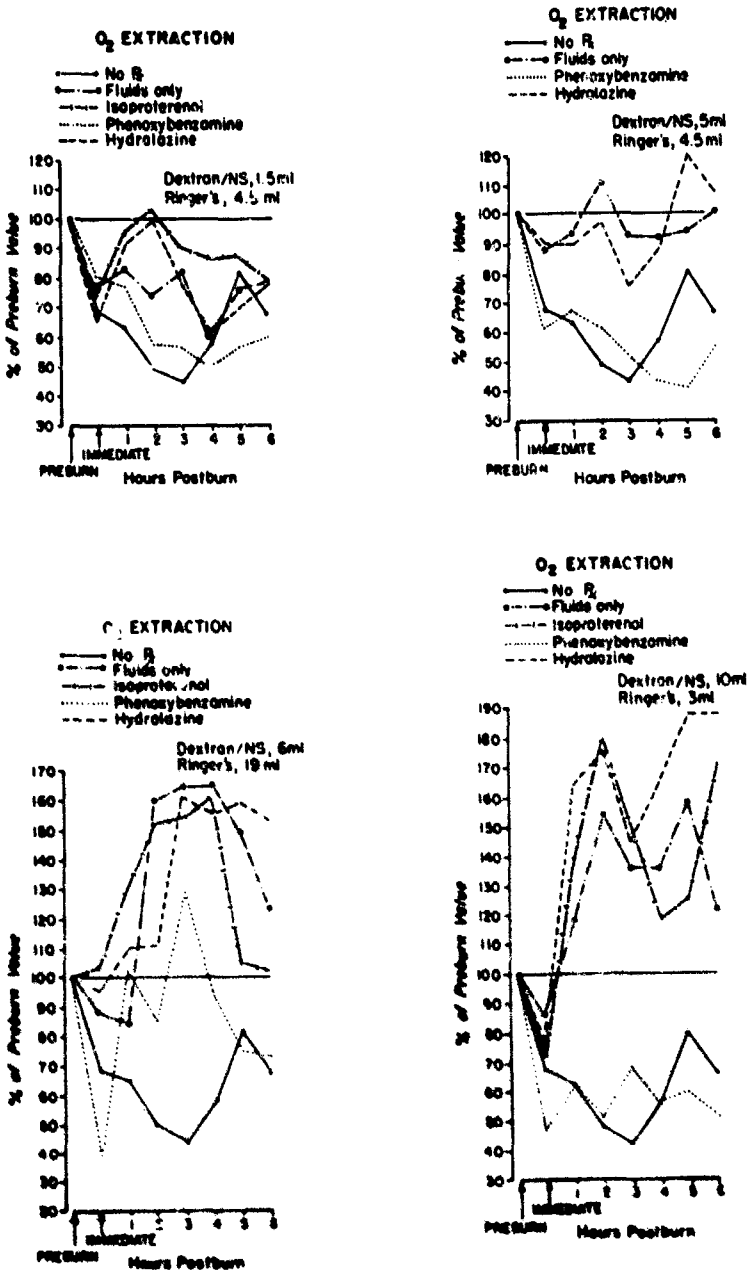


Figure 11. Oxygen extraction was found to be most efficient when fluid volumes were combined with hydralazine or Isoproterenol, particularly the former. Only in the very large fluid volume replacements was the administration of phenoxybenzamine associated with significant improvement. This is in contrast to the XSL values.

Legends for Figures 12A, B, C, and D

The inverse correlation of oxygen extraction and excess lactate obtains in all fluid volume and drug groups except the phenoxybenzamine groups. In the latter, arterial XSL declines in spite of a persistently low oxygen extraction. The A-V differences in XSL are difficult to explain.

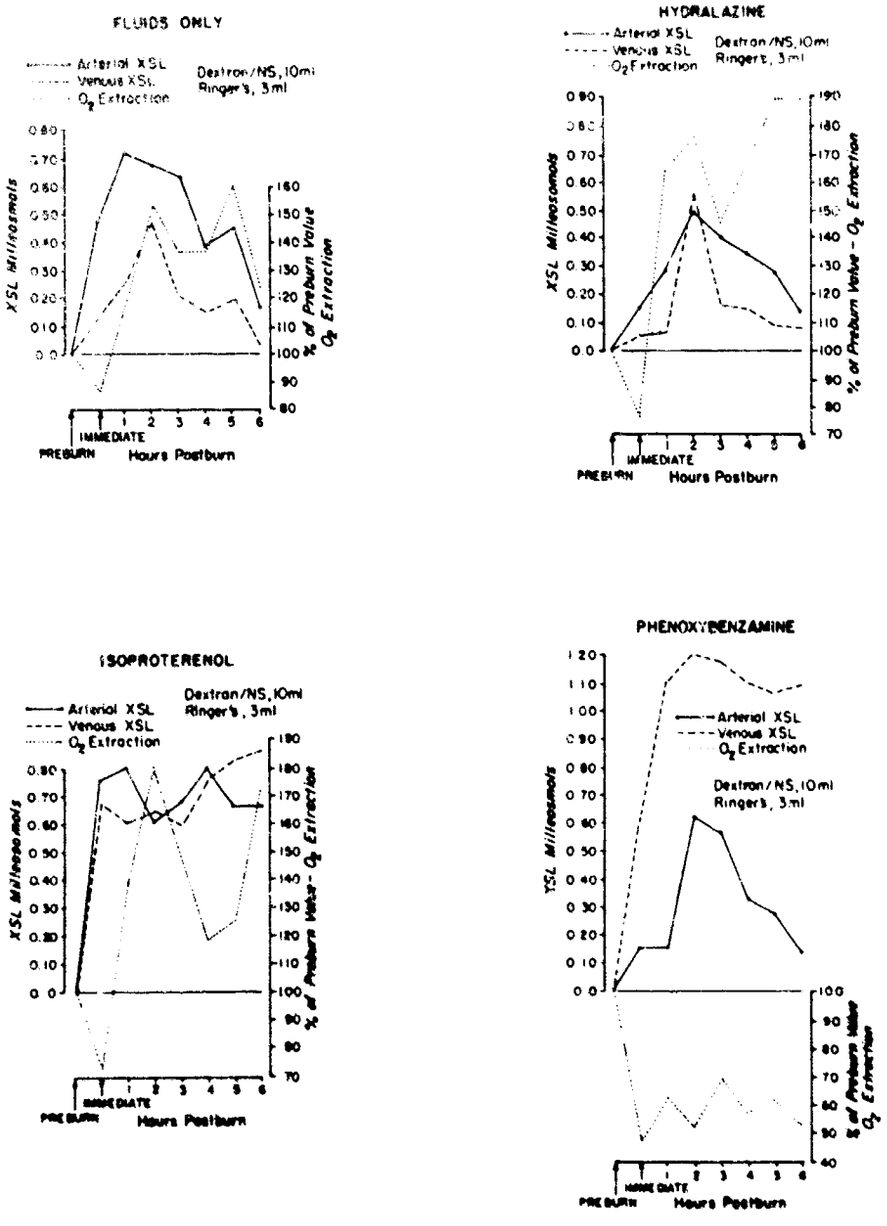

FLUIDS ONLY
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 10ml
Ringer's, 3ml
XSL Milliosmols
% of Preburn Value - O_2 Extraction
IMMEDIATE
PREBURN
Hours Postburn
HYDRALAZINE
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 10ml
Ringer's, 3ml
XSL Milliosmols
% of Preburn Value - O_2 Extraction
IMMEDIATE
PREBURN
Hours Postburn
ISOPROTERENOL
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 10ml
Ringer's, 3ml
XSL Milliosmols
% of Preburn Value - O_2 Extraction
IMMEDIATE
PREBURN
Hours Postburn
PHENOXYBENZAMINE
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 10ml
Ringer's, 3ml
YSL Milliosmols
% of Preburn Value - O_2 Extraction
IMMEDIATE
PREBURN
Hours Postburn

Figure 12A

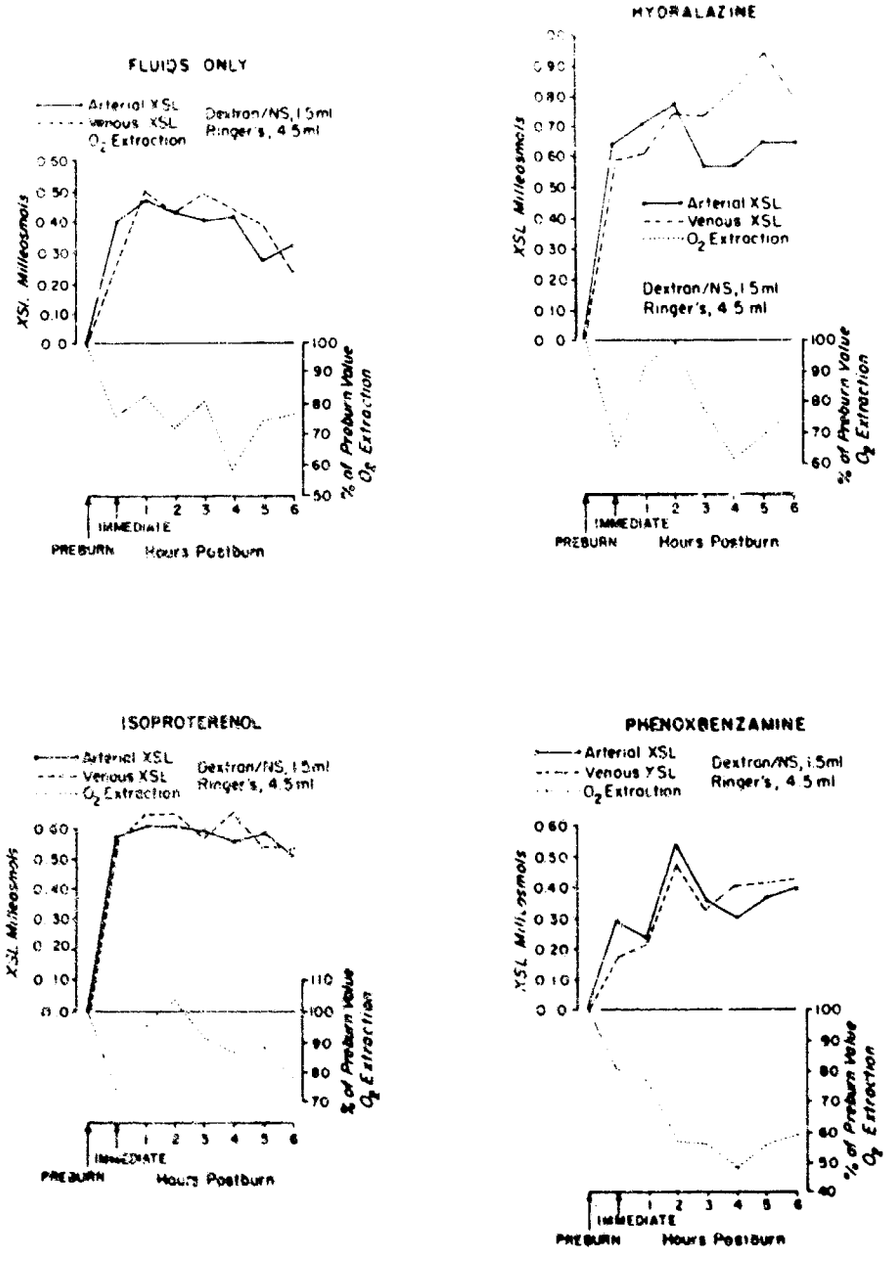

FLUIDS ONLY
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 1.5ml
Ringer's, 4.5ml
XSL Milliosmols
% of Preburn Value O_2 Extraction
IMMEDIATE
PREBURN
Hours Postburn
HYDRALAZINE
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 1.5ml
Ringer's, 4.5 ml
XSL Milliosmols
% of Preburn Value O_2 Extraction
IMMEDIATE
PREBURN
Hours Postburn
ISOPROTERENOL
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 1.5ml
Ringer's, 4.5ml
XSL Milliosmols
% of Preburn Value O_2 Extraction
IMMEDIATE
PREBURN
Hours Postburn
PHENOXBENZAMINE
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 1.5ml
Ringer's, 4.5 ml
XSL Milliosmols
% of Preburn Value O_2 Extraction
IMMEDIATE
PREBURN
Hours Postburn

Figure 128

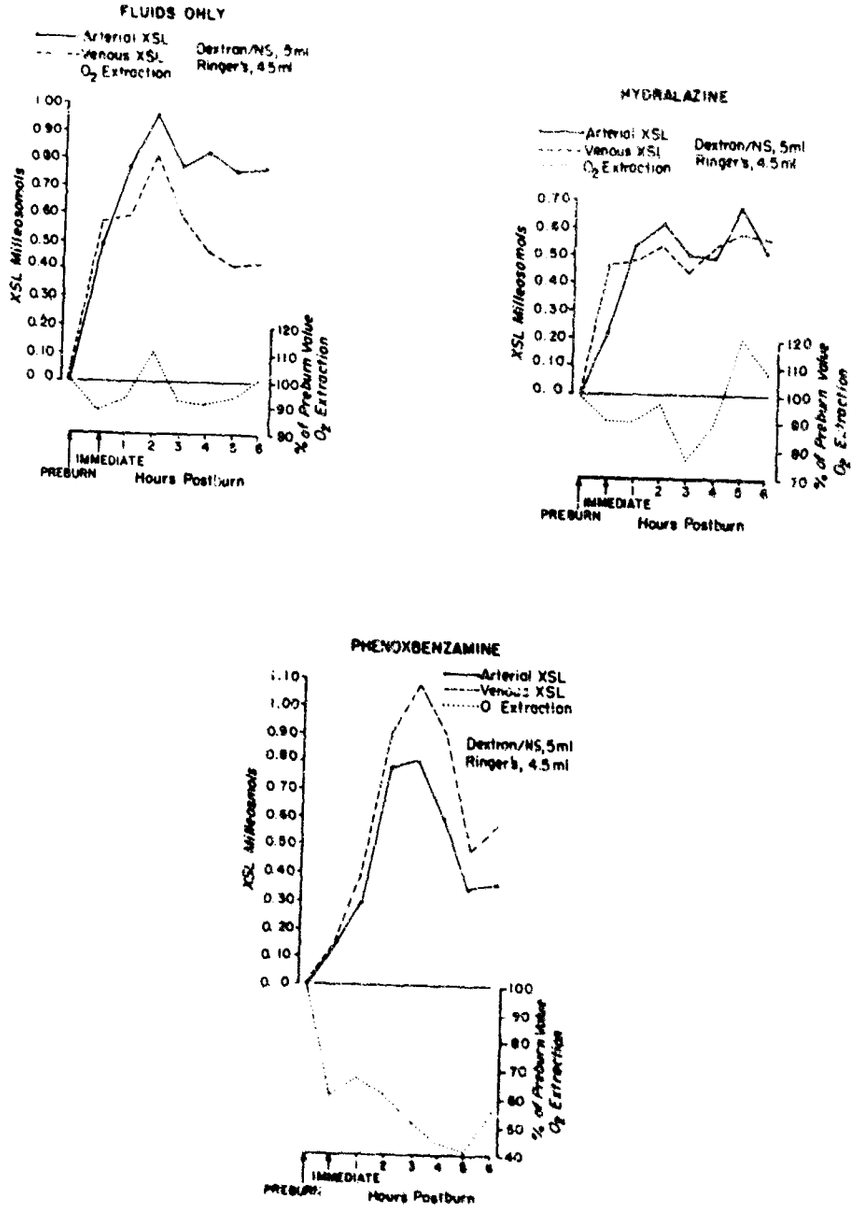
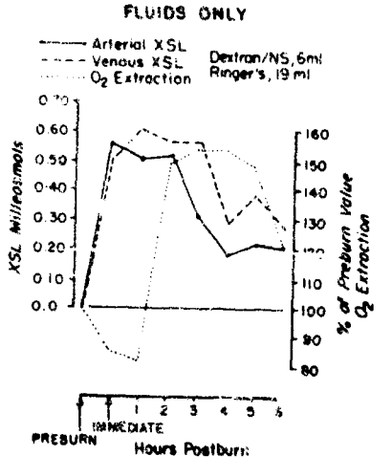
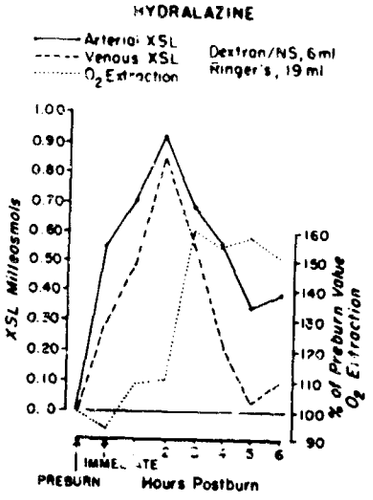
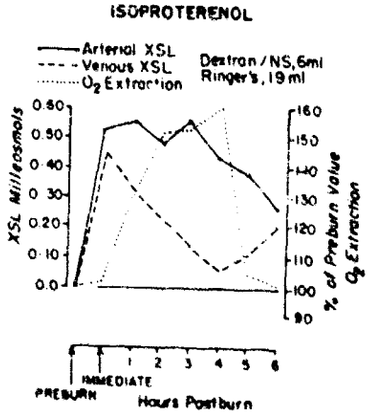

FLUIDS ONLY
Arterial XSL
Venous XSL
O2 Extraction
Dextran/NS, 5ml
Ringer's, 4.5ml
XSL Milliosmols
% of Preburn Value O2 Extraction
IMMEDIATE
PREBURN
Hours Postburn
HYDRALAZINE
Arterial XSL
Venous XSL
O2 Extraction
Dextran/NS, 5ml
Ringer's, 4.5ml
XSL Milliosmols
% of Preburn Value O2 Extraction
IMMEDIATE
PREBURN
Hours Postburn
PHENOXBENZAMINE
Arterial XSL
Venous XSL
O Extraction
Dextran/NS, 5ml
Ringer's, 4.5ml
XSL Milliosmols
% of Preburn Value O2 Extraction
IMMEDIATE
PREBURN
Hours Postburn

Figure 12C


FLUIDS ONLY
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 6ml
Ringer's, 19 ml
XSL Milliosmols
% of Preburn Value O_2 Extraction
PREBURN
IMMEDIATE
Hours Postburn


HYDRALAZINE
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 6ml
Ringer's, 19 ml
XSL Milliosmols
% of Preburn Value O_2 Extraction
PREBURN
Hours Postburn


ISOPROTERENOL
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 6ml
Ringer's, 19 ml
XSL Milliosmols
% of Preburn Value O_2 Extraction
PREBURN
IMMEDIATE
Hours Postburn

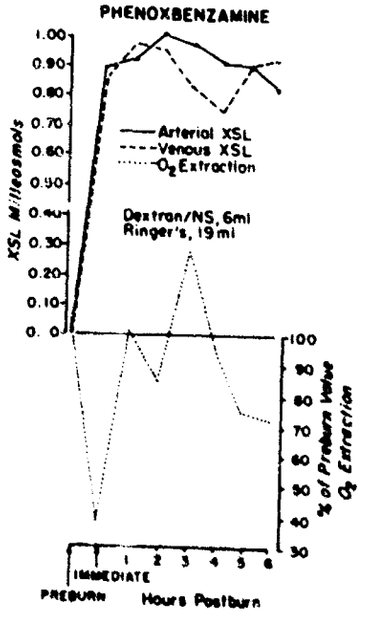

PHENOXBENZAMINE
Arterial XSL
Venous XSL
O_2 Extraction
Dextran/NS, 6ml
Ringer's, 19 ml
XSL Milliosmols
% of Preburn Value O_2 Extraction
PREBURN
IMMEDIATE
Hours Postburn

Figure 120

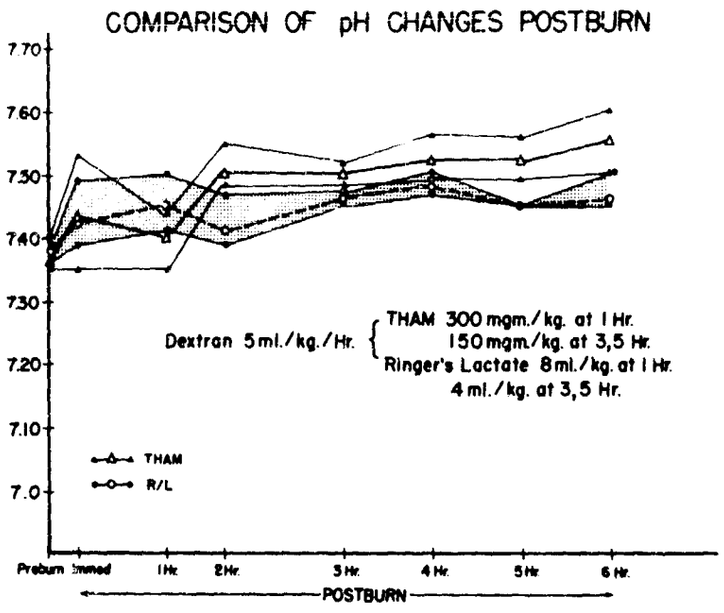


Figure 13. Figures are mean values and standard deviation. Equal volumes of fluid were given in each instance. Buffering action was essentially the same for Tris (hydroxymethyl) aminomethane and Ringer's lactate.

Legends for Figures 14A and B

The administration of Tris buffer resulted in no improvement over that of fluid volume alone.

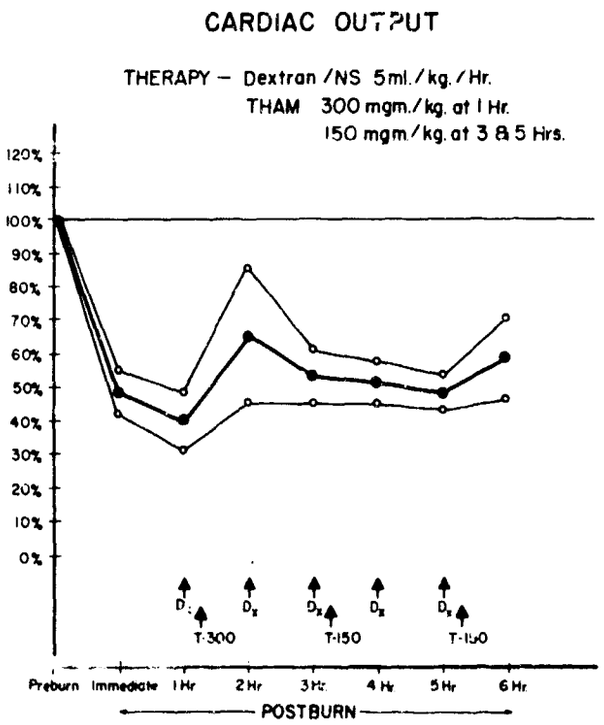

CARDIAC OUTPUT
THERAPY – Dextran /NS 5ml./kg./Hr.
THAM 300 mgm./kg. at 1 Hr.
150 mgm./kg. at 3 & 5 Hrs.
120%
110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
T-300
T-150
T-150
Preburn
Immediate
1 Hr
2 Hr
3 Hr
4 Hr
5 Hr
6 Hr
POSTBURN

Figure 14A

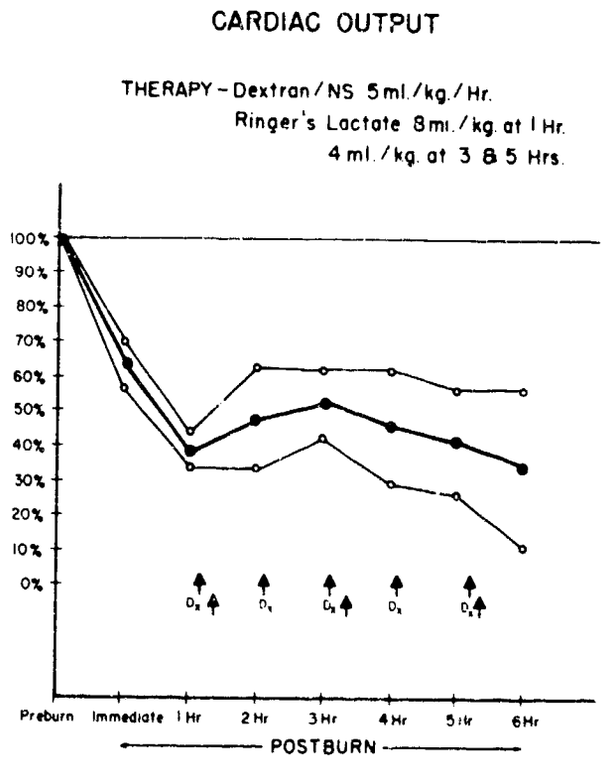

CARDIAC OUTPUT
THERAPY - Dextran / NS 5 ml./kg./Hr.
Ringer's Lactate 8 ml./kg. at 1 Hr.
4 ml./kg. at 3 & 5 Hrs.
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Preburn
Immediate
1 Hr
2 Hr
3 Hr
4 Hr
5 Hr
6 Hr
POSTBURN

Figure 14B

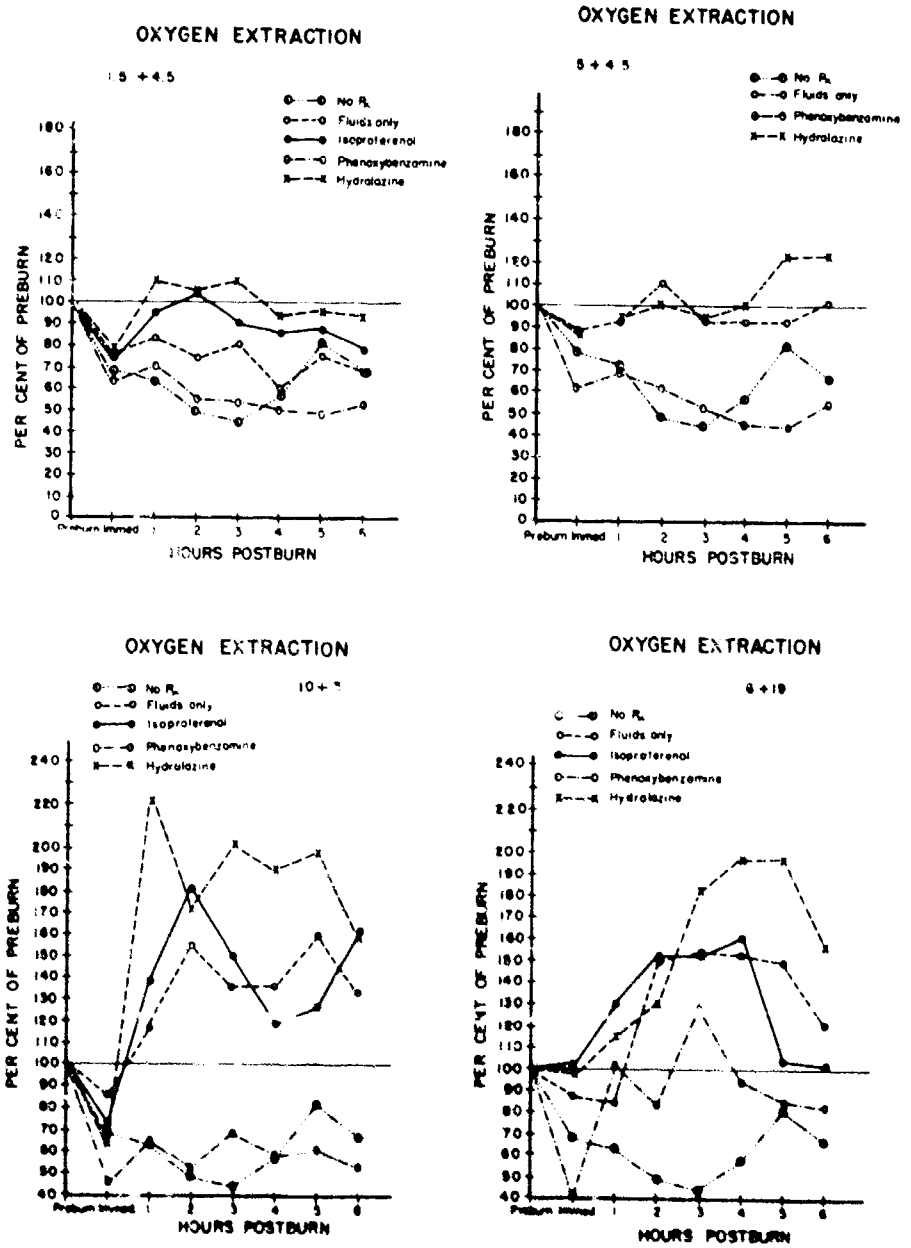


Figure 15. Oxygen extraction improved with increasing volumes of fluid replacement therapy except in the groups receiving phenoxybenzamine. This is in contrast to the XSL values.

Figures 16A and 16B

Increasing volume by increasing colloid resulted in no improvement within the period of observation until hydralazine was added.

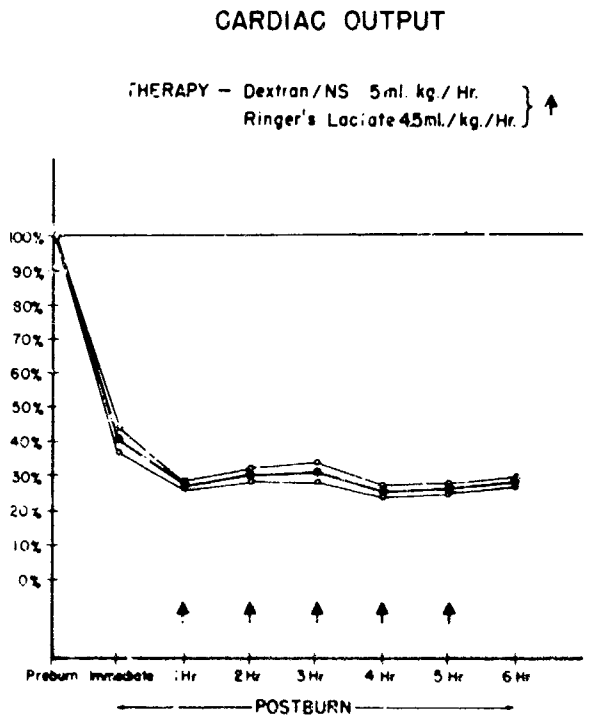

CARDIAC OUTPUT
THERAPY – Dextran/NS 5ml. kg./ Hr.
Ringer's Lactate 4.5ml./kg./Hr.
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Preburn
Immediate
1 Hr
2 Hr
3 Hr
4 Hr
5 Hr
6 Hr
POSTBURN

Figure 16A

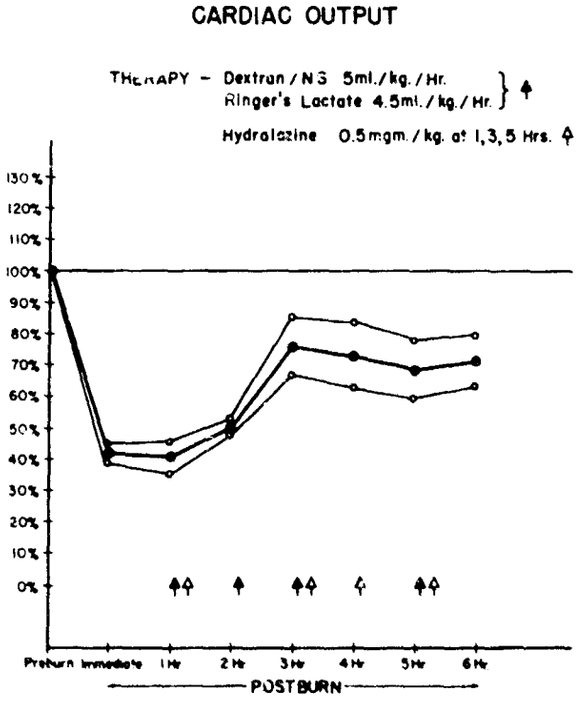

CARDIAC OUTPUT
THERAPY – Dextran / NS 5ml./kg./Hr.
Ringer's Lactate 4.5ml./kg./Hr.
Hydralazine 0.5mgm./kg. at 1,3,5 Hrs.
130%
120%
110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Preburn
Immediate
1 Hr
2 Hr
3 Hr
4 Hr
5 Hr
6 Hr
POSTBURN

Figure 168

Figures 17A and 17B

Administering the entire 24-hour fluid volume (Brooke formula) in four hours still results in only slight improvement during this period. However, the addition of hydralazine is associated with a tremendous increase.

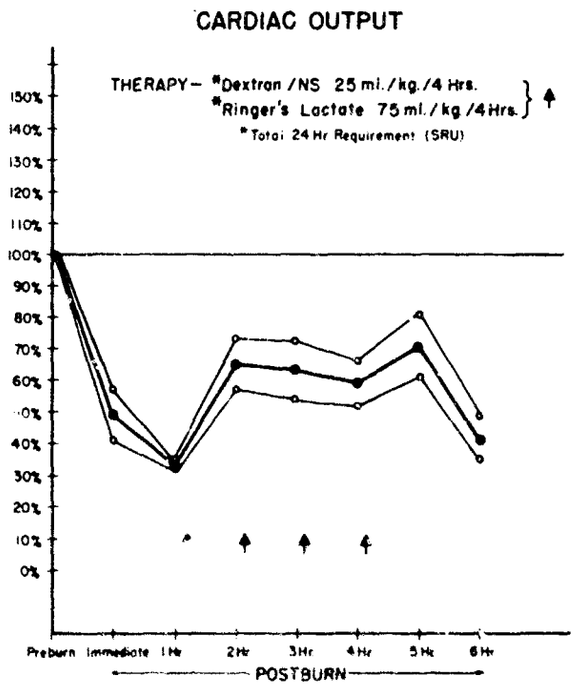

CARDIAC OUTPUT
THERAPY– *Dextran /NS 25 ml./kg./4 Hrs.
*Ringer's Lactate 75 ml./kg./4 Hrs.
* Total 24 Hr Requirement (SRU)
150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
40%
30%
20%
10%
0%
Preburn
Immediate
1 Hr
2 Hr
3 Hr
4 Hr
5 Hr
6 Hr
POSTBURN

Figure 17A

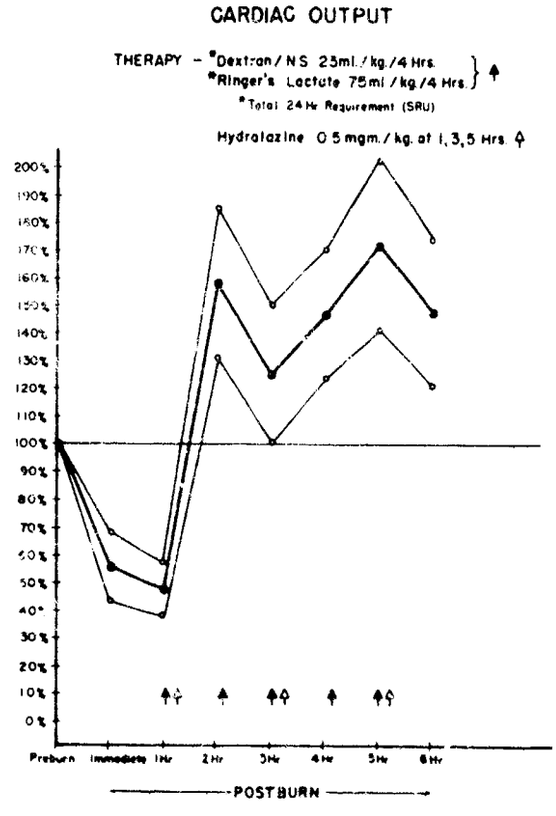

CARDIAC OUTPUT
THERAPY – *Dextran / N S 25ml. / kg. / 4 Hrs.
*Ringer's Lactate 75ml / kg. / 4 Hrs.
*Total 24 Hr Requirement (SRU)
Hydralazine 0.5 mgm. / kg. at 1, 3, 5 Hrs.
200%
190%
180%
170%
160%
150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Preburn
Immediate
1Hr
2Hr
3Hr
4Hr
5Hr
6Hr
POSTBURN

Figure 17B

Legend for Figures 18A and 18B

This volume is essentially the volume and rate advocated by the Brooke formula. No improvement is seen except with hydralazine. Values are mean and standard deviation.

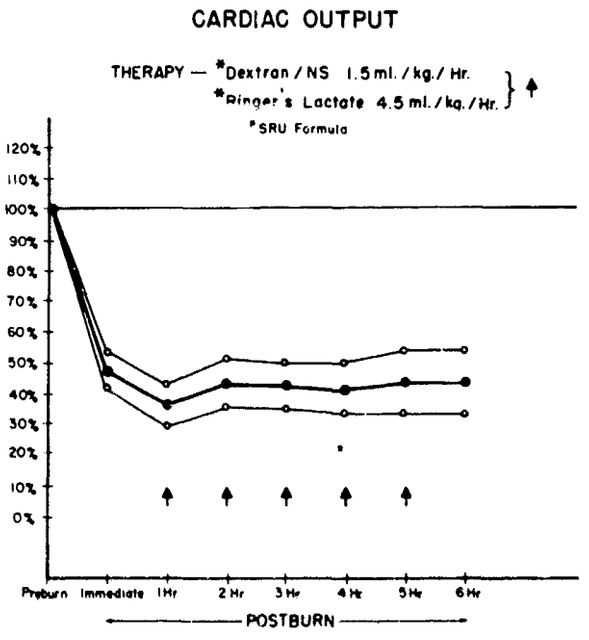

CARDIAC OUTPUT
THERAPY — *Dextran / NS 1.5 ml. / kg. / Hr.
*Ringer's Lactate 4.5 ml. / kg. / Hr.
*SRU Formula
120%
110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Preburn
Immediate
1 Hr
2 Hr
3 Hr
4 Hr
5 Hr
6 Hr
POSTBURN

Figure 18A

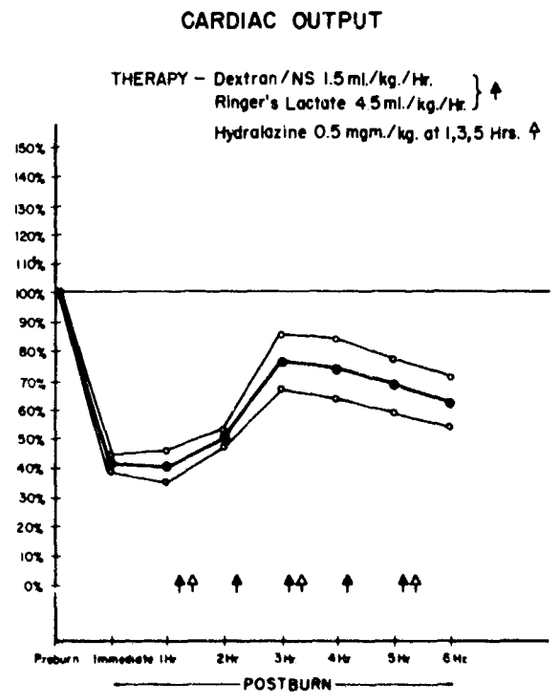

CARDIAC OUTPUT
THERAPY – Dextran/NS 1.5 ml./kg./Hr.
Ringer's Lactate 4.5 ml./kg./Hr.
Hydralazine 0.5 mgm./kg. at 1,3,5 Hrs.
150%
140%
130%
120%
110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Preburn
Immediate
1 Hr
2 Hr
3 Hr
4 Hr
5 Hr
6 Hr
POSTBURN

Figure 18B

individual appears well established within the surgical philosophy pertinent to this particular injury. Since the initial work of Evans[15] and the retrospective analysis of fluid therapy which resulted in the Brooke formula,[37] little has been done to methodically evaluate the efficacy of fluid replacement therapy in the burned patient. Rate of urinary output has been used as the primary guide to adequacy of replacement therapy and in major thermal injury in which all four extremities are usually involved, it often becomes the only indication of circulatory esponse. The appropriateness of this reliance upon urinary output may be seriously questioned when one observes patients with obviously poor peripheral flow and an extremely low measured cardiac output who still maintain a urinary output of 50 to 100 cc per hour. In addition, the often inappropriate but nevertheless occasional use of osmotic diuretic agents obviates any reliance upon rate of urine formation as an index of adequacy of fluid resuscitation therapy.

In clinical practice, the burn resuscitation formulae are properly used only as a guide to initiation of therapy and not adhered to rigidly.

The Evans formula advocates equal quantities of whole blood and salt water, while the Brooke formula advocates the same total volume of fluids but a redistribution so that three-fourths of the initial volume is salt water and one-fourth blood or colloids. Recently, Moyer[33] has advocated the use of salt solutions alone in the resuscitation of the burned patient in the immediate postburn period. While the latter has not gained widespread popularity, it nevertheless poses an intriguing hypothesis which deserves investigation if for no other reason than to attempt to clarify the confusion generated.

Within the obvious limitations of transposition of data from one species to another and the influence of anesthesia upon the preparation utilized, it appears reasonable to state that the circulatory response as manifested by cardiac output demonstrates an improvement in proportion to the volume and rate of fluid administration. The administration of a volume of fluid roughly equivalent to the Brooke formula results in minimal improvement in cardiac output over a period even as long as 36 hours postburn. If the volume of fluid is increased, particularly by increasing the volume of colloid, one gets very significant and rapid increase in cardiac output, reaching normal levels within eight hours postburn and being maintained there with a lesser volume of fluid. The only

advantage which accrues from further increases in volume replacement therapy, particularly in the colloid constituent, is a more rapid return to preburn levels. Increase in total volume above 10 ml/Kg/hr or colloid above 5 ml/Kg offers no significant improvement and often results in overload.

The response of the animals is the same as that of humans with large burns who invariably require larger total volumes and particularly an increase of colloid above that suggested by the Brooke formula (0.5 ml/Kg/hr).

The animals receiving Ringer's lactate alone without colloid received the same amount of sodium ions and 15 per cent more total fluid than did those receiving the moderate volume of fluids (10 + 3) with a heavy colloid load. The colloid utilized in the latter group was dextran in saline and it might be argued that it was actually the sodium ions in the dextran-saline solution that was important rather than colloid. However, the salt solutions alone did nothing to improve cardiac output and under these conditions failed to support the thesis of the salt water advocates. Volumes of salt water of the magnitude of 3-4 times the colloid volumes might be more effective.

The optimum cardiac output in the immediate postburn period is yet to be determined but it may well be in excess of normal.[5,6] A great deal has been said in recent months concerning the use of pharmacologic agents as adjunctive measures in the treatment of shock which has not responded adequately to volume replacement therapy. The alpha adrenergic blocking agent, phenoxybenzamine, and the beta adrenergic stimulant, Isoproterenol,[26,28] have been advocated in these situations. In addition, the steroids[27,38] have always enjoyed some mysterious popularity and are often used with little rationale in difficult situations. Hydralazine has long been used as an oral antihypertensive agent, and a recent report would indicate that its use with volume replacement therapy in the immediate postburn period might be beneficial.[30] This latter drug works directly on the smooth muscle of the vessels to result in dilatation of both the resistance and capacitance vessels, and also has some inotropic or central cardiac stimulating action.[1,3,12] Isoproterenol is also known to have an inotropic effect.

While the dosages utilized in this study, particularly Isoproterenol,[28] may be in some dispute, conclusions must be made on the basis of the data vailable. Steroids in the form of Methylprednisolone exert no demonstrable effects during the six-hour periods of observation utilized. Isoproterenol

results in some mild improvement in cardiac dynamics as demonstrated by improvement in cardiac output to a point approximately midway between the immediate postburn deficit and the preburn normal levels. The observation period was only six hours, however, and it is possible that further observation would have resulted in eventual improvement to preburn levels. However, during the last three hours of observation, values plateaued and there was no significant tendency towards improvement.

The use of phenoxybenzamine results in insignificant improvement in cardiac output, even over a 36-hour period of observation, except when the extremely large doses of fluid volume are utilized. Under these circumstances, the animals have wet lungs and fluids drip from the nose in large quantities. Overload is obvious.

Hydralazine, even with the smallest volumes of fluid, results in improvement in cardiac output and the return to preburn levels is generally maintained. Increasing the volume of fluid administered in combination with hydralazine results in an even more rapid return to normal preburn levels and subsequent outputs exceed baseline in proportion to the volume of fluid administered. If cardiac output is a reliable index of the adequacy of the circulatory response, the combination of hydralazine with resuscitative fluids is beneficial. The moderate fluid volumes (5 + 4.5) combined with hydralazine result in excellent output values without overload. It seems probable that in the first four to eight hours postburn a volume of fluid administration far in excess of that presently popular is advisable in order to return cardiac output to normal or supernormal levels. It would appear particularly appropriate in circumstances in which initiation of therapy has been delayed. Following the rapid return to these levels, the fluid therapy in all likelihood could be reduced sharply and still maintain essentially the same cardiac output.

Indeed, such conclusions are justified by recent observations that fluid loss from the vascular compartment is very greatly increased for the first 22-30 hours postburn, after which time vascular integrity is restored and subsequent volume replacement therapy remains mainly within the vascular compartment.[36]

The response of the heart to thermal injury has been investigated by others[16] with the implication that central myocardial failure is a predominant factor in postburn shock. This conclusion was based on simultaneous cardiac output and blood volume determinations. Since output decreases much more rapidly than volume, it was considered that central failure had occurred. However, such a conclusion is based on the assumption that blood volume deficit or myocardial failure are the only factors causing a deficiency in cardiac output. Such is not warranted. Sudden rises in peripheral resistance can be a major consideration.

The response of the myocardium in this study indicates that given a good fluid load the heart is quite capable of a respectable response. The increase in output with increasing fluid volumes and in the shunt animals is evidence for this. The fact that the administration of inotropic agents (hydralazine, Isoproterenol) are associated with a further increase in cardiac output is not necessarily indicative of an original limited response. It could also be purely a drug augmentation of the normal myocardium such as that seen with digitalis.[8] This study does not clearly delineate which is occurring. However, phenoxybenzamine, which has no inotropic effect, can be expected to result in an improvement in cardiac output only by decreasing peripheral resistance. It does in fact cause much less of a positive response, suggesting that myocardial depression may be a factor.

While the precise mechanism of action of these pharmacological agents under these specific circumstances is not known, it is apparent that phenoxybenzamine, Isoproterenol and hydralazine all decrease peripheral resistance. In addition, Isoproterenol and hydralazine have an inotropic effect which in the case of Isoproterenol is directly on the myocardium. It is not known whether hydralazine works directly on the myocardium or through a neural center. In contrast to Isoproterenol which works as a beta adrenergic stimulant to dilate the resistance vessels, hydralazine works directly on the smooth muscle of both the resistance and capacitance vessels.[1] Whatever the precise mechanisms, the hydralazine under the circumstances and in the dosage presented here appears superior. It may well be that an increased dose of Isoproterenol would result in findings similar to those presented for hydralazine. However, there are some dangers in increasing the dosage of the former drug.

The use of "excess lactate" (XSL) as a measure of anaerobic metabolism and thus an index of the tissue hypoxia has been popularized by Huckabee and others.[9,22,23,24] While the use of such determinations is disputed by some,[34] the controversy involves primarily the question of whether anaerobiosis may occur without the appearance of excess lactate. This does not concern us in this study, however, as it is ordinarily accepted that excess lactate does not occur in the absence of anaerobiosis. If this is true, the determination of excess lactate when it exists should be a rough index of at least the minimal degree of anaerobiosis, realizing that more may be occurring than is reflected in the excess lactate.

If in turn anaerobiosis is a measure of adequacy of peripheral circulation, then the measurement of excess lactate should be a reflection of this peripheral tissue circulation.[19] In addition, calculation of oxygen extraction as a measure of anaerobic tissue metabolism should also be a measure of the adequacy of peripheral circulation and should inversely correlate with excess lactate determinations.[7,11]

If one assumes that the foregoing relationship between excess lactate, oxygen extraction and peripheral circulation is correct, then the data obtained in this study becomes of greater significance. The use of fluid volume replacement alone results in increasing improvement in oxygen extraction in proportion to the volume of fluid administered. This becomes in excess of the baseline preburn values with the larger fluid volumes. If an oxygen debt had been incurred during the first hour after burning, it would be necessary to increase oxygen extraction above the preburn baseline values in order to make up for this deficit. It would thus appear that with fluid volume replacement alone an early repair of such an oxygen deficit would require the administration of dangerously large volumes of fluid.

Addition of hydralazine to the fluid replacement therapy results in mild improvement with the smaller volumes of fluid and a marked and rapid rise in oxygen extraction above the baseline values even with the moderate volumes. In these instances, the debt could be readily reversed. However, when one observes the effect on oxygen extraction by the addition of the phenoxybenzamine to the fluid resuscitation regimen, it is not until extremely large volumes of fluid replacements

are accomplished (6 + 19) that there is any significant improvement, and this does not appear adequate to overcome any oxygen debt in this early postburn period.

The inverse relationship between oxygen extraction and excess lactate[29] determination appears at least roughly present in these determinations. In the smaller fluid volume replacements, even with the drug additives, improvement in oxygen extraction does not appear to be enough to overcome the initial oxygen debt. Conversely, in these instances the excess lactate levels remain elevated with no tendency to drop significantly during the period of observation. As oxygen extraction improves to a sustained level well above baseline, the excess lactate determinations tend to decrease towards normal. Such a situation obtains in all cases except that in which phenoxybenzamine has been utilized as the pharmacologic agent. Under the latter circumstances, oxygen extraction does not appear adequate to overcome the initia oxygen debt. While arterial excess lactate in this larger volume replacement group does appear to return toward the normal baseline, the venous excess lactate, which is drawn from the inferior vena cava draining the area burned, remains elevated in all groups to a greater degree than the arterial.

Since oxygen extraction in these instances is calculated as a product of cardiac output, hematocrit and A-V oxygen saturation, the lowering of the hematocrit due to splenic engorgement subsequent to the administration of phenoxybenzamine might be a significant factor in decreasing the overall oxygen extraction value in that group. However, correction for this by substituting the hematocrit value of the hydralazine animals in the formula for the determination of oxygen extraction with phenoxybenzamine results in only a maximum increase in oxygen extraction of 10 per cent. This improvement would not be enough to make any significant improvement in the degree of oxygen extraction in the animals receiving phenoxybenzamine indicating RBC sequestration as being of little significance in this regard.

The persistent deficit in oxygen extraction, the sustained elevation of venous excess lactate values, and the gradual decline in arterial excess lactate values are possibly explained on the basis of an arteriovenous shunt subsequent to burning which is not reversed by the administration of phenoxybenzamine. Such a shunt mechanism would decrease both the A-V oxygen difference at the two ends of the capillary

bed as well as the pressure differential between inflow and outflow points. Under these circumstances, flow through the peripheral capillary bed would be diminished and explain the persistent elevation of the venous excess lactate levels. In addition, a decreased A-V oxygen difference would be reflected in a calculated decrease in total oxygen extraction and yet the shunt mechanism with flow through it increased by large vessel dilatation would provide for an improvement in cardiac output as measured centrally.

That such a shunting mechanism exists is strongly suggested by recent investigations of the vascular response to sympathetic nerve stimulation or norepinephrine infusion.[2] This shows that with nerve stimulation arterial and venous constriction and arteriolar dilatation occurs. With norepinephrine, arteriolar constriction and some venular constriction occurs, particularly in peripheral tissues. In both instances, there is a shunting of blood from the peripheral tissue to the deep circulation of the muscles. In the immediate postburn period, both sympathetic stimulation and norepinephrine release occur. Thus, it would be logical to expect shunting postburn.

If phenoxybenzamine were capable of dilatation of the entire peripheral vascular tree, one would expect a clear improvement in both peripheral flow and oxygen extraction. However, the drug is effective only as a dilator of large vessels, with the arterioles unaffected.[25] Such action leaves the arteriolar constrictor effect of norepinephrine intact and the shunting to deeper tissues continues. The large vessel dilatation would also increase rate of flow through the shunt and improve cardiac output without benefit to peripheral tissues.

If the foregoing is correct, phenoxybenzamine while improving somewhat the circulatory hemodynamics in respect to cardiac output perpetuates the deficit in peripheral circulation which exists in the immediate postburn period.

Hydralazine, acting directly on smooth muscle, could overcome the vasoconstrictor effect of both nerve stimulation and norepinephrine infusion, thus reversing the shunting effect and improving peripheral flow as well as cardiac output. It is possible that Isoproterenol may act in a similar manner.

It is of interest that hydralazine, while effectively dilating the peripheral vascular tree, does not interfere with the contraction of the spleen, an act accomplished by smooth muscle also. On the contrary, phenoxybenzamine administration results in a striking engorgement of the spleen, as if it were completely flaccid.

Summary

Dogs subjected to a 50 per cent scald burn were studied in an attempt to delineate the response to various fluid regimens and pharmacologic agents in the period 6-36 hours postburn.

Following burning,cardiac output decreased rapidly to 50 to 60 per cent of preburn levels and by one hour was 30 to 40 per cent of initial values. Without therapy, a gradual slight decline continued. With increasing volumes of fluid administration, output returned toward preburn levels. With addition of pharmacologic agents, further improvement was slight with phenoxybenzamine, moderate with Isoproterenol and marked with hydralazine. Steroids showed no beneficial effect. Salt water alone as a resuscitating agent had little effect in the volumes used and was greatly inferior to colloid.

Increases in early resuscitation volumes above 10 ml/Kg/hr and more than 5 ml/Kg/hr of colloid offered no significant advantage. This was particularly true when hydralazine was added as 0.5 mgm/Kg q 2h.

Results indicate the myocardium is capable of a good response to a fluid load but inotropic agents increase the response. Peripheral flow is most effectively increased with hydralazine, less so with Isoproterenol and least of all by phenoxylbenzamine. An explanation for this is offered.

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Publications

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(U) Approach - Catheterization of large artery, small artery, small vein and large vein, and measurement of point pressure changes, and change in pressure between above vessels following burn and following administration of various drugs.

25. (U) Progress (Jul 65 - Jun 66) - Results demonstrate marked increase in small vein pressures following burns. Greatest increase in resistance between small and large veins. Action of Hydralazine appears to be on small vessel segment. Action of Dibenzyline appears to be on larger arteries. Only denervation completely abolishes response.

Probable great increase in mean capillary pressure which may explain one mechanism of edema formation.

26.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501β71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: STUDY OF SEGMENTAL RESISTANCE CHANGES DURING BURN SHOCK

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Michael P. Kaye, Captain, MC
Arthur D. Mason, Jr., M.D.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288 (R1)

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Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: STUDY OF SEGMENTAL RESISTANCE CHANGES DURING BURN SHOCK

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Michael P. Kaye, Captain, MC
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6. Reports Control Symbol: RCS-MEDDH-288 (R1)

A study of small blood vessel pressures was undertaken in extremities subjected to scald injury to ascertain the site of changes in vascular resistance.

Results demonstrate a marked increase in small vein pressures following burns. The greatest increase in vascular resistance is noted in the small-to-large vein segment. Hydralazine appears to act primarily on the small vessel segment while phenoxybenzamine acts primarily on the larger arteries. Only denervation completely abolishes the veno-pressor response.

(1) Small vessel pressure
(2) Peripheral resistance
(3) Venule pressure

STUDY OF SEGMENTAL RESISTANCE CHANGES DURING BURN SHOCK

In an attempt to describe the mechanisms accounting for the greatly depressed cardiac output observed in untreated burned dogs, Michie[1] hypothesized that there was a decreased venous return and an increase in peripheral resistance. He also hypothesized, since the administration of Hydralazine plus a minimal fluid replacement restored the cardiac output to preburn levels, that the action of Hydralazine was primarily that of peripheral vasodilatation. Utilizing a technique, described by Haddy,[2] in which it was possible to measure pressures in the various vascular segments, an attempt was made to determine the site of vasoconstriction which occurs in the burned animal.

Methods

All studies were performed on mongrel dogs under light pentobarbital anesthesia. A superficial dorsal metatarsal vein was exposed in the paw and a PE-10 or a Teflon catheter, 0.6 mm OD, was inserted upstream through the small vein for a distance of 2-3 cm in the paw. A small superficial artery supplying the paw was similarly catheterized. After catheters were tied in place, blood could be aspirated and saline freely infused through all catheters, indicating adequate collateral circulation. The femoral artery and femoral vein were catheterized with polyethylene tubing placed through branches in these vessels. Burns were inflicted to the extremity by immersion in boiling water for 12 seconds. All drugs were administered by catheters placed in the jugular veins of these animals. Hydralazine, 0.5 mg/kg, was administered one hour postburn. Results referred to as post-Hydralazine were recorded 15-20 minutes following administration of the drug. Phenoxybenzamine (Dibenzyline) was administered in doses of 1 mg/kg or 5 mg/kg one hour prior to burning. Denervations were accomplished by sectioning of the sciatic nerve high in the leg.

Results

The first two tables demonstrate the responses obtained

in the burned as opposed to the unburned control extremity (Tables 1 and 2). Following burning, the pressure gradient from large to small artery of the injury extremity increased, the small artery - small vein gradient decreased and the gradient between small vein and large vein is markedly increased. In the unburned extremity, there is a general depression of pressure at all points measured. This change is compatible with decreased flow through the unburned area. Hydralazine appeared to reduce all pressures on the arterial side of the circulation while having only a small effect on the venopressor response of the burned extremity. Vasodilatation and increased flow are suggested in the control extremity by the decreased arterial and increased venous pressures.

The animals pretreated with phenoxybenzamine were so treated in an attempt to abolish the vasoconstrictor responses due to circulatory catecholamines and possibly at high doses to inhibit any neurogenically mediated constriction. As can be seen in Table 3, there is actually a decrease in large artery pressure following burn but an increase in both small artery and small vein pressure. Again, if mean capillary pressure is calculated as the mean of small artery and small vein pressures, then there is a marked increase in this pressure. The gradient from small vein to large vein is greatly increased.

An attempt was made to abolish this venopressor response by denervation. Since it is known that the paw is innervated primarily through the sciatic nerve, it was decided to section this nerve rather than to attempt to acutely denervate the entire extremity. Consequently, only pressures in the small arteries and small veins were recorded in this experiment. Following denervation, small artery pressure is reduced and small vein pressure is returned to control levels (Table 4). Although the amount of data recorded following the administration of atropine and propanolol is not significant, it was evident from the few experiments performed that neither atropine nor propanolol had any significant effect on the pressures recorded in the small vessels. In order to eliminate the possibility of this pressure change being due to

Table 1. Pressure (mmHg) Burned Extremity

	Preburn	Postburn	Post-Hydralazine Postburn
Large Artery	131	134	118
Small Artery	38	34	23
Small Vein	8.8	20.9	16.3
Large Vein	3.5	1.9	3.8

Table 2. Pressure (mmHg) Unburned Extremity

	Preburn	Postburn	Post-Hydralazine Postburn
Large Artery	140	133	110
Small Artery	35	34	20
Small Vein	8.6	4.4	5.9
Large Vein	3.5	2.4	3.8

Table 3. Pressure (mmHg) Burned Extremity
Dibenzyline Treated

	Pre-Treatment Control	Post-Treatment Preburn	Post-Treatment Postburn
Large Artery	130	95	82
Small Artery	38	31	34
Small Vein	8.8	5.6	17.6
Large Vein	3.5	1.6	1.2

Table 4. Pressure (mmHg) Denervated Burned Extremity

	Preburn	Postburn	Postburn Denervation
Small Artery	55	56	47
Small Vein	16	40	15

muscle contractions, a few animals were studied in which the muscle contractions were blocked by the administration of curare. Results in these animals were similar to those in the non-curarized animal. A series of four animals was examined in which small muscular arteries and small muscular veins were studied along with the cutaneous small veins in situations similar to the above. Following burning, there was a small decrease in muscle arterial pressure, which decreased further with the administration of Hydralazine (Table 5). Small venous pressure increased, but the magnitude of increase was not nearly that seen in the cutaneous small veins. The adminstration of Hydralazine was without effect on the muscle small veins. Again, in all these instances, the cutaneous small venous pressure was markedly increased.

Interpretation of the above results is extremely difficult because there was no control of flow to the studied extremity. However, since the circulation to the dog's hindpaw is known to be primarily distributed to skin and associated structures, the changes seen in the small vessels in these experiments reflect the changes seen in the skin of the burned extremity. In light of the recent paper by Abboud and Eckstein,[3] the results of pressure studies in our experiments become more significant. Quite similar to the changes seen in Abboud's study, under sympathetic stimulation, there was a marked constriction of the venous segments whereas small vessel segment usually dilated. It is believed that due to arterial constriction there occurred a shunt of blood from the skin into the muscle. The results following drug administration suggest that Dibenzyline acts primarily on the large arterial segment with no effect on the venous segment. Hydralazine, which is known to act directly on smooth muscle, appears to have its effect primarily in the small vessel segment in our experiments.

In order that the studies may become significant as far as alterations in blood flow through the various tissues in burned extremities are concerned, it is necessary that they be repeated using more refined techniques, such as described by Abboud and Eckstein, in which the circulation through the extremity is one of controlled flow.[3]

Table 5. Pressure (mmHg) Burned Extremity

	Preburn	Postburn	Postburn Post-Hydralazine
Muscle Small Artery	97	94.5	74.5
Muscle Small Vein	8.07	12.4	12.6
Cutaneous Small Vein	15.07	74.2	62.5

Furthermore, it would be necessary to control separately the flow through muscle and the flow through skin under our experimental conditions. It is quite possible that under these conditions the mechanism of edema formation might be explained, since there appears to be, even in our experiments, evidence to indicate significant elevation of the mean capillary pressures. Should this be the case, it is quite possible that by abolishing the venopressor response, and hence the marked elevation of the mean capillary pressure, that some part of the mechanism for the formation of edema might be abolished.

References

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Publications and/or presentations

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 0A6977	CSCRD - 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RES'ARE
30 06 66	D. CHANGE 27 08 65	U / U	NA	NL	A. WORK UNIT.

10a. CURRE.. NUMBER/CODE: 6114501i 3A014501B71R 01 162

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) Metabolic Studies of Pseudomonas aeruginosa Infection (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 64	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 65	2	14
		CURRENT FY 66	2	20

19. GOVT LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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Mason, A.D., Jr, M.D.
TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Endotoxin, carbohydrate metabolism, Pseudomonas aeruginosa

24. (U) Tech Objective - Since infection by gram negative organisms is a major problem in burn therapy, work is in progress in this laboratory to elucidate the mechanism by which death occurs in experimental Pseudomonas infection.

25. (U) Approach - Reports in the literature indicate that the carbohydrate stores of animals suffering from endotoxemia are depleted. Our previous work has shown a similar carbohydrate depletion occurs in Pseudomonas infected rats. There is evidence that the cause of this depletion is a decreated rate of gluconeogenesis. The liver enzymes responsible for synthesizing glucose from protein and glycolytic pathway intermediates may be impaired. The following work expands and confirms these observations.

26. (U) Progress (Jul65-Jun 66) - The capacity of surviving liver slices to produce glucose from 2-C^{14} pyruvic acid in vitro was studied. Isolation and determination of the specific activity of glucose in the media surrounding liver slices after a 2 hour incubation with 2-C^{14} pyruvic acid was employed as the technique for determining the extent of glucogenesis. Liver slices from rats infected with Pseudomonas aeruginosa produced only about 65% the amount of glucose produced by normal liver slices. Glucogenesis in liver slices from rats injected with E. coli endotoxin was also found to be significantly impaired. However, endotoxin added to the media of control liver slices had no effect.

27. COMMUNICATIONS SECURITY	28.	29. DDC CODE	30. AGENCY CODE
a. CLASSIFIED OR CONTROLLED b. NOT RELATED (X)		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. CAT. FUNDS (In thousands)	36.
CAT. 6.1	

DD FORM 1 JAN 66 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: METABOLIC STUDIES OF PSEUDOMONAS AERUGINOSA INFECTION

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Kathryn F. LaNoue, Ph.D.
Arthur D. Mason, Jr., M.D.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: METABOLIC STUDIES OF PSEUDOMONAS AERUGINOSA INFECTION

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Kathryn F. LaNoue, Ph.D.
Arthur D. Mason, Jr., M.D.

6. Reports Control Symbol: RCS-MEDDH-288(R1)

The capacity of surviving liver slices to produce glucose from 2-C^{14}-pyruvic acid in vitro was studied. Isolation and determination of the specific activity of glucose in the media surrounding liver slices after a two-hour incubation with 2-C^{14}-pyruvic acid was employed as the technique for determining the extent of glucogenesis. Liver slices from rats infected with Pseudomonas aeruginosa produced only about 65 per cent of the amount of glucose produced by normal liver slices. Glycogenesis in liver slices from rats injected with E. coli endotoxin was also found to be significantly impaired. However, endotoxin added to the media of control liver slices had no effect.

(1) Endotoxin
(2) Carbohydrate metabolism
(3) Pseudomonas aeruginosa

METABOLIC STUDIES OF PSEUDOMONAS AERUGINOSA INFECTION

We have shown in previous reports,[1,2] that animals suffering from a fatal gram negative infection die with depleted carbohydrate stores. This depletion is manifested by low blood sugar levels and greatly diminished glycogen levels in the liver and muscle. In vivo studies have indicated that the cause of the hypoglycemia is a failure of glucogenesis. The rats studied showed a diminished capacity in vivo to synthesize glucose from such precursors as pyruvic, fumaric and aspartic acids. Since the liver is the chief site of glucogenesis in vitro, studies of rat liver tissue were begun in order to find a more specific metabolic lesion.

Materials and Methods

Male, Sprague-Dawley, albino rats (200 gm - 300 gm) were given unlimited access to food and water until 24 hours before the experiment, when they were deprived of pellets but permitted to drink. They were killed after the 24-hour fast by a blow on the head, followed by exsanguination. The livers were quickly removed and placed in a slightly modified,[3] ice-cold Krebs-Ringer bicarbonate solution. A block of tissue was cut from the liver and sliced with a Stadie-Riggs tissue slicer. The slices (0.4 - 0.5 mm in thickness) were suspended in the Krebs solution and then two were placed in each Warburg vessel. The tissue used in each flask weighed between 100 - 250 mg wet weight and 15 to 40 mg dry weight.

The Warburg vessels each contained 2.5 ml of Krebs Ringer bicarbonate or Krebs Ringer phosphate with added glucose (100 mg per cent) and sodium pyruvate (10 mM). The side arm of the vessel held 0.5 ml of the same solution plus 0.25 µc of 2-C^{14}-pyruvate. The vessels were incubated for 10 minutes and gassed with 100 per cent O_2 or 95 per cent O_2 - 5 per cent CO_2 gas mixture. Then, the solution in the side arm with the 2-C^{14}-pyruvic acid was tipped into the main vessel and the reaction allowed to continue for two hours. Measurements of oxygen utilization were made when the media was Krebs Ringer phosphate.

At the end of the experiment, the tissue slices were quickly removed and placed on copper planchets. These were dried overnight in a vacuum dessicator and weighed the next

day. A two ml aliquot was taken from the reaction vessel, deproteinized with 0.6 ml BaOH followed by 0.4 ml $ZnSO_4$. The protein-free filtrate was first analyzed for glucose by the Nelson technique.[4] Then, a two ml aliquot was removed, and 4 mg of carrier glucose was added together with 48 mg phenylhydrazine hydrochloride and 41 mg of sodium acetate. Phenylglucosazones were prepared by placing the tubes on a boiling water bath for 2-3 hours.[5] The crystals were collected by centrifugation, washed with water and ether, and then recrystallized five times with 30 per cent ethanol. The purified glucosazones were plated on tared copper planchets, weighed and counted in a Nuclear, Chicago Model 183 Scaling Unit. The specific activity of the glucose in the media at the end of the two-hour experiment was calculated from the specific activity of the glucosazone by the formula:

$$\text{media glucose}^* = \text{glucosazone}^* \times \frac{\text{Mw(glucosazone)}}{\text{Mw(glucose)}} \times \frac{\text{mg carrier glucose} + \text{mg media glucose}}{\text{mg media glucose}}$$

The specific activity of the media glucose is multiplied by the total amount of glucose in the flask to obtain the total cpm transferred from pyruvate to glucose.

A blank vessel from which the slices had been omitted was processed with each experiment in order to check on the completeness of separation of the C^{14}-glucose from the 2-C^{14}-pyruvate. In each case the blank vessels produced glucosazone samples only a few (3 - 5) cpm above background (approximately 16 cpm).

The Walker technique[6] was used to produce infected rats. The animals were seeded with Pseudomonas aeruginosa, SRU strain 12-4-4(59), beneath a 20 per cent split-thickness skin graft. The animals were studied on the second day postgraft.

The endotoxin used was lipopolysaccharide W from E. coli 0111:B4, commercially available from Difco Laboratories.

Results

Experiments were carried out in phosphate media in order

that the oxygen uptake of the surviving liver slices could be measured. In this way the general viability of the tissue is determined and can be compared with the more specific process of glucogenesis.

Measurements were made on liver slices from infected rats and liver slices from control rats. One set of experiments using liver slices from control rats was carried out with endotoxin added to the media (0.2 mg/3 ml). The endotoxin was initially placed in the side arm of the vessel and added to the liver slices with the 2-C^{14}-pyruvic acid. The results of these three sets of experiments are shown in Table 1, and a statistical analysis of the results are shown in Table 2.

The media glucose concentration at the start of the experiment was 100 mg per cent. All normal liver slices and most of the infected liver slices caused an increase in this concentration during the two hour course of the experiment. The final glucose concentrations are recorded in Table 1. The amount of glucose required to raise the 3 ml of media to the measured amount is calculated, divided by the dried weight of the liver tissue and recorded in Table 1 under the heading, "Glucose Added to the Media, µmoles/mg". When the glucose concentration was lower than 103 mg per cent, no calculation was made. The amount of glucose added to the media during the course of the experiment represents a combination of the effects of glycogenolysis, glucogenesis, and glucose utilization. Glucogenesis is more accurately measured by determining the cpm of carbon 14 incorporated into the glucose in each flask. This is divided by the total cpm initially added to the flask as 2-C^{14}-pyruvate. The percentage is then divided by the weight of dry tissue and recorded in Table 1. The per cent of total cpm transferred is equal to the per cent of sodium pyruvate in the flask converted to glucose. This value in µmoles is divided by two in order to obtain the µmoles of C^{14}-glucose synthesized.

The values obtained for Q_{O_2} in this set of experiments agree well with literature values and the Q_{O_2}'s of the infected livers do not differ significantly from those of normal livers. The infected livers, however, produced significantly less glucose in two hours than the normal livers. In vivo studies have indicated that glucose production in the whole animal is reduced to about half normal. The liver slices from infected rats appear, from the data in Table 1, to synthesize

Table.1. Evaluation of Glucogenesis and Oxygen Uptake in Rat Liver Slices (Krebs-Ringer-Phosphate)

A. Control Rats

Flask No.	Q_{O_2} µL/hg/mg*	Final Media Glucose Conc. (mg%)	Glucose added to Media µmoles/mg*	C^{14}-glucose Synthesized µmoles/mg*	% Carbon-14 Converted to Glucose %/mg*
1	5.87	117	0.076	0.083	0.554
2	8.22	129	0.167	0.135	0.898
3	7.70	137	0.184	0.095	0.663
4	8.55	126	0.176	0.122	0.813
5	7.87	117	0.133	0.114	0.760
6	7.12	121	0.232	0.138	0.923
7	7.68	128	0.259	0.126	0.845
$\bar{Y}$	7.58			0.116	
S.D.	0.87			0.020	

Table 1. continued

B. Infected Rats

Flask No.	Q_{O_2} µL/hr/mg*	Final Media Glucose Conc. (mg%)	Glucose added to Media µmoles/mg*	C^{14}-glucose Synthesized µmoles/mg*	% Carbon-14 Converted to Glucose %/mg*
1	7.29	112	0.0678	0.069	0.465
2	8.27	100		0.094	0.630
3	8.45	100		0.063	0.422
4	8.39	108	0.0507	0.086	0.573
5	6.10	111	0.102	0.057	0.382
6	6.87	110	0.110	0.067	0.445
7	6.05	103		0.0363	0.243
8	7.05	112	0.0927	0.061	0.407
9	7.12	110	0.0935	0.098	0.645
10	8.16	116	0.144	0.111	0.740
$\bar{Y}$	7.41			0.074	
S.D.	0.97			0.022	

Table 1. continued

Control Rats with Endotoxin in Media (0.2 mg/3 ml)

Flask No.	Q_{O_2} µL/hr/mg*	Final Media Glucose Conc. (mg%)	Glucose added to Media µmoles/mg*	C^{14}-glucose Synthesized µmoles/mg*	% Carbon-14 Converted to Glucose %/mg*
1	7.95	124	0.170	0.142	1224
2	8.53	117	0.142	0.132	1144
3	8.80	150	0.306	0.125	1074
4	9.05	150	0.286	0.125	1076
$\bar{Y}$	8.58			0.131	
S.D.	0.47			0.008	

* mg of liver tissue after drying 24 hours in dessicator.

Table 2. Analysis of Variance for Studies in Krebs-Ringer-Phosphate Group A - Control rats. Group B - Control rats with endotoxin (0.2 mg/3 ml) added to incubation media. Group C - Infected rats.

ANOV	Degrees of Freedom DF	Sum of Squares SS	Mean Square M	F
A/B	1	0.00055	0.00055	1.38 N.S.
A,B/C	1	0.01163	0.01163	28.7***
Error	18	0.007305	0.000405	

glucose at 64.0 per cent of the normal rate. Endotoxin added to the media has no effect.

Since it has been suggested that endotoxin may be a factor in the pathogenesis of gram negative infection, the effects of endotoxemia on glucogenesis are of interest. It is known that endotoxin when injected into an animal may create severe hypoglycemias. It is likely that failure of the liver glucogenic enzymes is the cause.

A sample of E. coli endotoxin was injected intravenously into a series of fasted rats. Amounts of endotoxin varying from 0.1 mg/100 gm body weight to 5 mg/100 gm of body weight were given. Blood sugars were followed for a period of eight hours. Severe hypoglycemias occurred in all rats given over 0.5 mg/100 gm body weight. The hypoglycemias do not occur initially but rather about three hours after injection. The appropriate LD_{50} for the sample of endotoxin was 1 mg/100 gm body weight. Death, when it occurred, was at about four hours after injection, irrespective of dose.

The livers of five groups of animals were studied. The first group were control animals and the second were controls whose liver slices were studied in media containing 0.2 mg endotoxin/3 ml media. The endotoxin in these experiments was added to the main chamber of the Warburg vessel. Therefore, the liver slices were pre-incubated with endotoxin for about 15 minutes before the start of the experiment. The third group was made up of infected rats. A fourth consisted of rats given an intravenous injection of 1 mg/100 gm body weight endotoxin four hours before sacrifice. The last group received 2 mg/100 gm body weight four hours before sacrifice. The results are shown in Table 3. A statistical analysis of the results is shown in Table 4.

There is no statistically significant effect of endotoxin added to the media. The infected and endotoxin treated groups are different from both control groups. The infected rats' liver slices synthesize glucose at 67 per cent of the normal rate, in good agreement with the results in phosphate buffer.

Apparently the 1 mg dose of endotoxin mimics the effect of infection whereas the larger, 2 mg, dose produces a still more extreme reduction in the rate of glycogenesis. All

Table 3. Evaluation of Glucogenesis in Rat Liver Slices
(Krebs-Ringer-Bicarbonate)

A. Control Rats

Flask No.	Final Media Glucose (mg%)	Glucose added to Media µmoles/mg*	C^{14}-glucose Synthesized µmoles/mg*	% Carbon-14 Converted to Glucose %/mg*
1	121	0.318	0.145	0.970
2	138	0.264	0.103	0.692
3	125	0.185	0.122	0.817
4	120	0.183	0.137	0.915
5	127	0.238	0.129	0.862
6	123	0.206	0.113	0.757
7	108	0.081	0.134	0.892
8	116	0.163	0.107	0.718
9	121	0.175	0.121	0.807
10	117	0.191	0.153	1.02
11	113	0.137	0.087	0.582
12	119	0.273	0.137	0.916
13	141	0.278	0.180	1.20
14	125	0.188	0.081	0.54
15	134	0.266	0.099	0.662
16	97		0.104	0.698
17	109	0.11	0.102	0.682
18	106	0.064	0.124	0.830
19	141	0.186	0.138	0.925
20	136	0.142	0.094	0.630

Table 3. continued

Flask No.	Final Media Glucose (mg%)	Glucose added to Media µmoles/mg*	C^{14}-glucose Synthesized µmoles/mg*	% Carbon-14 Converted to Glucose %/mg*
21	140	0.168	0.117	0.782
22	146	0.175	0.119	0.798
23	143	0.346	0.156	1.04
24	147	0.450	0.213	1.42
25	136	0.253	0.149	0.993
26	150	0.295	0.146	0.978
$\bar{Y}$			0.127	
S.D.			0.026	
B. Infected Rats				
1	115	0.0509	0.0765	0.509
2	122	0.0822	0.0885	0.591
3	117	0.0742	0.0695	0.464
4	117	0.0880	0.0825	0.549
5	106	0.0428	0.0720	0.463
6	105	0.0412	0.0840	0.561
7	109	0.0788	0.118	0.792
8	105	0.0475	0.096	0.642
$\bar{Y}$			0.086	
S.D.			0.016	

Table 3. continued

C. Rats Injected with Endotoxin (1 mg/100 gm body wt)

Flask No.	Final Media Glucose (mg%)	Glucose added to Media µmoles/mg*	C^{14}-glucose Synthesized µmoles/mg*	% Carbon-14 Converted to Glucose %/mg*
1	109	0.0552	0.090	0.610
2	102		0.135	0.900
3	91		0.094	0.628
4	107	0.0635	0.097	0.650
5	90		0.0425	0.284
6	96		0.052	0.346
7	103		0.094	0.627
8	103		0.104	0.695
$\bar{Y}$			0.088	

D. Rats Injected with Endotoxin (2 mg/100 gm body wt)

1	90		0.078	0.52
2	88		0.079	0.528
3	91		0.067	0.449
4	105	0.0704	0.055	0.369
5	90		0.0449	0.299
6	100		0.0222	0.148
7	99		0.0446	0.297
8	99		0.0525	0.352
$\bar{Y}$			0.055	

Table 3. continued

E. Control Rats with Endotoxin Added to Media (0.2 mg/3 ml)

Flask No.	Final Media Glucose (mg%)	Glucose added to Media µmoles/mg*	C^{14}-glucose Synthesized µmoles/mg*	% Carbon-14 Converted to Glucose %/mg*
1	107	0.162	0.120	0.80
2	98		0.088	0.585
3	103		0.099	0.660
$\bar{Y}$			0.102	

* mg of liver tissue after drying 24 hours in dessicator.

Table 4. Analysis of Variance for Studies in Krebs-Ringer-Bicarbonate Group A - Control rats. Group B - Control rats plus endotoxin. Group C - Infected rats. Group D - Rats injected with endotoxin (1 mg/100 gm body wt). Group E - Rats injected with endotoxin (2 mg/100 gm body wt).

ANOV	Degrees of Freedom DF	Sum of Squares SS	Mean Square M	F
A,B/C,D,E	1	0.0306	0.0306	45.88***
A/B	1	0.00172	0.00172	2.57 N.S.
C/D,E	1	0.00102	0.00102	1.53 N.S.
D/E	1	0.00440	0.00440	6.60**
Error	48	0.03213	0.00067	

animals in this group were moribund, and it may be that if the infected animals were sacrificed at a similarly moribund stage, their results would have been closer to the 2 mg dosage group.

Discussion

Pseudomonas infection and endotoxemia both appear to reduce the ability of the liver to synthesize glucose from pyruvate. This may be due to any one of a number of factors. Some of the more obvious possibilities are failure in the synthesis of certain key enzymes in the pathway of reverse glycolysis, inhibition of one or all of these enzymes by a factor whose release is mediated by endotoxin or stress, or deficiency of a necessary cofactor. It is obvious from the results shown here that endotoxin itself does not inhibit the glucogenic enzymes.

We plan to assay certain pertinent enzymes,[7] such as glucose-6-phosphatase, fructose-1,6-diphophatase, and phosphopyruvate carboxykinase, under optimal conditions in order to further distinguish between these possibilities.

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2. LaNoue, K., and Mason, A.D., Jr.: U.S. Army Surgical Research Unit Annual Progress Report, 30 June 1965.

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7. Weber, G. (ed): Advances in Enzyme Regulation. Vol. 2. N.Y., The MacMillan Co. 1964, Chapter 1.

Presentations and/or Publications: None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6980	CSCRD-1L

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A" NEW"	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R C1 165

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) Studies of Protein Turnover in Burn Injury (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CMT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)		NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE b. NUMBER TYPE	d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA		PRIOR FY 65	1	6
			CURRENT FY 66	1	8

19. GOVT LAB/INSTALLATION/ACTIVITY
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TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Protein, burn, trauma, turnover

(U) Tech Objective - To determine the cause of the dysproteinemia observed following burn injury and to determine if the more marked dysproteinemia seen in the presence of infection of the burn wound is an effect of infection itself or merely the result of the combination representing a more severe injury. It is hoped that this will aid in understanding similar changes which are observed in the human burn patients.

(U) Approach - Glycine-2-C^{14} is injected into control, burned, and burned infected rats (using a 20% burn or a 20% burn which has been seeded with Pseudomonas aeruginosa) on the 6th day after injury. Blood samples are drawn and the amount of C^{14} incorporated into the serum protein fractions is determined by a combination of paper electrophoresis and liquid scintillation counting technics. The next step will be to inject purified serum protein fractions which are labelled with C^{14} into each of the animal models and measure their rate of disappearance from the blood to obtain turnover rates. The major problem is preparation of purified protein fractions with high specific activity for reinjection.

(U) Progress (Jul 65-May 66) - Preliminary results indicate that both the burned and burned infected animals are capable of synthesizing all five serum protein fractions at rates greater than those of the control animals. Since albumin and gamma globulin concentrations were not increased, this implies that there was a more rapid turnover of these proteins in the injured animals. Turnover studies will be required to confirm this. These studies are being planned.

27. COMMUNICATIONS SECURITY	28.	29. GSD CODE	30. SURVEY CODE
☐ a. CRYPTO OR CRYPTO RELATED ☒ b. NOT CRYPTO RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. FY FUNDS (In thousands)	36.
FY:	

DD FORM 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: STUDIES OF PROTEIN TURNOVER IN BURN INJURY

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Wanda L. Brown, M.S.
J. Wesley Alexander, Captain, MC
Eleanor G. Bowler, Ph.M.
Arthur D. Mason, Jr., M.D.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDCH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: STUDIES OF PROTEIN TURNOVER IN BURN INJURY

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Wanda L. Brown, M.S.
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The incorporation of glycine-2-C^{14} into the serum protein of control, burned, and burned-infected rats was studied utilizing paper electrophoresis for separation of the protein fractions, and liquid scintillation technics for counting. The amount of radioactivity incorporated into all of the serum protein fractions by the burned and burned-infected animals was greater than that incorporated by the control animals. The increased C^{14} incorporation paralleled a corresponding increase in the circulating levels of the alpha-2 and beta globulin. The increased incorporation of C^{14} into the albumin and gamma globulin in the presence of decreased or normal serum concentrations would indicate a more rapid turnover of these proteins in the injured animals. Further studies will be required to confirm this.

(1) Protein
(2) Trauma
(3) Burn
(4) Turnover

STUDIES OF PROTEIN TURNOVER IN BURN INJURY

The serum protein changes which occur in man following burn injury have been well documented by several groups of investigators.[1-3] There is a progressive decrease in the albumin, an increase in the alpha-1 and alpha-2 globulins, and either no change or a slight increase in the beta and gamma globulin concentrations. These changes are not specific for burn injury, but are observed in many kinds of trauma, in malignancy, and in acute infections.[4]

In rats which were burned over 20 per cent of the body surface, serum protein changes similar to those in man occurred except that there was essentially no increase in the alpha-1 globulin concentration.[5] When a similar burn injury was seeded with Pseudomonas aeruginosa immediately after burning, the depression of the albumin, and the elevation of the alpha-2 globulin concentrations in the serum were more marked than those observed in the animals which had received only burn injury.[5]

The decrease in the serum protein concentrations following burn injury have been attributed to loss of protein into or from the burn wound,[6,7] increased catabolism,[8-10] or to decreased synthesis of albumin.[11] The increase in the concentration of the alpha globulin is in part due to the increased synthesis of glycoproteins which occurs after injury.[12]

The availability of a reproducible animal model for burn and burn infection has enabled us to study the C^{14} incorporation into serum protein in an attempt to explain the more marked protein changes which occur in the presence of burn injury plus infection when compared to those seen in burn injury alone. A preliminary report of the results is given.

Methods and Materials

Young, healthy Sprague-Dawley rats weighing 180 to 200 gm were used throughout this experiment. One group was subjected to a full-thickness burn of 20 per cent of the body surface by placing the rats in a fixed-area shield and immersing in water at 100° C for 10 seconds. Another group was given the same burn injury; then the burned area was immediately seeded with 0.1 ml of an 18-hour broth culture of Pseudomonas aeruginosa (SRU 12-4-4-(59) by the technic developed by Walker, et al.[13] The animals were then housed in individual cages

and given Raulston Chow and water ad libitum until they were injected on the sixth day postburn. Control rats were of equivalent size and age.

Twenty microcuries of glycine-2-C^{14} (specific activity 21-22 MC/mM) in 0.5 ml of saline was injected into the tail vein of each rat. Two and one-half hours after the injection the rats were exsanguinated by cardiac puncture. The serum was separated and stored at 4° C.

Total radioactivity in the serum was determined by placing 0.05 ml of serum in one ml of saline in a counting vial. Fourteen ml of Bray's scintillator solution was added and the samples were counted in a Packard Tricarb Liquid Scintillation Counter.

The radioactivity which was incorporated into the protein was determined on a serum sample after passage through a Sephadex G-25 column (1 x 10 cm) which was equilibrated with 0.15 M saline. The fraction from the column containing the protein was collected, and the protein concentration was determined by a biuret procedure.[14] A volume of the eluate containing protein approximating the amount contained in 0.05 ml of the untreated serum was added to enough saline in a counting volume to give a total volume of 1.05 ml. Fourteen ml of Bray's solution was added and the samples counted as before. Background corrections were made by subtracting the counts measured on samples containing 1.05 ml of saline and 14 ml of Bray's solution. The radioactivity of the samples containing only protein-incorporated C^{14} was converted to c.p.m. per ml of serum by using the ratios of the protein concentrations of the treated and untreated serum. A standard solution of C^{14} was then added to all the vials and they were recounted to test for quenching. None was observed in these samples.

To determine the amount of radioactivity in the different protein fractions, the serum fractions were separated by electrophoresis on S and S 2043A mgl paper strips in barbital buffer, pH 8.6, 0.075 ionic strength, at a constant current of 3.5 ma per tank in a Spinco Electrophoresis Apparatus. The use of this higher current was required to accomplish complete separation of the albumin and alpha-1 globulin in rat serum. Papers moistened with buffer were placed inside the ends of the cover of the tanks to prevent drying of the electrophoresis strips during the run due to the heat generated

by the higher current.

Four electrophoresis strips were prepared for each serum sample. Ten microliters of serum were placed on one strip and 20 microliters of serum on each of the other three. After the separation was completed, the strips were dried in an oven at 110-120° C for 30 minutes. The strip with 10 microliters of serum and one of the strips with 20 microliters were stained with alcoholic bromphenol blue by Spinco Procedure B.[15] The 10 microliter strip was scanned in a Spinco Analytrol integrating densitometer and the percentage of protein in each fraction was determined. The concentrations of protein were determined by multiplying the percentages by the total protein concentration of the serum.

After they had been washed to remove unincorporated radioactive compounds, the other two strips from each sample were used to measure the radioactivity of each protein fraction. The strips were rinsed in absolute methanol six minutes to remove residual buffer. They were then transferred to 5% acetic acid for 15 minutes; 0.002% nigrosin in 2% acetic acid for 15 minutes; followed by three 15 minute rinses in 2% acetic acid. The strips were blotted between paper towels and dried in an oven at 110-120° C for 30 minutes. This dilute nigrosin penetrates only the thinner areas of the protein on the strip, but was dark enough to demarcate the margins of the bands without interfering with the scintillation counting as the bromphenol blue did. These strips were aligned with the 20 microliter strips which had been stained with bromphenol blue; the fractions were delineated and then cut apart. The paper containing each fraction was placed in a counting vial and 10 ml of toluene scintillation solution was added. The samples were counted in a Packard Tricarb Liquid Scintillation Counter. Portions of the paper strips containing no radioactivity and which had been treated in the same way were used for background measurement. After correction for background, the counts obtained from the five fractions of each strip were totalled and the percentage of counts present in each fraction was calculated. These results were converted to c.p.m per ml of serum for each fraction by multiplying the percentages by the total protein c.p.m. per ml of serum which had been previously determined. The specific activity of the protein fractions was determined by dividing the c.p.m. per ml by the concentration in mg per ml of protein in each fraction.

An additional calculation was made to correct for differences in weights of the animals. This calculation is:[16]

$$\text{Relative specific activity} = \frac{\text{c.p.m. per 100 mg protein}}{\text{c.p.m. injected per gm wt}}.$$

Results

The mean concentrations of the serum protein fractions in the control, burned, and burned-infected groups are shown in Table 1. The decrease in albumin concentration and the increase in alpha-2 concentration were more marked in the burned-infected animals than in those having burn injury alone. The alpha-1 and beta globulin concentrations in the burned animals were essentially the same as those of the controls while the concentrations of both of these fractions were nearly doubled in the burned infected animals. There was no significant difference between the gamma globulin concentrations of the three groups. These results agree with one observed previously in this laboratory using the same animal model.[5]

The mean relative specific activities of the serum proteins of the three groups are shown in Table 2. The amount of radioactivity incorporated into all of the serum protein fractions by the burned and burned-infected animals was greater than that incorporated by the control animals. The relative specific activity of all of the serum protein fractions except that of the alpha-1 globulin, was higher in the burned-infected animals than in those with burn injury only. Results of a few experiments in which blood samples were drawn during the first hour after glycine-2-C^{14} injection indicate that these differences may be even greater.

The increased C^{14} incorporation into the alpha-2 and beta globulin fractions paralleled a corresponding increase in the circulating levels of these fractions in the serum of the burned and burned-infected rats. However, although the relative specific activities of the alpha-1 globulins were similar in the burned and burned-infected rats, the serum concentration of this fraction in the burned-infected rats was twice that of those which were only burned.

The increased rate of incorporation of C^{14} into the

Table 1. Mean Protein Concentrations in Grams per 100 ml in Serum from Control (C), Burned (B), and Burned-Infected (BI) Rats. Determinations made on the 6th day postburn. Probability values were obtained by analysis of variance (ANOV) between C vs (B + BI) and between B vs BI.

Group	No. in Group	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin
Control	9	5.80	3.54	0.58	0.49	0.70	0.47
Burned	11	5.35	3.09	0.51	0.57	0.74	0.42
Burned-infected	9	5.60	1.42	1.20	1.14	1.45	0.42
ANOV C vs (B + BI)		$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	NS
ANOV B vs BI		$0.05 < p < 0.1$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	NS

Table 2. Relative Specific Activity of Protein Fractions in Serum From Control (C), Burned (B) and Burned-Infected (BI) Rats. Samples were drawn from six days postburn and two and one-half hours after injection of glycine-2-C14. Probability values were obtained by analysis of variance between C vs (B + BI) and B vs BI.

Group	No. in Group	Total Protein	Albumin	Alpha-1 Globulin	Alpha- 2 Globulin	Beta Globulin	Gamma Globulin
Control	9	0.960	0.?10	2.441	2.739	1.628	1.357
Burned	11	1.510	0.592	3.533	3.509	2.462	1.718
Burned-infected	9	3.199	0.765	3.585	5.662	3.260	3.121
ANOV C vs (B + BI)		p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001
ANOV B vs BI		p < 0.001	p < 0.01	NS	p < 0.001	p < 0.01	p < 0.001

albumin and gamma globulin fractions in both injured groups in the presence of normal or decreased concentrations of serum protein probably indicates a more rapid turnover of these fractions. This has been shown to occur in burned humans using disappearance rates of injected I^{131} labelled albumin and gamma globulin.[9,10] Davies also noted increased catabolism of gamma globulin in one of these patients during a period of Pseudomonas septicemia.[10]

Whether the more marked changes in the serum protein concentrations in the animals in which infection was superimposed upon burn injury are caused by the infection, or are merely a result of the combination representing a more severe injury, cannot be determined from these studies. These studies do show that at this point all of the animals are still capable of synthesizing protein at a rapid rate.

We plan to complete the studies of the rate of C^{14} incorporation during the first hour after glycine-2-C^{14} injection at which time the rate of incorporation is maximal, and to study turnover rates of some of the fractions in an attempt to determine if the differences observed in the burned and burned-infected animals are a result of a different mechanism or merely an enhancement of a similar one.

Summary

The incorporation of glycine-2-C^{14} into the serum protein of control, burned, and burned-infected rats was studied utilizing paper electrophoresis for separation of the protein fractions, and liquid scintillation technics for counting. The amount of radioactivity incorporated into all of the serum protein fractions by the burned and burned-infected animals was greater than that incorporated by the control animals. The increased C^{14} incorporation paralleled a corresponding increase in the circulating levels of the alpha-2 and beta globulin. The increased incorporation of C^{14} into the albumin and gamma globulin in the presence of decreased or normal serum concentrations would indicate a more rapid turnover of these proteins in the injured animals. Further studies will be required to confirm this.

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Presentations and/or Publications

None.

RESEARCH AND TECHNOLOGY RESUME

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10e. PRIOR NUMBER CODE: NONE

11. TITLE: (U) Isolation and Identification of Antigenic Materials Associated with Immunity to Infection by Pseudomonas aeruginosa (44)

12. SCIENTIFIC OR TECH AREA	13. START DATE	14. CRIT COMPL DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	09/64	NA	Other | DA

16. PROCURE METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	1	4
		CURRENT FY 66	1	8

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TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Pseudomonas, infection, antigen, immunity, burn injury

24. (U) Tech Objective - This study was designed to investigate possible use of immunotherapy for prevention and treatment of sepsis from Pseudomonas aeruginosa. The first phase of this work was to isolate and identify the infection protective antigen and classify the Pseudomonas aeruginosa organisms into groups to determine whether or not broad spectrum immunization could be accomplished.

25. (U) Approach - Initial work was done with a single strain of Pseudomonas aeruginosa. Various fractions of disrupted bacilli were tested for their antigenicity in experimental animals. Later, saline washings of the bacteria were used, and specific infection protective antigens were isolated by means of chemical and chromatographic means. Immunodiffusion, bacterial agglutination and in vivo protection were used to test the efficiency and purity of antigenic materials.

26. (U) Progress (Jul 65-Jun 66)- A high molecular weight material of low toxicity was obtained which gave immune protection in microgram quantities. A wide degree of cross-antigenicity was demonstrated by agglutination tests, and cross protection with the flap-infection model was demonstrated. Recent studies indicate that a multivalent antigen of very broad spectrum activity can be obtained to immunize animals. Protection by passive administration of specific antibody has also been shown. It is felt the use of this antigenic material will find wide application in prevention of fatal Pseudomonas infection in susceptible individuals, such as those having burns, mucoviscidosis, debilitating illnesses and iatrogenic-induced susceptibility. Technical report entitled "Studies on the Isolation of an Infection-Protective Antigen from Pseudomonas aeruginosa" has been submitted for publication.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
a. b. RELATED			

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

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35. EST. FUNDS (In thousands)	36.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501871R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: ISOLATION AND IDENTIFICATION OF ANTIGENIC MATERIALS ASSOCIATED WITH IMMUNITY TO INFECTION BY PSEUDOMONAS AERUGINOSA

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

J. Wesley Alexander, Captain, MC
Wanda L. Brown, M.S.
Harrel L. Walker, M.S.
Arthur D. Mason, Jr., M.D.
John A. Moncrief, Colonel, MC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: ISOLATION AND IDENTIFICATION OF ANTIGENIC MATERIALS ASSOCIATED WITH IMMUNITY TO INFECTION BY PSEUDOMONAS AERUGINOSA

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: J. Wesley Alexander, Captain, MC
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John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

Invasive infection caused by Pseudomonas aeruginosa continues to be a problem following severe thermal injury and results in an extremely high mortality once septicemia becomes established. Any means for the effective prevention or treatment, therefore, merits consideration. This study was carried out to evaluate the effectiveness of immunization with various fractions from a single strain of Ps. aeruginosa and to determine whether immunization with purified antigens against one strain of Pseudomonas would protect against invasive infection by other strains.

A high molecular weight material of low toxicity was obtained from Pseudomonas strain SRU 12-4-4(59) which gave immune protection in microgram quantities in the standard rat flap model infected with Pseudomonas strain, SRU 12-4-4(59). This material was found to induce protection with a single injection of 12 nanograms per kilogram in mice who were given a subsequent 100 LD_{50} challenge of organisms.

Tests for antigens common to Pseudomonas strain SRU 12-4-4(59) were made with 113 other strains of Pseudomonas, and cross-antigenicity was demonstrated by agglutination tests in 51 of these. Cross-protection was demonstrated with all of the eight

organisms tested which showed cross-reaction by agglutination, and to two of three which did not.

The ease of production of this highly antigenic and effective antigen from a single strain of Pseudomonas gives encouragement that immunotherapy can be used effectively for the prevention and treatment of infection from Pseudomonas aeruginosa.

(1) Pseudomonas
(2) Ps. Infection
(3) Antigen
(4) Immunity
(5) Burn injury

ISOLATION AND IDENTIFICATION OF ANTIGENIC MATERIALS ASSOCIATED WITH IMMUNITY TO INFECTION BY PSEUDOMONAS AERUGINOSA

The present methods of treatment for an established septicemia caused by Pseudomonas aeruginosa are almost without effect on mortality.[5,17,21] Although the incidence of this complication in burned patients has been diminished by the routine use of topical Sulfamylon prophylaxis at this installation,[14] it has not been completely eradicated, and such prophylactic measures cannot be used in other circumstances, such as in certain leukemias, mucoviscidosis, and various diseases where debilitation favors an opportunistic invasion by Pseudomonas.[5] The use of systemic antibiotics for the prevention of Pseudomonas infections in burns has been without benefit.[12] Despite the development of numerous antibacterial compounds and antibiotics, it is remarkable that only a few of these are effective with Pseudomonas, and these few often fail therapeutically in the face of established septicemia.

In recent years there has been interest in the prevention and treatment of Pseudomonas infections by immunologic measures. This interest was fostered to a large extent by the work of Rosenthal and his colleagues [22] at the National Institutes of Health, who demonstrated that experimental infections in mice were susceptible to serotherapy. Independently, a similar observation was made by Fisher and Manning,[7] who also demonstrated that specific antibody was involved. More recently, Feller and Kamei [6] have reported on attempts at the prevention of Pseudomonas septicemia in burned patients with a program of combined active and passive immunization using a heat-killed vaccine made from a single strain of Pseudomonas, but to our knowledge this work has not been documented elsewhere. Other investigators have demonstrated the effectiveness of specific antisera in the prevention and treatment of experimental Pseudomonas infections from the same strain.[18,20] Except for some limited studies,[11] experimental evaluation has not been reported concerning cross-protection between strains.

An extensive experience with experimental Pseudomonas infections has been accrued in this laboratory following the development of an animal model in which progressive Pseudomonas infection occurs which is somewhat comparable to human Pseudomonas burn sepsis. It was felt that this knowledge would aid in a further attempt to characterize the antigens of Pseudomonas aeruginosa which are associated with inducing protection from infection. If adequate protection from Pseudomonas infection

can be obtained by either active or passive immunization, a useful tool will be added to the surgeon's therapeutic armamentarium. This report presents experimental data which indicate that such is possible.

Materials and Methods

Healthy, adult, male Sprague-Dawley (Holtzman strain) rats were used throughout the investigations. Except where indicated, the experimental model described by Walker, Mason and Raulston [25] was used to evaluate the effectiveness of the antigenic materials prepared for protection against invasive infection by Ps. aeruginosa. This "standard flap model" was created by the excision of the skin and attached panniculus carnosis on the dorsal aspect of a rat, representing 20 per cent of the body surface area. The wound was then seeded with approximately 10^8 viable organisms, and the entire flap replaced and held in position to the excised wound edges with Michel clips. This, in effect, created a large area of devitalized tissue which was secondarily infected by a controlled inoculum. Using this model and Pseudomonas strain SRU-12-4-4(59) as the challenging organism, there has been 100 per cent mortality in an experience of approximately 200 nonimmunized animals, the average day of death being on the fourth postoperative day. All surviving animals in the following experiments were observed for a 21-day period before sacrifice since it has been previously found by Walker, et al.,[25] that animals destined to succumb did so before this time. The day of death was recorded, and the average survival time is listed at the top of each bar graph in the figures to follow.

All extracts and antigenic materials used throughout this study were prepared from 18 to 24 hour cultures of a single strain of Ps. aeruginosa SRU-12-4-4(59), which were harvested from surface growth on trypticase soy agar.

Chromatographic separations of antigens or extracts were performed at 4° C on 2.5 x 50 cm columns of G-200 Sephadex equilibrated with 0.15M NaCl. The fractions were eluted with 0.15M NaCl and collected with a Beckman, Model 132, Fraction Collector contained in a mobile refrigeration unit. A drop-counting head was used to determine the sample volume. The ultraviolet absorption of each sample was measured at 280 microns and 260 microns in one cm silica cells in a Beckman DU Spectrophotometer. The approximate protein concentration of each

sample was calculated using an equation derived from the data of Warburg and Christian:[26]

$$\text{Protein concentration (mg/ml)} = 1.55\ D_{280} - 0.76\ D_{260}.$$

An estimation of the purity of the protein was obtained by using the ratio D_{280}/D_{260} and referring to tables for mixtures of protein and nucleic acids.[13] This same method was used for estimating the protein content of the crude preparations. Exact weight measurements were made on the residue of selected samples after dialysis and lyophilization.

Approximate molecular weights of the fractions were obtained by plotting the O.D. vs. the elution volume. The volume at the absorption peak of the fractions was compared to a graph made by running proteins of known molecular weight through the same procedure and plotting elution volume vs. log molecular weight.[3]

Antisera to the Sephadex fraction Part 1 from Experiment 2 (whole bacteria supernate) was prepared by a course of immunization in rabbits, using four weekly intravenous injections of the antigen. The animals were exsanguinated one week after the last injection. These sera were used for immunodiffusion tests for purposes of identification of common antigens and partial quantitation. A microtechnic of double diffusion in agar gel was used similar to that described by Crowle,[4] with modification of the template wells so that greater quantities of reactants could be introduced.

For simplicity, the methods of preparation of the antigenic materials are included in the results.

Results

Experiment 1. A comparison of immunization with live organisms and crude extracts was made with and without Colistin therapy. Seven and one-half grams of organisms (wet weight) were suspended in three volumes of normal saline and frozen in a Hughes press. The cells were then disrupted in the frozen state with the press. Recovered material was centrifuged, and the residue was washed several times with normal saline. After final centrifugation, the volume of the combined supernates was approximately 100 ml; this contained 2.8 mg of nitrogen per ml.

Nine precipitin bands were found by immunodiffusion tests of the crude supernate against antisera specific for the material. Insoluble residue was suspended in three volumes of normal saline, and this material contained 4.2 mg of nitrogen per ml. The experimental animals were divided into four groups.

Group A. Ten animals were immunized with the supernate according to the following dosage schedule. Each rat was given an initial subcutaneous injection of 0.13 ml of antigen mixed with 0.07 ml of complete Freund's adjuvant. At subsequent weekly intervals, each animal was given 0.2, 0.4, 0.8, and 1.0 ml of antigen without adjuvant intraperitoneally, the dose increasing each week.

Group B. One-tenth ml of the suspended residue and 0.1 ml of complete Freund's adjuvant were administered subcutaneously initially to 10 animals. At subsequent weekly intervals, 0.1, 0.2, 0.4, and 0.5 ml of residue was administered intraperitoneally.

Group C. Eleven animals were immunized with live organisms. An initial dose of 0.025 ml of an 18-hour trypticase soy broth culture was given intraperitoneally. The volume was doubled at subsequent weekly intervals for a total of five doses.

Group D. Sixteen animals received no immunization and served as controls.

Each of the four groups was further divided into two subgroups. In Subgroup A1, B1, C1, and D1, the animals were treated with Colistin sulfate, 5 mg/kg/day, given in three divided doses for a period of four days beginning at the time of operation. Subgroups A2, B2, C2, and D2 received no antibiotics. All of the animals were challenged using the standard flap model and Pseudomonas strain SRU-12-4-4(59) five weeks after the initiation of the immunization procedure.

The survival rates are shown in Figure 1. None of the animals in Subgroup A1, A2, B1, C1, or C2 died. Eighty per cent survival was seen in Group B2, with the sole death occurring on the second day. None of the controls, with or without Colistin sulfate therapy, survived and the average survival time was 5.1 days in each subgroup. This experimentally confirms the ineffectiveness of prophylactic antibiotics for Pseudomonas infections.

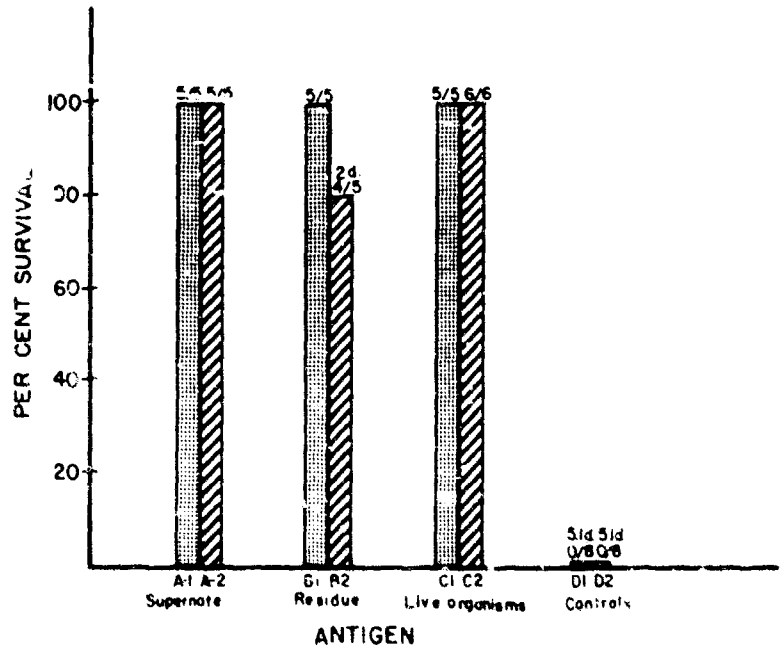

Figure 1. Comparison of immunization with live organisms and crude extracts with and without Colistin therapy.

Experiment 2. In this experiment, antigenic materials of different molecular weight were compared. Supernatant material containing approximately 7.1 mg protein per ml was prepared as described in Experiment 1, and 50 ml were used for separation of the components by column chromotography. Fractions were collected in 10 ml aliquots. Three rather distinct portions were obtained. The first part (50 ml) consisted of the material with a molecular weight greater than 200,000. There was an average protein content of 0.309 mg/ml with 24 per cent appearing as nucleic acid, as estimated by the ultraviolet absorption ratio at 280 and 260 microns.[12] The second part had a protein content ranging from 0.73 to 2.01 mg/ml (average 1.168), with 15.3 per cent appearing as nucleic acid. The third part (90 ml) had a protein content ranging from 1.9 to 5.6 mg/ml (average 3.19), with 17 per cent nucleic acid. The last represented the fraction with the lowest molecular weight compounds.

Twenty-five rats were divided into five groups. Group A was the control group and received no immunization. Groups B, C, D, and E were then immunized on a dosage schedule which consisted of an initial injection of 0.13 ml of antigen mixed with 0.07 ml of complete Freund's adjuvant subcutaneously, with subsequent weekly intraperitoneal injections of the antigen of 0.2 ml, 0.4 ml, and 0.8 ml, respectively. Group B was immunized with Part I from the Sephadex eluate, Group C with Part II, Group D with Part III, and Group E with the whole supernate.

Figure 2 gives the per cent survival in each of these groups, with the average length of survival of those animals which died. All of the animals were challenged with the standard flap model and Pseudomonas strain SRU-12-4-4(59) four weeks after the initiation of the immunization schedule. It is apparent that the best survival occurred with the high molecular weight portion from the Sephadex separation.

Experiment 3. Several other methods of preparation of antigenic materials were tried in this experiment. Since the work of Alms and Bass[2] and Liu[15,16] indicated that the slime layer contained the most important antigenic substances which were associated with protection from infection, simple washings of the organisms were included.

Group A. Five animals were immunized by a single intraperitoneal injection of 0.5 ml of material taken from

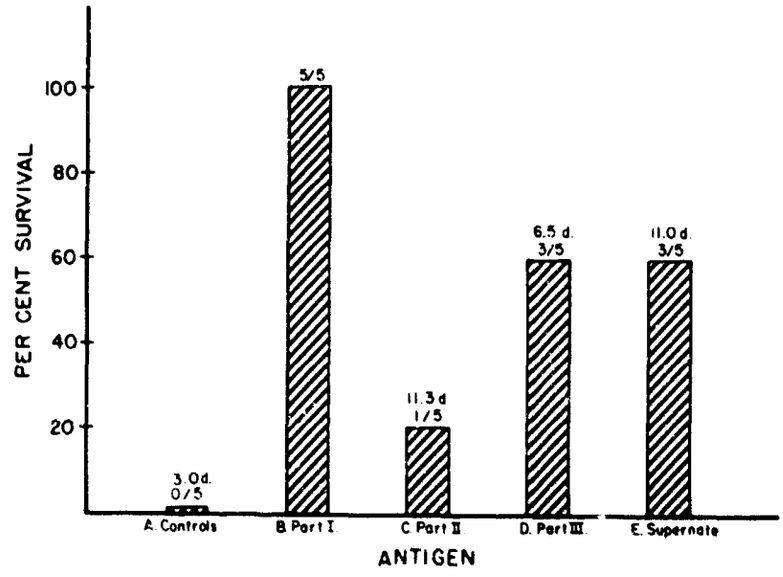

Figure 2. Comparison of antigenic materials separated by molecular weight.

the peak of a second Sephadex G-200 separation of the Part 1 material from the Sephadex separation in Experiment 2.

Group B. Organisms were subjected to four episodes of repeated freezing and thawing. A saline extract was made of this material, and after centrifugation it was sterilized by filtration through a sintered glass filter. The five animals in this group were immunized by a single intraperitoneal injection of 0.5 ml.

Group C. Five animals were immunized intraperitoneally with 0.5 ml of a material prepared by agitating live organisms with 0.15 molar KCl followed by centrifugation and finally filtration through sintered glass for sterilization. This material produced four bands by gel-diffusion test against antisupernate antiserum.

Group D. Five animals were immunized intraperitoneally with 0.5 ml of a saline solution of a 60 per cent ethanol precipitate of the KCl wash from Group C.

Group E. Five animals served as controls without immunization.

All animals were subjected to challenge with the standard flap model infected with Pseudomonas strain SRU-12-4-4(59) 10 days after the single immunization.

Figure 3 shows the results and indicates that full protection is afforded with the freeze-thaw preparation and the KCl wash, with good protection provided by both the Sephadex preparation and the 60 per cent ethanol precipitate of the KCl wash. All of the controls died.

Experiment 4. A comparison of various ethanol precipitation fractions was made. A suspension of live organisms in 0.15 molar KCl was agitated with a Virtis "45" homogenizer, running at the lowest speed setting for 10 minutes. The suspension was centrifuged at 17,000 r.p.m. for 20 minutes, and the supernatant material was fractionated by serial precipitation with absolute ethanol at increasing concentrations beginning with a 40 per cent concentration. The material was then centrifuged and the precipitate dissolved in KCl. By UV reading, the concentration of protein was estimated to be 0.30 mg/ml, with 15.5 per cent appearing as nucleic acid.

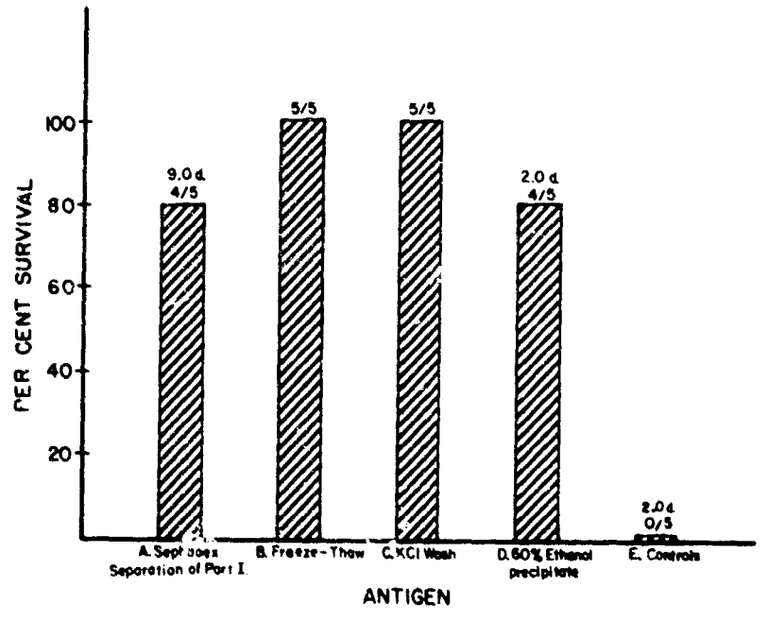

Figure 3. Comparison of several methods for the preparation of antigenic materials.

The concentration of ethanol in the supernate was then increased to 60 per cent, and the precipitate again removed. This precipitate, dissolved in KCl, had a concentration of 0.201 mg/ml, with 5.8 per cent appearing as nucleic acid. After increasing the concentration of ethanol in the supernate to 80 per cent, a heavier flocculant precipitate formed, which, when dissolved in KCl, had a concentration of 0.523 mg/ml, with a nucleic acid content of 4.2 per cent. The original KCl wash contained 2.05 mg protein per ml, with 13 per cent appearing as nucleic acid. Twenty-five animals were divided into five groups, and the first four of these were immunized with a single intraperitoneal dose of 0.5 ml of the antigenic material seven days prior to the time of challenge with the standard flap model which was infected with Pseudomonas strain SRU-12-4-4(59). Group A was immunized with the KCl wash; Group B was immunized with the material from the 40 per cent ethanol fraction; Group C was immunized with the material from the 40 per cent to 60 per cent alcohol fraction; and Group D was immunized with the material from the 60 per cent to 80 per cent alcohol fraction. Group E served as controls and received no immunization.

Four of the animals died of anesthetic complications at the time of operation. The survival of the remaining animals is shown in Figure 4. It is readily apparent that some protection was afforded by the KCl wash, the 40 per cent ethanol precipitate, and the 60 per cent ethanol precipitate. All of the animals immunized with the 60 per cent to 80 per cent ethanol precipitate died, and their average time of survival was not significantly greater than the controls. It was felt that the latter afforded little or no protection against active infection from Pseudomonas. The short interval between immunization and challenge was felt to contribute to the deaths which occurred in Groups A, B, and C.

Experiment 5. In this experiment, an attempt was made to determine the stability of the antigenic material which induced protection. A 60 per cent ethanol precipitate was made of a sodium chloride washing of Pseudomonas strain SRU-12-4-4(59), and this was dissolved in normal saline and sterilized by sintered glass filtration. Twenty animals were divided into four groups. All animals were immunized with 0.5 ml of the material given intraperitoneally 10 days prior to challenge with the standard flap model and Pseudomonas strain SRU-12-4-4(59).

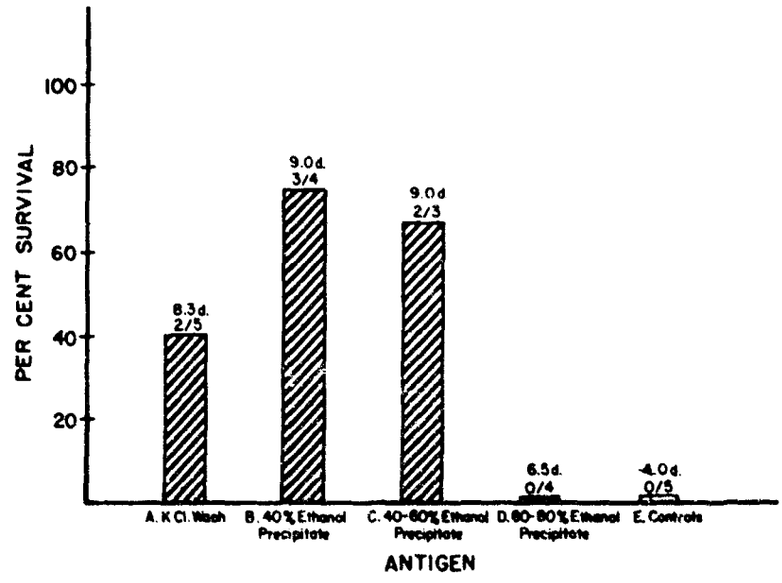

Figure 4. Comparison of serial ethanol fractions with the KCl wash.

Group A. These animals received the untreated antigen.

Group B. A portion of the antigen which had been frozen and thawed three times was given.

Group C. These animals were immunized with a portion of the material which had been heated at 56° C for one hour.

Group D. This group was immunized with the same material which had been heated for one hour at 100° C.

The results are shown in Figure 5. Good protection was found in Groups B and C, where 50 per cent and 80 per cent of the animals survived, respectively; and, of those animals that died, survival was considerably prolonged. Prolonged heating of the material at a higher temperature resulted in incomplete loss of activity indicating a fairly high degree of stability.

Experiment 6. Preparation of the antigenic material by means utilized for polysaccharides was done in this experiment. A 60 per cent ethanol precipitate of a sodium chloride wash was prepared as described above, and this was dissolved in normal saline. One per cent cetyltrimethylammonium bromide was added until a flocculant precipitate was obtained. The complex was partly dissociated by use of the critical solubility temperature after dissolving the precipitate in 1.5 molar KCl, as described by Scott.[23] After centrifugation, two volumes of 95 per cent ethanol saturated with KCl was added to the supernate to precipitate the polyanion. The final precipitate was dissolved in 30 ml of distilled water and will be referred to as the purified CTAB extract hereafter. Serial ethanol cuts were made from this purified CTAB extract, using 30, 40, 50, and 60 per cent ethanol concentrations, respectively. Between each cut, the precipitate was removed by centrifugation before additional alcohol was added, and and then it was dissolved in a volume of 15 ml of 0.15 molar KCl. Approximately four hours at 4° C were allowed for precipitation with each of the steps. Thirty animals were divided into six groups to determine the effectiveness of the antigen prepared by this method. Group A was immunized with the CTAB extract, Group B with the 30 per cent cut, Group C with the 40 per cent cut, Group D with the 50 per cent cut, and Group E with the 60 per cent cut. Group F served as controls. All test animals were immunized with a single intraperitoneal

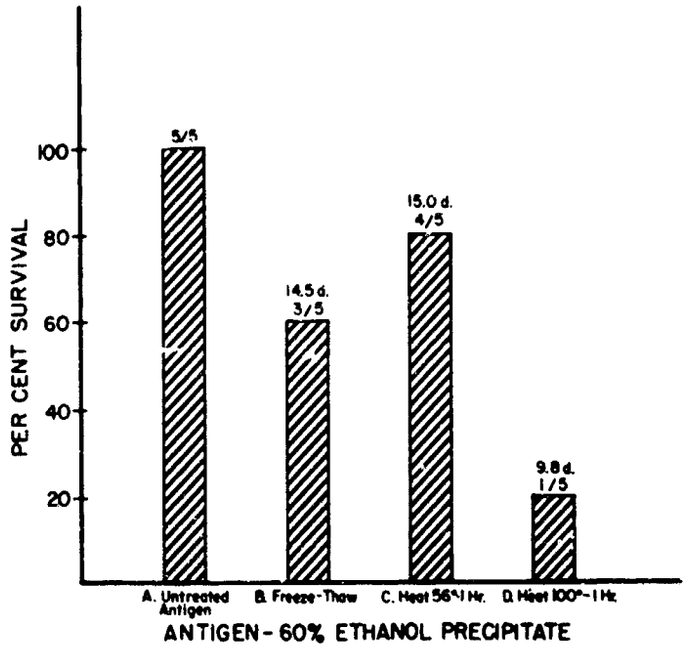

Figure 5. Determination of antigen stability.

injection of 0.5 ml of the respective material given 12 days prior to a challenge with the standard flap model which was infected with Pseudomonas strain SRU 12-4-4(59).

The results are shown in Figure 6. A rather disappointing amount of protection was afforded by the purified CTAB extract, but, surprisingly, serial cuts of this material gave better protection. The best protection was afforded by the 50 per cent to 60 per cent ethanol fraction. Dr. M. W. Fisher of Parke, Davis & Co., tested this fraction in mice and found that a single subcutaneous injection of 0.25 ml of a 10^{-5} dilution (approximately 0.0005 µg) gave protection against a 100 LD_{50} challenge of organisms one week later.

Experiment 7. In this experiment, the effectiveness of antigens separated by chromatographic means was tested. The material obtained by 60 per cent ethanol precipitation of a sodium chloride wash of live Pseudomonas strain SRU-12-4-4(59) organisms was separated chromatographically as described before and collected in 4.2 ml aliquots. Two distinct peaks were obtained by ultraviolet absorption. The first represented materials with an average molecular weight of 180,000, whereas compounds in the second were well below that limit, with an average of 15,000. A 0.5 ml aliquot of material was used to immunize all animals, each with a single intraperitoneal dose given 10 days prior to the challenge with the flap model infected with Pseudomonas strain SRU-12-4-4(59). All groups had five rats each except Group A, which had 10. Group A was immunized with material from the first peak, and Group B from the second. The material used for immunization for Group A contained 369 µg of material per ml by weight. A 1:10 dilution of the material used in Group A which contained approximately 36.9 µg per ml was used to immunize Group C. In Group D, immunization was accomplished with a 1:100 dilution of the material from Peak 1, which contained approximately 3.69 µg protein per ml. In Group E, further dilution to 1:1000 was made. Five animals served as controls in Group F.

The results are shown in Figure 7. Progressive decrease in protection was seen with serial dilutions of the antigen from Peak 1, with full protection afforded by the undiluted material. In Group E, all of the animals died. The average length of survival, however, was prolonged considerably when compared with the controls.

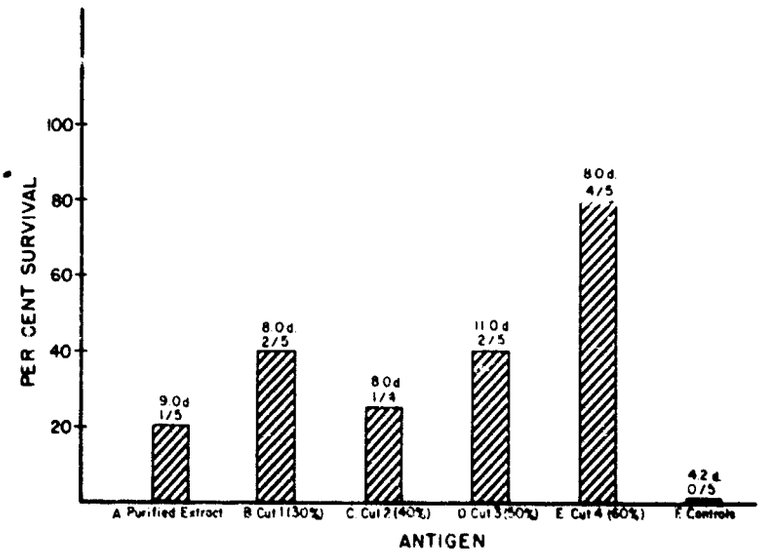

Figure 6. Preparation of the antigenic material by methods used for preparation of polysaccharides.

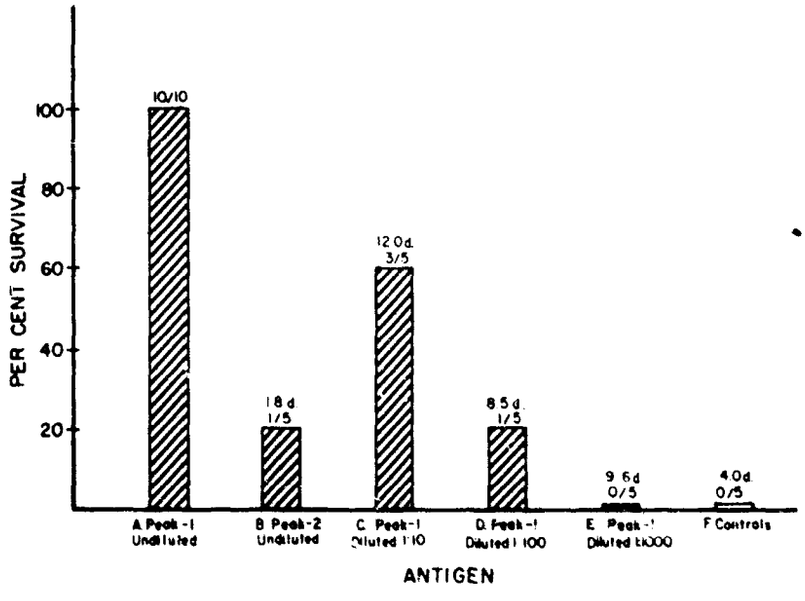

Figure 7. Effectiveness of antigens separated by chromatographic means.

The material from Peak 1 was tested in mice and found to be effective at a 50 per cent protection level against a 100 LD_{50} challenge in a dose of 12 nanograms per kilogram (0.00024 µg/mouse).

Experiment 8. The important question of cross protection was studied in this experiment. Additional antisera were made in two rabbits with a series of weekly intravenous injections of the material present in a single tube at the apex of the first peak from the Sephadex separation of the 60 per cent ethanol precipitate. Double diffusions in agar gel with the antigen against these antisera showed one heavy precipitin band and two faint ones. This antiserum was used to do immunodiffusion studies for the detection of common antigens from saline washes from 46 other strains of Pseudomonas. Bands of common identity were seen in 22 (47.8 per cent) of these (Figure 8). Agglutination tests were done with these and an additional 67 strains of Ps. aeruginosa, using the same antisera and live organisms suspended in saline as the antigen. Of the 113 strains, 51 (45.1 per cent) gave a positive agglutination reaction at a serum dilution of 1:100. When the results of the agglutination tests were compared with those obtained by gel-diffusion, exact confirmation was obtained.

Using the above results as a guide, eight organisms which showed cross identity with Pseudomonas strain SRU-12-4-4(59), and three which did not, were selected. Cross protection was tested in 140 animals which were divided in 11 groups, each with subgroups for test and control. Groups A-H showed cross antigenicity by agglutination and gel-diffusion, whereas Groups I - K did not. All of the test animals were immunized (as indicated in Figure 9) with antigens prepared from Pseudomonas strain SRU-12-4-4(59). None of the controls were immunized. The results are shown in Figure 9. The number of survivors over the total number in each subgroup is shown as a fraction. Evidence of cross protection was found not only in Groups A - H, but also in Groups I and J where it was not expected. There was no evidence of cross protection with Pseudomonas VA-134. In Groups A and B, it was apparent that an increase in survival followed purification of the antigen used for immunization. There was an overall survival of 54 per cent in the test group compared to a 10 per cent survival in the nonimmunized control group.

Legend for Figure 8A

Immunodiffusion tests showing reactions of cross identity with Pseudomonas strain SRU-12-4-4(59).

Center well - freeze-thaw preparation of Pseudomonas strain SRU-12-4-4(59).
Wells B & E - antiserum to Pseudomonas strain SRU-12-4-4(59).
Well A - freeze-thaw preparation of Pseudomonas strain SRU-3-9-4(64).
Well C - freeze-thaw preparation of Pseudomonas strain SRU-8-28-3(63).
Well D - freeze-thaw preparation of Pseudomonas strain SRU-7-29-2(63).
Well F - freeze-thaw preparation of Pseudomonas strain Whittington-11-15-2(60).

All four organisms tested show cross identity with Pseudomonas strain SRU-12-4-4(59). Small pieces of agar adjacent to Wells E & F were removed with the template.

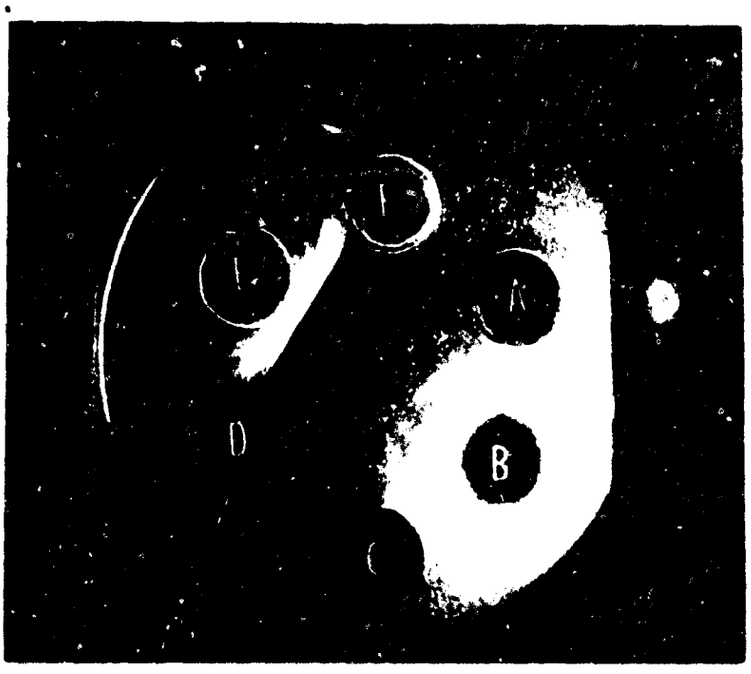

Figure 8A

Legend for Figure 8B.

Immunodiffusion tests showing reactions of cross identity with *Pseudomonas* strain SRU-12-4-4(59).

Center well - freeze-thaw preparation of *Pseudomonas* strain SRU-12-4-4(59).
Wells B & E - antiserum to *Pseudomonas* strain SRU-12-4-4(59).
Well A - saline wash of *Pseudomonas* strain UCF-274.
Well C - saline wash of *Pseudomonas* strain UCF-501.
Well D - saline wash of *Pseudomonas* strain UC-1009.
Well F - saline wash of *Pseudomonas* strain UC-7710.

Only *Pseudomonas* strain UCF-501 shows cross identity with *Pseudomonas* strain SRU-12-4-4(59).

Figure 8B

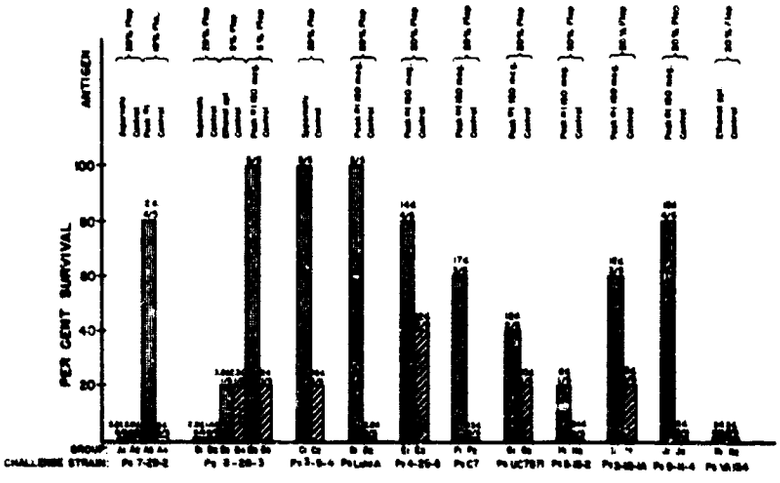

Figure 9. Cross protection studies with animals immunized with antigens prepared from Pseudomonas strain SRU-12-4-4(59) and challenged with 11 different strains of Pseudomonas.

Discussion

Several studies have been conducted which demonstrate the effectiveness of active immunization with live or killed Ps. aeruginosa in the prevention of infection by the same strain. Walker, Mason, and Raulston [25] show that rats immunized with multiple injections of live Pseudomonas organisms were protected against infection with a rather severe challenge using their standard flap model. Merthiolate-killed organisms in the same dosage failed to elicit protective antibodies. Millican and Rust [20] were also able to effectively immunize against Ps. aeruginosa using live organisms in albino rabbits, but the mortality for the series of immunizations was almost 50 per cent. Fox and Lowbury [8] have used formol-killed organisms for immunization of rabbits; and McMeel, Wood, and Senterfit [19] were able to use heat-killed organisms for active immunization for the prevention of infection of the cornea from the same strain of Ps. aeruginosa in rabbits. Liu, et al.,[15,16] in two reports, tried to demonstrate the roles of various fractions of Ps. aeruginosa in its virulence and in pathogenesis. They concluded that extracellular enzymes produced by the organisms were species specific, but that the slime layer was type specific. Homolysins were nonantigenic. They felt that the ability to produce extracellular toxins was important to virulence, but that the slime layer was important in the production of antibodies which prevented invasive infection. They further concluded that antibody to the slime layer was responsible for both agglutination and protection against invasive infection by the organism, although they felt that the slime layer was poorly antigenic.

The study reported herein represents an attempt to isolate and identify the antigenic substance or substances from a single strain of Ps. aeruginosa which are responsible for the elicitation of an immune response protective against active invasive infection. Since a great deal of experience had been accrued in this laboratory with infection in the standard flap model in the rat with Pseudomonas strain SRU-12-4-4(59), this organism was selected for study. By avoiding harsh chemical separations, it was felt that destruction of possible labile antigenic materials or moieties could be avoided. The initial experiments were done by disrupting intact live organism in a frozen state with a Hughes press with subsequent extraction with normal saline, maintaining the materials at a cold temperature. Immunization of rats with the material

obtained gave good protection against invasive infection which was far superior to that which could be obtained with antibiotic therapy. Separation of this material chromatographically with Sephadex G-200 and immunization of animals with the fractions therefrom indicated that the material which elicited the protective antibody was of a molecular weight approximating 200,000. It had been shown by Alms and Bass [2] that a protective antigen could be obtained simply by using a saline washing of cells. Following this lead, an antigenic material was obtained by both saline wash and subsequent alcohol precipitation, which was found to be an effective immunizing agent. Gel-diffusion studies showed that the same substances present in the dissolved ethanol precipitant were present in the extract from the whole organism, and, furthermore, several nonessential antigenic materials had been removed. It was felt that the purification of the antigen to one or two specific substances was most desirable because of the problem of competition of antigens, as discussed by Adler. [1] Further separation of this material by Sephadex chromatography again showed that the active substance or substances were in the high molecular weight fraction, and that further purification had been obtained. The partially purified antigen obtained by chromatographic separation of the 60 per cent ethanol precipitate was found to be highly active against a severe challenge with Ps. aeruginosa in rats using the standard flap model, even after a single injection of a few micrograms. A single dose as low as 12 nanograms per kilogram proved to be protective in mice. The antigen was found to be fairly stable, although some activity was lost both by heating and repeated freezing and thawing. The ability to extract it with cetyltrimethylammonium bromide indicated that it was a large molecular weight polysaccharide material. It is possible that peptide linkages are present in the antigen since proteolysis with Pronase will destroy its ability to induce the formation of protective antibody.

Antisera prepared against the purified antigen was shown to be cross-reactive with several strains of Ps. aeruginosa, both by immunodiffusion studies of saline washes from intact organisms and by agglutination of live organisms in saline. This indicates that it may be possible to group Pseudomonas into several distinct categories for purposes of classification. Verder and Evans [24] and Gould and McLeod [10] were able

to obtain a certain degree of classification with agglutinating sera against Ps. aeruginosa using killed organisms as antigen. Hopefully, antisera to a highly purified antigen may be even more effective in distinct classification into agglutinating groups. It is encouraging that some evidence of cross-protection was demonstable in all eight strains which showed both cross-reactivity by agglutination and gel-diffusion tests, and also in two strains which did not. In one strain, where no demonstrable cross-reaction was obtained by the tests, there was no cross protection obtained in rats immunized against Pseudomonas strain SRU-12-4-4(59).

These studies suggest that an effective polyvalent and nontoxic antigen can be prepared for the prevention of infection from Ps. aeruginosa. Early studies with passive protection, which are being extended, indicate that purified gamma globulin and hyperimmune sera may offer an adjunctive means not only for the prevention of infection, but also for the active treatment of established infection.

In most instances, the small number of animals used for each group within this series has been found to be sufficient for evaluation of antigenic materials since there is a 100 per cent mortality in the standard flap model infected with Pseudomonas strain SRU-12-4-4(59). Any survival, therefore, is significant. Prolongation of life may be of significance, but it would be unwise to draw conclusions using this as the sole basis for evaluation of the effectiveness of antigenic materials when so few animals are used. Total survival of five animals, as compared to death in five controls, is highly significant.

Summary

Invasive infection caused by Ps. aeruginosa continues to be a problem following severe thermal injury and results in an extremely high mortality once septicemia becomes established. Any means for the effective prevention or treatment, therefore, merits consideration. This study was carried out to evaluate the effectiveness of immunization with various fractions from a single strain of Ps. aeruginosa and to determine whether immunization with purified antigens against one strain of Pseudomonas would protect against invasive infection by other strains.

A high molecular weight material of low toxicity was obtained from Pseudomonas strain SRU-12-4-4(59) which gave immune protection in microgram quantities in the standard rat flap model infected with Pseudomonas strain SRU-12-4-4(59). This material was found to induce protection with a single injection of 12 nanograms per kilogram in mice who were given a subsequent 100 LD_{50} challenge of organisms.

Tests for antigens common to Pseudomonas strain SRU-12-4-4(59) were made with 113 other strains of Pseudomonas, and cross-antigenicity was demonstrated by agglutination tests in 51 of these. Cross protection was demonstrated with all of the eight organisms tested which showed cross-reaction by agglutination, and two of three which did not.

The ease of production of this highly antigenic and effective antigen from a single strain of Pseudomonas gives encouragement that immunotherapy can be used effectively for the prevention and treatment of infection from Ps. aeruginosa.

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Presentations

Alexander, J. W.: Studies on the isolation of an infection-protective antigen from Pseudomonas aeruginosa. Presented at Surgical Seminar meeting, Department of Surgery, University of Cincinnati, Cincinnati, Ohio, 3 November 1965.

Publications

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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Immunity, Pseudomonas, burns, hemagglutination

24. (U) Tech Objective - The successful application of topical Sulfamylon to the control of burn wound sepsis leaves unanswered the question of the role of immunity in the resistance of Sulfamylon-treated burn patients to invasive infection. Such a factor undoubtedly is important in these injuries, but no information regarding immune response in topically treated burns exists. The initial state of this study was intended to acquire such data.

25. (U) Approach - A species-specific thermolabile soluble antigen was extracted from Pseudomonas aeruginosa strains and was used to sensitize sheep erythrocytes. Sera from Pseudomonas-infected burned rats were studies for agglutinin at intervals, with and without topical treatment.

26. (U) Progress (Jul 65-Jun 66) - Antibody response was demonstrated in as little as 2 to 3 days post burn. The least virulent strains incited highest antibody response, while virulent strains incited less antibody. Treatment with Sulfamylon resulted in higher antibody to the more virulent strains. Antibody response varied directly with the concentration of the drug. Protective role of such antibody remains to be assayed.

27. COMMUNICATIONS SECURITY	29.	30. DOD CODE	31. BUDGET CODE
☐ a. CLASSIFIED RELATED ☒ b. NOT RELATED		AR	1

32. MISSION OBJECTIVE	33. PARTICIPATION
NA	NA

34. REQUESTING AGENCY	35. SPECIAL EQUIPMENT

36. EST. FUNDS (In Thousands)	37.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: IMMUNE RESPONSE IN EXPERIMENTAL BURNS TREATED WITH TOPICAL SULFAMYLON

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert B. Lindberg, Colonel, MSC
Lawrence K. Inouye, B.S.
Russell E. Brame, M.S.
John A. Moncrief, Colonel, MC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: IMMUNE RESPONSE IN EXPERIMENTAL BURNS TREATED WITH TOPICAL SULFAMYLON

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period Covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert B. Lindberg, Colonel, MSC
Lawrence K. Inouye, B.S.
Russell E. Brame, M.S.
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

Successful control of burn wound sepsis with topical sulfamylon* prompted assessment of the immune response in rats burned, then seeded with virulent Pseudomonas aeruginosa strains and topically treated. It was found that the most rapid and highest level of antibody appeared with strains of weak virulence. The most virulent strains elicited the least and slowest antibody response. When topical therapy was applied to infections with virulent strains, the effect on antibody response varied with concentration of the drug. Ten per cent Sulfamylon treatment resulted in a rapid, high-titer response in a test system which used hemagglutination of red cells sensitized with a thermostable aqueous extract of Ps. aeruginosa. Reduced concentration of 5% Sulfamylon altered the immune response radically. With this concentration, antibody appeared more slowly with a lower, biphasic peak titer, and reflected an antigenic stimulus falling between that of a low- and high-virulence strain.

(1) Immune response (2) Virulence studies (3) Ps. aeruginosa

* Sulfamylon furnished by the Sterling-Winthrop Research Institute, Rensselaer, New York.

IMMUNE RESPONSE IN EXPERIMENTAL BURNS TREATED WITH TOPICAL SULFAMYLON

Infection in severe burns has been a major cause of death in patients who survive the initial trauma of injury. Burn wound sepsis, as the definitive wound lesion, appears as an overwhelming bacterial growth in devitalized tissue of the full-thickness areas,[1] with invasion of second-degree burn areas and rapid destruction of the surviving vascular bed.[2] The protective action of 10% Sulfamylon HCl (p-aminomethylbenzenesulfonamide hydrochloride)* in a water dispersible base has been demonstrated,[10] with virtual elimination of the burn wound sepsis syndrome and a greatly reduced mortality in severe burns.[3] The mechanisms involved in this treatment are not, as yet, understood; for example, the bacterial content of severely burned tissues topically treated is usually lowered in comparison with that of uncontrolled septic burns, but the difference is not necessarily beyond one order of magnitude. Similarly, in experimentally burned rats seeded with Pseudomonas aeruginosa and treated with 10% Sulfamylon burn cream, some survivors exhibit tissue bacterial contents of up to 10^7 organisms per gram of tissue.[4] This level is in the range of that found in untreated fatal infections, yet such animals showed no overt difference in recovery from others with tissue levels of 10^3 to 10^4 organisms per gram. It has been suggested frequently that immunity factors play a significant role in defense of the patient against invasive infection, and an assessment of such mechanisms in the experimental burned seeded rat was conducted.

Materials and Methods

A sensitive and specific measure of antibody response was needed. After trial of such systems as direct agglutination, precipitin, and bactericidal antibody,[5] a hemagglutination procedure, modelled after that employed by Neter, and co-workers, for measurement of antibody to enteropathogenic Escherichia coli, was selected.[6] The sensitizing antigen was prepared by suspending cells of an 18-hour culture of Ps. aeruginosa in physiologic saline solution at a concentration of 10^{10} per ml. The washed cells were heated in 0.9 per cent NaCl solution for one hour at 100° C.

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* Sulfamylon furnished by Sterling-Winthrop Research Institute, Rensselaer, New York.

The suspension was centrifuged one hour at 17,000 G to remove particulate matter and the supernatant fluid was designated as stock antigen. Sheep red blood cells in 10% concentration were sensitized by exposure to the antigen for one hour at 37° C, followed by washing three times to remove excess antigen. The use concentration was 1%. Serums to be tested were diluted from 1:2 to 1:1056. The optimal sensitizing concentration of antigen was determined by sensitizing sheep red blood cells with serial dilutions of the sensitizing extract and testing it against homologous hyperimmune rabbit serum. Figure 1 illustrates the pattern of reaction observed with strain SRU-12-4-4(59), and the manner in which an optimal sensitizing dose was selected.

The antigen is referred to as Polysaccharide; its chemical composition has not been determined as yet, but it unquestionably contains a large polysaccharide moiety, together with lipid, some protein, and an appreciable component of nucleic acids. Dilutions of the antigen were used to sensitize sheep red blood cells which were then washed and tested against dilutions of rabbit hyperimmune serum. As figure 1 shows at dilutions of from 1:100 to 1:1000 a maximum titer with the immune serum for strain SRU 12-4-4(59) was recorded. A working strength of 1:3200 as a sensitizing strength was selected. With strain VA-134, (Figure 2), the sensitization curve had a slightly different form: in the optimal combining zone, from 1:100 to 1:10,000 of the cell extract hemagglutinating titers of 1:6400 to 1:12,800 were obtained with the homologous serum. Again, a median dilution for sensitizing of 1:3200 was selected.

With the optimal sensitizing concentration of 1:3200 of antigen, each test rat was checked for pre-existing antibody prior to seeding with Ps. aeruginosa. No antibody was detected in any normal rat by this method. The reaction was thus highly specific. Titers of groups of rats were averaged by the method of least squares.

Experimental burn sepsis was created by the standard seeded burn model.[7] Rats were kept, when possible, in the 190 to 220 gm weight range. Burns were 20 per cent body surface, for 10 seconds at 98° - 100 ° C. Seed inocula were 10^8 cells unless otherwise noted.

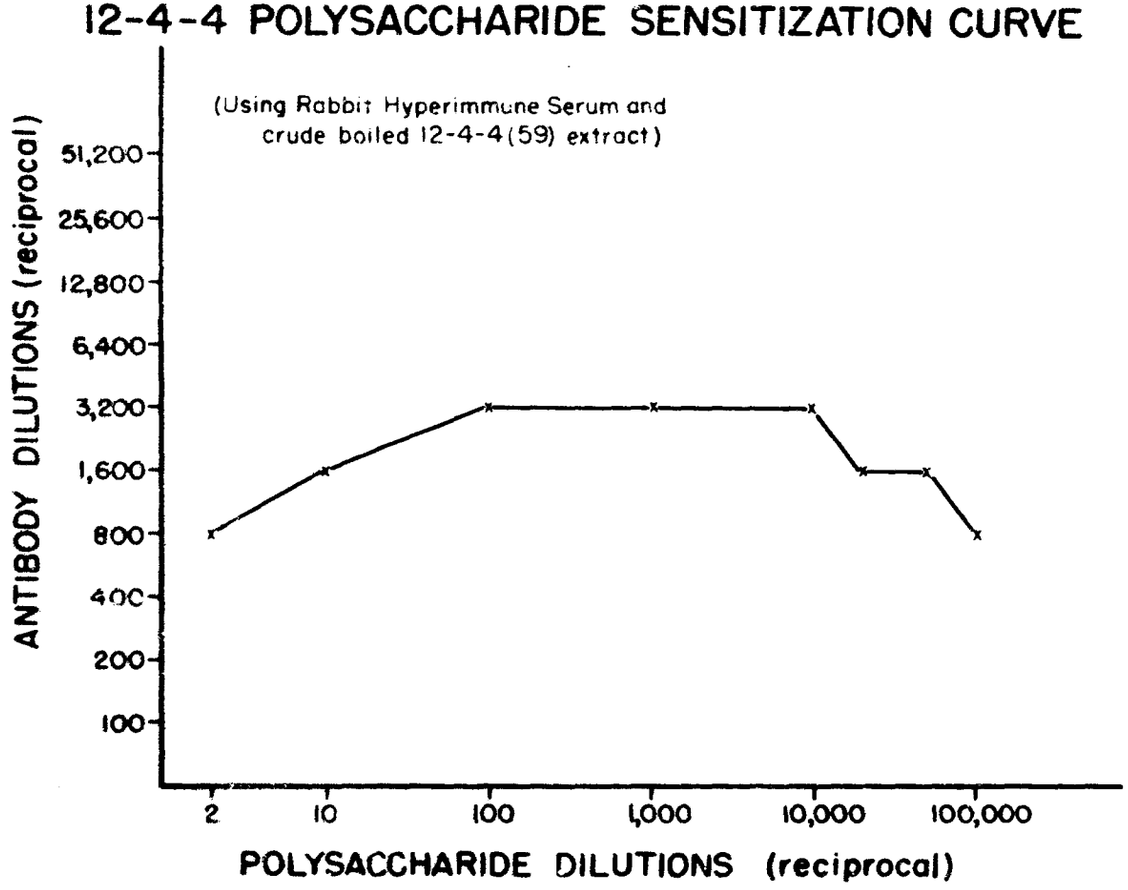

12-4-4 POLYSACCHARIDE SENSITIZATION CURVE
(Using Rabbit Hyperimmune Serum and crude boiled 12-4-4(59) extract)
ANTIBODY DILUTIONS (reciprocal)
51,200
25,600
12,800
6,400
3,200
1,600
800
400
200
100
2
10
100
1,000
10,000
100,000
POLYSACCHARIDE DILUTIONS (reciprocal)

Figure 1.

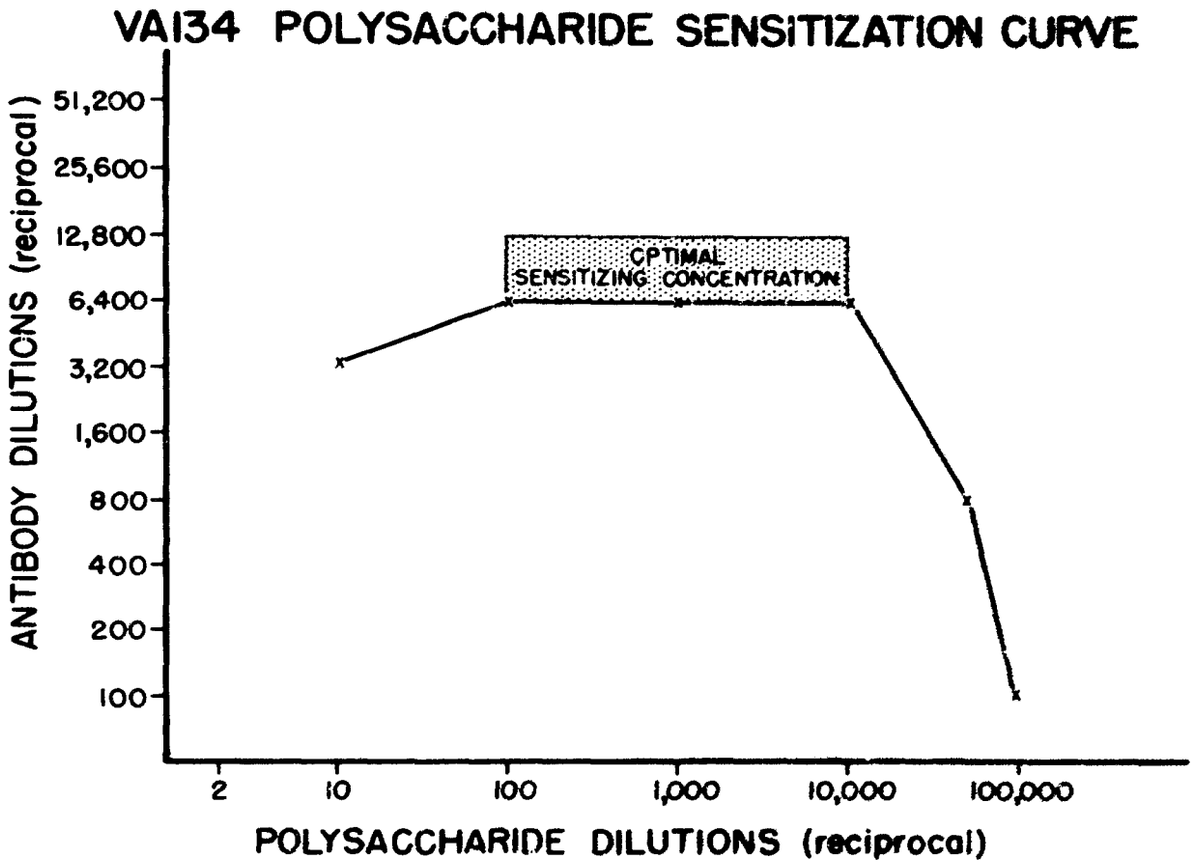

VA134 POLYSACCHARIDE SENSITIZATION CURVE
ANTIBODY DILUTIONS (reciprocal)
51,200
25,600
12,800
6,400
3,200
1,600
800
400
200
100
OPTIMAL SENSITIZING CONCENTRATION
2
10
100
1,000
10,000
100,000
POLYSACCHARIDE DILUTIONS (reciprocal)

Figure 2.

Results

Response in relation to virulence of infecting strain. A wide variation in virulence of Ps. aeruginosa, as assessed by burn wound invasion, has been demonstrated in this laboratory.[8,9] Four strains designated VA-134, SRU 8-28-3(63), SRU 12-4-4(59), and SRU 12-2-2(65) were tested. In virulence, these ranged as follows:

VA-134 : 100% virulent; death from 3 to 7 days, depending on size of rat.

SRU 8-28-3(63): 100% virulent; death in 5 to 10 days.

SRU 12-4-4(59): Virulence varying between 100% and 75% in different sets of animals.

SRU 12-2-2(65): Lethal for 10 to 20% of rats seeded.

An inverse relationship in antibody response with relation to virulence was observed (Figure 3). VA-134, the most virulent strain, effected agglutinin at 1:2 level at 72 hours after seeding. The titer rose rapidly to a mean level of 1:14, then declined to approximately 1:8 at the time of death. The rise in titer was the slowest of any strain observed. With strain SRU 8-28-3(63), equally virulent but slower to kill, a titer of 1:2 was observed at the third postburn day. This level rose somewhat more rapidly than with VA-134 to a mean level of 1:40 at the fifth post-infection day, then more slowly to a peak level of 1:90, after which a drop occurred in surviving rats up to the longest survivals at day 10.

The fastest antibody response appeared in animals seeded with strain SRU 12-4-4(59). At the third postseeding day a titer of 1:3.5 was found; this rose rapidly to 1:16 at four days postseeding, to 1:64 at day five, and reached a first peak at eight days postburn, at 1:164. A slight but consistent drop in titer occurred by day 10; it is tempting to speculate that such a fall coincides with a period of proliferation of bacteria in the tissues. In any event, survivors in this series exhibit a later rise to a titer of 1:256. Observations on survivors of this strain are continuing, but at this time were completed only through day 30. The drop in titer, it will be noted, resembles that seen with 100% lethal strains in the two days preceding death. The least

ANTIBODY RESPONSE TO PSEUDOMONAS AERUGINOSA IN SEEDED BURNED RATS TREATED WITH SULFAMYLON 10% STRAIN: 8-28-3

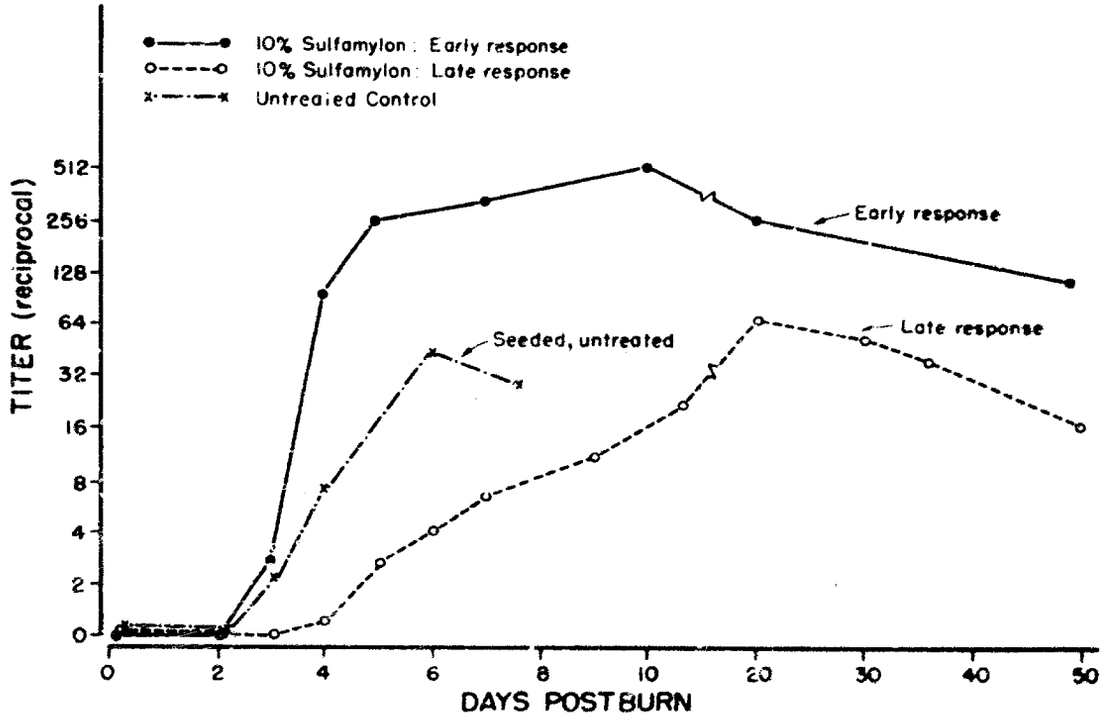


Figure 3.

virulent strain, killing never more than one-fifth of the test animals, prompted the highest antibody response, although the rise was slightly slower than that of SRU 12-4-4(59). Peak titer was reached at day nine, and declined only from 1:320 to 1:128 at 50 days postburn. Strains of this order of virulence are capable of penetrating viable tissue but do not go on to a lethal sepsis, unlike the behavior of virulent strains.[8]

Effect of treatment on antibody response. When rats were infected with virulent strains of Pseudomonas and treated in the standard manner as developed in this laboratory (3 gm of 10% Sulfamylon burn cream once daily, starting at 24 hours postseeding and continuing for 10 daily treatments), the rats survived the infection. Antibody response was measured in 20 rats selected from successive therapy studies. Figure 4 shows the antibody responses observed. The response grouped clearly in two clear-cut increments, designated here as "early" and "late" antibody rise. This pattern appears to be strain-related. Other virulent strains, on preliminary observations, have not shown this paradoxical behavior.

The antibody response, in relation to resistance of the animals to a potentially lethal burn infection, indicates that survivors of such a challenge are potentially capable of putting forth a much more rapid and a higher antibody response than occurs with the unprotected burn. The response with this lethal strain resembles that seen in infection with a strain of limited lethality. To the extent that the burned human patient contributes a defense mechanism to the control of burn wound sepsis, this response could represent an optimal immune reaction in the treated burn. An explanation for late responding animals is not yet available; it is, indeed, possible that the challenge presented by this virulent organism is such that a slight difference in early invasion would result in a more intense proliferation of bacteria and a reduction in circulating antibody. The two response zones are valid entities with the strain being used. It is not certain that all virulent strains, when topically treated, call forth two different patterns of response in different groups of rats.

Antibody differences with different dosage levels of Sulfamylon. The selection of optimal dosage of Sulfamylon

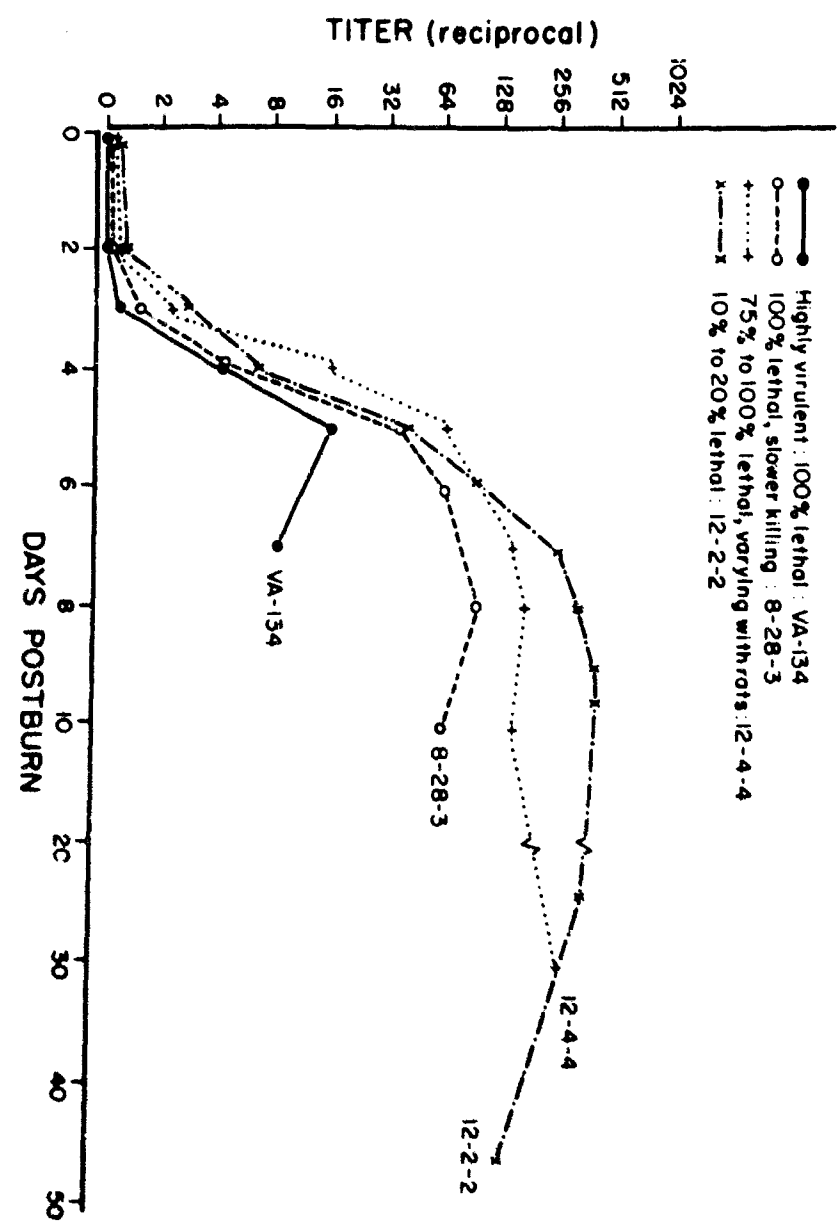
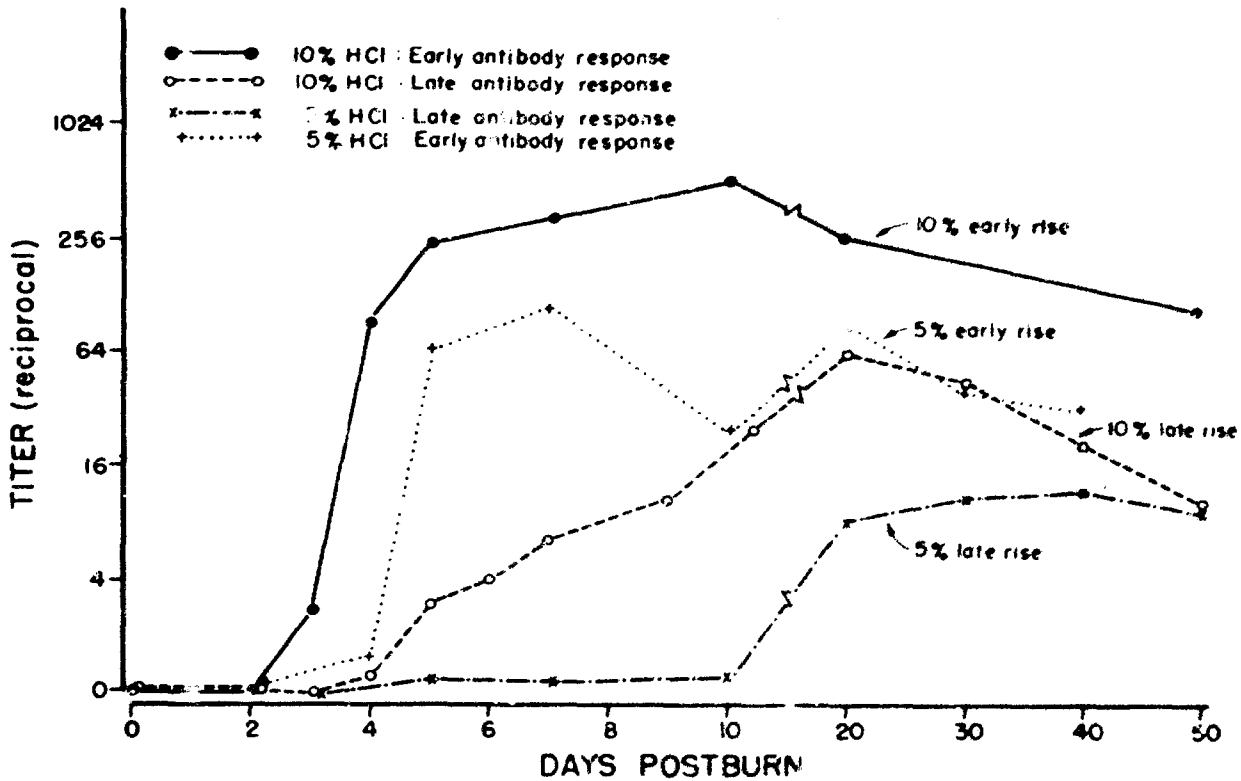

ANTIBODY RESPONSE IN BURNED RATS SEEDED WITH
PSEUDOMONAS AERUGINOSA STRAINS OF VARYING VIRULENCE
TITER (reciprocal)
0
2
4
8
16
32
64
128
256
512
1024
DAYS POSTBURN
0
2
4
6
8
10
20
30
40
50
Highly virulent: 100% lethal: VA-134
100% lethal, slower killing: 8-28-3
75% to 100% lethal, varying with rats: 12-4-4
10% to 20% lethal: 12-2-2
VA-134
8-28-3
12-4-4
12-2-2

Figure 4.

was originally made [10] on the basis of survival of seeded rats, treated with graded amounts of drug. The question remained, however, as to whether less than an optimal dose of chemotherapy would be actually detrimental to the patient. The nature of the protective mechanism with partial bacteriostasis is of practical, as well as theoretical importance. The conditions of burn management often result in presence on the wound of reduced concentrations of drug, and in practice, one must assume that reduced amounts of medication are present on many burn wounds. A comparison of immune response on burned, seeded rats was carried out using a succession of strains of varying virulence. Treatment consisted of 10% and 5% Sulfamylon on groups of rats burned and seeded with Ps. aeruginosa strain SRU 8-28-3(63), VA-134, and SRU 12-4-4(59). The animals were treated for 10 days starting on the first postburn day with the customary 3 gm of Sulfamylon burn cream. The antibody response patterns for SRU strain 8-28-3(63) are shown in Figure 5.

The antibody response is based on equal numbers of surviving rats. However, a striking difference in antibody response appeared when therapy at a reduced intensity was employed. The 10% Sulfamylon permitted a very rapid antibody rise, with a peak titer approached in seven days. The response resembled that caused by infection with a weak virulent strain. Antibody level fell slowly over a two-month period, to a level of one-third the peak titer. Again, two forms of antibody response occurred, one early, the other late. All animals survived. With 5% Sulfamylon, 80% of the treated rats survived, and survivors resembled those surviving with 10% Sulfamylon treatment. But antibody response began more slowly than with the 10% drug, rose to only one-eighth the titer reached with the 10% treatment, and showed a sharp fall in titer at 10 days, with a secondary rise to a peak at 20 postburn days. This fall in titer appeared two days after the most common time of death in unprotected control rats, and it seems probable that an exacerbation of infection occurred in the rats, reaching a peak at which point the protective action of the drug still effected survival of most of the rats. With the 5% Sulfamylon-treated rats, no antibody appeared at all until postburn day 10, at which point a weak rise to a 1:8 titer was recorded. The antibody roughly paralleled the course of the early-rise set of rats under 5% concentration, but the amount of antibody was vastly decreased.

ANTIBODY RESPONSE IN RATS TO CHALLENGE OF SEEDED BURNS WITH PSEUDOMONAS AERUGINOSA 8-28-3. TREATMENT WITH SULFAMYLON H CL 10% AND 5% CONCENTRATION



Discussion

The inverse relationship between virulence of strain and rate and degree of antibody response had not been previously known. The phenomenon offers an insight into possible explanations for the virulence mechanism with tissue infection involving Pseudomonas. Virulent strains may proliferate more rapidly (although this has not been demonstrated as yet) so that antibody as formed is found by the proliferating cells. Another possibility is the absence of effective antigen in the more virulent strains, although these strains in rabbits called forth effective antibody response.

It is possible that this procedure could point to maximally effective antigens, if search for an immunizing antigen were being conducted.

When the factor of topical treatment is imposed on the burn infection, a marked alteration in antibody response occurs. With the 10% Sulfamylon dosage, antibody rose rapidly in a manner strikingly similar to that seen with untreated infections with strains of low virulence, while 5% Sulfamylon called forth a delayed, weaker antibody response. For this reaction, there is no counterpart in the trials with antibody response in untreated infections.

A plausible hypothesis, prompted by this admittedly small number of strains, is that the nature of the infection, in the presence of Sulfamylon, is altered, but remains an active infectious process which brings bacteria into contact with viable tissues. Either the actual number of organisms is greatly reduced, or an alteration in growth or metabolism of the Pseudomonas strains is imposed by the Sulfamylon. Thus the presumed overwhelming infection may, in fact, be exposure to an altered organism, which prompts a lessened antibody response.

The anomolous antibody response demonstrated in this experimental burn infection calls for further study to delineate the reaction in animals, and to assess Sulfamylon-treated burned patients to determine the nature of their response to Pseudomonas infection. As shown in other sections of this Annual Report, the control of burn wound sepsis has not eliminated Ps. aeruginosa from the patients' environment. In view of the experimental evidence that heightened

antibody response is effected by reducing the concentration of bacteria in the wound, the clinical exposure of burned patients today may constitute a potent immunizing factor for Pseudomonas aeruginosa.

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Presentations

Lindberg, R.B.: The immune response in Pseudomonas aeruginosa infected burns with sulfamylon treatment. Presented at American Association of Immunologists, Atlantic City, New Jersey, 15 April 1966.

RESEARCH AND TECHNOLOGY RESUME	1.	2. SECURITY CLASSIFICATION	3. AGENCY ACCESSION DA OB 6394	REPORT CONTROL SYMBOL CSCRD-103
4. DATE OF RESUME 30 06 66	5. KIND OF RESUME "A. NEW"	6. SECURITY (a) U (b) U	7. REGRADING NA	8. RELEASE LIMITATION NIL	9. LEVEL OF RESUME A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 185

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) A New Technic for Culturing Burn Wound and other Surfaces (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE b. NUMBER c. TYPE NA d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House		PRIOR FY 65	0	0
		CURRENT FY 66	1	4

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21. TECHNOLOGY UTILIZATION
Life Sciences

22. COORDINATION
None

23. KEYWORDS
Infection, Pseudomonas, culture technic

24. (U) Tech Objective - A basic need in laboratory procedures for burn wound study is a simple valid method for determining qualitatively and quantitatively the surface flora of the burn and correlating this information with the threat of infection on the progress of healing of the burn wound. Swab technic is inadequate for this purpose; biopsy is too complex and may be hazardous. A technic to accomplish the desired objectives was sought.

25. (U) Approach - Bacteriologic culture plates were modified by incorporation of a gauze strip projecting out of the Petri dish to permit removal of the agar and its application to tissue surfaces. An impression culture of wound surfaces was obtained, and quantitation of surface flora is made by colony count.

26. (U) Progress (Jul 65-Jun 66) - The contact plate technic has furnished quantitative data on the rate and extent of colonization of the burn wound, the nature of the surface flora which connotes invasive infection and conversely the bacterial content and growth relationships which accompany a satisfactorily healing wound. The effect of topical therapy on the burn wound flora has been thus assessed. It is an essential tool in the laboratory evaluation of burn wound treatment.

27. COMMUNICATIONS SECURITY ☐ a. CLASSIFIED OR CONTROLLED ☒ b. NOT CLASSIFIED OR CONTROLLED	28.	29. DOD CODE AR	30. BUDGET CODE 1
31. MISSION OBJECTIVE NA		32. PARTICIPATION NA	
33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT		
34. SEY. FUNDS (In thousands) SEY + 1	35.		

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501871R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: A NEW TECHNIC FOR CULTURING BURN WOUNDS AND OTHER SURFACES

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert B. Lindberg, Colonel, MSC
Walter E. Switzer, Lt Colonel, MC
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: A NEW TECHNIC FOR CULTURING BURN WOUNDS AND OTHER SURFACES

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert B. Lindberg, Colonel, MSC
Walter E. Switzer, Lt Colonel, MC
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.

6. Reports Control Symbol: RCS-MEDDH-288(R1)

A method has been perfected for taking impression plates of burn wounds and tissue surfaces. The procedure displays the entire flora of the burn wound, demonstrates relative concentrations of the different organisms present, delineates patterns of growth as they may be related to the state of healing or infection present in the wounds, and is ideally suited to culturing homograft beds and other exposed tissue surfaces. It has been extensively applied in several hundred burn cases.

(1) Burn wounds
(2) Culturing

A NEW TECHNIC FOR CULTURING BURN WOUNDS AND OTHER SURFACES

Conventional technic in wound culture consists of rubbing a swab over the area in question, then streaking the swab on culture media. The swab is usually sent to the laboratory to be applied to culture media. Transport media, hopefully, are used to maintain the flora in an unaltered state, but this is uncertain at best; at worst, drastic changes occur in recoverable bacterial content before the specimen reaches culture media. The swab offers little information as to the quantity of bacteria on a wound, nor their proportionate distribution as to species and strains. Quantitation of flora from swab cultures is strikingly variable since technic of taking swabs varies widely. The technic is virtually impossible to standardize. Many hospitals encourage ward personnel--doctors, nurses, or attendants--to plate out swabs, but in the hands of the inexperienced, this is seldom a success. The usual result of plating out cultures by non-laboratory personnel is an over-inoculated plate, subject to considerable delay in assessment of its growth.

Prompted by the need for a more informative technic for sampling burn and operative wounds, a technic was devised for applying the sheet of agar in the petri dish direct to the surface to be cultured. This procedure has been carried out on over 2000 cultures; it is described and evaluated herein.*

Method

Petri dishes were prepared with strips of surgical gauze lining the dish, with tabs of gauze extending outside the petri dish. Trial has shown the two layers (not more) of gauze are the right amount to hold the agar solidly. The strips are cut two-inches wide. The media is poured in the dish as usual; about 18 ml of blood agar is optimal. Any agar substrate can be used. The dishes can be stored with no contamination problem. To use the agar sheet, the dish cover is

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* A year after this procedure was developed, the senior author found a description of a similar procedure by Foster.[1] It is possible that the idea has been applied elsewhere; it is by its nature difficult to categorize and hence tends to be overlooked.

removed,and the agar removed by holding the two gauze ends and lifting the sheet out. No indication of aerial contamination has occurred. The cultures are taken at the bedside, in the operating room, or wherever a wounded surface is of interest. The flexible agar sheet conforms readily to a variety of surfaces. If it is obvious that the sheet cannot adequately contact all surfaces, it is a simple matter to use a Kelly forceps or other sterile instrument to push the agar sheet against the surface.

Figure 1 shows the agar sheet being removed from the petri dish. Figure 2 illustrates the taking of a surface culture; while Figure 3 shows a culture being taken on a burned child on admission. The agar adheres readily to tissue surfaces; the gauze "handles", can then be tugged gently to move tissue and agar sheet together. This apparently has the effect of opening crevices in the wound surface whence bacteria are picked up by the agar sheet. The sheet is ordinarily kept in contact with the wound surface for one to two seconds. It is then lifted off, turned so that the inoculated side is up, and placed in the cover of the petri dish; the bottom of the dish is then inverted over the agar sheet. The plate is incubated without further manipulation.

The occasional presence of spreading colonies presents a problem; for this reason, as well as in the primary objective of obtaining prompt information on the wound flora, plates are usually taken with two media. Routine use calls for Eosen Methylene Blue and Blood Agar; any other formula can be used. The method is suited to almost any surface: floors, furniture, equipment, faucets, door knobs - all can be covered.

Results

The plates are read in the customary manner, and subcultured for identification as needed. Overgrowth may be a problem in heavily contaminated wounds, but in a series of 500 plates (250 surfaces cultured with two plates each) there were fewer than five per cent of the specimens in which massive overgrowth precluded prompt assessment of the growth. The quantity of growth actually serves as an indicator of the degree of infection. Plates with two media appear as replicates; thus the absence of a colony on selective medium and its presence on Blood Agar may furnish all the information

Figure 1. The agar sheet being removed from the petri dish.

Figure 2. Taking of a surface culture.

Figure 3. Culture being taken on a burned child at admission.

needed to effect a prompt identification.

A facet of wound flora previously unsuspected has been observed in the pattern of bacterial growth. A wound subject to unchecked bacterial growth, particularly a burn wound sepsis, may initially have a sparse flora on contact culture. Subsequent developments have shown that the wound reverted to a septic invasive process and simultaneously exhibited a tremendous increase in surface flora. Thus, although it can not be said that the technic furnishes a generally applicable tool for clinical guidance, it has proven to be so in several instances.

Discussion

The surface culture technic described has been applied to over 2000 wound surfaces. A subsequent development has been the perfecting of a simple 35 mm camera assembly for photographing selected plates at the time they are catalogued. This permits the bacteriologist to retain, not only a log of the identity and approximate concentration of specific organisms, but a visualization of the manner of growth and the colonial morphology.

The procedure has permitted the development of a rationale regarding the sequence in which the burned patient is colonized after his arrival on the ward. It has clarified the relationship of various manipulative procedures to the burn wound flora; (reference here is to the effect of scrubbing, hydrotherapy, and other manipulations on the surface flora of the burn). The procedure is extremely well suited to assessing the bacterial flora of homograft beds, and has been used for this purpose in surgery. It can be suited to a variety of surfaces and situations, and in recent months several surgeons on the Surgical Research Unit staff have begun to collect cultures at times when the question of surface flora was of particular interest.

The interpretation of burn wound culture in relation to the state of the patients' illness is a topic that cannot be summarized at this time. Based on the surface culture technic, analysis of this information is to be carried out.

References

1. Foster, C.H.: A method for obtaining surface cultures on agar plates. Appl. Microbiol. 46:1207, 1954.

Presentations and/or Publications

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 08 6398	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A0145018718 01 189

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (U) A Comparison of Sulfamylon Hydrochloride and Sulfamylon Acetate in Control of Experimental Burn Wound Infections (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE b. NUMBER c. TYPE d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	2	8
		CURRENT FY 66	2	12

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TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: NONE

25. KEYWORDS: Experimental burn wounds, chemotherapy formulations, Sulfamylon

23. (U) Tech Objective - Search for a means of preventing burn wound sepsis (BWS) led to development of a bacteriostatic burn cream with Sulfamylon (supplied by Sterling Winthrop Res. Inst.) hydrochloride (p-amino methyl benzene sulfonamide HCl) 10% in a water-dispersible ointment base. The clinical use of this material since 1 Jan 1964 has resulted in virtual elimination of BWS as a clinical entity and cause of death with a corresponding lowering of mortality rate in burn patients thus treated. A side effect of hyperchloremia, with an increased acid load, was encountered in some extensive, Sulfamylon-treated burns, and search for a modification of the drug which would reduce this effect was made.

(U) Approach - Sulfamylon modifications included a-lactate, -gluconate, and -acetate. The acetate proved to be most stable and was supplied by Sterling Winthrop Res. Inst. Pseudomonas burn rat model used as screening device. End point: survival of an LD-100 preparation.

(U) Progress (Jul 65-Jun 66) - Sulfamylon acetate was slightly less active in vitro against Pseudomonas aeruginosa than Sulfamylon HCl. In the burned seeded rat, prevention of deaths was slightly less with acetate than with HCl. The difference in effectiveness was more apparent with highly virulent challenge strains. The behavior of Sulfamylon acetate justified its trial in human burn patients, but a comparative trial was strongly indicated.

27. COMMUNICATIONS SECURITY	28.	29. DDC CODE	30. SUPPL. CODE
a. CLASSIFIED b. NOT CLASSIFIED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

34. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

36. EST. FUNDS (In thousands)	38.

DD FORM 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: A COMPARISON OF SULFAMYLON HYDROCHLORIDE AND SULFAMYLON ACETATE IN CONTROL OF EXPERIMENTAL BURN WOUND INFECTIONS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert B. Lindberg, Colonel, MSC
John A. Moncrief, Colonel, MC
Russell E. Brame, M.S.
Arthur D. Mason, Jr., M.D.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: A COMPARISON OF SULFAMYLON HYDROCHLORIDE AND SULFAMYLON ACETATE IN CONTROL OF EXPERIMENTAL BURN WOUND INFECTIONS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert B. Lindberg, Colonel, MSC
John A. Moncrief, Colonel, MC
Russell E. Brame, M.S.
Arthur D. Mason, Jr., M.D.

6. Reports Control Symbol: RCS-MEDDH-288(R1)

A comparative trial of Sulfamylon hydrochloride (p-aminomethylbenzenesulfonamide hydrochloride)* and Sulfamylon acetate (p-aminomethylbenzenesulfonamide acetate)* was carried out using burned rats seeded with Pseudomonas aeruginosa and treating them with the two forms of the drug. The prevention of deaths was similar, but the hydrochloride was slightly superior to the acetate, especially in treatment of the more virulent forms of Ps. aeruginosa. In vitro comparison revealed a similar difference in inhibitory action between the hydrochloride and acetate. The Sulfamylon HCL was slightly more effective in inhibiting Ps. aeruginosa.

(1) Sulfamylon HCl

(2) Sulfamylon acetate

(3) Pseudomonas aeruginosa

(4) Infection

* Sulfamylon furnished by Sterling-Winthrop Research Institute, Rensselaer, New York.

A COMPARISON OF SULFAMYLON HYDROCHLORIDE AND SULFAMYLON ACETATE IN CONTROL OF EXPERIMENTAL BURN WOUND INFECTIONS

Early in the course of treatment of patients with Sulfamylon hydrochloride (HCl)*,(p-aminomethylbenzenesulfonamide hydrochloride), the impression arose that, in very large burns or in patients with a considerable component of respiratory impairment, a chloride load arising from the breakdown of the compound could appear and act as a factor in producing or enhancing acidosis with deleterious effects. In the search for formulations to avoid this effect, an acetate was, at the request of this Unit, prepared by the Sterling-Winthrop Research Institute. It has been tested extensively in the SRU rat model, and is currently undergoing clinical trial. The animal trials are summarized in this report.

Methods

The Sulfamylon acetate (p-aminomethylbenzenesulfonamide acetate) represents a slightly larger proportion of the molecule as acetate in contrast to the molecular weight of the HCl. Hence, to provide equi-molar concentrations of the sulfamylon itself, allowance was made for the added weight of the acetate, which was made up in weight per volume to a concentration of 11 per cent. This represents the same amount of sulfamylon that is contained in 10% Sulfamylon HCl. To avoid confusion, the acetate concentration will be referred to as "10% sulfamylon acetate".

The initial experiments were directed toward learning whether the acetate was the equal of the hydrochloride in preventing burn wound sepsis in the seeded burned rat. The criterion was death or survival, as it was in the development of the Sulfamylon HCl as a therapeutic agent. The trial concentrations of the drug were ultimately reduced to increase the mortality rate and offer a more intelligible zone of difference should such be present. The standard procedure was to seed rats, then start topical treatment 24 hours later and continuing for 10 daily treatments of 3 gm per rat.

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* Sulfamylon furnished by Sterling-Winthrop Research Institute, Rensselaer, New York.

The conditions of the test system are difficult to accomplish at all times, and in some experiments the optimal rat weight was not available. Over 25 different experiments, using different concentrations of drug and different challenge strains, were performed. A total of 788 rats to date have been utilized.

Results

The results of the numerous experiments comparing the two drugs are summarized in Table 1.

It is evident that in general the two forms of sulfamylon here tested were similar in their effect on infected burns, but they were not identical. With the VA-134, which is the most virulent test strain in use, the 10% hydrochloride permitted 4 out of 9 survivals, while only one of 12 survived with the acetate. Seven of 25 rats survived with 5% treatment with HCl; only two of 25 survived with acetate. One of five even survived treatment with 2½% HCl; none survived with acetate. Survival times were similar with both drugs.

With SRU 8-28-3(63), a 100 per cent virulent strain but less deadly to burned rats than VA-134, results were more closely matched, but slight differences appeared. Forty-four rats survived out of 52 at 10% treatment with HCl; 42 survived of 51 with the acetate. Survival with the 5% emulsion was similar for both drugs; with the 2.5% concentration, a slight increase in survivals with the HCl over the acetate was found.

With strain SRU-12-4-4(59), the least virulent of the test strains, results were almost identical for both drugs. Survival times were essentially identical.

In vitro tests of Sulfamylon HCl and Sulfamylon acetate showed that the former is slightly more inhibitory to Pseudomonas aeruginosa than is the acetate. The comparison is presented in Table 2.

The test population for this series was collected from wound cultures, blood cultures, and urine cultures during 1965. It is apparent that the two compounds have a similar degree of activity in suppressing Ps. aeruginosa, but the HCl once again is slightly more effective than the acetate. This

Table :. Comparison of Sulfamylon Hydrochloride and Sulfamylon Acetate in Capacity for Preventing Burn Wound Sepsis

Strain	Concentration	Hydrochloride			Acetate		
		Deaths/ Total	Survival - Days		Deaths/ Total	Survival - Days	
			Average	Range		Average	Range
VA-134 (most virulent)	10%	5/9	8.0	4-12	11/12	5.8	3-9
	5%	18/25	5.8	3-13	23/25	5.9	3-12
	2.5%	4/5	3.7	3-5	5/5	3.6	3-5
	Control	33/33	4.9	3-13	-	-	-
SRU 8-28-3(63) (highly virulent)	10%	8/52	5.7	6-11	9/51	7.3	3-9
	5%	42/87	6.6	3-12	37/79	7.2	3-16
	2.5%	9/17	6.6	4-9	7/10	9.4	4-15
	Control	123/124	6.2	3-13	-	-	-
SRU 12-4-4(59)	10%	3/31	12.5	9-16	1/26	13.0	-
	5%	9/57	11.9	8-13	8/50	12.5	8-15
	2.5%	3/14	12.1	10-13	2/8	11.0	-
	Control	65/68	8.5	5-12	-	-	-

Table 2. The *In Vitro* Inhibitory Action of Sulfamylon Hydrochloride and Sulfamylon Acetate on *Pseudomonas aeruginosa*

Strains Tested		Per Cent Inhibited by Not Less Than:*								
		5.0	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.19
HCL:	72	0	0	0	0	5.5	16.6	11.1	66.6	0
Acetate:	72	0	0	0	1.3	16.6	6.9	18.0	58.3	0

* Expressed in gm/per cent.

distinction is more readily recognized in graphic form (Fig.).

In the figure it is apparent that 66% of the strains are inhibited by 0.039% of the hydrochloride, which level of acetate inhibits 58%. Below this concentration all are tolerant. At approximately 0.1% concentration (or 100 mg%) the hydrochloride would inhibit 84% of the strains tested. The acetate would inhibit 78%. At 0.156% concentration, the difference is greatest. Here the acetate inhibited 83% of the strains, while the HCl inhibited 93%.

The difference is not great but it is real. It is of particular interest since the difference in protection with the acetate and the HCl is also slight but real. It must be assumed that a slight but real difference will exist in antibacterial action in the human as well. Whether this would have a significant effect on the clinical outcome in treating burn cases can only be learned by experience, but the results enjoin caution in using the Sulfamylon acetate until its efficacy in patients at maximum risk is established.

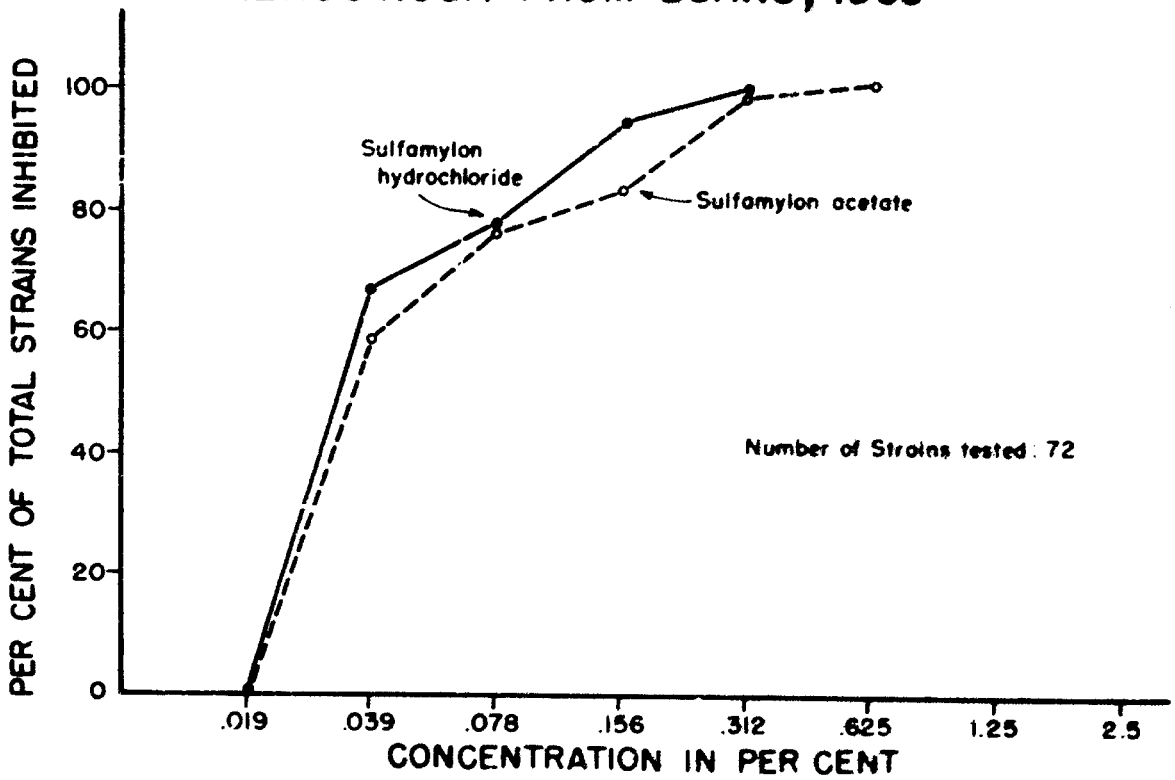
Discussion

The consolidation of comparative trials with Sulfamylon HCl and Sulfamylon acetate permits the conclusion that the two drugs do affect the experimental burn wound sepsis model in a comparable manner. There are several areas, however, where the hydrochloride looks slightly more effective. It would appear valid, with these data, to permit the results of clinical comparative trial to be the deciding factor. Even if the hydrochloride is slightly more effective in control of infection, the acetate, if otherwise preferable, would be likely to control infection in a manner acceptably equivalent to that demonstrated by the Sulfamylon hydrochloride.

Presentations and/or Publications

None.

INHIBITORY ACTION OF SULFAMYLON HYDROCHLORIDE AND SULFAMYLON ACETATE ON PSEUDOMONAS AERUGINOSA FROM BURNS, 1965



RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 0B 6393	CSLAD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 184

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (U) Biopsy of the Burned Seeded Rat as a Means of Evaluating Burn Therapy and Pathogenesis of Infecting Microorganisms

12. IENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE b. NUMBER c. TYPE d. AMOUNT	18. RESOURCES EST.	a. PROFES ONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	4

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INVESTIGATORS PRINCIPAL ASSOCIATE: Lindberg, R.E., Col., MSC; Brame, R.E., MS
TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Burns, pathogenesis, Pseudomonas, chemotherapy

24. (U) Tech Objective - To determine the relationship between density of seeding of burned rats with Pseudomonas aeruginosa, virulence, and lethality. To evaluate intensity and spacing of topical therapy in presence of strains of varying virulence.

(U) Approach - Burned seeded rats were punch biopsied at intervals to determine bacterial population of the burn as established by different strains and regimens of treatment.

(U) Progress (Jul 65-Jun 66) - Strains of lesser degrees of virulence established fixed levels of population in tissues without correlation to intensity of chemotherapy. Highly lethal strains with reduced inocula produced varying levels of tissue invasion as a strain-related attribute. Dosage schedules permitting 100% survival with a lethal strain did not of necessity reduce the bacterial content by more tha 1.5 logs below that seen in controls.

25

27. COMMUNICATIONS SECURITY	28.	29. GSD CODE	30. BUDGET CODE
a. CLASSIFIED [X] b. NOT CLASSIFIED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT
35. EST. FUNDS (In thousands)	36.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A[illegible]4501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: BIOPSY OF THE BURNED,SEEDED RAT AS A MEANS OF EVALUATING BURN THERAPY AND PATHOGENESIS OF INFECTING MICROORGANISMS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert B. Lindberg, Colonel,MSC
Russell E. Brame, M.S.
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: BIOPSY OF THE BURNED,SEEDED RAT AS A MEANS OF EVALUATING BURN THERAPY AND PATHOGENESIS OF INFECTING MICROORGANISMS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert B. Lindberg, Colonel, MSC
Russell E. Brame, M.S.
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.

6. Reports Control Symbol: RCS-MEDDH-288(R1)

A punch biopsy procedure has been developed for determining the bacterial content of the eschar of the burned rat. The procedure made possible serial observations on treated, infected rats, and revealed that, particularly with highly virulent strains, a dosage schedule which will save most rats will not, of necessity, reduce the wound content more than 1.5 logs below the level reached in the lethal control infection. With less virulent strains, a low constant level of Pseudomonas persists in the treated rat. Infections set up with 10^4 organisms instead of the usual 10^8, showed diverse patterns of proliferation in the burn. Some remained at a low level throughout the course of healing; others produced levels of 10^7 but survival still occurred. Highly lethal strains can readily kill even when reduced by 4 logs in inoculum.

(1) Burn infection

(2) Bacterial count

BIOPSY OF THE BURNED, SEEDED RAT AS A MEANS OF EVALUATING BURN THERAPY AND PATHOGENESIS OF INFECTING MICROORGANISMS

The development of the burn wound sepsis concept, and of topical sulfamylon cream for treatment of burns were carried out with observations on groups of burned and seeded rats, in which survival of the treated animal was the principal criterion of therapeutic effectiveness.[1] Quantitative studies were made by sacrificing animals out of a series, collecting tissues in gram amounts, and conducting the quantitative autopsy as would be done on a human subject. A quantitative serial sampling technic which could assess progress of infection and the animal's response to chemotherapy without dispatching the rat, was needed. Thus serial observations could be made at a lesser expense of material and effort, and with continuous observation of each individual animal. Such a procedure has been developed and validated and is being used to study response to chemotherapy, to evaluate new drugs, and to elaborate the pathogenic mechanisms of burn wound infection.

Materials and Methods

The established method of quantitative bacteriology has involved dispatching the animal, collecting one gram amounts of burn wound, grinding the sample in a Ten Broeck grinder, and making serial dilutions of the homogenate. An initial dilution of two volumes of broth per volume of eschar is needed to effect the grinding. The process is time-consuming and must be carried out in the cold to minimize error due to growth of bacteria in the tissue slurry.

The biopsy procedure, attempted first with excision of burn tissue with scalpel, has been carried out with a Cutaneous Biopsy Punch Set, FSN 6515-299-8716. Plugs of skin and eschar can be readily collected; after initial reliance on the smaller diameters, the 8 mm punch is now used with complete success. The surface to be sampled is cleansed with 70 per cent isopropyl alcohol and sponges, blotted dry, and the punch is passed down to the muscle or fascial plane. Trial sizes of the punch led to selection of the 8 mm size, which collected a block of tissue of approximately 0.05 gm. Since personnel cannot always be spared for final culturing when samples are taken, a systematic procedure of freezing all blocks has been established. Fifty c plastic dishes, with tight-fitting lids, are tared to facilitate weighing the sample. The biopsies (which can be collected very quickly) are placed in the dishes, sealed,

weighed and frozen at -70 until ready for assay. Grinding is far easier than is the case with an entire gram of tissue.

The dilution sequence is carried out by adding 1.8 ml of broth to each tube, assessing an empirical volume of 0.2 ml to each biopsy. With an initial dilution of 1:10 serial dilutions of 10^{-1}, 10^{-3}, etc., are carried out. Correction of count based on the actual weight of the tissue block is done after the count is made.

Results

An obvious question is the relative accuracy of biopsy samples in comparison with autopsy samples. Parallel counts on a series of rats, with one gram of eschar removed adjacent to the biopsy site, gave the parallel counts noted in Table 1. Further experience validates the accuracy of the procedure in rats. It is proposed to test the procedure further in patients; it will be of value to have quantitative evidence of the bacterial counts in topically treated burns.

The precise extent of bacterial suppression in the burn therapy model as now used is not known. The original data on bacterial content of sulfamylon-treated experimental wounds was based on treatment of sufficient intensity to save all the test rats;[3] this led to the twice-daily "buttering" routine on rats seeded with strains of 100 per cent virulence. The twice-daily treatment routine in patients was based on this conclusion.

It has subsequently become apparent that no single strain, in the infected burn model, offers a comprehensive tool for analyzing the process of wound infection and its control by topical therapy. Table 2 summarizes an experiment in which biopsy procedure was used to interpret the action of sulfamylon on sets of Pseudomonas-infected rats, and to obtain insight into the nature of its therapeutic action. The three strains of Ps. aeruginosa were used. SRU 8-28-3(63) is a highly virulent strain; it killed unprotected rats in this set in an average of 5.4 days, and the wound content went from 10^5 to 10^8 in the first five days. Once-daily applications of 10% Sulfamylon HCl spared one-half the test rats; the survival of treated rats which died was extended to 8.0 days average. The wound content in treated rats fluctuated widely, but mean values ranged from 10^5 to 10^6. Thus suppression occurred even

Table 1. Biopsy and Autopsy Samples from Burned Rats Seeded with *Pseudomonas aeruginosa*

Strain	Rat No.	Days Postburn	Treatment	Biopsy	1 gm Autopsy Sample
SRU 12-4-4(59)	1	2	None	8.3×10^6	5×10^6
SRU 12-4-4(59)	2	2	None	6×10^5	5.7×10^5
SRU 12-4-4(59)	3	5	None	2.1×10^7	3.0×10^7
SRU 12-4-4(59)	4	5	None	7×10^8	6.7×10^8
SRU 8-28-3(63)	5	2	None	1.7×10^7	2.0×10^7
SRU 8-28-3(63)	6	2	Sulfamylon HCl	8.0×10^3	8.4×10^3
SRU 8-28-3(63)	7	5	None	9.0×10^7	1.2×10^8
SRU 8-28-3(63)	8	5	Sulfamylon HCl	2.6×10^7	2.3×10^7
SRU 12-2-2(65)	9	2	None	1.5×10^8	1.3×10^8
SRU 12-2-2(65)	10	2	Sulfamylon HCl	3.1×10^2	2.9×10^2
SRU 12-2-2(65)	11	5	None	3.5×10^8	3.6×10^8
SRU 12-2-2(65)	12	5	Sulfamylon HCl	1.5×10^3	1.5×10^3

Table 2. Response to Therapy with Sulfamylon with Pseudomonas Strains of Varying Virulence: Biopsy Measurements

Strain	No. Rats	Treated	Mean Biopsy Count, Eschar Day Postburn			Death/ Total	Survival (Days)	
			1	2	5		Average	Range
SRU 8-28-3(63) (highly virulent)	7	Sulfamylon HCl 10% once daily	3.0×10^5	1.1×10^5	3×10^6	4/8	8.0	6-10
	7	Control	1×10^5	5.0×10^7	1.4×10^8	7/7	5.4	5-10
SRU 12-4-4(59) (virulent)	7	Sulfamylon HCl 10% once daily	1×10^5	1.1×10^7	1.0×10^6	1/7	9.0	-
	7	Control	8.1×10^5	4×10^7	8.5×10^7	7/7	8.3	7-9
SRU 12-2-2(65) (moderately virulent)	4	Sulfamylon HCl 10% once daily	5×10^4	2×10^4	7×10^4	0/4	-	-
	3	Control	1.2×10^5	4×10^6	6×10^7	2/3	14.5	13-16

though therapy did not save all the rats.

With strain SRU 12-4-4(59), the virulence was again 100 per cent, although this is not always the case. Treatment saved six rats out of seven. The counts, however, suggest that while survival is unequivocal in the seeded population, it evidently is not solely dependent on reducing the numbers of pseudomonads in the challenged animal. The counts for the first two days were comparable; at day five, there was a difference of only 1.8 logs between the two sets.

When a moderately virulent strain, SRU 12-2-2(65) was tested, the picture once again changed. This strain, on larger assays, was lethal for about 70 per cent of test rats. Treatment permitted all rats to survive. The counts here resemble those that one might have assumed would be typical of all treated animals: levels of 10^4 in treated rats, and a rise from 10^5 to 10^7 in the controls. Similar data have been accumulated in other sets of rats during recent therapy trials, and it is evident that in the animal model at least the topical therapy with sulfamylon may exert its effect by direct suppression of growth in the burn, or it may permit growth of the virulent strain and still prevent lethal invasion.

The biopsy technic has offered an improved tool for studying another facet of the burn infection problem, which is the relationship between size of inoculum and the likelihood of lethal invasion. This subject was touched on by Order,[2] but, as with other aspects of the burn problem, has not been studied with several strains of varying virulence.

Table 3 presents the behavior of three Pseudomonas strains of recent acqusition and of two standard strains, when inocula of different densities were employed. The new strains, like several of older pedigree in this laboratory, would be rated as virulent; yet with reduced inoculum, they were not. However, the numbers appearing in the burn wound are not necessarily similar. The more highly virulent strains (SRU 8-28-3(63) and SRU 12-4-4(59)) may start with reduced count for two days, followed by a rise to a lethal level. Or, with SRU 12-4-4(59) (less virulent than SRU 8-28-3(63)) the reduced inoculum may set up an infection which even at five days was still at the 10^5 level. Nevertheless, death supervened at 11 days.

Table 3. Quantitative Bacteriology by Biopsy on Conventional and Reduced Inocula in Burned Rats Seeded with Pseudomonas aeruginosa

Strain	Inoculum	Biopsy Counts of Eschar Postburn Day			Day Died
		1	2	5	
SRU 8-28-3(63)	10^8	3.4×10^2	1.1×10^8	1.2×10^9	12
	10^4	4.0×10^1	5.0×10^3	2.3×10^7	9
SRU 12-4-4(59)	10^8	4.5×10^4	8.0×10^4	1.5×10^8	10
	10^4	3.0×10^3	3.0×10^7	6.0×10^5	11
SRU 12-19-2(65) (new strain)	10^8	1.5×10^8	5.2×10^6	6.8×10^8	10
	10^4	4.6×10^2	1.5×10^2	1.8×10^4	Survived
SRU 10-29-17(65) (new strain)	10^8	3.0×10^9	1.5×10^8	4.2×10^8	14
	10^4	6.8×10^8	5.6×10^6	6.0×10^7	Survived
SRU 12-2-2(65) (new strain)	10^8	2.8×10^6	5.0×10^2	9.0×10^7	16
	10^4	5.0×10^3	5.0×10^3	5.0×10^3	Survived

The new strains exhibit two patterns in the response to small inoculum. SRU 12-.9-2(65) and SRU 12-2-2(65) failed to proliferate in the eschar, while SRU 10-29-17(65) promptly produced a count up to one-tenth that found in the rat with a lethal 10^8 inoculum. It is of interest to note that the continuance on a fixed level of bacterial content often seen in rats with topical treatment sufficient to ensure survival but at a sub-optimal dosage level.

The serial biopsy technic can evidently be employed without effecting the survival potential of the burned rat. It offers a means of observing the course of events in the tissues of individual rats during infection, and its use has revealed a level of complexity hitherto unsuspected, of the bacterial population dynamics in infected, treated animals. It furnishes also a means of assessing the behavior of non-lethal strains in burned rats. Its use should further our understanding of the dynamics of infection in burns and provide a useful adjunct to the criteria of effectiveness of topical therapy in infected burns.

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1. Lindberg, R.B., Brame, R.E., Moncrief, J.A., and Mason, A.D., Jr.: Prevention of invasive Pseudomonas aeruginosa infection in seeded, burned rats by use of topical Sulfamylon cream. Fed. Proc. 23:1725, 1964.

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3. Lindberg, R.B., Brame, R.E., Latta, R.L., Moncrief, J.A., and Mason, A.D., Jr.: Experimental therapy with a topical sulfamylon cream in burned Pseudomonas-infected rats. U.S. Army Surgical Research Unit, Annual Progress Report, 30 June 1963.

Publications

None.

Presentations

Lindberg, R.B.: The current status of topical chemotherapy in burns. Presented at Symposium on Recent Advances in Burn Treatment, New England Medical Center, Boston, Mass, May 1-3, 1965 (convened by Dr. Don Dressler).

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RESEARCH AND TECHNOLOGY RESUME			2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
				DA 0A6398	CSCRD-103
4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY AR / WHS	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
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10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 133

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (U) Further Studies on Development of Topical Therapy for Severe Burns: Variations in Virulence of Pseudomonas Aeruginosa (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 61	NA	Other | DA

16. PROCURE METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	2	4
		CURRENT FY 66	2	5

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TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: NONE

23. KEYWORDS: Pseudomonas, virulence, infection, sepsis

24 (U) Tech Objective - To determine the relationship between virulence of Pseudomonas aeruginosa strains and invasive burn wound infection.

(U) Approach - Clinically effective control of burn infection by use of Sulfamylon burn cream has been established. The method was based on control of Pseudomonas - infection in seeded burned rats, using selected strains of Pseudomonas aeruginosa. 25 However, a wide variation in virulence of different strains for burned rats was found, and since clinical burn infection is caused by a heterogeneous population of bacterial strains, it was deemed urgent that the virulence of strains of Pseudomonas aeruginosa be correlated with clinical state of the patient, as well as with phage type, and with invasive behavior in the experimental animal.

(U) Progress (Jul 65-Jun 66) - Existence of a wide spectrum of phage types in burn patients was confirmed. Virulence ranged from zero to 100% lethal, but has not as 26 yet been correlated with clinical severity of the illness. Phage type and virulence are not correlated closely. The role of virulence in contributing to spread of a particular strain remains to be assessed.

27. COMMUNICATIONS SECURITY	28	29. DOD CODE	30. PROJECT CODE
a. COMSEC OR COMSEC RELATED (X) b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT
35. EST. FUNDS (In thousands)	36

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A0;4501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: FURTHER STUDIES IN VARIATIONS IN VIRULENCE OF PSEUDOMONAS AERUGINOSA IN THE BURN WOUND

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert B. Lindberg, Colonel, MSC
Russell E. Brame, M.S.
Ruth L. Latta, B.S.
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: FURTHER STUDIES IN VARIATIONS IN VIRULENCE OF PSEUDOMONAS AERUGINOSA IN THE BURN WOUND

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert B. Lindberg, Colonel, MSC
Russell E. Brame, M.S.
Ruth L. Latta, B.S.
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.

6. Reports Control Symbol: RCS-MEDDH-288(R1)

A collection of 30 strains, including 10 from fatal burn wound cases, were assessed for virulence by seeding the burned rat model. A spread of virulence from 100 per cent to zero was observed, with 11 strains in the 80 per cent virulence range. The strains did not show correlation between source or phage type and virulence in burns.

(1) Virulence studies

(2) Burn studies

FURTHER STUDIES IN VARIATIONS IN VIRULENCE OF PSEUDOMONAS AERUGINOSA IN THE BURN WOUND

The laboratory model of Pseudomonas aeruginosa infection in burned, seeded rats has been intensively used in the development of a topical formulation for preventing infection in burn wounds. The virulence of the test strain is a vital factor in assessing topical therapy, but in addition, the as yet unexplained differences in virulence of Ps. aeruginosa strains constitute a major point of interest in the whole question of burn wound infection. It is still not clear whether the differences noted in the experimental model are paralleled in the human. Further study of strain virulence has been carried on.

Materials and Methods

The collection of strains has been directed in part by the history of the strain, by phage pattern, and by morphologically distinct characters in some instances. The technic of handling strains and of seeding, observing and sampling rats has been previously described. The phage typing of the strain is of added interest in establishing another parameter between strain identity and virulence.

The group here reported had no water and soil strains included.

Results

A detailed summary of sources of strains is set down in Table 1, with the nature of the lethal behavior and the phage type. Although the group of patients is relatively homogeneous, the spread of strains and sources is as diverse as was seen when various sources were sampled. Of the 100 per cent virulent strains, four came from blood cultures, the rest from wounds. Three patients in this group died, and of these, two harbored type 21,68. This is not surprising; the type was very common in 1965.

The 50 to 99 per cent virulent group came from four blood cultures, one wound, one urine, and one lung at autopsy. In this group of seven, three patients died. Two of these harbored 21,68; so did a third patient who survived.

The weakly virulent strains, most numerous with 11 strains,

Table 1. Virulence of Patient *Pseudomonas* Strains

Strain	Patient No.	Source	Death/ Total	Survival Days Average	Survival Days Range	Phage Type
GROUP I						
SRU 4-30-3(65)*	1	B/C**	10/10	6.8	5-9	44,1214
SRU 10-29-17(65)	2	Surface	4/4	11.5	9-16	N.T.#
SRU 3-14-4(65)	3	Surface	7/7	9.4	7-15	44,1214,352
SRU 6-26-1(65)	4	B/C	4/4	8.5	6-12	21,68
SRU 11-1-4(65)	5	Surface	4/4	3.5	3-5	2,7,31,44,1214, 68,109,F8,119X
SRU 10-29-1(65)	6	B/C	4/4	10.0	6-16	21,68
SRU 3-16-8(66)	7		8/8	5.7	4-8	Unknown
SRU 12-7-13(65)	8	Surface	6/6	4.8	3-7	2,7,21,24,44,68, 109,352,119X,F7, M4
SRU 12-7-9(65)	9	Surface	6/6	5.1	5-6	2,7,44,109,119X, F8
SRU 10-29-10(65)	10	B/C	4/4	6.7	5-8	2,7,31,73,M4
GROUP II						
SRU 10-29-3(65)	11	Lung	3/4	8.0	-	N.T.
SRU 5-12-1(65)	12	B/C	9/10	9.4	7-11	21,31,68
SRU 8-5-8(65)	13	B/C	3/4	8.6	7-10	21,68
SRU 9-18-6(65)	14	B/C	3/4	9.6	3-14	7,31
SRU 12-6-11(65)	15	Urine	5/6	10.2	7-12	N.T.
SRU 12-22-4(65)	16	Surface	5/6	10.8	8-13	21,68
SRU 12-6-12(65)	17	B/C	5/6	10.4	9-13	21,68
GROUP III						
SRU 12-24-14(65)	18	Rt.foot	3/6	13.6	8-20	N.T.
SRU 4-26-5(65)	19	B/C	2/10	20.0	13-28	31
SRU 3-27-8(65)	20	Surface	2/10	11.0	10-12	31,68
SRU 4-3-10(64)	21	Surface	1/11	21.0	-	31
SRU 4-22-6(65)	22	Rt.arm	1/4	20.0	-	24,31,44,1214,352, 119X,M6,F7
SRU 8-2-6(65)	23	Lt.arm	2/4	14.0	12-16	119X,M6
SRU 8-23-5(65)	24	B/C	1/4	3.0	-	2,7,73
SRU 12-2-2(65)	25[A]	Liver	1/6	13.0	-	Unknown
SRU 8-9-2(65)	26	Rt. ear	1/6	12.0	-	68,F7
SRU 12-19-2(65)	27	Urine	1/6	12.0	-	119X
SRU 12-9-6(65)	28	Urine	3/5	11.2	9-13	21,68
GROUP IV						
SRU 4-19-5(65)	29[B]	B/C	0/13	-	-	109
SRU 12-2-11(65)	25	Rt.lung	0/6	-	-	21,68,F10

SUMMARY:	No. Of Strains	Mortality: Total	Per Cent Mortality	Avg.Surv. Time	Survival Range
I. 100	10	52/52	100	7.2	5.3 - 10.2
II. 51-99	7	33/40	80	9.5	7.0 - 13.2
III. 1-50	11	18/72	25	13.7	9.7 - 15.0
IV. Avirulent	2	0/19	-	Less than 21 days	Less than 21 days

* Suffix (65) connotes strain isolated in 1965, etc.

** B/C : Blood culture

\# N.T.: Non typable

[A] Pt. 25 shows two entries; two different isolates from same patient.

included three fatalities. One of these strains came from blood, one from autopsy, one from urine.

The two non-virulent strains each came from a fatal case, one of whom contributed a strain also in Group III.

The phage patterns do not represent a particularly selected group. They are, in fact, representative of the entire collection of strains observed in the unit in 1965. As in previous collections, there is no striking correlation between source and virulence. All of these patients were treated with sulfamylon.

Killing times increase as the severity of virulence decreases. This is entirely logical but merits noting, since there is actually no basis yet known for correlating these two attributes.

The attributes of the entire collection are summarized in Table 2. The earlier presumptive distribution of virulence over a spectrum of degrees appears to have been substantiated by this series.

The basis for virulence has not been uncovered. The collection presented will contribute to the further evaluation of new agents, and to the separation of strains varying in tissue localization. The chemical or cultural basis for virulence has not yet been recognized. Such collections as these contribute, however, to its recognition and thus to better understanding of the basis for pathogenesis of Pseudomonas burn wound infection.

Presentations and/or Publications

None.

Table 2. Summary of Individual Strains Tested for Virulence, 1965

	Degree of Virulence	No. Strains	Death/ Total	Per Cent Mortality	Survival Time - Days	
					Average	Range
I	100%	10	57/57	100	7.2	5.3 - 10.2
II	51-99%	7	33/40	80	9.5	7.0 - 13.2
III	1-50%	11	18/72	25	13.7	9-7 - 15.0
IV	Avirulent	2	0/9	-	< 21	< 21

GPO 770864

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 08 6395	CSCRD-103

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11. TITLE: (U) Assessment of Virulence using Experimental Burn Infections with Proteus, Aerobacter and Serratia Strains (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 65	NA	Other DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	2

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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS

Infection, burns, Aerobacter, Proteus, Serrata

24. (U) Tech Objective - Effective topical chemotherapy, preventing burn wound sepsis, has been evolved using Pseudomonas aeruginosa as the test organism. Burn wounds treated effectively with Sulfamylon HCl still harbor fecal bacteria in most instances, and it is probable that the Enterobacteriaceae contribute to the morbidity and mortality of large burns. An experimental infection model is needed if the pathogenic role of Aerobacter, Proteus and other Enterobacteriaceae is to be understood, as a necessary prerequisite for its treatment. Demonstration of burn wound virulence and acquisition of an experimental burn wound model for Enterobacteriacea are the objectives of this study.

(U) Approach - Burned rats are to be seeded with suitable wound strains of Enterobacteriacea in a system resembling the Pseudomonas aeruginosa model.

(U) Progress (Jul 65-Jun 66) - Proteus spp (23 strains) Aerobacter (11 strains) and Serratia sp (5 strains) were tested on the burned rat. Eleven (47%) of Proteus strains showed in some degree capacity to produce lethal septicemia; 6 of these killed 50% or more of test animals. Wound sepsis was not evident. Only 2 strains (18%) of Aerobacter showed even minimal killing ability. Two of the 5 Serratia marcessens were virulent; one produced burn wound sepsis in 75% of animals seeded. The demonstrations of Enterobacteriaceae in burn wounds makes plausible their contribution to burn morbidity, and makes possible experimental chemotherapy studies to achieve control of this aspect of the burn wound problem.

27. COMMUNICATIONS SECURITY	28.	29. DOD CLASS	30. SUBJECT CODE
a. COMSEC INFO b. NOT COMSEC RELATED X		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
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35. [illegible] FUNDS (In thousands)	36.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: ASSESSMENT OF VIRULENCE USING EXPERIMENTAL BURN INFECTIONS WITH PROTEUS, AEROBACTER AND SERRATIA STRAINS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert B. Lindberg, Colonel, MSC
Russell E. Brame, M.S.
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

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ABSTRACT

1. PROJECT NO. 3A014501B/1R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: ASSESSMENT OF VIRULENCE USING EXPERIMENTAL BURN INFECTIONS WITH PROTEUS, AEROBACTER AND SERRATIA STRAINS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert B. Lindberg, Colonel, MSC
Russell E. Brame, M.S.
John A. Moncrief, Colonel, MC
Arthur D. Mason, Jr., M.D.

6. Reports Control Symbol: RCS-MEDDH-288(R1)

A series of 23 strains of Proteus spp, 11 Aerobacter-Klebsiella group, and 5 Serratia marcessens were tested on the burned rat for evidence of potential invasive capability. Eleven Proteus strains of different species were capable in some degree of producing lethal septicemia. Aerobacter spp. furnished only two strains with marginal invasive capability. Serratia marcessens produced two strains of five with potential for tissue invasion, with one lethal for three-fourths of the test animals. The relationship of Enterobacteriaceae to burn wound infection is further validated by these findings, and increased scope in exploring chemotherapy of the burn wound is obtained.

(1) Virulence studies
(2) Proteus spp
(3) Aerobacter Klebsiella
(4) Serratia marcessens

ASSESSMENT OF VIRULENCE USING EXPERIMENTAL BURN INFECTIONS WITH PROTEUS, AEROBACTER AND SERRATIA STRAINS

Since the delineation of burn wound sepsis as the major lethal mechanism in burn infections, virtually all emphasis has been placed on the role of Pseudomonas aeruginosa as the major offending bacterium. However, with effective topical chemotherapy now established as the optimal method for prevention of burn wound sepsis, the burned patient displays a bacterial flora, and the clinical signs of a wound infection which, while it may be less virulent than Pseudomonas invasion, still plays a major role in burn morbidity, and may indeed represent a factor in the mortality of severe burns. The wound flora includes, in addition to Pseudomonas, chiefly, Aerobacter, Proteus, and other Enterobacteriaceae strains during the first two weeks postburn. The numbers of these strains are reduced during intensive sulfamylon therapy but readily reappear after hydrotherapy sessions and at the latter part of each "buttering" period. The total of E. coli, Proteus, and Aerobacter strains in the blood stream in terminal illness in major burns constitutes at least half of the species appearing in these blood cultures, and the assessment of the invasive potential of such organisms in experimental burn wounds thus appears to be justified. The uncontrolled burn wound sepsis case itself seldom represents a pure culture of Pseudomonas aeruginosa, and the mixed nature of these infections calls for better understanding of the role of Enterobacteriaceae in burn wounds.

Materials and Methods

The objective of this study has been to determine ability of strains to produce burn wound invasion, not merely to demonstrate toxicity of the strains. The basic technic has been to seed 200-220 gm rats prepared by the standard Surgical Research Unit burn preparation. Inoculation of one ml of broth culture, suitably diluted was made within 90 minutes after burning. Rats were observed for 21 days for evidence of lethal wound invasion. Seedings were made on blocks of four or five animals at a time. The strains tested included those recovered from the blood stream, burn wounds, respiratory tract and autopsy tissues when available.

Proteus Strain. This study included 23 strains of Proteus, consisting of 9 Proteus mirabilis, 4 Proteus morganii, 1 Proteus

rettgeri, and 9 undifferentiated strains. Sources included wound surfaces 12, urine 4, respiratory tract 3, and 4 from other sources. The blood sources included one septicemia regarded as the cause of death, in a severe burn of 65 per cent of body surface.

Results

The virulence evidence for Proteus strains was greater than had been anticipated. Out of 23 strains tested, 11 were capable of killing in at least 20 per cent of the animals seeded. Extremely virulent strains were not found as often as was the case with Ps. aeruginosa,[1,2] but invasive infection leading to septicemia unquestionably occurred.

Table 1 summarizes the results with non-virulent Proteus strains. It will be seen that inocula up to 10^{11} were used, with no indication that the animals were affected. Cultures of the lesions up to eight days post-seeding showed that Proteus strains were present on the wounds, but no evidence of penetration of viable tissues was seen.

The virulent strains were recognized by death of the inoculated rats. The results of seeding the 11 virulent strains are summarized in Table 2.

The virulence of the killing strains varied in intensity. Five strains killed one-fourth or less of the rats, two killed 50 per cent, three killed 75 per cent, and one strain (two clones) was 100 per cent lethal. Survival times varied from one to 21 days, with mean values from 2.8 days to 17.5 days.

The weak virulent strains appeared to kill with a septicemia following relatively little change in the wound appearance. The survival times of fatally infected rats extended up to two weeks. The more lethal strains (50%) still did not kill more rapidly, but when three-fourths of the seeded rats died, the death time shortened the range of one week or less.

The most virulent strain, SRU 1-23-1(64), was recovered from the blood of a patient with an 84 per cent body surface burn. The patient was treated with Sulfamylon burn cream for eight days postburn, but treatment was suspended when the patient developed pulmonary dysfunction and a metabolic acidosis. At autopsy, burn wound sepsis was not present.

Table 1. *Proteus spp* Non-virulent in Burned Seeded Rats

Strain	Accession No. (SRIJ)	Source	Inoculum	Death/Total
Proteus spp	10-23-19(65)	Urine	10^{11}	0/8
Proteus spp	1-18-4 (64)	Wound	10^{8}	0/8
Proteus spp	1-18-4 (b)	Wound	10^{6}	0/4
Proteus mirabilis	3-24-10(64)	Wound	10^{11}	0/6
Proteus mirabilis	6-29-18(65)	Urine	10^{8}	0/8
Proteus mirabilis	3-11-5 (64)	Surface	10^{8}	0/6
Proteus mirabilis	3-17-1 (64)	Wound	10^{8}	0/6
Proteus morganii	3-16-5 (64)	Wound	10^{11}	0/6
Proteus morganii	3-19-1 (64)	Wound	10^{11}	0/6
Proteus morganii	2-30-3 (64)	Wound	10^{8}	0/6
Proteus morganii	2-20-4 (64)	Wound	10^{8}	0/4
Proteus rettgeri	2-20-4 (64)	Wound	10^{8}	0/6

Strains tested = 12
Total rats seeded = 74

Table 2. Behavior of Virulent *Proteus* Strains on Burned Rats

Strain	Accession No. (SRU)	Source	Seeded With	Death/Total	Survival (Days)	
					Range	Average
Proteus spp	8-4-6 (65)	Trachea	10^{12}	1/4	-	11.0
Proteus spp	2-11-9(66)	Rat biopsy	10^{11}	1/4	-	9.0
Proteus spp	11-29-8(65)	Sputum	10^{8}	2/4	14-21	17.5
Proteus spp	2-8-9 (66)	Urine	10^{8}	2/15	8-11	9.5
Proteus spp	11-22-3(65)	Urine	10^{8}	6/8	2-12	7.6
Proteus spp	11-22-7(64)	Trachea	10^{7}	4/7	11-14	13.0
Proteus mirabilis	5-10-6(65)	Wound	10^{8}	1/4	-	12.0
Proteus mirabilis	2-15-6(64)	Leg wound	10^{8}	2/8	12-16	14.0
Proteus mirabilis	1-23-1a(64)	Blood	10^{8}	16/16	2-10	3.2
Proteus mirabilis	1-23-1b(64)	Blood	10^{6}	6/8	1-4	3.5
Proteus mirabilis	8-5-6 (65)	Wound	10^{6}	3/4	2-3	2.8

The patient had seven positive blood cultures between the 7th and 16th postburn day, and died on the 19th postburn day. There were three cultures positive for Aerobacter, two for staphylococci, two (on the 14th and 16th postburn days) for Pseudomonas, and on the 8th postburn day, a mixed Aerobacter and Proteus blood culture. The strain thus can not be said to have played a conspicuous part in his illness, but its behavior in the burned rat was remarkable. The rats, seeded with as few as 10^6 organisms per burn, became moribund in as little as 18 hours, showed bleeding at the nostrils, were lethargic, passed no feces, and expired within three days. Later passages showed a wider range of killing; one rat survived for 10 days. The wound itself did not show the hemorrhagic surface appearance which typifies Pseudomonas infections.

Quantitative autopsies of moribund rats showed bacterial content much lower than would be found in moribund Pseudomonas-seeded rats. Table 3 summarizes this information.

The bacterial counts are on the order of two to four logs lower than would be expected in a series of rats moribund with a Pseudomonas-seeded septic burn. Further studies of the Proteus strains in the infected rat are projected. One indication to be pursued further was the observation that when certain Proteus strains are seeded simultaneously with virulent Pseudomonas cultures, the rats fail to succumb, although the Pseudomonas alone is lethal.

Aerobacter-Klebsiella Group. Organisms of the Aerobacter Klebsiella group are ubiquitous in severe burns, but do not present an unequivocal picture of invasion in human wounds. Eleven strains of Aerobacter were tested on the burn rat model, including two which were sent to this laboratory as allegedly invasive for burned rats.

Table 4 summarizes the results of tests on 11 strains of Aerobacter-Klebsiella.

To date there is little indication that the Aerobacter Klebsiella group possesses marked potential for invading the rat burn model wound. Two strains produced one death each, and have been retested without any evidence of a greater virulence potential than was initially shown. The absence of invasive activity is consistent with the observation that

Table 3. Proteus, SRU 1-23-1(64), in Burned Rats Sacrificed at Two Days Postseeding

Rat No.	Blood	Skin (eschar)	Lung	Liver	Spleen	Kidney
1	Proteus mirabilis	9.9×10^8	4×10^8	4.1×10^5	7×10^3	3.4×10^1
2	Proteus mirabilis	1.2×10^9	3.2×10^6	3×10^5	7×10^5	3×10^5
3	Proteus mirabilis	5.5×10^8	6×10^5	7×10^5	7.6×10^5	1.6×10^6

Table 4. Virulence of Aerobacter-Klebsiella Strains Isolated from Burned Patients 1965-1966

Organism	Accession No.	Source	Inoculum	Death/ Total	Survival Days Postburn
A. aerogenes	SRU 4-20-3 (65)	Lung	2.0×10^8	0/4	-- *
A. aerogenes	SRU 6-26-6 (65)	Left calf	1.2×10^8	1/4	12
A. aerogenes	SRU10-25-11(65)	Trachea	2.0×10^8	0/4	-- *
A. aerogenes	SRU10-22-2 (65)	Cut down	7.0×10^8	1/4	6
A. aerogenes	SRU 1-15-5 (66)	Abd.eschar	2.1×10^8	0/4	-- *
A. aerogenes	SRU 2-7-22 (66)	Rt. arm	4.0×10^8	0/3	-- *
A. aerogenes	SRU 2-12-16(66)	Rt. lung	2.0×10^8	0/4	-- *
A. aerogenes	SRU 2-15-9 (66)	Spleen	5.0×10^8	0/4	-- *
A. aerogenes	3-16-9 (66)	Wound	4.0×10^8	0/8	-- *
A. aerogenes	SRU 2-7-22 (66)	Rt. arm	1.3×10^9	0/4	-- *
A. aerogenes	3-22-22(66)	Wound	6.0×10^{10}	0/10	-- *

* Rats survived beyond the duration of experiment.

wounds heavily colonized with Aerobacter may show little indication of active infection. The alleged invasive strains from another burn center showed no sign of virulence.

Serratia marcessens. Invasive behavior of the chromagen Serratia marcessens is rare but has been reported. When several wounds revealed Serratia marcessens present in the burn ward, five strains were collected and tested on the burn wound rat model for virulence. One strain was found to possess a significant degree of virulence as shown in Table 5.

The lesions in the rats dying of Serratia infection resembled those of Pseudomonas aeruginosa seeded rats. As a further confirmation of the potential burn lethality of this species, the strains were studied for penetration of viscera and the burn wound. It was found that visceral penetration did, indeed, occur, as summarized in Table 6. The concentration of organisms rose in the eschar to the 10^8 - 10^9 level, much as the count does in the Pseudomonas model. In the viscera, quantities with the SRU-McK(65) strain, which is virulent, were lower than they were with the non-lethal SRU 11-9-2(65). It had been hoped that the lethality of this species would permit it to be used for further definitive animal studies, but this does not appear possible with the strains thus far studied.

Summary

Virulence studies have revealed that almost half of 23 Proteus strains studied are capable of invading the burned rat with lethality varying from 20 per cent to 100 per cent. The most lethal strain found produces a fulminating septicemia that is the most rapid yet seen in a burned animal model.

Aerobacter-Klebsiella strains appear to be almost devoid of ability to invade the burned rat.

Serratia marcessens include lethal strains and others which invade the burned tissue and the viscera without killing.

The behavior of these Enterobacteriaceae strains broadens the concept of burn wound sepsis by revealing the potential role of other burn wound flora. The strains studied offer added tools for the study of topical chemotherapy of the burned wound.

Table 5. Virulence of Strains of Serratia marcessens in Burned Rats

Strain	Source	Inoculum	Death/Total	Survival (Days)	
				Average	Range
SRU McK (65)	Burn wound	10^8	7/15	3.7	2-5
SRU 9-1-4 (65)	Autopsy eschar	10^8	0/5	-	-
SRU 9-1-16(65)	Autopsy eschar	10^8	0/5	-	-
SRU 9-1-13(65)	Autopsy eschar	10^8	0/5	-	-
SRU 11-9-2(65)	Urine	10^8	0/5	-	-

Table 6. Penetration of Tissues of Seeded Burned Rats by Strains of *Serratia marcessens*

Strain	Postburn Day	Tissue Content					
		Blood	Eschar	Lung	Liver	Spleen	Kidney
SRU McK (65)	1	0	1×10^6	1×10^1	0	0	0
	2	+	4×10^8	1.5×10^3	4.8×10^3	1×10^4	6×10^4
	3	+	8×10^8	3×10^5	3.4×10^1	5×10^2	0
	5	+	3×10^8	2×10^2	1.1×10^3	5×10^4	4×10^2
SRU 11-9-2 (65)	1	0	1×10^6	0	0	0	0
	2	+	7×10^8	3×10^0	5×10^6	2×10^2	1.3×10^1
	3	0	1×10^9	1×10^2	3.8×10^5	5×10^2	7.4×10^1
	5	+	8×10^8	3×10^4	5×10^2	6×10^2	2×10^2

References

1. Lindberg, R.B., Moncrief, J.A., Brame, R.E., and Mason, A.D., Jr.: Variation in virulence of Pseudomonas aeruginosa from severe burns. Fed. Proc. 24:571,1965.

2. Lindberg, R.B., Brame, R.E., Moncrief, J.A., and Latta, R.L.: Further studies on development of topical therapy for severe burns: Variation in virulence Pseudomonas aeruginosa. U.S. Army Surgical Research Unit Annual Progress Report, 30 June 1965.

Presentations

Lindberg, R.B.: The animal model in the study of burn wound therapy. Presented at the Ninth Annual Burn Symposium, Chicago, Illinois, 14 November 1965.

Publications

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 0A6979	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A, NEW"	U U	NA	NL	A, WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 164

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) Evaluation of Dimethyl Sulfoxide in the Burned Rat with Pseudomonas Aeruginosa (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	10 65	12 65	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE b. NUMBER c. TYPE d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	2

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INVESTIGATOR PRINCIPAL ASSOCIATE: Harrel L. Walker
TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Dimethyl Sulfoxide, burned rat model, Pseudomonas Aeruginosa

24. (U) Tech Objective - To establish the value of Dimethyl Sulfoxide in burn wound sepsis in rats.

25. (U) Approach - The use of Dimethyl Sulfoxide as a therapeutic agent on Pseudomonas Aeruginosa burn wound sepsis model. Treatment of the model of burn wound sepsis was with DMSO, Sulfamylon, or both, incorporated in a water-soluble base. The treatment agents were applied topically on post burn days 1, 3 and 5 in 3 gm. aliquots.

(U) Progress (Oct 65-Dec 65) - Treatment of the burn model animals with DMSO fails to protect the animal with burn wound sepsis. Pseudomonas aeruginosa produces a consistent, lethal infection in rats that can be reversed when treated with Sulfamylon.

26.

27. COMMUNICATIONS SECURITY	28.	29. DOD TYPE	30. SUMMARY CODE
☐ a. CONTROL OF CLASSIFICATION ☒ b. NOT CLASSIFIED		AP	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. DEV FUNDS (In thousands) (FY)	36.

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FINAL REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EVALUATION OF DIMETHYL SULFOXIDE IN THE BURNED RAT WITH PSEUDOMONAS AERUGINOSA

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 October 1965 to 30 June 1966

Investigators:

John A. Moncrief, Colonel, MC
Harrel L. Walker, M.S.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: EVALUATION OF DIMETHYL SULFOXIDE IN THE BURNED RAT WITH PSEUDOMONAS AERUGINOSA

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 October 1965 to 30 June 1966

5. Investigators: John A. Moncrief, Colonel, MC
Harrel L. Walker, M.S.

Treatment of the burn model animals with dimethyl sulfoxide fa 's to protect the animal with Pseudomonas burn wound sepsis. Pseudomonas aeruginosa produces a consistent, lethal infection in rats that can be reversed when treated with Sulfamylon.

The treatment of burn infected animals with a combination of dimethyl sulfoxide and high and low doses of Sulfamylon fails to improve treatment with Sulfamylon alone.

(1) DMSO
(2) Burned Rat Model
(3) Pseudomonas aeruginosa

EVALUATION OF DIMETHYL SULFOXIDE IN THE BURNED RAT WITH PSEUDOMONAS AERUGINOSA

Dimethylsulfoxide (DMSO) has been advocated for many conditions, particularly painful arthralgias. While supporters of DMSO have little data to substantiate their claims, their claims are loud and demand reclamer. Since DMSO is an exceptionally good organic solvent, it has been suggested it be used as an effective vehicle for various biologically effective agents including the antibacterial ones. In addition, DMSO is supposedly effective against microorganisms.

To test the effectiveness of DMSO as a vehicle for antibacterial agents as well as a suppressive agent itself, a study was designated utilizing the burn wound sepsis model.[1]

Materials and Methods

Adult, Sprague-Dawley (Holtzman strain) rats were used throughout this investigation. The rats were stabilized on Ralston Purina Lab Chow and water ad libitum for at least two weeks prior to any experiment. The weight range of each group was within ± 10 gm from the average for the group.

Experimental and control animals were anesthetized with sodium pentobarbital (1 mg/25 gm) for the burning procedure.[1]

Test organism Pseudomonas aeruginosa (SRU 12-4-4(59) was the microorganism employed throughout the study.[1]

Treatment of the model of Pseudomonas burn wound sepsis was with DMSO, Sulfamylon, or both, incorporated in a water-soluble base. The treatment agents were applied topically on postburn days 1, 3 and 5 in 3 gm aliquots. The aliquots were weighed on tongue blades from the following preparations:

(1) 33% DMSO + 66% base.
(2) 33% DMSO + 65% base + 1% Sulfamylon.

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* Sulfamylon used in these experiments was furnished Sterling-Winthrop Research Institute, Rensslaer, New York.

(3) 95% base + 1% Sulfamylon
(4) 33% DMSO + 56% base + 10% Sulfamylon
(5) 90% base + 10% Sulfamylon

Results

1. Treatment of the burn model with DMSO alone. A group of 20 animals was subjected to a 20% total body burn. These animals were then treated with preparation (1) on postburn days 1, 3 and 5. Of these 20 animals, one died on day 11. The remaining 19 animals lived through a 30-day observation period (see Table).

2. Treatment of the Pseudomonas-seeded burn model with DMSO alone. Another group of 20 animals was subjected to a 20% total body burn and seeded with Ps. aeruginosa. These animals were then treated with preparation (1) on postburn days 1, 3 and 5. Of these 20 animals, 18 died between days 6 and 11. The remaining two animals lived through a 30-day observation period.

3. Treatment with DMSO and 1% Sulfamylon of the burn model seeded with Pseudomonas aeruginosa. A group of 19 animals was subjected to a 20% total body burn and seeded with Ps. aeruginosa. These animals were then treated with preparation (2) on postburn days 1, 3 and 5. Of these animals, 18 died between days 5 and 9. The remaining one animal lived through a 30-day observation period.

4. Treatment with 1% Sulfamylon of the burn model seeded with Ps. aeruginosa. A group of 20 animals was subjected to a 20% total body burn and seeded with Ps. aeruginosa. These animals were then treated with preparation (3) on postburn days 1, 3 and 5. Of these animals, 14 died between days 5 and 10. The remaining six animals lived through a 30-day observation period.

5. Treatment with DMSO and 10% Sulfamylon of the burn model seeded with Ps. aeruginosa. A group of 19 animals was subjected to a 20% total body burn and seeded with Ps. aeruginosa. These animals were then treated with preparation (4) on postburn days 1, 3 and 5. Of these animals, one died on day 16. The remaining 18 lived through a 30-day observation period.

Treatment of 20 Per Cent Total Body Burn Rats with DMSO and Sulfamylon

	No. of Animals	No. Died	Time of Death (days)	Volume of 18-hour Culture (ml)	Strains of Pseudomonas aeruginosa (SRU)
33% DMSO (1)	20	1	11		
33% DMSO (1)	20	18	6-11	0.1	12-4-4(59)
DMSO + 1% Sulfamylon (2)	19	18	5-9	0.1	12-4-4(59)
1% Sulfamylon (3)	20	14	5-10	0.1	12-4-4(59)
33% DMSO + 10% Sulfamylon (4)	19	1	16	0.1	12-4-4(59)
10% Sulfamylon (5)	10	2	8-11	0.1	12-4-4(59)
Burn control	18	0			
Infection control	10	10	6-8	0.1	12-4-4(59)

6. Treatment with 10% Sulfamylon of the burn model seeded with Ps. aeruginosa. A group of 10 animals was subjected to a 20% total body burn and seeded with Ps. aeruginosa. These animals were then treated with preparation (5), on postburn days 1, 3 and 5. Of these animals, two died between 8 and 11 days. The remaining eight animals lived for a 30-day observation period.

7. Burn control. A group of 18 animals were subjected to a 20% total body burn. Of these animals, all 18 lived for a 30-day observation period.

8. Burn control seeded with Ps. aeruginosa. A group of 10 animals was subjected to a 20% total body burn. Of these 10 animals, all died between days 6 and 8.

Discussion

Treatment of the burn model animals with dimethyl sulfoxide fails to protect the animal with Pseudomonas burn wound sepsis. Pseudomonas aeruginosa produces a consistent, lethal infection in rats that can be reversed when treated with Sulfamylon.

The treatment of burn infected animals with a combination of dimethyl sulfoxide and high and low doses of Sulfamylon fails to improve treatment with Sulfamylon alone.

References

1. Walker, H.L., Mason, A.D., Jr. and Raulston, G.L.: Surface infection with Pseudomonas aeruginosa. Ann. Surg., 160:297-305, 1964.

Publications and/or Presentations

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6392	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 183

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (U) The Effect of Sulfamylon, 0.5% Silver Nitrate and Open Therapy on Eschar and Granulation Tissue in the Burned Rat (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	09 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	a. DATE b. NUMBER NA c. TYPE d. AMOUNT	PRIOR FY 65	0	0
		CURRENT FY 66	1	2

19. GOVT LAB/INSTALLATION/ACTIVITY
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TEL.: 512 - CA1 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Silver nitrate, Sulfamylon, rats, escher, granulation

24. (U) Tech Objective - The objective was to evaluate in rats the claim that 0.5% silver nitrate therapy of third degree burns results in earlier eschar separation, less granulation tissue and less subsequent wound contracture.

(U) Approach - Standard uninnoculated 30% third degree burn wound rats were treated with open therapy alone, Sulfamylon applied twice daily, silver nitrate soaks twice daily and a combined regimen of silver nitrate for three weeks followed by Sulfamylon for three weeks. All treatment was given for six weeks and weekly eschar biopsies were taken for evaluation.

(U) Progress (Sep 65-Jun 66) - There was no measurable difference in the granulation layer thickness. The eschar did separate more rapidly in the silver nitrate and control groups, but this was associated with increased intra-eschar bacterial proliferation and with more rapid gross wound contracture.

25.

27. COMMUNICATIONS SECURITY	28.	29. GSD CODE	30. BUDGET CODE
☐ a. CONFIDENTIAL OR CRYPTO RELATED ☒ b. NOT CRYPTO RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. GOVT. FUNDS (In thousands)	36.

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FINAL REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECT OF SULFAMYLON, 0.5 PER CENT SILVER NITRATE AND OPEN THERAPY ON ESCHAR AND GRANULATION TISSUE IN THE BURNED RAT

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 September 1965 to 30 June 1966

Investigators:

Lawrence H. Foster, Jr., Major, MC
Franklin D. Foley, Captain, MC
Harrel L. Walker, M.S.
Beverly L. Worley, B.S.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: THE EFFECT OF SULFAMYLON,0.5 PER CENT SILVER NITRATE AND OPEN THERAPY ON ESCHAR AND GRANULATION TISSUE IN THE BURNED RAT

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 September 1965 to 30 June 1966

5. Investigators: Lawrence H. Foster, Jr., Major, MC
Franklin D. Foley, Captain, MC
Harrel L. Walker, M.S.
Beverly L. Worley, B.S.

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

In rats with uninoculated 30 per cent full-thickness burns treated by (1) open method, (2) twice-a-day Sulfamylon * application, or (3) twice-a-day silver nitrate soaks, there is no gross difference in the thickness of the granulation layer, although the surface area of the granulation bed is greater in the Sulfamylon-treated group. The eschar fragments sooner in silver nitrate-treated and control groups, but this is associated with increased intra-eschar bacterial proliferation; and the wound contracts faster with control or silver nitrate treatment than with Sulfamylon.

(1) Sulfamylon
(2) Silver nitrate
(3) Burns, experimental

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* Supplied by Sterling-Winthrop Research Institute, Rennslaer, New York.

THE EFFECT OF SULFAMYLON, 0.5 PER CENT SILVER NITRATE AND OPEN THERAPY ON ESCHAR AND GRANULATION TISSUE IN THE BURNED RAT

The suppression of bacteria in the burn wound by topical antibacterial agents has prolonged eschar adherence which may permit more growth of granulation tissue before the wound comes to autografting. Our present policy of accelerating eschar separation once it begins, by wet dressings, does allow limited bacterial proliferation and may further stimulate granulation overgrowth which increases the subsequent scar contracture. Other methods of accelerating eschar separation without destroying viable tissue or allowing bacterial proliferation have been investigated without success.

The hypotonicity of 0.5% $AgNO_3$ has been said to result in sufficient osmotic water absorption by the eschar collagen to cause fragmentation and earlier separation, thereby permitting earlier autografting on a less developed granulation base and therefore less eventual scar contracture. It is also possible, knowing that 1% $AgNO_3$ is inhibitory to regenerating epithelium, that 0.5% $AgNO_3$ is inhibitory to the development of excess granulation tissue. This may explain the observations that there is little or no grossly evident granulation tissue during burn wound treatment with $AgNO_3$.[1] If this is correct, the substitution of 0.5% $AgNO_3$ for saline soaks may further accelerate eschar separation, while suppressing bacterial proliferation, and result in less subsequent contracture.[2]

Purpose and Methods

It was the purpose of this investigation to compare histologically the fragmentation and separation of eschar at 1, 2, 3, 4, 5 and 6 weeks postburn and to observe final contracture and granulation layer thickness in four groups of 10 rats, subjected to 30 per cent full-thickness burns and treated as shown in the following schedule.

Treatment and Biopsy Schedule

R_x Schedule	Biopsy Schedule					
	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.
10 Controls Open therapy						
10 Sulfamylon b.i.d. x 6 wks.						
10 0.5% $AgNO_3$ b.i.d. x 6 wks.						
10 Sulfa 3 wks., then $AgNO_3$ 3 wks.						

Results

Gross

The eschar separated one to two weeks sooner in control and silver nitrate-treated groups, with more rapid subsequent wound contracture. At six weeks, the control and $AgNO_3$ groups had scars which measured 4-8 mm in width which were almost completely reepithelialized, whereas the Sulfamylon * group had an area of clean granulation measuring 10-12 mm in width with an equal margin of reepithelialization. The combined Sulfamylon-silver nitrate-treated group had a degree of granulation tissue and contracture intermediate between the group treated with Sulfamylon and that treated with silver nitrate. A total of 11 rats succumbed during the experiment: four in the control group, four in the Sulfamylon group, one in the silver nitrate group, and two in the silver nitrate-Sulfamylon group. All were autopsied and found to have murine pneumonia probably related to repeated general anesthetics. The burn wounds were not inoculated, and no quantitative or qualitative cultures were done. The animals were isolated from infected animals; no gross evidence of diffuse burn wound sepsis was seen.

Microscopic

All biopsies were stained with hematoxylin and eosin and a modified buffered Giemsa stain to facilitate visualization of bacteria.[3] In addition, the biopsies of all groups taken through the fourth week postburn were stained with Movat's[4] Pentachrome I and II, but this was not found to substantially enhance the interpretation of routinely stained hematoxylin and eosin sections in this material.

At one week, there was an equivocal increase in visualized bacteria within the eschar of the $AgNO_3$-treated group; however in the biopsies from the end of the second week there was unequivocal intra-eschar proliferation of

* Supplied by Sterling-Winthrop Research Institute, Rensselaer, New York.

bacteria in the $AgNO_3$-treated and control groups, with negligible numbers of bacteria in the Sulfamylon-treated group.

At three weeks, there was focal bacterial burn wound infection in the $AgNO_3$-treated and control groups, but the Sulfamylon-treated group remained virtually free of invasive bacterial growth.

At four weeks, the eschar was off or in the process of separating in most of the biopsies from the $AgNO_3$-treated and control groups, with considerable intra-eschar bacteria and sub-eschar suppuration. The eschar of the Sulfamylon-treated group contained few bacteria and was still adherent to sub-eschar granulation tissue at this time.

At five and six weeks, eschar had separated in biopsies from all groups, and the wound surface was composed of neutrophiles, granulation and connective tissue in various proportions. Throughout the study period, the thickness of granulation tissue was a variable, dependent on the depth of biopsy, and therefore did not lend itself to meaningful histological quantitation.

Biopsies from the combined $AgNO_3$-Sulfamylon-treated group exhibited a degree of intra-eschar bacterial proliferation and temporal eschar separation somewhere between the other groups, e.g., at four weeks, four of eight eschars had separated in biopsies from this combined treatment group.

Remarks

The method of Sulfamylon application worked well in these rats; however the silver nitrate application was not so successful. Most mornings, two or three rats out of the 20 receiving silver nitrate would be found to have pulled the dressings out from under the plastic cover. This was immediately replaced, but there was no way of knowing how many hours of effective contact the wound had had with the silver nitrate the previous night. The twice-a-day soaking

with silver nitrate and once-a-week dressing change do not compare with the recommended clinical soaking every two hours and dressing change every day.

Summary

We can only conclude that in these rats with uninoculated 30 per cent full-thickness burns treated by (1) open method, (2) b.i.d. Sulfamylon application, or (3) b.i.d. silver nitrate soaks, there is no gross difference in the thickness of the granulation layer, although the surface area of the granulation bed is greater in the Sulfamylon-treated group. The eschar fragments sooner in silver-nitrate-treated and control groups, but this is associated with increased intra-eschar bacterial proliferation; and the wound contracts faster with control or silver nitrate treatment than with Sulfamylon.

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Publications and/or Presentations

None.

GPO 779304

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6382	CSCRD - 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 01 173	NONE

11. TITLE:
(U) Skin Homograft Survival in the Burned Rat (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	11 65	NA	Other DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House		PRIOR FY 65	0	0
		CURRENT FY 66	1	5

19. GOVT LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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	TEL. 512 - CAI 2720 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	NONE

23. KEYWORDS
Experimental burn, homograft, immunologic paralysis

24. (U) Tech Objective - To determine whether prolonged skin graft survival postburn is related to immunologic paralysis.

25. (U) Approach - Rats were pre-sensitized with skin homografts prior to experimental burn. If prolongation of graft survival is due to immunologic paralysis, then pre-sensitization should abolish or modify this phenomenon.

(U) Progress (Nov 65 - Jul 66) - Data currently available are insufficient to justify a detailed report at this time.

26.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.

DD FORM 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: SKIN HOMOGRAFT SURVIVAL IN THE BURNED RAT

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert M. Vertel, Captain, MC
Franklin D. Foley, Captain, MC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963.)

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: SKIN HOMOGRAFT SURVIVAL IN THE BURNED RAT

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert M. Vertel, Captain, MC
Franklin D. Foley, Captain, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

The phenomenon of prolonged skin homograft survival has frequently been observed in burned patients at the US Army Surgical Research Unit. There are a number of reports describing prolonged survival of skin homografts in extensively burned patients. Altered graft reactivity can be produced in burned rats. This animal work, however, is not convincing and it has been suggested that homograft reaction time in the burned rat may actually be shortened. These conflicting findings probably stem from poorly controlled variables such as graft size and crude methods for determining graft viability. The most sensitive criterion for graft rejection is capillary stasis in the graft, observed by stereo-dissecting microscopy. Our study utilizes this sensitive indicator of homograft rejection.

Burned patients do not suffer a reduction in resistance to infection or a loss of capacity to form serum antibody. Burned rats may suffer impaired antibody response to a primary tetanus toxoid injection while their anamnestic response to this antigen is normal or even augmented. These findings are consistent with previous experimental work defining immunologic paralysis. Using pneumococcal polysaccharide as antigen, pre-sensitized animals were quite resistant to induction of immunologic paralysis, requiring much larger doses

of antigen to achieve this result. In the burned animal, the burned tissue is presumably the source of this antigen although bacterial antigens may play a role. It seems likely that prolonged skin homograft survival in burned recipients is also due to some form of immunologic paralysis. If this is the case, then pre-sensitization with skin homografts should abolish or modify this phenomenon.

A study designed to test the above hypothesis is now underway at the Surgical Research Unit. However, there are insufficient data to warrant a detailed report at this time.

(1) Experimental Burn
(2) Homograft
(3) Immunologic Paralysis

GPO 770004

RESEARCH AND TECHNOLOGY RESUME	1.	DOD ACCESSION	DA OB 6387	REPORT CONTROL SYMBOL CSCRD-103
4. DATE OF RESUME: 30 06 66	5. KIND OF RESUME: "A. NEW"	6. SECURITY: U	7. GRADE: NA	8. RELEASE LIMITATION: NL	9. LEVEL OF RESUME: A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 178

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) The Effects of Burns and Sepsis on Secretion of Gastric Acid (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	Nov 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	8

19. GOVT LAB/INSTALLATION/ACTIVITY
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TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Curling's ulcer, gastric acid, gastric physiology

24. (U) Tech Objective - Since there is still no adequate explanation for the association between burns and gastroduodenal ulceration, direct studies of gastric acid secretion were initated. If a pattern of gastric hyperacidity is found, this would provide a basis for both conservative and surgical therapy of Curling's ulcer.

25. (U) Approach - The plan utilizes studies in both burned dogs and patients. Dogs with isolated innervated and denervated gastric pouches are being studied before and following burning to determine whether burning is associated with increases in acid output. Overnight gastric collections have been made in a series of burned patients, and further collections are being made. Thus far, no major problems have been encountered.

(U) Progress (Nov 65-Jun66) - The dog studies are in progress, but no results are available at the present time. Studies in twenty patients do not indicate a consistent pattern of gastric hypersecretion following burns; however, many more studies are necessary before definite conclusions can be drawn.

27. COMMUNICATIONS SECURITY	28.	29. DOD CODE	30. SUBKEY CODE
[ ] a. COMSEC OR COMSEC RELATED [X] b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT
35. EST. FUNDS (In thousands)	36.

DD FORM 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECTS OF BURNS AND SEPSIS ON SECRETION OF GASTRIC ACID

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 November 1965 to 30 June 1966

Investigator:

James A. O'Neill, Jr., Captain, MC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-HEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: THE EFFECTS OF BURNS AND SEPSIS ON SECRETION OF GASTRIC ACID

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 November 1965 to 30 June 1966

5. Investigator: James A. O'Neill, Jr., Captain, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Twelve-hour nocturnal collections of gastric secretion were made in a series of patients with varying-sized burns. Only an occasional patient had evidence of acid hypersecretion. These studies are being continued.

Experimental studies in dogs prepared with innervated and denervated gastric fundic pouches are currently in progress.

(1) Curling's Ulcer
(2) Gastric Acid
(3) Gastric Physiology

THE EFFECTS OF BURNS AND SEPSIS ON SECRETION OF GASTRIC ACID

Gastroduodenal ulceration and gastrointestinal bleeding are commonly associated with thermal burns. Despite the fact that this association has been a recognized clinical entity for many years and despite the fact that many theories have been offered to explain it, the basic etiology is still unknown. Since acid-peptic hypersecretion is known to be the responsible factor in chronic peptic ulcer disease, this seemed to be a logical starting point. Previous studies have been contradictory. Friesen was unable to demonstrate hyperacidity in his experimental burn preparations.[1] On the other hand, Hummel and co-workers reported elevated uropepsin levels in burned patients, especially if their injuries were over 35 per cent of the body surface.[2]

Since indirect indications of gastric activity such as uropepsin output are subject to error, and since they do not necessarily reflect the gastric functional state at the time of collection, direct measurements of gastric secretory activity seemed to be warranted. Little or no information of this sort is available.

Several methods for the estimation and direct determination of gastric acid output are currently available.[6] Direct measurement is the only method which gives some idea of the net effect in the patient or experimental subject. Much information is available on levels of total and free acidity in nocturnal collections from normal individuals and those with peptic ulcer. Hence, this would appear to be a satisfactory basis for comparison with other disease states which are associated with gastric secretory changes. In addition to this, the augmented histamine test has proved to be valuable in the estimation of total secretory potential.[3]

Methods

1. Experimental Studies

Isolated innervated and denervated gastric pouches are being constructed in two groups of dogs, with four in each group. After a one-month recovery period, standard fasted three-hour collections will be made, followed by a Kay test daily for one week. The dogs will then be subjected to 20 per cent full-thickness burns and similar collections made for another week. The collected gastric juice will be analyzed for volume, pH, total titratable acidity (pH 7.0) and free acid.

2. Clinical Studies

Patients with varying-sized burns, sepsis, a past history of ulcer or evidence or suspicion of current gastrointestinal bleeding are being studied. Collections of 12-hour nocturnal gastric secretory output have been made, with special attention given to technical considerations such as tube placement, patient position, tube patency, fractionation of collection periods, and others. Although augmented histamine tests were originally planned for patient study, it has not proved feasible to stimulate acutely ill patients in this fashion so they have not been performed. The nocturnal collections have been analyzed for volume, pH, bile, blood, total titratable acidity and free acid.

Results

1. Experimental Studies

These studies have been initiated, but no results are available at this time.

2. Patient Studies (see Table)

Thus far, 20 patients have had 12-hour nocturnal collections of gastric secretory output made. The patients ranged from nine months to 75 years in age. The total extent of burn ranged from 7 to 95 per cent, with a mean of 49 per cent. Eleven of the 20 patients had a diagnosis of Curling's ulcer made. Six were proven at postmortem examination; one was found at operation, and the other

Summary of 12-Hour Nocturnal Gastric Secretory Studies in Patients

Patient	Per Cent Burn	Volume (ml)	Total Acid (mEq)	Free Acid (mEq)
1	60	165	10.2	5.0
2	24	1200	96.5	55.4
3	39	440	45.6	17.7
4	78½	75	4.4	2.1
5	78½	165	4.1	0.0
6	60	330	15.3	14.3
7	43	155	11.5	3.6
8	36½	410	16.1	6.4
9	71½	555	17.4	3.3
10	24	107	0.7	0.0
11	34	340	9.5	5.1
12	24	204	9.2	5.6
13	36	90	2.6	0.8
14	36	191	0.0	0.0
15	80	180	3.7	0.5
16	22	78	0.0	0.0
17	7	520	1.7	0.0
18	37	310	3.8	0.0
19	77	425	30.6	21.8
20	95	110	6.2	2.2

four were diagnosed clinically because of massive hemorrhage.

If 18 mEq of free acid in a 12-hour collection of gastric secretions is considered to be the upper limit of normal, only two or possibly three of these patients can be considered to have hypersecretion. The patient who had a 12-hour output of 55.4 mEq was 28 years old, had a 24 per cent total body burn and a crushed chest, and bled massively on the third postburn day. The patient with a high normal output of 17.7 mEq of free acid was 75 years of age, had severe chronic pulmonary disease and a 39 per cent total body burn, bled massively, and had multiple gastric and duodenal ulcerations at autopsy. The third patient with hypersecretion put out 21.8 mEq of free acid. He was 20 years old, had a 77 per cent total body burn and bled massively. The other eight patients with ulcers had no evidence of acid hypersecretion. All 20 patients had evidence of gross or occult blood in their collections.

Comment

Up to this point, there does not seem to be any consistent pattern of hypersecretion in association with the early postburn period. Also, although the numbers are small, there does not seem to be evidence for acid hypersecretion in patients with known Curling's ulcer. Only three of the 11 patients with ulcers had hyperacidity. Two of these had associated pulmonary problems, one acute and the other chronic. It is known that both acute and chronic hypercarbia are associated with hypersecretion, so the hypersecretion seen in these patients may not necessarily be related to their burns.[4,5]

It is planned that many more patients will be studied, and it is hoped that the experimental studies will shed further light on the subject.

Summary

Limited studies of nocturnal gastric secretory output in burned patients, half of which had Curling's ulcer,

give no support to the theory that acid-peptic hypersecretion is related to this entity. Additional studies are planned.

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1. Friesen, S.R.: The genesis of gastroduodenal ulcer following burns. An experimental study. Surgery, 28:123, 1950.

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Publications and/or Presentation

None.

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RESEARCH AND TECHNOLOGY RESUME			2. GOVT ACCESSION	3. AGENCY ACCESSION	RES-ENG'G PROJ SYMBOL
				DA UB 6381	CSCRD - 103
4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	NA	NL	A. WORK UNIT

10A. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 172
10B. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) The Influence of Thermal Burns on Gastric Mucous Production (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	11 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE b. NUMBER c. TYPE d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	8

19. GOV'T LAB/INSTALLATION/ACTIVITY
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TEL: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION
Life Sciences

22. COORDINATION
None

23. KEYWORDS
Curling's ulcer, gastric mucin, gastric physiology

24. (U) Tech Objective - This study was undertaken to determine whether unfavorable alterations of the gastric mucous barrier occur following thermal injury. Should such changes be found, further studies will be performed to determine whether mucigogues can be utilized to protect against gastroduodenal ulceration seen so frequently in association with burn injury.

25. Approach - The plan utilizes studies in normal and burned rats. Both histologic and chemical analytic quantitations of gastric mucus are being made. Dogs with isolated antral pouches are also being utilized, and analyses of hexosamine, fucose and sialic acid are being made before and after burning. Thus far there have been no major problems.

26. Progress (Nov 65 - Jun 66) - The initial studies have almost been completed and indicate that the rate of renewal of gastric mucus is markedly diminished following burns and this correlated with a marked increase in the incidence of ulceration. Thus far no definite change in the chemical quality of the gastric mucus secreted has been found. It is hoped that a method can be found to reverse this unfavorable change in mucus output.

27. COMMUNICATIONS SECURITY	28.	29. ORG CODE	30. SUBKEY CODE
		AR	1
31. MISSION OR S/T TYPE		32. PARTICIPATION	
	NA	NA	
33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT		
35. DEF FUNDS (In thousands)	36.		

DD FORM 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE INFLUENCE OF THERMAL BURNS ON GASTRIC MUCUS PRODUCTION

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 November 1965 to 30 June 1966

Investigators:

James A. O'Neill, Jr., Captain, MC
Charles R. Ritchey, Captain, MSC
Arthur D. Mason, Jr., M.D.
Ysidro Villarreal, B.A.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-28 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A0145C1B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: THE INFLUENCE OF THERMAL BURNS ON GASTRIC MUCUS PRODUCTION

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 November 1965 to 30 June 1966

5. Investigators: James A. O'Neill, Jr., Captain, MC
Charles R. Ritchey, Captain, MSC
Arthur D. Mason, Jr., M.D.
Ysidro Villarreal, B.A.

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

No consistent relationship between gastric hyperacidity and Curling's ulcer has been demonstrated. Since gastric mucus has a known protective function, studies were made in this regard.

Histologic and chemical studies of gastric mucosa were made in control and burned rats in order to quantitate the total amount of mucus present. Similar studies were made in dogs with isolated antral pouches, from which collections of mucus were made before and following burning.

The results indicate that following thermal injury there is a marked decrease in the rate of gastric mucous production.

Additional studies in humans are planned, as well as studies related to the use of mucigogues.

(1) Curling's ulcer
(2) Gastric mucin
(3) Gastric physiology

THE INFLUENCE OF THERMAL BURNS ON GASTRIC MUCUS PRODUCTION

The relationship between severe thermal injury and gastroduodenal ulceration is well known. However, the mechanism of ulcer production has not been fully elucidated. The studies of Friesen have indicated the importance of hemoconcentration and resultant mucosal congestion as an etiologic factor.[2] He noted no increase in secretion of gastric acid, although later studies by others have yielded different findings.[5] On this account, it seemed warranted that other aspects of gastric physiology should be investigated in addition to acid-peptic mechanisms.

One possible alternate explanation for gastroduodenal ulceration in the presence of normal gastric acid output is an unfavorable alteration of the gastric "mucous barrier", a well-recognized protective mechanism against mucosal autodigestion.[4] This has been well demonstrated by Wolff and by others who have shown that the gastric mucosa is much more susceptible to damage from gastric juice or various forms of trauma when the mucous layer is removed.[6] This "mucous barrier" may be deficient in volume and thus have a lessened acid-neutralizing ability; it may not be renewed often enough to cover the mucosal surface adequately; or its components may be altered so that the mucus undergoes enzymatic hydrolysis and no longer acts as an acid barrier. The mean buffering capacity of gastric mucus is only approximately 50 mEq/L of gastric mucus, so it is felt that its protective action is primarily explained by its physical properties of viscosity, adhesiveness, and cohesiveness.[4]

Methods

1. Rat Studies

a. Control group. Thirty 250-gm Sprague-Dawley rats were fasted for 24 hours. They were then sacrificed and the stomachs examined for the presence of ulcers. Sections were taken from the glandular stomach for microscopic examination using hematoxylin and eosin and Alcian blue-P.A.S. stains. In addition, the mucosa was scraped from the submucosa and the material subjected to chemical analysis. Determinations of hexosamine, sialic acid, and L-fucose content were made.

b. Experimental group. Thirty 250-gm Sprague-Dawley rats were subjected to standard 20 per cent full-thickness scald burns utilizing boiling water at 99°C. applied for 10 seconds. Twenty-four hours later, they were sacrificed and identical studies performed.

2. Dog Studies

Isolated, denervated antral pouches were constructed in six healthy, adult, mongrel dogs whose weight averaged 20 kilograms. Gastrointestinal continuity was reestablished by means of gastroduodenal anastomosis. Specially constructed Teflon cannulae were utilized in order to insure adequate pouch drainage of the tenacious mucous secretions. After a one-month recovery period, a series of daily three-hour pouch mucous collections was made for one week. During the collection periods, the animals were placed in specially constructed cages to insure that complete standard collections would be made.

Following this period of control collections, a standard 20 per cent full-thickness burn was inflicted utilizing an iron heated to 350°C. and applied for 12 seconds. Daily three-hour pouch collections were made for seven days following burning. Care was taken to keep the dogs well hydrated during this period. Determinations of volume, total dry weight after lyophilization, and hexosamine, sialic acid and L-fucose content were made on each pouch collection.

The dogs were then sacrificed and a complete autopsy was performed. Sections were taken from the area of burn and from the antral pouch. The latter were compared with sections of antrum from normal dogs sacrificed for other reasons. All sections of stomach were stained with both hematoxylin and eosin and Alcian blue-P.A.S.

Results

1. Rat Studies

a. Gross examination of the stomach.

On gross examination of the stomach, the control rats had a 17 per cent incidence of gastric mucosal erosion, whereas 73 per cent of the burned rats had ulcers.

b. Microscopic examination of the stomach.

No difference was noted between the stomachs of the control and burned rats on examination of the sections stained with hematoxylin and eosin. However, a marked difference was noted in the stainable acid and neutral mucopolysaccharide in the gastric sections stained with Alcian blue-P.A.S.

All sections were examined on a blind basis and a rating given for the amount of stainable surface, intraglandular and intracellular mucus in comparable microscopic fields. Only one-third as much stainable mucus was found in the stomachs of the burned rats as compared with the controls.

c. Chemical analyses (Table 1)

Chemical analyses performed on the gastric mucosal scrapings demonstrated significant decreases in sialic acid and L-fucose content but an increase in hexosamine content. Analyses are available on 25 control and 17 burned rats.

2. Dog Studies

a. Gross examination of the stomach

None of the six dogs had any gastrointestinal abnormality noted at the time of construction of the antral pouch. At postmortem examination, one dog had an acute perforation of a duodenal ulcer. The others had no abnormality.

b. Histologic examination of the antrum

No significant difference was noted between mucous-stained sections of the burned animals' antral pouches and sections of antrum taken from normal dogs.

c. Chemical analyses (Table 2)

All six dogs had a marked uniform decrease in the rate of mucous secretion after burning, both in terms of total volume and of the total weight of the freeze-dried mucosubstances. Concentration of the freeze-dried mucosubstances tended to increase, though not in every instance. Chemical determinations are available on four of the six dogs at this time. No consistent change occurred in the content of hexosamine, sialic acid or L-fucose, and their molar ratios remained essentially the same.

Table 1. Mean Content of Cardohydrate Constituents of Mucosal Scrapings in Rats

	No. Animals	Hexosamine*	L-Fucose*	Sialic Acid†
Control Rats	25	10.1	5.9	6.4
Burned Rats	17	36.4	3.7	4.0

* Expressed as milligrams per gram of freeze-dried mucosubstance
† Expressed as micrograms per gram of freeze-dried mucosubstance

Table 2. Mean Antral Pouch Output and Content of Carbohydrate Constituents of Pouch Mucus in Dogs

Dog No.		Volume (ml/3 hr)	Dry Wt (mg)	Hexosamine*	L-Fucose*	Sialic Acid†	SA/HAM	F/HAM
#1	Preburn	11.0	229	28.5	14.4	4.9	0.17	0.50
	Postburn	4.2	134	24.6	16.7	4.5	0.18	0.68
#2	Preburn	6.8	291	25.4	16.3	3.6	0.14	0.64
	Postburn	3.2	105	21.3	11.4	3.3	0.15	0.53
#3	Preburn	8.5	240	56.6	17.9	5.6	0.10	0.32
	Postburn	2.2	188	96.8	36.1	8.7	0.09	0.37
#4	Preburn	12.3	342	32.8	19.5	5.6	0.17	0.59
	Postburn	5.2	178	51.7	31.1	8.7	0.17	0.60

* Expressed as milligrams per gram of freeze-dried mucosubstance
† Expressed as micrograms per gram of freeze-dried mucosubstance

Comments

These studies indicate that a marked decrease in the rate of gastric mucous secretion occurs following thermal injury in rats and in dogs. This is felt to be a significant finding, since absolute hyperacidity has not been implicated in the etiology of Curling's ulcer and since gastric mucus has a known protective function. As mentioned, it has been demonstrated that the local absence of mucus leaves the stomach and duodenum vulnerable to acid-peptic digestion and ulceration. If the rate of renewal of the mucous layer lags behind the rate of destruction, mucosal damage may then occur from acid-peptic activity or mechanical trauma. These animals had both a decreased rate of mucous secretion and an increased incidence of ulceration following burning in support of the above hypothesis.

An additional factor that may be involved is relative hyperacidity, although acid measurements were not made on any of these animals. Since mucus does buffer some quantity of gastric acid, a fall in the total amount of mucus would lead to a higher acid content. Thus, even "normal" amounts of acid secretion might conceivably lead to ulceration.

It is difficult to conclude from the chemical determinations that any consistent change occurs in the quality of gastric mucus secreted following burning. The increase in burned rat gastric mucosal hexosamine content reflects not only the amount of mucus present but also increases in ground substance and tissue fluid mucopolysaccharides. The decreases in burned rat gastric mucosal sialic acid and fucose content are indications of decreased total mucous secretion. Also, it has been pointed out that sialic acid is responsible for some of the important physicochemical properties of the glycoprotein molecule.[1] It is hydrophilic and would tend to increase the volume of mucus if its concentration was increased. Also, Gottschalk has shown that sialic acid hinders enzymatic hydrolysis of the mucoprotein molecule.[3] Thus, a decrease in sialic acid would tend to lower the volume of mucus secreted and also would make the mucus more susceptible to enzymatic destruction.

No consistent change occurred in thechemical pattern of pure mucus secreted from the canine antral pouches. The molar ratios of the mucoprotein carbohydrate moieties varied very little, indicating that essentially the same kind of mucus was being secreted although much less in amount. Since the total dry weight consistently decreased, it is felt that the

decreased secretion rate cannot be accounted for by mere changes in water content.

The most striking finding following thermal injury, then, was a marked decrease in the rate of gastric mucous secretion. It is suggested that these marked decreases in the rate of renewal of gastric mucus occurring after burn injury may be important in the etiology of Curling's ulcer.

Summary

Burn trauma caused a decrease in the mucous content of rat stomachs and also marked decreases in the rate of mucous secretion in dogs prepared with isolated, denervated antral pouches. These changes correlated with an increased incidence of postburn gastric and duodenal ulceration. No consistent change in the character of mucus secreted could be determined from chemical analysis.

It is proposed that the burn-induced decrease in the rate of renewal of gastric mucus is an important factor in gastroduodenal ulceration following thermal injury.

References

1. Dische, Z.: Fucose and sialic acid in glycoproteins of the mucus of the digestive tract. Fed. Proc.,19:904, 1960.

2. Friesen, S.R.: The genesis of gastroduodenal ulcer following burns. An experimental study. Surgery, 28:123, 1950.

3. Gottschalk, A.: Sialic acids: Their molecular structure and biological function in mucoproteins. Bull. Soc. Chim. Biol.,42:1387, 1960.

4. Hollander, F.: The two-component mucous barrier. Arch. Int. Med., 93:107, 1954.

5. Hummel, R.P., Lanchantin, G.F., and Artz, C.P.: Clinical experiences and studies in Curling's ulcer. J. Amer. Med. Assoc., 164:141, 1957.

6. Wolf, S., and Wolff, H.G.: Human Gastric Function. Oxford University Press, New York, 1943, pp. 167-169.

Publications and/or Presentations

None.

GPO 779064

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OR 6958	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 199

10b. PRIOR NUMBER/CODE: None

11. TITLE:

(U) A Search for an Antidote for Burning White Phosphorus (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	03 66	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	0	0
		CURRENT FY 66	0	0

19. GOVT LAB/INSTALLATION/ACTIVITY

NAME: Headquarters
ADDRESS: US Army Medical Res & Dev Command
Washington, DC 20315

PRIN. INVSTR.: Kovaric, J.J., Lt Col
TEL.: 8 22 63737

20. PERFORMING ORGANIZATION

NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas

INVESTIGATORS PRINCIPAL: Summerlin, W.T., Capt, MC
ASSOCIATE: Ritchey, C.R., Capt, MSC
TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Antidote, burns, white phosphorus

(U) Tech Objective - A search is being made for an antidote, which can be carried ideally by the individual soldier in his first aid packet, for treatment on exposure to white phosphorus.

(U) Approach - A variety of chemicals are to be tested to find a satisfactory antidote. When a chemical is found which reacts relatively rapidly and completely with white phosphorus and which produces non-toxic materials as a result of the reaction, it will be tested on animals. Following this, a means for delivering the antidote to the burning white phosphorus will be developed.

(U) Progress (Mar 66 - Jun 66) - Several oxidizing-neutralizing agents such as sodium percarbonate and sodium carbonate peroxide have been tried in the laboratory thus far with no success under our conditions. Additional agents will be tested in an attempt to find a satisfactory one.

31. CLASSIFICATION/DOWNGRADING SECURITY	32.	33. KIND CODE	34. DURATION CODE
		AR	1

35.	36. PARTICIPATION
NA	NA

37.	38. SPECIAL EQUIPMENT

39. DEV FUNDS IN THOUSANDS	40.
(1)	

DD FORM 1498

PREVIOUS EDITIONS OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: A SEARCH FOR AN ANTIDOTE FOR BURNING WHITE PHOSPHORUS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

William T. Summerlin, Captain, MC
Charles R. Ritchey, Captain, MSC
Franklin D. Foley, Captain, MC
Arnold I. Walder, Captain, MC
Ami Giladi, Lt Colonel, MC (Israeli Army)
John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: A SEARCH FOR AN ANTIDOTE FOR BURNING WHITE PHOSPHORUS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

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John A. Moncrief, Colonel, MC

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: A SEARCH FOR AN ANTIDOTE FOR BURNING WHITE PHOSPHORUS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: William T. Summerlin, Captain, MC
Charles R. Ritchey, Captain, MSC
Franklin D. Foley, Captain, MC
Arnold I. Walder, Captain, MC
Ami Giladi, Lt Colonel, MC (Israeli Army)
John A. Moncrief, Colonel, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

The Surgical Research Unit is seeing an increasing number of patients with white phosphorus burns. Since white phosphorus ignites spontaneously in air, a means must be found to cover the burning area with a coating impervious to air and/or to remove the phosphorus through a chemical reaction.

At the present time a solution of cupric sulfate is being applied to areas burned by white phosphorus. The cupric sulfate reacts with the surface of the phosphorus to form a protective coating, but only a small amount of the total phosphorus present is reacted upon. Therefore, we are searching for a compound which will react with the whole phosphorus mass.

Since the products of the reaction must cause no further damage to the burned area and must be nontoxic, it was decided to search for a compound that would oxidize the phosphorus to phosphorus oxides which could be neutralized by an appropriate alkali. There are several compounds available that are in themselves both oxidizing and neutralizing agents. Two of them (sodium percarbonate and sodium carbonate peroxide) have been tried in vitro, but with little success since the rate

of oxidation of phosphorus using these compounds is too slow to be effective. Other oxidizing agents, catalysts to speed up the rate of oxidation, and other classes of compounds remain to be tried. Once an effective antidote is found, a means for easy application of this antidote will be sought.

(1) White phosphorus

(2) Chemical oxidizing agents

RESEARCH AND TECHNOLOGY WORK UNIT — DA OA 0960 — CSCRD - 103

3. DATE OF WORK UNIT	4. DATE OF WORK UNIT	5. SECURITY CLASSIFICATION	6. REGRADING	7. TYPE OF WORK UNIT	8. WORK UNIT
30 06 66	"D. CHANGE" 27 08 65	U U	NA	M1	A. WORK UNIT

10a. CURRENT NUMBER CODE	10b. PREVIOUS NUMBER CODE
61145011 3A014501871R 01 145	NONE

11. TITLE

(U) Acute Renal Insufficiency in Experimental Animals (44)

12. SCIENTIFIC OR TECH AREA	13. START DATE	14. CMPT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	07 55	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE	b. NUMBER	c. TYPE	d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House		NA			PRIOR FY 65	15	10
					CURRENT FY 66	2	4

19. GOV'T LAB/INSTALLATION/ACTIVITY

NAME Headquarters
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Washington, DC 20315

RESP. INDIV. Kovaric, J.J., Lt Col
TEL. 8 22 63737

20. PERFORMING ORGANIZATION

NAME US Army Surgical Research Unit
ADDRESS Fort Sam Houston, Texas

INVESTIGATOR PRINCIPAL ASSOCIATE Mason, A.D., Jr, MD
Bowler, E.G., Ph.M
TEL. 512 - CA1-2720 TYPE DA

21. TECHNOLOGY UTILIZATION

Life Sciences

22. COORDINATION

None

23. KEYWORDS Acute renal failure, renal physiology, renal pathology, hemoglobinuria, hemoglobin metabolism

24. (U) Tech Objective - The purpose of this study is to extend understanding of the mechanisms and management of acute renal failure. The immediate intention is a study of the renal handling of hemoglobin under conditions in which renal failure may be produced.

25. (U) Approach - It appears that native hemoglobin, which is soluble, becomes less soluble under conditions leading to acute renal failure. The plan for exploring this change in solubility examines two possible modes of hemoglobin precipitation: (1) oxidative denaturation with the production of a less soluble pigment molecule and (2) the existence of a co-precipitant molecule capable of reacting with hemoglobin to produce a gel. The first of these is approached through a study of oxidative denaturation of hemoglobin in vitro and in vivo. The major technical problem is the identification of early oxidative denaturation of the globin moiety of hemoglobin when such denaturation involves only a small fraction of the total pigment present. The second area is approached through the examination of a variety of possible physiologic

26. polyanionic precipitants in vitro and in vivo. We have attempted to define such material in the urine of animals subjected to tourniquet injury or renal ischemia and have also explored the consequences of injecting such materials along with hemoglobin.

(U) Progress (Jul 65 - 30 Jun 66) - The behavior of hemoglobin in various redox situations has been explored. Some of these in vitro systems have appeared promising in that coupled oxidation and accelerated production of insolub hemoglobin derivatives has been possible in systems containing physiologically achievable concentrations of reactants. In vivo studies of these systems have not been successful, however. Some polyanionic materials have functioned as co-precipitants in vivo, but have not identified comparable concentrations of polyanionic materials under physiologic conditions.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MILESTONE OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY + 1	

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to NASA Form 1121) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: ACUTE RENAL INSUFFICIENCY IN EXPERIMENTAL ANIMALS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Arthur D. Mason, Jr., M.D.
Eleanor G. Bowler, Ph.M.
Wanda L. Brown, M.S.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: ACUTE RENAL INSUFFICIENCY IN EXPERIMENTAL ANIMALS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Arthur D. Mason, Jr., M.D.
Eleanor G. Bowler, Ph.M.
Wanda L. Brown, M.S.

6. Reports Control Symbol: RCS-MEDDH-288(R1)

Studies of hemoglobin denaturation by physiological systems have been continued. The production of insoluble hemochromogens has been possible with reasonable concentrations of oxidants and in physiologically possible systems with coupled oxidation in vitro. In vivo production of acute renal failure by the injection of hemoglobin and oxidant systems has not yet been possible.

Occasional cast production has been possible in vivo, using injections of hemoglobin and polyanionic mucopolysaccharides. Renal failure per se has not occurred with these materials, however.

Several technics for the measurement of urine glucose concentration have been examined and a method has been selected for future studies.

(1) Acute renal failure
(2) Renal physiology
(3) Renal pathology
(4) Hemoglobinuria
(5) Hemoglobin metabolism

ACUTE RENAL INSUFFICIENCY IN EXPERIMENTAL ANIMALS

In the course of these studies of experimental acute renal failure, the nephropathy has been produced by injecting ferrocyanide together with either hemoglobin or methemoglobin, by injecting globin alone, or by injecting diazo derivatives of methemoglobin or globin. The physiological derangements and the morphological lesions produced by these injections are similar in many respects to those observed in lower nephron nephrosis. An important gap still present in our knowledge, however, lies in the fact that each of these experimental situations begins with an altered heme pigment, while the clinical syndrome appears to follow the release into plasma of native hemoglobin. It has been repeatedly shown in animals and occasionally demonstrated in man that simple injection of native hemoglobin is relatively innocuous. Some additional physiologic factor must exist which enhances the nephrotoxic potential of simple hemoglobinemia if renal failure is to follow its existence. Others have sought this enhancing factor in dehydration and in hemorrhagic shock. The dehydration must be unrealistically severe to contribute in any major sense and hemorrhagic shock has been a singularly unproductive approach.

It seems quite likely that enhancement of nephrotoxicity occurs when hemoglobin, which is relatively soluble, becomes less soluble for any reason. One possibility suggested by our studies is that hemoglobin is subjected to oxidative denaturation either before or after it enters the renal tubule in those conditions where hemoglobinemia leads to renal failure. During the reporting period we have explored a number of systems which oxidize hemoglobin in vitro. These systems accelerate hemoglobin denaturation and the production of insoluble hemochromogens in vitro. Ferrocyanide accelerates oxidative denaturation of hemoglobin in an oxygen containing atmosphere. We have been able to produce occasional episodes of renal failure in animals by the simultaneous injection of hemoglobin and certain oxidants, and have reported in the past the occurrence of renal failure in appropriately prepared animals following the simultaneous injection of human hemoglobin in ferrocyanide. So far, however, we have not found any physiologic oxidant which will make native hemoglobin nephrotoxic. The possibility is attractive and these studies

will be continued.

A second possibility suggested by our studies is the occurrence of a co-precipitant capable of gelling with hemoglobin and appearing in the tubular fluid under those conditions permitting hemoglobinemia to lead to renal failure. Pilot experiments have been carried out in which the ability of urine to cause precipitation of hemoglobin has been examined in normal animals and in animals subjected to tourniquet injury. Similar studies have also been carried out in animals subjected to varying intervals of interruption of renal blood flow. In none of these have we been able to discover any urinary co-precipitant or any acceleration of hemoglobin precipitation.

Theoretical considerations suggest that the co-precipitant, if it exists, should be polyanionic in character and this requirement is met by certain of the mucopolysaccharides. Pilot experiments involving the injection of native hemoglobin together with polyanionic mucopolysaccharides have resulted in some cast formation within the collecting tubules but have not led to frank acute renal failure. More severe impairment has been produced by injecting these materials with methemoglobin. This group of studies, too, appears promising and will be continued.

During the reporting period, several methods for the measurement of urine glucose concentration have been examined. This measurement is necessary for the examination of reabsorptive mechanisms in acute renal failure. None of the measurement technics has been ideal when considered in regard to blank, specificity and sensitivity. Of the technics tried, the most promising appears to be that reported by Hyvarinen[1] which uses ortho-toluidine in glacial acetic acid for the determination of aldosaccharides, and we have selected this method for further development and use.

Reference

1. Hyvarinen, A., and Nikkila, E.A.: Specific determination of blood glucose with o-toluidine. Clin. Chim. Acta. 7:140-142, 1962.

PRESENTATIONS

Mason, A. D., Jr.: Acute renal insufficiency in experimental animals.Seminar on the Surgical and Orthopaedic Aspects of Trauma, BGH, BAMC, Fort Sam Houston, Texas,8-12 Mar. 1965.

Publications

None.

RESEARCH AND TECHNOLOGY RESUME		2. GOVT ACCESSION	3. AGENCY ACCESSION	3a. PROJECT CONTROL SYMBOL
			DA OB 6954	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER CODE: 61145011 3A014501B71R 01 195

10b. PRIOR NUMBER CODE: NONE

11. TITLE: (U) Studies of the Peritoneal Membrane. The Factors Influencing Movement of Uric Acid Across the Peritoneal Membrane (44)

12. SCIENTIFIC OR TECH AREA	13. START DATE	14. CRIT COMPL DATE	15. FUNDING AGEN
Clinical Medicine (003500)	08 65	NA	Other / DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	1	4
		CURRENT FY 66	1	4

19. GOV'T LAB/INSTALLATION/ACTIVITY
NAME: Headquarters
ADDRESS: US Army Medical Res & Dev Command
Washington, DC 20315
RESP. INDIV.: Kovaric, J.J., Lt Col
TEL.: 8 22 63737

20. PERFORMING ORGANIZATION
NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas
INVESTIGATORS PRINCIPAL ASSOCIATE: Knoche., J.P., Maj, MC
TEL.: 512 - CA1-2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Uric acid, peritoneal diffusion, glucose

24.
(U) Tech Objective - To determine the influence of glucose on uric acid movement across the peritoneal membrane.

(U) Approach - Mongrel dogs underwent peritoneal dialysis with solutions containing uric acid which were made isotonic with either urea or glucose. Diffusion constants were established for uric acid movement from peritoneal fluid to plasma under the influence of these solutions.

(U) Progress (Aug 65-Jun 66) - All studies completed thus far indicate no effect of glucose per se on uric acid movement across the peritoneum.

26.

27. COMMUNICATIONS SECURITY	28	29. OSD CODE	30. BUDGET CODE
[ ] a. COMSEC OR COMSEC RELATED [X] b. NOT RELATED		BR	5

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT
35. EST FUNDS (In thousands) FY ( )	36

DD FORM 1 AUG 66 1498 (Items 1 to 36 identical to NAVY Form 11321 REPLACES DD Forms 611 & 612 WHICH ARE OBSOLETE)

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501871R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: STUDIES OF PERITONEAL MEMBRANE. PART I. THE FACTORS INFLUENCING MOVEMENT OF URIC ACID ACROSS THE PERITONEAL MEMBRANE

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigator:

James P. Knochel, Major, MC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501871R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: STUDIES OF PERITONEAL MEMBRANE. PART I. THE FACTORS INFLUENCING MOVEMENT OF URIC ACID ACROSS THE PERITONEAL MEMBRANE

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigator: James P. Knochel, Major, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

Studies of the mechanisms by which uric acid moves across the peritoneal membrane were continued during the past year. These studies have demonstrated that glucose has no apparent augmentative or suppressive action on uric acid movement across the peritoneal membrane.

(1) Uric acid
(2) Peritoneal diffusion
(3) Glucose

STUDIES OF PERITONEAL MEMBRANE. PART I. THE FACTORS INFLUENCING MOVEMENT OF URIC ACID ACROSS THE PERITONEAL MEMBRANE

A previous study demonstrated that alkalinization of peritoneal fluid augmented net movement of uric acid from blood to peritoneal fluid (PF). Failure of pyrazinamide or lactate to impede movement of uric acid across the peritoneum suggested that an active transport mechanism similar to that found in the mammalian kidney was not implicated. Since glucose administration reestablishes uricosuria in hyperuricemic patients with starvation ketoacidosis, the effect of glucose on movement of uric acid across the peritoneum was examined and compared with that occurring from equimolar solutions of urea.

Increasing the concentration of uric acid in the dialyzing solution showed a suppressive effect on relative diffusion of uric acid from PF to blood.

Methods

Mongrel, fasted, non-Dalmatian dogs were dialyzed against solutions of various composition listed in Table 1. Arterial blood and peritoneal fluid samples were collected simultaneously throughout each experiment and analyses performed as previously reported (1).

Table 2 shows that the per cent of uric acid remaining in the peritoneal cavity was the same in equimolar solutions of urea or glucose except for the final value. Although rate constants (Table 4) for uric acid movement became divergent toward the end of the experimental period, they were not significantly different.

Table 3 shows a significantly greater retention of uric acid if the original concentration was 500 mg/100 ml rather than 50 mg/100 ml. Similarly, rate constants (Table 4) for uric acid movement from PF to blood were also significantly less in the high concentration group.

Discussion

Since uric acid diffusion from PF to blood was virtually identical in equimolar solutions of urea or glucose, it is reasonable to conclude that glucose, under these circumstances neither impedes nor augments transport of uric acid by the

Table 1. Composition of Solutions for Peritoneal Dialysis

	Uric Acid mg/100 ml	$LiCO_3$ mg/100 ml	Glucose gm/100 ml	Urea gm/100 ml	Osmolality mOsm/Kg H_2O
A	50.0	150	4.64	-	301
B	50.0	150	-	1.48	301
C	500.0	150	4.28	-	320

Table 2. Per Cent Uric Acid Remaining in Peritoneal Cavity

Solution		Minutes After Instillation							
		2.5	5.0	7.5	10	12.5	15	20	30
Glucose Uric acid 50 mg/100 ml n = 4	Mean	88	84	79	75	72	69	59	47
	Range	80 - 95	78 - 87	72 - 83	72 - 77	69 - 73	65 - 74	54 - 64	43 - 52
Urea Uric acid 50 mg/100 ml n = 4	Mean	92	88	83	78	72	66	56	36
	Range	90 - 93	85 - 92	78 - 86	73 - 82	67 - 77	61 - 70	54 - 57	32 - 38

Table 3. Per Cent Uric Acid Remaining in Peritoneal Cavity

Solution		Minutes After Instillation							
		2.5	5.0	7.5	10	12.5	15	20	30
Glucose Uric acid 50 mg% n = 4	$\bar{Y}$	88	84	79	75	72	69	59	47
	Range	(80-95)	(78-87)	(72-83)	(72-77)	(69-73)	(65-74)	(54-64)	(43-52)
Glucose Uric acid 500mg% n = 3	$\bar{Y}$	98	94	93	92	92	88	85	76
	Range	(98-98)	(93-96)	(92-95)	(91-94)	(91-92)	(84-90)	(82-86)	(72-81)

Table 4. Rate Constants (mg/min/mg Δ) Uric Acid From Peritoneal Fluid to Blood

Solution		Minutes After Instillation							
		2.5	5.0	7.5	10	12.5	15	20	30
Glucose 4.28%	$\bar{Y}$	0.015	0.015	0.015	0.015	0.015	0.014	0.015	0.016
n = 3 Uric acid 500 mg%	Range	(0.013-0.016)	(0.013-0.016)	(0.014-0.015)	(0.014-0.015)	(0.015-0.015)	(0.013-0.015)	(0.014-0.018)	(0.010-0.020)
Glucose 4.6%	$\hat{Y}$	0.035	0.033	0.031	0.031	0.030	0.030	0.030	0.031
n = 5 Uric acid 50 mg%	Range	(0.032-0.047)	(0.030-0.042)	(0.025-0.038)	(0.027-0.035)	(0.028-0.034)	(0.027-0.035)	(0.025-0.039)	(0.017-0.060)
Urea	$\bar{Y}$	0.041	0.040	0.041	0.044	0.049	0.055	0.065	0.082
n = 4 Uric acid 50 mg%	Range	(0.033-0.046)	(0.032-0.048)	(0.030-0.051)	(0.031-0.060)	(0.031-0.068)	(0.031-0.077)	(0.033-0.099)	(0.039-0.126)

peritoneal membrane. Nonetheless, this does not exclude an effect of glucose per se since in this in vivo experiment, glucose was present in normal concentration on the opposite side of the membrane. To demonstrate dependence upon glucose would necessitate a different experimental approach.

Of more possible importance in this preliminary study was the demonstration that increasing the concentration of uric acid in PF significantly impeded its diffusion constant. Ordinarily, if simple chemical or electrochemical diffusion down a concentration or electrical gradient is the mode of transport, as in the case of urea, the rate constant will be approximately the same regardless of the concentration. If an active transport system is involved, one might expect eventual saturation of the carrier and thereafter, as the concentration is raised, the diffusion constant becomes progressively lower. In this instance, the net quantity transported per unit time will be constant. The results presented herein are remotely suggestive of this characteristic, although considerably more experimentation is required to garner more support for such a hypothesis.

A practical question concerning the possibility of an active transport mechanism for uric acid in the peritoneum entails the reason why the peritoneal membrane should possess such a seemingly useless property. Although the existence of this mechanism is indeed unlikely, it is known from recent work in starvation ketoacidosis in man that uric acid transport in the kidney is somehow interrelated with that of hydrogen ion, potassium, glucose, ketone bodies and lactate. Perhaps a similar interrelationship exists in other cells.

Reference

Knochel, J.P., and Mason, A.D., Jr.: The effect of alkalinization on peritoneal diffusion of uric acid. Am. J. Physiol. (In press).

Publications

None.

Presentations

Knochel, J.P.: A portion of this work was presented at the Annual Meeting, American Federation for Clinical Research and American Society for Clinical Investigation, Southern Section, New Orleans, 29 January, 1966.

GPO 710004

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6953	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U APT U WRK	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 194

10b. PRIOR NUMBER/CODE: NONE

11. TITLE: (U) Studies of the Peritoneal Membrane. Effects of Intraperitoneal Glucose and Urea Solutions on Solute and Water Shifts (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	08 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE b. NUMBER c. TYPE NA d. AMOUNT	18. RESOURCES EST.	d. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House		PRIOR FY 65	1	4
		CURRENT FY 66	1	4

19. GOVT LAB/INSTALLATION/ACTIVITY
NAME: Headquarters
ADDRESS: US Army Medical Res & Dev Command
Washington, DC 20315
RESP. INDIV.: Kovaric, J.J., Lt Col
TEL.: 8 22 63737

20. PERFORMING ORGANIZATION
NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas
INVESTIGATORS PRINCIPAL ASSOCIATE: Knochel, J.P., Maj, MC
TEL.: 512 - CA1-2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: None

23. KEYWORDS: Peritoneal diffusion, glucose vs urea, sodium and water flux

24. (U) Tech Objective - To define the dynamics of solute and water flux underlying compartmental expansion following instillation of isotonic glucose solutions. Extravascular infiltration or intraperitoneal instillation of isotonic dextrose solutions may lead to serious depletion of extracellular fluid volume and hyponatremia. This study was undertaken to examine the dynamics of solute and water flux underlying this compartmental expansion following instillation of isotonic glucose solutions.

25. (U) Approach - Mongrel dogs underwent peritoneal dialysis with isotonic solutions of glucose, urea or distilled water. Serial changes of osmolality, urea, sodium, glucose and total protein concentrations were made at close intervals. Intraperitoneal volume was measured directly immediately upon completion of each experiment.

26. (U) Progress (Au Jun 66) - Intraperitoneal instillation of isotonic glucose solutions results in d loss of sodium and water from the extracellular space to the peritoneal cavi Although peritoneal fluid (PF) volume expands, the quantity of water accompanying this solute shift is inadequate to maintain isotonicity of the PF. On the other hand, while the diffusion coefficient for Na+ into an isotonic urea solution is quantitatively similar, it is unaccompanied by PF volume expansion. Serial measurements of PF osmolality and concentrations of glucose, urea and Na+ suggest that the development of an osmotic gradient in the PF results not only from the rapid influx of Na+ with its accompanying anions and the relatively slow efflux of glucose, but also suggests that water diffusion is independent to that of solute and occurs down its own concentration gradient created by solute diffusion.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) FY	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1121) REPLACES DD Forms 613 & 613C WHICH ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: STUDIES OF THE PERITONEAL MEMBRANE. PART II. EFFECTS OF INTRAPERITONEAL GLUCOSE AND UREA SOLUTIONS ON SOLUTE AND WATER SHIFTS

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigator:

James P. Knochel, Major, MC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: STUDIES ON THE PERITONEAL MEMBRANE. PART II. EFFECTS OF INTRAPERITONEAL GLUCOSE AND UREA SOLUTIONS ON SOLUTE AND WATER SHIFTS

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigator: James P. Knochel, MC

6. Reports Control Symbol: RCS-MEDDH-288(R1)

Intraperitoneal instillation of isotonic glucose solutions results in rapid loss of sodium and water from the extracelluiar space to the peritoneal cavity. Although peritoneal fluid (PF) volume expands, the quantity of water accompanying this solute shift is inadequate to maintain isotonicity of the PF. On the other hand, while the diffusion coefficient for Na^+ into an isotonic urea solution is quantitatively similar, it is unaccompanied by PF volume expansion. Serial measurements of PF osmolality and concentration of glucose, urea and Na^+ suggest that the development of an osmotic gradient in the PF results not only from the rapid influx of Na^+ with its accompanying anions and the relatively slow efflux of glucose, but also suggests that water diffusion is independent to that of solute and occurs down its own concentration gradient created by solute diffusion.

(1) Peritoneal diffusion (2) Glucose vs. urea

(3) Sodium and water flux

STUDIES OF THE PERITONEAL MEMBRANE. PART II. EFFECTS OF INTRAPERITONEAL GLUCOSE AND UREA SOLUTIONS ON SOLUTE AND WATER SHIFTS

It is a well recognized clinical observation that extravascular introduction of isotonic glucose solution may lead to serious hemodynamic disturbances as a consequence of solute and water shifts into the glucose solution prior to its absorption.[1-3] The studies reported herein were designed to examine the kinetics underlying this observation. They point out the rapid formation and magnitude of the marked osmotic gradient which develops under these circumstances.

Methods

Isotonic solutions of glucose or urea were rapidly instilled into the peritoneal cavity of fasted, mongrel, non-diuretic dogs in a dosage of 50 ml/kg body weight. Collections of arterial blood were made before fluid instillation, after which samples of peritoneal fluid (PF) and arterial blood were collected simultaneously at close intervals. Upon completion of the last collection, the animals were killed with either 20 molar KCl intravenously or a large intravenous dose of pentobarbital. The peritoneal cavity was immediately opened and fluid collected by aspiration and sponging. The difference in weight between the solution instilled and that harvested was assumed to be the total volume. Corrections were not made for specific gravity. Osmolality of plasma and peritoneal fluid was estimated from the freezing point depression using the Fiske Osmometer. Na^+ was determined by flame photometry using an internal lithium standard. Urea was determined by a modification of the Berthelot reaction. Glucose was determined by the method adapted to the Technicon Autoanalyzer. Recovery of glucose from solutions of high concentration, after appropriate dilution, averaged 96 per cent. Total proteins were estimated by the spectrophotometric method.

An experiment was designed to study the effect of isotonic glucose and NaCl solutions separated by a cellophane membrane in a rigid dialysis chamber which allowed serial measurement of volume changes.

Results

Table 1 compares mean serial determinations for peritoneal fluid and plasma osmolality between the two solutions. PF

Table 1. Comparison of Serial Osmolality Determinations on Peritoneal Fluid and Plasma

		Minutes								
		0	2.5	5.0	7.5	10	12.5	15	20	30
Peritoneal Fluid										
Glucose n = 5 mMol/kg H_2O										
	Mean	301	320	325	328	331	333	332	330	322
	Range	295-311	312-329	319-333	321-336	324-336	327-338	326-337	324-336	313-329
Urea n = 4 mMol/kg H_2O										
	Mean	301	303	303	303	304	305	304	303	301
	Range	298-304	302-304	302-305	302-306	303-306	304-307	303-304	302-305	299-304
Plasma										
Glucose										
	Mean	302	299	299	297	297	295	296	294	292
	Range	296-307	292-303	291-302	291-302	290-302	290-302	288-301	290-300	284-299
Urea										
	Mean	306	308	308	306	306	306	307	305	304
	Range	303-307	305-311	305-311	305-307	304-308	304-308	305-308	303-307	301-305

osmolality with the glucose solutions reached a maximum value 15 to 20 minutes after introduction. Osmolality of the urea solution showed no remarkable change from that determined before introduction. Plasma osmolality showed a consistent fall in the glucose experiments, but no remarkable change in the urea experiments.

Table 2 shows individual and mean values for PF volumes determined immediately upon completion of the experiments. The average volume increased 21 per cent in the dogs receiving the glucose solutions but showed no change in those receiving urea solutions.

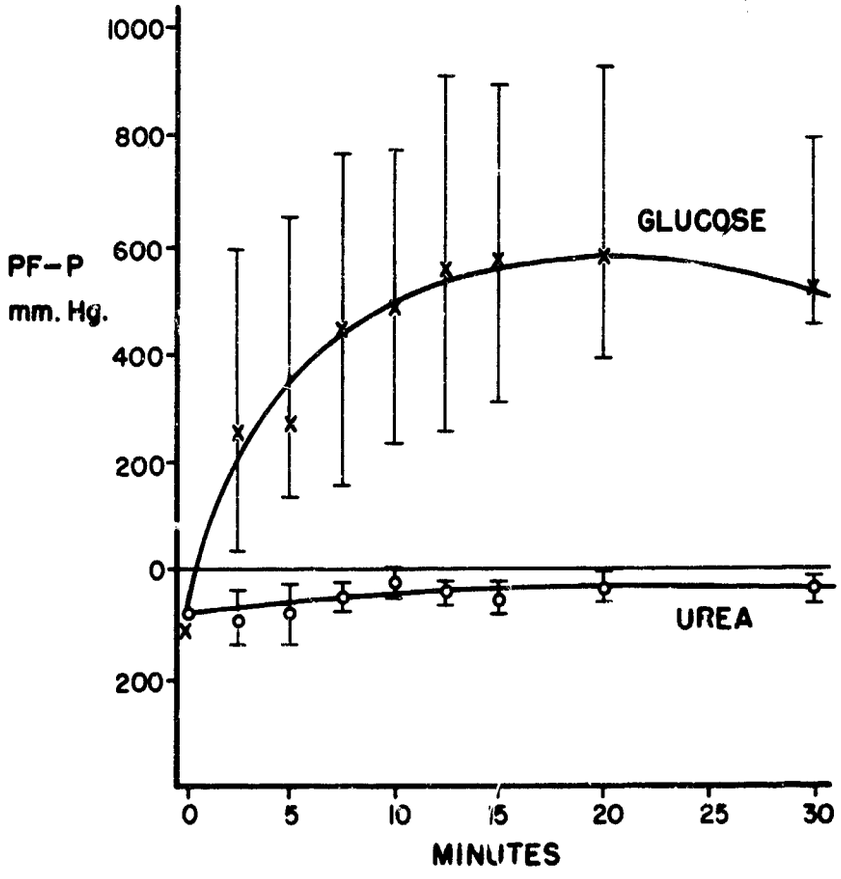
The mean and range of values for osmotic pressure gradient across the peritoneum were estimated from the difference between Pf and plasma osmolality[6], and are shown in Figure 1. The speed with which this remarkable pressure gradient developed outstripped that of the corresponding volume change shown in Table 2. Accompanying this change in PF osmolality and PF volume was a mean increase of total serum protein concentration of 10 per cent (Table 3) which similarly agrees with the simultaneous changes of PF osmolality and volume. Total plasma protein concentration in dogs receiving urea solutions showed no change.

Table 4 compares serial changes of sodium concentration in peritoneal fluid in animals dialyzed against glucose or urea solutions. The values are not remarkably different, indicating that development of the PF osmotic gradient was not due to more rapid influx of Na^+ in dogs receiving glucose solutions.

Figure 2 illustrates the serial fall of glucose concentration compared to the simultaneous rise of Na^+ concentration in a representative experiment. In a similar manner, Figure 3 compares the fall of urea concentration with the rise in Na^+ concentration in another experiment. The diffusion coefficient for Na^+ influx into the Pf is nearly identical with either glucose or urea solutions and suggests that rapidity of Na^+ influx into the PF occurs down its own concentration gradient independently of the type of solute present in the PF or the degree of PF hypertonicity. The diffusion coefficient for urea in Figure 3 was greater than that for glucose illustrated in Figure 2.

Table 2. Comparison of Peritoneal Fluid Volume Changes

Glucose		Urea	
Dog No.	Fraction Final/Initial Volume	Dog No.	Fraction Final/Initial Volume
5T7	1.26	5T16	0.97
5K44	1.18	5T17	0.96
5T41	1.22	5T8	0.97
5T37	1.18	5T38	1.04
5S23	1.22		
Mean	1.21		0.99



MEAN AND RANGE OF OSMOTIC PRESSURE GRADIENT BETWEEN PERITONEAL FLUID AND PLASMA.

Figure 1.

Table 3. Change in Total Plasma Protein Concentration During Dialysis with Glucose Solution (n = 8)

	Minutes								
	0	2.5	5.0	7.5	10	12.5	15	20	30
Total Protein gm/100 ml									
Mean	6.85	7.02	7.13	7.11	7.20	7.27	7.41	7.48	7.56
% Increase	-	102	104	104	105	106	108	109	110

Table 4. Change of Sodium Concentration in Peritoneal Fluid (mEq/L)

		Minutes								
		0	2.5	5.0	7.5	10	12.5	15	20	30
Glucose										
	Mean	0	26	35	45	55	63	72	86	104
	Range	-	19-30	26-40	41-52	48-60	60-69	67-78	81-93	94-108
Urea										
	Mean	0	20	32	41	51	65	76	92	116
	Range	-	18-24	28-41	36-45	48-56	59-73	72-83	91-92	112-120

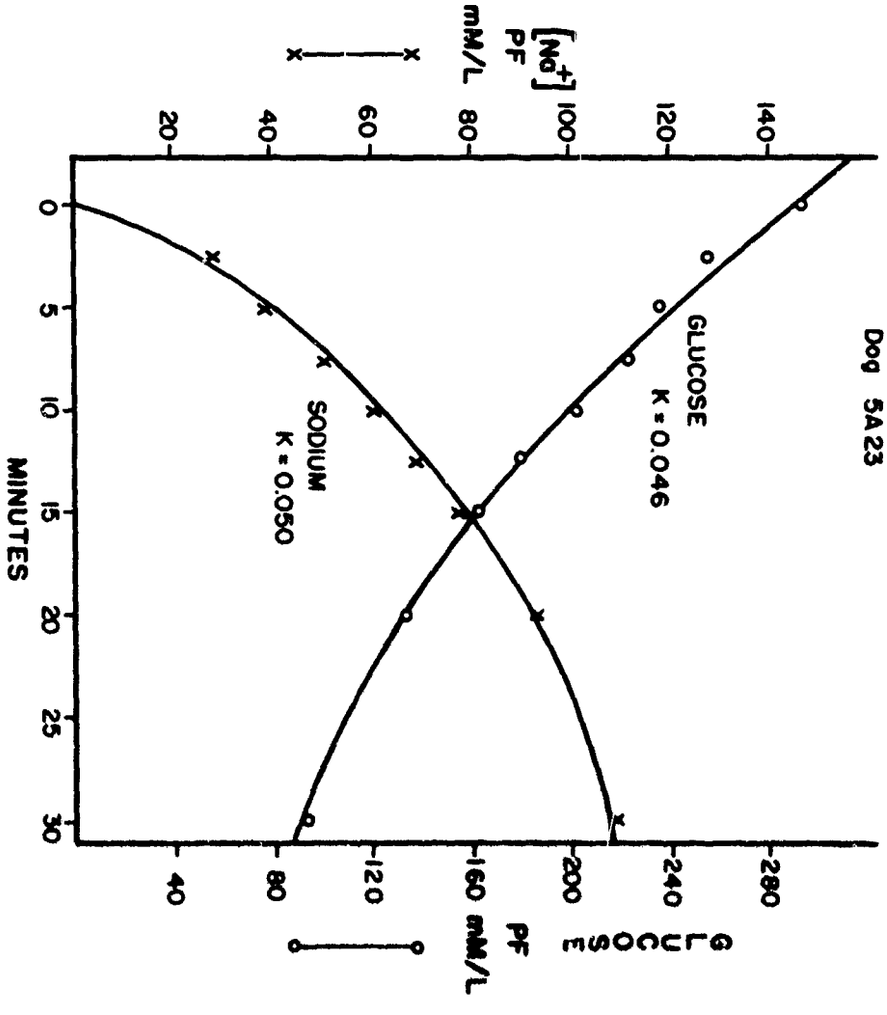

Dog 5A23
GLUCOSE
K = 0.046
SODIUM
K = 0.050
MINUTES
[Na+] PF mM/L
GLUCOSE PF mM/L

Figure 2.

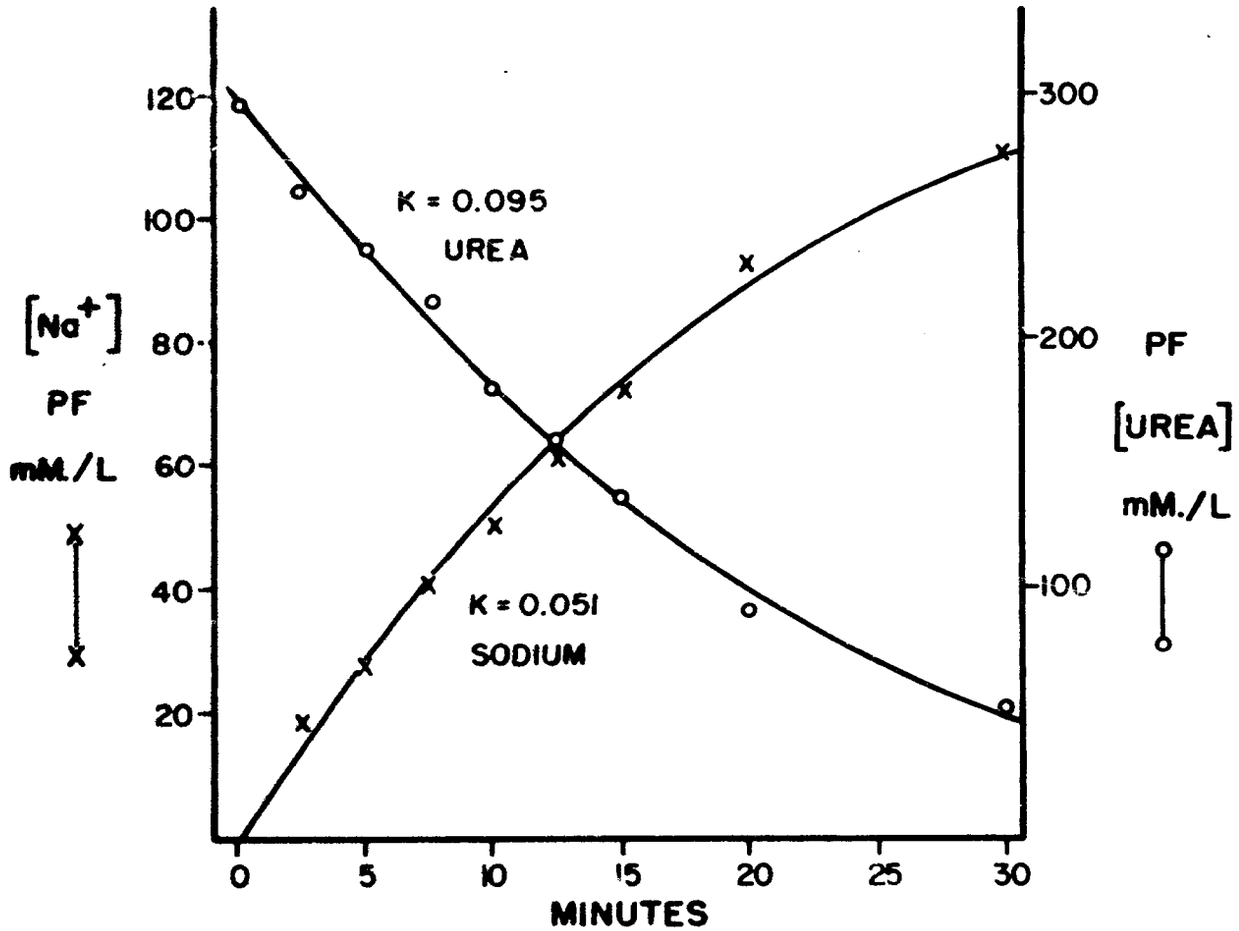

120
100
80
60
40
20
300
200
100
K = 0.095
UREA
K = 0.051
SODIUM
[Na+]
PF
mM./L
PF
[UREA]
mM./L
0
5
10
15
20
25
30
MINUTES

Figure 3

Table 5 shows the mean and range of per cent glucose and urea remaining in the peritoneal cavity, corrected for change in volume, at similar intervals. This confirms the greater diffusivity of urea under these conditions.

Manometric recordings indicative of fluid shifts during dialysis of normal saline against isotonic glucose across cellophane are illustrated in Figure 4. There was a gain of volume on the glucose side. similar qualitatively to that occurring in vivo.

Discussion

The mechanism underlying the accumulation of water and salt into subcutaneous, intraperitoneal or intraintestinal spaces following introduction of solutions containing poorly absorbed solutes has been studied by several groups of investigators.[1-5]

In these studies it was shown that after introduction of an isotonic glucose solution into the peritoneal cavity, extracellular electrolytes diffuse from blood to peritoneal fluid at rates determined by their individual coefficients for diffusion. Ultimately, extracellular electrolytes are sequestered and the associated hypovolemia leads to circulatory embarrassment. Although these consequences are well understood, interesting observations concerning their kinetics were made during the experiments reported herein. In this study, it was disclosed that despite an increase in PF volume, osmolality increased.*

Net solute concentration changes in peritoneal fluid were unaccompanied by an appropriate quantity of water to preserve isotonicity. The simultaneous fall in plasma osmolality in the face of an expanding PF volume and increasing PF osmolality indicates that net solute influx from plasma was greater than the simultaneous influx of water.

There is no PF-plasma gradient for water at zero time

---

* One group of investigators (Schechter, et al.[5]) stated that introduction of isotonic glucose into the peritoneum produced no changes of peritoneal fluid osmotic pressure since solute and water shifts in both directions were balanced. This conclusion was based upon osmolality estimated from serial changes in glucose and sodium concentration, and therefore was probably erroneous.

Table 5. Comparison of Glucose and Urea Retention in Peritoneal Cavity

	Minutes								
	0	2.5	5.0	7.5	10	12.5	15	20	30
Per cent Glucose									
Remaining*	100	92	88	83	77	75	70	61	47
Range		38-98	86-91	80-88	70-84	66-81	61-75	52-68	38-57
Per cent Urea									
Remaining	100	89	83	76	65	58	49	43	24
Range		80-93	72-91	70-86	59-82	50-75	37-70	36-57	17-37

* Corrected for total volume change.

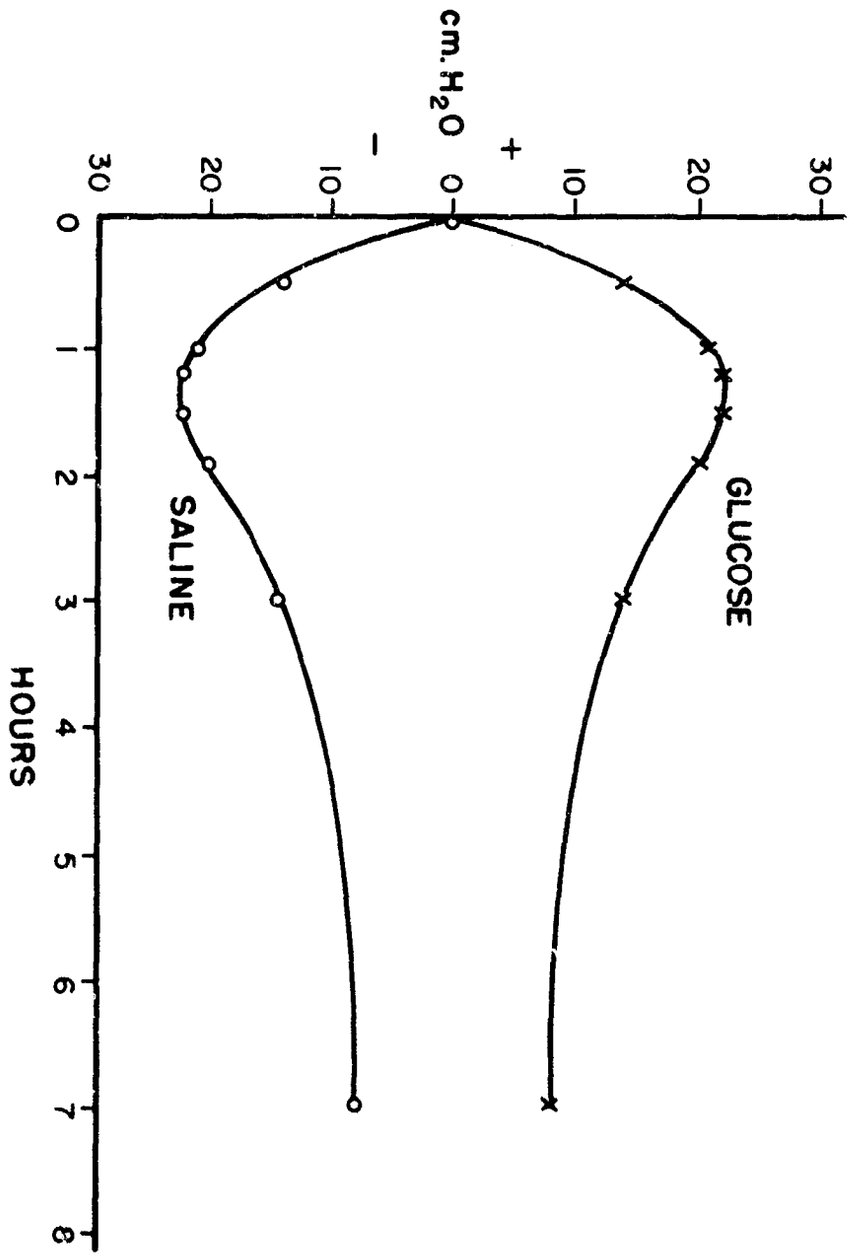


Figure 4

since the glucose solution was isotonic to plasma. However, a concentration gradient for water was created by diffusion of sodium and its accompanying anions from plasma, thus indicating that movement of solute is apparently independent of water movement. In the urea experiments, because of the relatively more rapid diffusion of urea to plasma, water gradient formation is prevented, thus a net flux of water does not occur.

Although diffusion coefficients for sodium influx into PF containing glucose and urea (Figure 5) and that for glucose efflux in terms of concentration gradient between plasma and PF were virtually identical (Figure 2), the total quantity of solute which diffused into PF (sodium and its accompanying anions) was greater into glucose than urea. Using data from tables 2 and 4, it is apparent that sodium and its anions accounted for a solute influx of 252 mMols,* whereas efflux of glucose was 159 mMols. This indicates that for each liter of isotonic glucose introduced into the peritoneal cavity, within 30 minutes approximately 93 mMols of solute and 210 ml of water entered from plasma. Normal plasma water concentration in terms of solute is approximately 3.3 ml/mOsm.** The observed ratio under these circumstances is 2.2 ml/mOsm which coincides well with the development of PF hypertonicity. Similar calculations from the data concerning exchange of solutes in the urea experiments show that an average of 232 mMols of Na^+ and its anions entered the PF while 228 mMols of urea entered the plasma. Thus an osmotic gradient favoring water movement was not produced.

References

1. Danowski, T.S., Winkler, A.W., and Elkinton, J.R.: Biochemical and hemodynamic changes following subcutaneous injection of glucose solution. J. Clin. Invest. 26:887-891, 1947.

2. Elkinton, J.R., Danowski, T.S., and Winkler, A.W.: Hemodynamic changes in salt depletion and in dehydration. J. Clin. Invest. 25:120-129, 1946.

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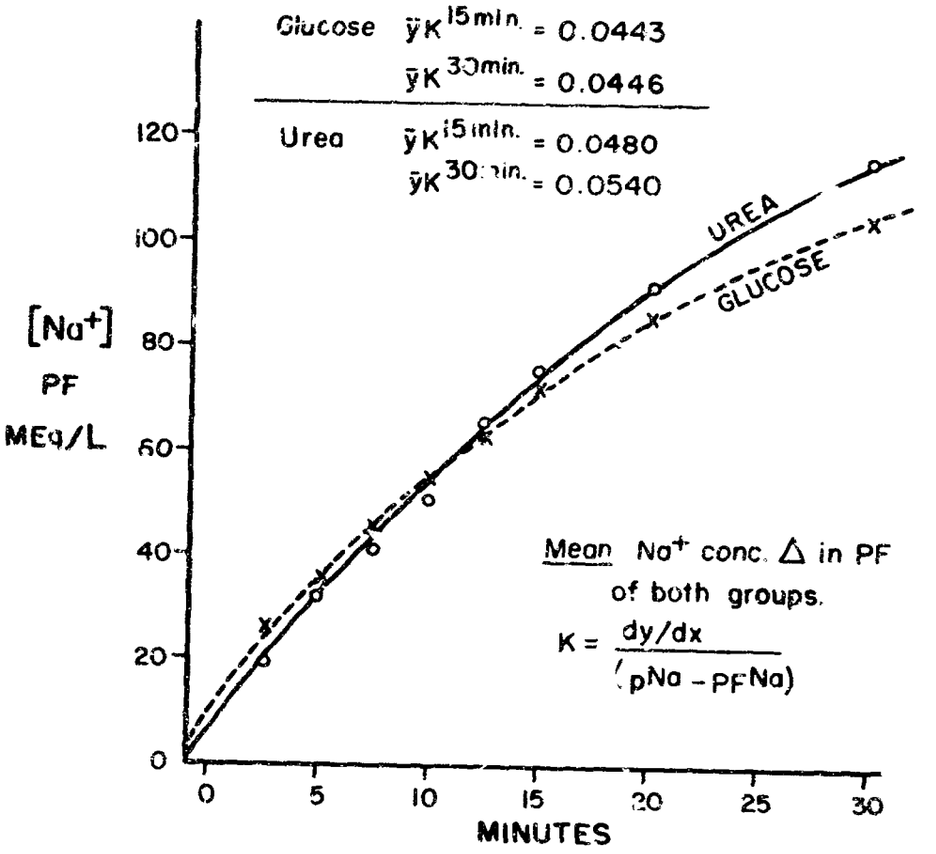
* Twice sodium concentration at 30 minutes.

** Reciprocal of plasma osmolality.


Glucose $\bar{y}K^{15min.} = 0.0443$
$\bar{y}K^{30min.} = 0.0446$
Urea $\bar{y}K^{15min.} = 0.0480$
$\bar{y}K^{30min.} = 0.0540$
UREA
GLUCOSE
[Na+] PF MEq/L
120
100
80
60
40
20
0
0
5
10
15
20
25
30
MINUTES
Mean Na+ conc. Δ in PF of both groups.
$K = \frac{dy/dx}{(pNa - PFNa)}$

3. Webb, W.R., Lemmer, R.A., and Elman, R.: Absorption rates, electrolyte and volume changes following subcutaneous intraperitoneal injections of solutions containing salt, glucose and amino acids. Surg.Gynec. Obstet. 91:265-270,1950.

4. Abbott, W.E., Levey, S., Foreman, R.C., Krieger, H., and Holden, W.D.: Danger of administering parenteral fluids by hypodermoclysis. Surg.32:305-315, 1952.

5. Schechter, A.J.,Cary, M.K., Carpentier, A.L., and Darrow, D.C.: Changes in composition of fluids injected into the peritoneal cavity. Amer. J. Dis. Children 46:1015-1026, 1933.

6. Karger, S. (ed): Scientific Tables. 5th ed. New York, 1959.

Presentations and/or Publications

None.

RESEARCH AND TECHNOLOGY RESUME		2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6952	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501D71R 01 193	NONE

11. TITLE:

(U) Factors Influencing Disposition of Uric Acid (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	11-65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	c. TYPE NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	4

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME Headquarters	NAME US Army Surgical Research Unit
ADDRESS US Army Medical Res & Dev Command Washington, DC 20315	ADDRESS Fort Sam Houston, Texas
RESP. INDIV. Kovaric, J.J., Lt Col	INVESTIGATORS PRINCIPAL ASSOCIATE Knochel, J.P., Maj, MC
TEL. 8 22 63737	TEL. 512 - CA1-2720 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS

Uric acid, acidosis, alkalosis, urinary NH_3

24. (U) Tech Objective - Studies were undertaken to determine acute effects of varying urine and plasma composition on uric acid excretion and the effect of urinary ammonia on hydrolysis of uric acid.

25. (U) Approach - It was discovered that uric acid concentration in urine or aqueous solutions showed a sharp decrease if exposed to ammonium hydroxide vapor. This finding suggested the possibility that urinary ammonia produced endogenously or that produced secondary to bacterial splitting of urea might alter uric acid content and consequently affect its values for clearance or excretion. A clinical corollary of this observation may be the recent report that certain patients who produce uric acid stones as well as patients with gout excrete less urinary NH_3 relative to hydrogen ion after receiving a standard acid load than normal subjects under the same circumstances. It was therefore reasoned that perhaps therein lies the explanation of increased uric acid excretion in certain patients with gout or urate-nephrolithiasis.

26. (U) Progress (Nov 65-Jun 66) - Studies are in progress involving both acute and long term manipulation of both plasma and urinary pH and urinary NH_3 concentration to determine if such a relationship exists. Sufficient data are not yet available to warrant discussion or conclusions.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC or COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY + 1	

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to NASA Form 1127) REPLACES DD Forms 61 WHICH ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: FACTORS INFLUENCING DISPOSITION OF URIC ACID

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 November 1965 to 30 June 1966

Investigator:

James P. Knochel, Major, MC

Reports Control Symbol: RCS-MEDDH-28(R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: FACTORS INFLUENCING DISPOSITION OF URIC ACID

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 November 1965 to 30 June 1966

5. Investigator: James P. Knochel, Major, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Studies were undertaken to determine acute effects of varying urine and plasma composition on uric acid excretion and the effect of urinary ammonia on hydrolysis of uric acid.

It was discovered in this laboratory that uric acid concentration in urine or aqueous solutions showed a sharp decrease if exposed to ammonium hydroxide vapor. This finding suggested the possibility that urinary ammonia produced endogenously or that produced secondary to bacterial splitting of urea might alter uric acid content and consequently affect its values for clearance or excretion. A clinical corollary of this observation may be the recent report (Henneman, P.H., S. Wallach and E.F.Dempsey: The metabolic defect responsible for uric acid stone formation. J. Clin. Invest. 41:537, 1962) that certain patients who produce uric acid stones as well as patients with gout excrete less urinary NH_3 relative to hydrogen ion after receiving a standard acid load than normal subjects under the same circumstances. It was therefore reasoned that perhaps therein lies the explanation of increased uric acid excretion in certain patients with gout or urate-nephrolithiasis.

Studies are in progress involving both acute and long term manipulation of both plasma and urinary pH and urinary NH_3 concentration to determine if such a relationship exists. Sufficient data are not yet available to warrant discussion or conclusions.

(1) Uric Acid (2) Acidosis (3) Alkalosis (4) Urinary NH_3

GPO 779884

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA 08 6951	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	6. SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 192

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) Factors Influencing Dialysance of Uric Acid (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	02 66	NA	Other | DA

16. PROCURE. METHOD	17 CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	c. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	c. TYPE NA	PRIOR FY 65	0	0
		CURRENT FY 66	1	4

19. GOVT LAB/INSTALLATION/ACTIVITY
NAME: Headquarters
ADDRESS: US Army Medical Res & Dev Command
Washington, DC 20315
RESP. INDIV.: Kovaric, J.J., Lt Col
TEL.: 8 22 63737

20. PERFORMING ORGANIZATION
NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas
INVESTIGATORS PRINCIPAL ASSOCIATE: Knochel, J.P., Maj, MC
TEL.: 512 - CA1-2720 TYPE: DA

21. TECHNOLOGY UTILIZATION	22 COORDINATION
Life Sciences	None

23. KEYWORDS: Uric acid, protein-binding, binding-displacement

24. (U) Tech Objective - Studies were initiated to determine simultaneous dialysance rates of uric acid, creatinine and urea across the twin-coil artificial kidney and whether changes in concentration of other endogenous or exogenous substances suspected to compete with uric acid for similar protein binding sites influence this measurement.

25. (U) Approach - Detailed investigation of methods for determination of uric acid binding have been completed. In normal individuals, approximately 25% or one mole of uric acid is bound to every two moles of serum proteins. Electrophoretic analysis of carbon-14 labelled uric acid incubated with normal plasma indicate that albumin is the major carrier protein with a smaller but significant fraction bound to an alpha globulin.

26. (U) Progress (Feb 66-Jun 66) Having established normal data, current studies are in progress to determine the simultaneous effects of substances which are known to react reversibly with serum proteins. Currently under investigation are fatty acids, triglycerides and certain drugs which, if shown to compete for similar binding sites, may be amenable to therapeutic manipulation to enhance uric acid removal during hemodialysis. This project has not been in effect for an adequate time nor have adequate data been obtained to allow a conclusive report.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
[ ] a. COMSEC or COMSEC RELATED [X] b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) FY	36.

DD FORM 1 AUG 64 1498

(Items 1 to 28 identical to AMXRD Form 1123) SUPERSEDES DA FORM 1498, 1 JUL 63, WHICH MAY BE USED.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: FACTORS INFLUENCING DIALYSANCE OF URIC ACID

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

2 January to 30 June 1966

Investigators:

James P. Knochel, Major, MC

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: FACTORS INFLUENCING DIALYSANCE OF URIC ACID

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 2 January to 30 June 1966

5. Investigator: James P. Knochel, Major, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Studies were initiated to determine simultaneous dialysance rates of uric acid, creatinine and urea across the twin-coil artificial kidney and whether changes in concentration of other endogenous or exogenous substances suspected to compete with uric acid for similar protein binding sites influence this measurement.

Detailed investigation of methods for determination of uric acid binding have been completed. In normal individuals, approximately 25 per cent or one mole of uric acid is bound to every two moles of serum proteins. Electrophoretic analysis of carbon-14 labelled uric acid incubated with normal plasma indicate that albumin is the major carrier protein with a smaller but significant fraction bound to an alpha globulin.

Having established normal data, current studies are in progress to determine the simultaneous effects of substances which are known to react reversibly with serum proteins. Currently under investigation are fatty acids, triglycerides and certain drugs which, if shown to compete for similar binding sites, may be amenable to therapeutic manipulation to enhance uric acid removal during hemodialysis.

This project has not been in effect for an adequate time nor have adequate data been obtained to allow a conclusive report.

(1) Uric Acid (2) Protein-binding (3) Binding-displacement

GPO 770864

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OB 6399	CSCRD-103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	"A. NEW"	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 01 190

10b. PRIOR NUMBER/CODE: NONE

11. TITLE:
(U) The Influence of Urinary Ammonia on Uric Acid Excretion (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (003500)	10 65	NA	Other | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 65	1	5
		CURRENT FY 66	2	10

19. GOVT LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
NAME: US Army Surgical Research Unit
ADDRESS: Fort Sam Houston, Texas
INVESTIGATORS PRINCIPAL ASSOCIATE: Knochel, J.P., Maj, MC; Seraile, L.G., MS
TEL.: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

22. COORDINATION: NONE

23. KEYWORDS: Uric acid, ammonia excretion, EDTA

24. (U) Tech Objective - In vitro studies show that ammonia produces a prompt fall in uric acid concentration in plasma or aqueous solutions containing uric acid and recent studies show that patients with gout produce less ammonia for a given degree of acidosis than normal subjects and suggest that renal excretion of ammonia may somehow be related to the disposition of uric acid. This study was designed to determine the effect of enhanced ammonia concentration on the renal excretion of uric acid while on a constant purine intake. In addition, the effect of EDTA on urine uric acid levels was examined, since evidence was elicited suggesting that EDTA protects uric acid in alkaline solutions from auto-oxidation.

25. (U) Approach - The basic plan was to influence the urinary ammonia output of test subjects by urinary alkalinization for a one-week period with Shohl's solution, followed by urinary acidification for a one-week period with ammonuim chloride. During this period, urinary output of uric acid an plasma uric acid levels were measured. Test subjects were maintained on a constant purine intake. Each voiding of urine was measured and divided into two equal aliquots. One was kept under refrigeration in a container with EDTA. Obtaining a sufficient number of test subjects who tolerate the regimen of the study has been difficult.

26. (U) Progress (Oct 65-Jun 66) - Test subjects studied seem to show no relation between their urinary uric acid output and their states of alkalinization or acidification. Plasma uric acid levels seem to fall near the end of the seven-day period of alkalinization. Uric acid levels measured in urine with no preservative were the same as those measured in urine containing EDTA.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY + 1	

DD FORM 1498

(Items 1 to 36 continued on NAVY Form 11227) REPLACES DD FORM 611 & 613C WHICH ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE INFLUENCE OF URINARY AMMONIA ON URIC ACID EXCRETION

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 October 1965 to 30 June 1966

Investigators:

James P. Knochel, Major, MC
Leonard G. Seraile, M.S.
James M. Glennon, SFC E7

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: THE INFLUENCE OF URINARY AMMONIA ON URIC ACID EXCRETION

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 October 1965 to 30 June 1966

5. Investigators: James P. Knochel, Major, MC
Leonard G. Seraile, M.S.
James M. Glennon, SFC E7

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Studies in progress in this laboratory have shown that exposure of aqueous solutions, urine or plasma containing uric acid to ammonia produces a prompt fall in uric acid concentration. Although the final products of this chemical reaction are unknown, chromatographic, radioautographic and electrophoretic studies have shown evidence of new product formation.

This finding, considered in light of recent studies showing that patients with gout produce less ammonia for a given degree of acidosis than normal subjects, suggests that ammonia in urine under certain circumstances may have an important role in disposition of uric acid.

A pilot study was performed using convalescent burned patients to determine the effect of urinary alkalinization as compared to acidification on renal uric acid excretion.

(1) Uric acid
(2) Ammonia excretion
(3) EDTA

THE INFLUENCE OF URINARY AMMONIA ON URIC ACID EXCRETION

In a study of peritoneal diffusion of uric acid in dogs,[1] it was necessary to determine the pK value of uric acid in solutions having the ionic strength of plasma at body temperature. The spectrophotometric method was chosen as the means of determining this value; this entails measuring the optical density of uric acid in buffers of carefully measured pH values at a preselected wavelength. It is necessary to use buffers with no significant absorbency at the wavelength under investigation. At the point of maximum absorbency for uric acid-pH9, the recommended buffer is one containing ammonium chloride and ammonium hydroxide in solution.

In our early attempts at measuring the absorbency of uric acid in the ammonium chloride-ammonium hydroxide buffer, we noted on several occasions that the absorbency of uric acid at pH9 was less than that in the buffers of lower pH at a wavelength of 292 mu, a fact which is inconsistent with theoretical considerations. When the same concentration of uric acid in buffers at pH9 of different composition was measured, the expected maximum absorbency at 292 mu was recorded. From this experience, it seemed that ammonia or ammonia-like compounds were responsible for the decomposition of uric acid.

To further investigate the effect of ammonia or uric acid, two similar aliquots of a standard solution of uric acid were each placed under a bell jar in an open container. Under one jar was placed a container of water, under the other was placed an open container of a solution of ammonium hydroxide. After overnight exposure at room temperature, the uric acid content of the two standard aliquots was measured. Practically 100 per cent of the uric acid remained in the container exposed to water vapor, whereas only about 10 per cent of the original uric acid remained in the container exposed to ammonia vapors. Similar results were observed when uric acid in aqueous solution or in .05M sodium borate buffer at pH 9.0 was exposed to ammonia vapors. There are rather casual references to the

instability of uric acid in alkaline solutions,[2-4] but for the most part degradative changes seem to have been observed in solutions of pH12 and above, more alkaline solutions than those in this study. It seems unlikely that the changes in uric acid concentration caused by ammonia are due to pH effects alone since uric acid in buffers of equivalent pH and ionic strength of the ammonia containing buffers does not seem to undergo the rather prompt decomposition observed in the ammonia buffers.

Equimolar solutions of uric acid in ammonium chloride buffers of pH 5.3, 6.7, and 9.0 in addition to an aqueous solution of uric acid were prepared and the optical densities at 292 recorded. The solutions were placed in the refrigerator over a four-day period, then their absorbencies again recorded. Only the uric acid at pH9 showed a change in reading.

To test further the action of ammonia on uric acid, an aqueous solution of uric acid was spotted onto the origin of Whatman No. 1 chromatographic paper. One paper was hung in a chromatographic chamber and exposed to water vapor overnight, the other paper was hung in a chromatographic chamber and exposed to ammonia vapors. Then both papers were air dried and chromatographed in a butanol-pyridine-water solvent. Upon examination of the chromatograms, there was some evidence of decomposition of uric acid in the ammonia atmosphere, but it was not of the order observed in the aqueous ammonia buffers. Chromatograms of uric acid developed in an ammonia-containing solvent also showed evidence of uric acid decomposition amounting to 10-15 per cent of the original concentration.

We tested the behavior of uric acid in an ammonia-like buffer of pH 8.9 containing Tris and EDTA and found that no spontaneous changes in absorbency occurred in this solution. Furthermore, when this solution was exposed to ammonia vapors overnight at room temperature, it showed less than a two per cent change in absorbency at 292 mu. Of the buffers tested in this study, only this one showed no change in absorbency after exposure

to ammonia vapors. This suggested that whatever the effect of ammonia on uric acid in solution, EDTA is inhibitory toward it.

The following experiment seemed to confirm the above hypothesis. A .05 M ammonium chloride-ammonium hydroxide buffer of pH 8.97 was prepared. An aliquot of this buffer was made 01 M with respect to EDTA and the pH adjusted to 8.97 with ammonium hydroxide. Equimolar quantities of uric acid were dissolved in each of the two buffers, a portion of each buffer being saved as a blank. The absorbency of each solution was read against its blank at selected time intervals. Between readings, the solutions were stored in the refrigerator. Over a period of four days, the absorbency of the uric acid solution containing EDTA showed no change, whereas that of the buffer without EDTA had decreased in absorbency by 36 per cent. At the end of 10 days, the EDTA containing solution still had not changed in absorbency whereas the other solution had decreased by 68 per cent. After 20 days when only four per cent of the original uric acid remained in the solution without EDTA, 96 per cent of the original uric acid was still present in the buffer containing EDTA.

Uric acid solutions labeled with C^{14} tracer were exposed to ammonia vapors and water and the resulting solutions chromatographed. The radioautographs of the chromatograms showed that while uric acid exposed to ammonia vapors underwent a form of decomposition, solutions exposed to water vapor alone also showed unexpected changes. Similar experiences were encountered with plasma samples containing C^{14} labeled uric acid when exposed to ammonia vapor and water. Until these experiments with C^{14} labeled uric acid, it was felt that ammonia containing solutions of alkaline pH had some unique oxidative effect on uric acid, but the ease with which carbon-labeled uric acid exposed to water vapor was oxidized caused some consternation.

The nature of the products formed from uric acid seems to vary with the conditions of the experiment. When exposed to water vapor, uric acid showed

radioautographic evidence of one major oxidation product whereas when exposed to ammonia vapor, several oxidative compounds were formed. Praetorius[5] and Canellakis[6] in their studies of the action of uricase on uric acid found also that the nature of end products formed is dependent on pH and type of buffer employed.

There remained the possibility that the loss of absorbency of ammonia-containing solutions of uric acid was due to formation of ammonium urate which either did not absorb in the ultraviolet region or was precipitated from solution. Solutions of authentic ammonium urate were prepared, however, and showed the absorption curve characteristic of uric acid. No visible precipitate was detectable in the ammonia-containing uric acid buffer, nor could any ultraviolet-absorbing material be eluted from a sintered glass filter through which the uric acid solution had been passed. Ammonium urate solutions left at room temperature overnight lost 84 per cent of their original absorbency at 292 mu whereas ammonium urate solutions prepared in pH9 buffer containing ammonium hydroxide, ammonium chloride and EDTA showed no changes in absorbency when left at room temperature overnight.

Recent studies by Henneman, et al.,[7] and by Gutman and Yu[8] show that patients with gout produce less ammonia for a given degree of acidosis than normal subjects. These observations along with our experimental studies of the action of ammonia on uric acid in solutions suggest that ammonia in urine under certain circumstances might have an important role in the disposition of uric acid. A pilot study was designed to determine the effect of urinary alkalinization against acidification on renal uric acid excretion while on a constant purine intake.

Normal subjects on a constant purine diet were alkalinized by daily oral administration of Shohl's solution for a period of seven days. Following this period, the next seven days each subject was given a daily oral dose of 7.5 gm of ammonium chloride in tomato juice. Urine samples were collected daily during the study.

Blood samples were collected every second day. Each voiding of urine was measured, and half the quantity was placed in a bottle containing 300 mg of EDTA as preservative; the other half was placed in a container with no preservative. Bottles were kept refrigerated during the collection period. At the end of each daily collection, each aliquot of urine was measured and analyzed for uric acid and electrolytes. Blood plasma was also analyzed for uric acid and electrolytes.

Total daily urinary output of uric acid seemed to be no different during the alkalinization period than during the period of acidification. The aliquots of urine containing EDTA had the same uric acid content as the corresponding aliquots without preservative. Random samples which were saved and stored in the refrigerator for several days then reanalyzed for uric acid content also showed no difference between aliquots containing EDTA and those with no preservative. The only notable change was an apparent fall in plasma uric acid level near the end of the seven-day period of alkalinization.

On the basis of the work to date, it is not possible to say just what effect urinary ammonia or alkalinization does have on the excretion of uric acid. Uric acid as it occurs in urine does not seem to undergo the spontaneous decomposition that was noted in alkaline ammonia containing systems. The protective action of EDTA toward uric acid in alkaline solution suggests that the autodecomposition might be a metal-catalyzed reaction enhanced by the presence of ammonia. Copper has been implicated as catalyst in both enzymatic[9] and nonenzymatic[10] oxidations of uric acid. Another study[11] suggests that iron may play a role in certain oxidative reactions of uric acid. Further work is needed to elucidate the nature of the action of ammonia on uric acid in solution.

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Presentations and/or Publications

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
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4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
30 06 66	A. NEW	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 01 177	NONE

11. TITLE:
(U) Erythrocythemia Secondary to an Ectopic Site of Erythropoietin Production (44)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CMPT. COMPL. DATE	15. FUNDING AGENCY
Clinical Medicine (0U3500)	07 65	NA	Other DA

16. PROCURE. METHOD	17. CONTRACT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	N/A	PRIOR FY 65	0	0
		CURRENT FY 66	2	10

19. GOVT LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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ADDRESS US Army Medical Res & Dev Command Washington, DC 20315	ADDRESS Fort Sam Houston, Texas
RESP. INDIV. Kovaric, J.J., Lt Col	INVESTIGATORS PRINCIPAL Vertel, R.M., Capt, MC
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	TEL. 512 - CAI 2720 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Life Sciences	None

23. KEYWORDS
Erythropoietin, polycythemia, renal cyst

24. (U) Tech Objective - To confirm the reliability and sensitivity of the polycythemic mouse bioassay of erythropoietin activity.

(U) Approach - Hypertransfused mice were injected with cyst fluid, serum and urine. Erythropoietin activity was gauged by percent incorporation of radioiron.

25. (U) Progress (Jul 65-Jun 66) - Preliminary studies have confirmed the reliability and sensitivity of this assay. Erythropoietin activity has been found in cyst fluid and in preoperative serum and urine of a patient with polycythemia associated with a simple renal cyst. Three weeks postoperatively, the hemoglobin and hematocrit had returned to normal and the urinary erythropoietin level.

26.

27. COMMUNICATIONS SECURITY	28. DOD CODE	29. SUMRY CODE
☐ a. CLASSIFIED OR CLASSIFIED RELATED ☐ b. NOT RELATED TO	8R	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL DISTRIBUTION

35. EST. FUNDS (In thousands)	36.
FY + 1	

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: ERYTHROCYTHEMIA SECONDARY TO AN ECTOPIC SITE OF ERYTHROPOIETIN PRODUCTION

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert M. Wertel, Captain, MC
Arthur D. Mason, Jr., M.D.

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: ERYTHROCYTHEMIA SECONDARY TO AN ECTOPIC SITE OF ERYTHROPOIETIN PRODUCTION

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert M. Vertel, Captain, MC
Arthur D. Mason, Jr., M.D.

6. Reports Control Symbol: RCS-MEDDH-288(R1)

At present, the most sensitive reliable method for determining erythropoietin activity is a bioassay utilizing radioiron incorporation into red blood cells as a gauge of erythropoiesis in hypertransfused or hypoxia-induced plethoric mice. Preliminary studies in the US Army Surgical Research Unit Renal Laboratory had confirmed the reliability and sensitivity of this assay method.

A case of polycythemia associated with a simple renal cyst is presented. Three weeks following excision of the renal cyst, the patient's hemoglobin had fallen to normal levels. Cyst fluid, serum, and urine were assayed for erythropoietin activity in the hypoxia-induced polycythemic mouse. Erythropoietin activity was found in the cyst fluid and in preoperative serum and urine. Three weeks postoperatively, the urinary erythropoietin level had returned to normal.

While there is abundant evidence that erythropoietin originates primarily in the kidney, the site of production within the kidney has not been conclusively determined. This patient's cyst wall shows only compressed tubules; no glomeruli or juxtaglomerular tissue is present. A

portion of this cyst wall is currently being propagated in tissue culture media in the Cytokinetics Laboratory of the USAF School of Aerospace Medicine. When sufficient tissue culture supernatant fluid has been harvested, this material will be assayed for erythropoietin activity. If activity is found, it would suggest that the renal tubular cell is indeed a site of erythropoietin production.

(1) Erythropoietin
(2) Polycythemia
(3) Renal Cyst

ERYTHROCYTHEMIA SECONDARY TO AN ECTOPIC SITE OF ERYTHROPOIETIN PRODUCTION

A humoral regulator of erythropoiesis was first suggested by Carnot and Deflandre in 1906 based on studies of rabbits with posthemorrhagic anemia.[1] In 1950, Reissman reported the first convincing evidence of such a humoral agent.[2] He demonstrated that if one of a pair of parabiotic rats were made hypoxic, erythroid hyperplasia developed in both animals, implying that a humoral agent had crossed the anastomotic vascular channels. Erslev, in 1953, was the first to provide direct demonstration of a plasma factor.[3] He injected large amounts of plasma from severely bled rabbits into normal rabbits. After four daily injections of 50 ml of plasma, he observed a reticulocytosis; when plasma was given over a longer period, polycythemia developed.

To date, erythropoietin has not been crystallized, but substantial purification has been achieved. The hormone is thought to be a glycoprotein with a molecular weight of about 28000.[4] Schooley and Garcia have produced anti-erythropoietin serum in rabbits and goats against concentrated partially purified human urinary erythropoietin.[5] Their serum produces distinct bands of precipitation on Ouchterlony plates and neutralizes biologic acactivity of erythropoietin specifically. This work may form the basis for an accurate immunoassay in the near future. Attempts to develop an *in vitro* assay have thus far met with little success.[6] At present, the most sensitive reliable method for determining erythropoietin activity is a bioassay utilizing radioiron incorporation into red blood cells as a gauge of erythropoiesis in hypertransfused or hypoxia-induced plethoric mice.[7]

Preliminary studies in the US Army Surgical Research Unit Renal Laboratory have confirmed the reliability and sensitivity of this assay method.

Erythropoietin Bioassay

Adult female Swiss-Webster mice are observed for a

period of four weeks for evidence of disease or failure to thrive. Any animal losing weight is not used in the assay. On Day 1 of the assay, 1.0 ml of washed, packed, homologous red blood cells are injected intraperitoneally. On Day 2, this intraperitoneal transfusion is repeated. On Day 7, the average hematocrit for the group has been 72% (average pretransfusion hematocrit 48%). The material to be assayed is injected intraperitoneally on Days 7 and 8. On Day 9, radioiron (ferrous citrate) is injected intraperitoneally (about 150,000 counts/minute/animal). On Day 11, the animals are bled, using the axillary pooling method, and 0.5 ml of blood is counted in a well counter. Animals with a hematocrit less than 55% on the day of counting are discarded. The % radioiron incorporation is determined by the formula shown in Table 1.

Erythropoietin is currently quantitated in terms of standard units set by the Medical Research Council of England. This agency has a large pool of concentrated, partially purified urinary human erythropoietin which it assays in polycythemic mice and distributes to investigators for assay standardization. They have recommended that investigators prepare their own erythropoietin laboratory control and calibrate the bioassay in terms of units of the International standard. Having established the potency of the laboratory standard, this material can then be used for further laboratory quantitation. The SRU assay has been calibrated with a laboratory standard provided by Dr. Peter Lowy of the California Institute of Technology (Table 2). Since this material has been calibrated with the International standard, it has been serving as an approximate gauge of activity, pending shipment of the International standard from England. These values plotted on semi-log paper yield a linear log dose-response curve.

The transfusions required to produce polycythemia in these mice are tedious, time consuming, and costly, since an average of four mice must be sacrificed to produce enough red cells to transfuse one test animal. We are, therefore, currently using the hypoxia-induced plethora technique. Adult female Swiss-Webster mice are kept at a simulated chamber altitude of 18,000 feet for 17 days. The average

Table 1. Formula for Calculation of Per Cent Radioiron Incorporation

$$\text{Per cent } Fe^{59} \text{ Incorporation} = \frac{(\text{Net Counts Blood/Min/0.5 ml})\ (\text{Body Weight})\ (15.0)}{\text{Net Counts of Standard Injected}}$$

(1) Net Counts = Ratemeter reading minus Background Counts/Min

(2) Body Weight in Grams

(3) $15.0 = \frac{7.5\ (\text{Blood Volume per cent of Total Body Weight})}{0.5\ (\text{Volume of Blood Counted})}$

Table 2. Dose-Response with Laboratory Standard EP

Mg of Laboratory Standard Erythropoietin	% Fe^{59} Incorporation
Saline control	0.28 ± 0.04
0.05	0.88 ± 0.39
0.20	3.83 ± 0.51
1.00	13.21 ± 4.20
3.00	22.78 ± 4.02

(1) Plotting on semi-log paper yields a linear log dose-response curve

(2) Five mice/test group

hematocrit at the end of this period is 68% (average prehypoxia hematocrit 48%). Three days after return to ambient pressure, the assay is begun as described above.

A patient with polycythemia was recently seen in consultation by the Renal Branch. The clinical usefulness of erythropoietin quantitation is illustrated by this case.

Case History

In November 1965, the Renal Branch was asked to see a patient in whom polycythemia had been recently diagnosed. The patient was a 46-year-old white male active duty Army sergeant major who had been well up to that time. Previous hemoglobin and hematocrit determinations throughout the patient's Army career had been normal. However, in November 1965, the patient was found to have a hemoglobin of 18 gm% with a hematocrit of 60%, WBC 8200, platelet count 212,000. Leukocyte alkaline phosphatase staining was normal. Chronium 51 red cell mass was at upper limit of normal and RISA plasma volume was normal. Pulmonary function studies showed a mild obstructive defect unchanged with IPPB, Arterial oxygen saturation pre- and post-exercise was normal. Nephrotomograms following the intravenous injection of 30% Hypaque showed a large midrenal mass which distorted the calyceal system of the left kidney and appeared to be of the same density as the renal parenchyma (Fig. 1). At surgery, the patient was found to have a large renal cyst lying on the anterior surface of the left kidney, measuring about 8 cm in diameter. The aspirated cyst fluid was clear. Following aspiration, the cyst wall was excised sharply and the edge oversewn. Microscopic examination of the cyst wall revealed compressed renal tissue with many identifiable tubules, most of which contained eosinophilic colloid-like material. No glomeruli were identified; no inflammatory infiltrate was present (Fig. 2). Three weeks following excision of the renal cyst, the patient's hemoglobin had falled to 14.6 gm with a hematocrit of 46% (Table 3). The patient's cyst fluid, serum and urine were assayed for erythropoietin activity in the hypoxia-induced polycythemic mouse (Table 4). Erythropoietin activity was found in the cyst fluid and in

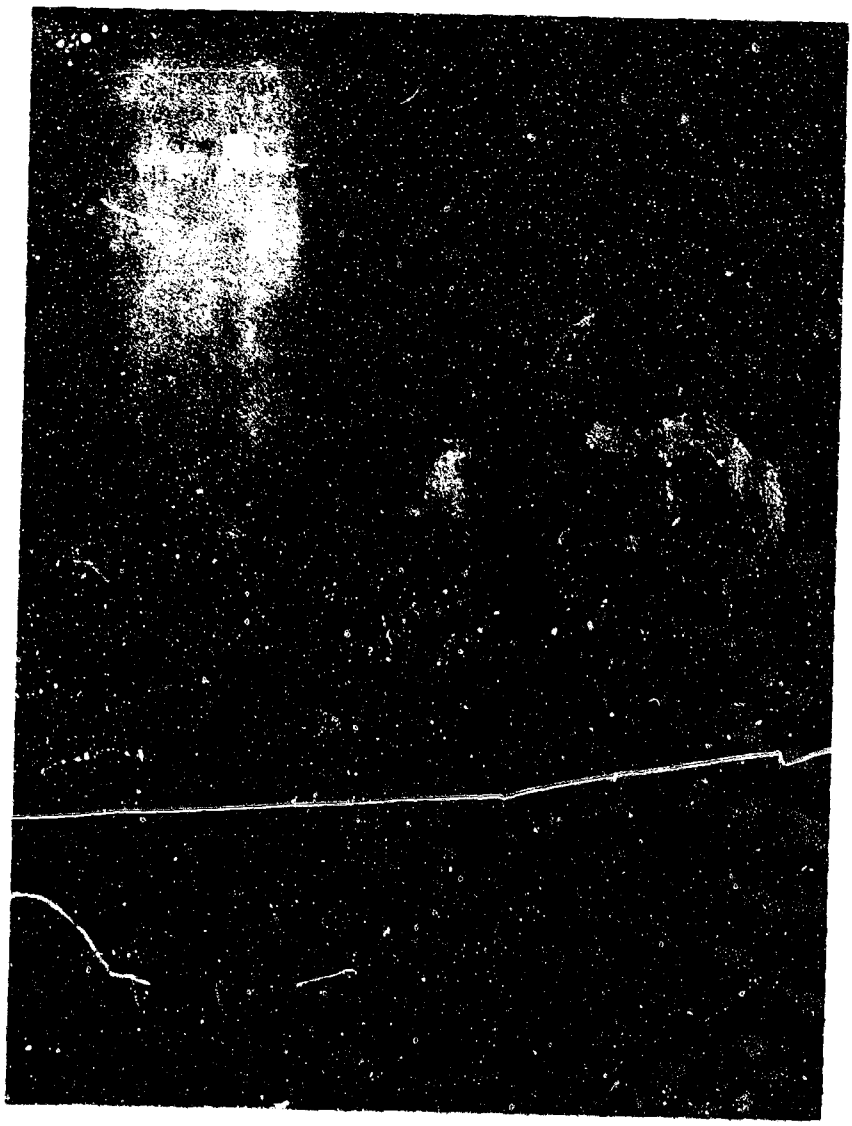

Figure 1

Figure 2

Table 3. Hematologic Data

	Hemoglobin Gm %	Hematocrit %	WBC	Platelets
November 1959	16.6	50	-	Adequate
November 1965	18.0 17.5	60 60	8200	212,000
February 1966 (preoperative)	18.0	54	6500	Adequate
March 1966 (3 weeks postexcision of renal cyst)	14.6	46	6200	Adequate
March 1966 (5 weeks postexcision of renal cyst	14.4	45	7200	220,000

Table 4. Renal Cyst with Polycythemia: Erythropoietin Assay

Material Assayed	% Fe^{59} Incorporation
Saline	0.95 ± 0.37
Serum 13 Feb 66	3.73 ± 0.99
Urine 13 Feb 66	6.96 ± 1.08
Renal Cyst Fluid 15 Feb 66	21.91 ± 4.35
Urine 18 Feb 66	2.24 ± 1.25
Urine 6 Mar 66	1.47 ± 0.32

(1) Excision renal cyst 15 Feb 66

(2) All specimens frozen immediately after collection and thawed just prior to injection

(3) Saline; urine: 1.0 cc injected daily on two successive days
Cyst fluid; serum: 0.5 cc injected daily on two successive days

(4) Five mice/test group

preoperative serum and urine. Urine obtained three days following surgery showed a striking fall in activity to a level only slightly above normal. Three weeks post-operatively the urinary erythropoietin level had returned to normal.

Discussion

During the past decade, important advances have been made in our understanding of the physiology of erythropoietin and normal regulation of erythropoises. The study of erythropoietin has likewise provided a fresh approach to the study of polycythemia.

Polycythemia Vera. This myeloproliferative disorder is usually manifested by a pancytosis; however, erythrocythemia may occur without associated thrombocytosis and leukocytosis. Splenomegaly is a common finding. Although evidence of primary hypoxemia is usually lacking, Lertzman demonstrated that the arterial oxygen tension may be low in polycythemia vera with no demonstrable coexistent cardiorespiratory disease and presented evidence that the hypoxia may be the result of the abnormality of the pulmonary vasculature.[8] However, conclusive evidence of hypoxia as a factor of etiologic significance in polycythemia vera is lacking. Erythropoiesis measured by plasma radioiron turnover rate is not suppressed by oxygen inhalation in polycythemia vera as it is in patients with hypoxia-induced plethora. The major question in this disorder has been whether the inappropriately increased production of red blood cells is caused by an exaggerated hormonal stimulation of the marrow, or does the marrow proliferate and differentiate at an increased rate without physiologic regulation? While earlier workers reported finding erythropoietin in plasma from polycythemia vera patients, Dogowin and Gurney, using the plethoric mouse, currently the most sensitive assay available, have never been able to demonstrate erythropoietin in plasma from polycythemia vera patients.[9] Van Dyke has clearly demonstrated that erythropoietin activity is routinely measurable in urinary concentrates in normal people. However, patients with polycythemia vera do not have measurable erythropoietin activity in urinary

concentrates.[10] Phlebotomy, usually a potent stimulus of erythropoietin production, fails to produce measurable activity in serum or urine in patients with polycythemia vera.[11] To date, two patients with polycythemia vera have been studied with the SRU erythropoietin assay. No measurable activity was found in the pre- or post-phlebotomy serum of either patient.

Hypoxia-Induced Plethora. The patient with chronic pulmonary insufficiency frequently develops secondary polycythemia as the result of an increased hormonal stimulation of a normal marrow. Hypoxia of the sensor organ, presumably the kidney, augments the secretion of erythropoietin which induces differentiation of stem cells to erythrocyte precursors. Erythrocytosis without accompanying thrombocytosis and leukocytosis is present in these patients. Erythropoiesis is suppressed by oxygen inhalation in patients with hypoxia-induced plethora. Prentice and Mirand have shown that in rabbits a significant rise in erythropoietin titer occurs between 4-8 hours from the onset of hypoxia, reaching a peak at 24-48 hours and returning to normal at 72 hours.[12] Siri, et al., demonstrated that a human subject exposed to low oxygen tension shows a maximum erythropoietin activity on the third day of hyoxia, followed by a rapid fall to unmeasurable levels during the fourth day.[13] Stohlman suggested that the fall in erythropoietin concentration with continued exposure to low oxygen pressure may result from increased utilization of the hormone by the activated marrow.[14] However, it is more likely that reduction of functional hypoxia produced by an increased red cell mass results in the attenuation of erythropoietin synthesis. Thus, it is not surprising that erythropoietin activity is seldom measurable in serum or urine of patients with compensated secondary polycythemia. However, if the compensatory equilibrium is modified by phlebotomy, measurable erythropoietin activity promptly appears in the serum and urine.[11] Thus, erythropoietin response to phlebotomy may differentiate polycythemia vera from secondary polycythemia.

Erythrocythemia Secondary to an Ectopic Source of Erythropoietin Production. In 1964, Waldman reviewed

238 cases of tumors associated with erythrocythemia (Table 5). Two-thirds of these reported cases were associated with renal pathology.

It appears from the literature that erythrocythemia occurs in approximately 2% of patients with hypernephroma.[16] Conversely, 1-2% of polycythemic patients are estimated to have an underlying renal carcinoma. Lawrence and Donald found that renal carcinoma occurred in 0.93% of 325 polycythemic patients, whereas the expected incidence of renal carcinoma in the United States is 0.003%.[17] Many laboratories have demonstrated erythropoietin activity in saline extracts of renal cell carcinomas.[15,16] Reversal of erythrocytosis has occurred in 31 of the 33 patients with renal tumors where the neoplastic lesions could be completely resected. In several cases, erythrocytosis recurred with the appearance of metastatic disease.

In 1958, McFadzean, et al., reported 17 cases of erythrocythemia in 176 patients with primary carcinoma of the liver.[18] Brownstein and Ballard recently added 11 new cases of hepatoma associated with unexplained erythrocytosis.[19] They found a 9.4% incidence of polytythemia among 213 patients with hepatoma at the Metropolitan VA Hospital in New York, which is similar to the 9.9% incidence among the 176 cases reported by McFadzean in Hong Kong. Erythropoietin assays of plasma have demonstrated an increased activity in two cases and a normal activity in one. Tumor assay has demonstrated an increased activity in one case, and normal activity in another.[19] Since the association of polycythemia with cirrhosis is extremely rare, the appearance of an elevated hemoglobin in a cirrhotic patient renders the possibility of neoplastic transformation very likely.

Nine to twenty per cent of patients with cerebellar hemangioblastomas have associated polycythemia.[20] In several cases cyst fluid from the cerebellar tumors have shown positive erythropoietin activity.[20]

There are several reported cases of hydronephrosis associated with erythrocytosis. Jaworski and Wolan

Table 5. Tumors Associated with Polycythemia

Lesion	Cases Reported
Renal carcinoma	110
Benign renal lesions (cysts, hydronephrosis, adenoma)	38
Cerbellar hemangioblastoma	50
Uterine fibroma	15
Adrenal cortical carcinoma or hyperplasia	8
Ovarian tumors	7
Hepatoma	7
Pheochromocytoma	3

described a young man with a huge hydronephrotic kidney and severe erythrocytosis.[21] The hydronephrotic kidney was drained and decompressed through a ureteral catheter for 12 days and then nephrectomy was performed. In this patient, decompression of the hydronephrotic kidney resulted in the withdrawal of the stimulus to excessive erythropoiesis as rapidly and effectively as might be expected following nephrectomy. Seven of nine cases of polycythemia and hydronephrosis reviewed by these authors had remission following nephrectomy. One of three had positive erythropoietin in plasma; hydronephrotic fluid was negative in the one patient assayed. Toyama and Mitus produced erythrocytosis in 63% of rabbits with experimental unilateral hydronephrosis.[22] This phenomenon was correlated with pressure atrophy of the renal medulla. Van Lessen, et al., were able to produce erythrocytosis in dogs by partial unilateral renal vein ligation and concluded that the engorged kidney produced erythropoietin.[23] These studies all suggest that increased intrarenal pressure may somehow trigger erythropoietin production. An alternative explanation would be that tissue ischemia produced by increased pressure is the stimulus for erythropoietin release.

In 1960, Nixon, et al., reported the first case of polycythemia associated with a simple solitary renal cyst.[24] Saline extract of the cyst wall, which contained only a single layer of cuboidal epithelium, demonstrated erythropoietin activity as did the cyst fluid. Since that time there have been several additional reports of reversible erythrocythemia related to renal cysts. In 1963, Rosse assayed cyst fluid from nine patients with renal cysts.[25] Three of these patients had normal hematocrits, six had polycythemia. The renal cyst fluid from the three normal patients showed erythropoietin activity while the renal cyst fluid from three of the six polycythemic patients showed erythropoietin activity. The authors concluded that the only certain evidence of a causal relationship between erythropoietin renal cyst fluid and polycythemia is remission of the polycythemia on removal of the cyst. Plzak obtained cyst fluid from 20 random patients undergoing renal cyst decompression.[26] Only one of these patients was polycythemic. Nevertheless, nine of the 20 patients studied showed erythropoietin activity in the cyst fluid, indicating that erythropoietin may be produced in renal cysts without causing polycythemia. The presence of erythropoietin in cyst fluid suggests

either that erythropoietin is formed by the cyst wall or concentrated from the serum. Renal cysts have been shown to concentrate creatinine against a gradient between the contents of the cysts and the serum. However, if renal cysts merely concentrate erythropoietin made elsewhere, one would not expect to observe remission of polycythemia after extirpation of the cyst. It is more likely that the cyst is the site of erythropoietin production.

While there is abundant evidence that erythropoietin originates primarily in the kidney, the site of production within the kidney has not been conclusively determined. The juxtaglomerular apparatus would seem a logical source of erythropoietin production within the kidney since these specialized secretory cells have a proved endocrine function. Hirashima and Takaku demonstrated that when rats were rendered anemic, whether by bleeding or by administration of phenylhydrazine, the juxtaglomerular index increased significantly.[27] When rats were made polycythemic by transfusion, the juxtaglomerular index was significantly reduced. Hansen demonstrated that constriction of one renal artery in rabbits caused a marked increase in juxtaglomerular granules in the clamped kidney; one animal developed erythrocythemia.[28] Takaku, et al., subsequently demonstrated that unilateral constriction of the renal artery resulted in an immediate increase in erythropoietin titer and reticulocytosis in in rabbits.[29] However, Goldfarb and Tobian showed that hypoxia without hypovolemia will not change the granularity of the juxtaglomerular cells in the rat.[30]

Fisher performed immunofluorescent studies using antisheep erythropoietin serum prepared in rabbits.[31] Sections of anemic sheep kidney showed intense fluorescence of the capillary walls of the glomerular tufts. No staining was present in the juxtaglomerular cells. The fluorescence seen in the capillary walls of the glomerular tufts suggested to the authors that erythropoietin is either produced or stored in these cells. Baum, et al., using a similar technique, showed that kidneys from a previously bled sheep demonstrated staining in virtually every glomerulus with the stain in intracellular globules in cells

along the outer periphery of the glomerulus.[32] No staining was present in the juxtaglomeru'ar area, liver, lymph nodes or spleen. No stain was seen in normal sheep kidney. This specific staining could be removed by absorption with commercially purified sheep erythropoietin.

Renal explant work by Muirhead, et al., clearly demonstrated that erythropoietin activity resides in both the cortex and medullary tissue.[33] These authors suggested the tubules as a logical source of erythropoietin production. Perfusion of th dog kidney with anemic or hypoxic blood has been shown to stimulate erythropoietin production.[34] It is of interest that Merailuride, a mercurial diuretic which is known to inhibit sulfhydryl containing enzymes in the renal tubules, can abolish the erythropoietin release in this experimental model.[35] This would also suggest that the renal tubule is somehow concerned with erythropoietin production or activition.

The cyst wall of the patient reported in this paper shows only compressed tubules; no glomeruli or juxtaglomerular tissue is present. A portion of this cyst wall is currently being propagated in tissue culture media in the Cytokinetics Laboratory of the US Air Force School of Aerospace Medicine. At the present time, this tissue had adjusted to tissue culture life and there is abundant growth of cells which strongly resemble renal tubular cells. When sufficient tissue culture supernatant fluid has been harvested, this material will be assayed for erythropoietin activity. If activity is found, it would suggest that the renal tubular cell is indeed a site of erythropoietin production.

Summary

At present, the most sensitive reliable method for determining erythropoietin activity is a bioassay utilizing radioiron incorporation into red blood cells as a gauge of erythropoiesis in hypertransfused or hypoxia-induced plethoric mice. Preliminary studies in the US Army Surgical Research Unit Renal Laboratory had confirmed the reliability and sensitivity of this assay method.

A case of polycythemia associated with a simple renal cyst is presented. Three weeks following excision of the renal cyst, the patient's hemoglobin had fallen to normal levels. Cyst fluid, serum, and urine were assayed for erythropoietin activity in the hypoxia-induced polycythemic mouse. Erythropoietin activity was found in the cyst fluid and in preoperative serum and urine. Three weeks post-operatively, the urinary erythropoietin level had returned to normal.

While there is abundant evidence that erythropoietin originates primarily in the kidney, the site of production within the kidney has not been conclusively determined. This patient's cyst wall shows only compressed tubules; no glomeruli or juxtaglomerular tissue is present. A portion of this cyst wall is currently being propagated in tissue culture media in the Cytokinetics Laboratory of the US Air Force School of Aerospace Medicine. When sufficient tissue culture supernatant fluid has been harvested, this material will be assayed for erythropoietin activity. If activity is found, it would suggest that the renal tubular cell is indeed a site of erythropoietin production.

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Presentations

1. Vertel, R.M.: Erythrocythemia secondary to an ectopic source of erythropoietin production. Department of

Medicine Grand Rounds, Brooke General Hosp., Fort Sam Houston, Texas, 18 March 1966.

2. Vertel, R.M.: Erythrocythemia secondary to an ectopic source of erythropoietin production. Department of Medicine Grand Rounds, Wilford Hall USAF Hosp., Lackland AFB, Texas, 5 May 1966.

Publications

None.

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19. GOVT LAB INSTALLATION ACTIVITY
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20. PERFORMING ORGANIZATION
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INVESTIGATORS PRINCIPAL: Vertel, R.M., Capt, MC
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TEL: 512 - CAI 2720 TYPE: DA

21. TECHNOLOGY UTILIZATION: Life Sciences

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23. KEYWORDS: Erythropoietin, parotid fluid

24(U) Tech Objective - Preliminary studies in the Renal Laboratory of the Surgical Research Unit have shown the erythropoietin bioassay in the polycythemic mouse to be a sensitive and reliable method. The purpose of this study is to determine if measurable erythropoietin activity is present in parotid fluid. There is some precedence for believing that erythropoietin might be found in a glandular secretion such as parotid fluid. If activity can be demonstrated, the relationship between 25 plasma and parotid fluid levels of erythropoietin activity will be quantitated. If a reproducible correlation is observed, the measurement of parotid fluid erythropoietin activity will be utilized as an investigational tool.

(U) Approach - In selected patients from whom plasma is obtained for the measurement of erythropoietin activity, simultaneous samples of parotid fluid will be collected and measured for erythropoietin activity.

26(U) Progress (Sep 65-Jun 66) - Data currently available are insufficient to justify a detailed report at this time.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A014501B71R, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: DETERMINATION OF THE ERYTHROPOIETIN ACTIVITY OF PAROTID FLUID

US ARMY SURGICAL RESEARCH UNIT
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS

1 July 1965 to 30 June 1966

Investigators:

Robert M. Vertel, Captain, MC
Richard M. Freeman, Captain, MC

The principles of laboratory animal care as established by the National Society for Medical Research were observed (AR 70-18, dated 29 March 1963).

Reports Control Symbol: RCS-MEDDH-288 (R1)

UNCLASSIFIED

Qualified requesters may obtain copies of this report from DDC.

ABSTRACT

1. PROJECT NO. 3A0I450:B71R, RESEARCH IN BIOMEDICAL SCIENCES

2. REPORT TITLE: DETERMINATION OF THE ERYTHROPOIETIN ACTIVITY OF PAROTID FLUID

3. US Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas

4. Period covered in this report: 1 July 1965 to 30 June 1966

5. Investigators: Robert M. Vertel, Captain, MC
Richard M. Freeman, Captain, MC

6. Reports Control Symbol: RCS-MEDDH-288 (R1)

Preliminary studies in the Renal Laboratory of the US Army Surgical Research Unit have shown the erythropoietin bioassay in the polycythemic mouse to be a sensitive and reliable method.

The purpose of this study is to determine if measurable erythropoietin activity is present in parotid fluid. There is some precedence for believing that erythropoietin might be found in a glandular secretion such as parotid fluid. In 1955, it was reported in the literature that erythropoietin-like activity could be produced in mice exposed to anoxic environments. This activity was transmitted to offspring not exposed to anoxia by breast feeding. This study therefore suggests that at least one glandular secretion, namely, milk, contains erythropoietin activity.

If activity can be demonstrated, the relationship between plasma and parotid fluid levels of erythropoietin activity will be quantitated. If a reproducible