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Resistance to Infection in Burned Patients *

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SEPTICEMIA is a major complication in burned patients who survive burn shock. It is more common in severely burned patients, and micro-organisms isolated from the blood stream are usually found in the burn wound.

The purpose of the present investigation was to determine whether severely burned patients were more susceptible to invasive infection because of an associated impairment of the natural antibacterial defense systems, and, to this end, eight severely burned patients were studied, six of whom were admitted to hospital within 18 hours of injury.

The basic defect promoting infection in the burn wound is the destruction of the remarkably protective epithelial barrier. The human host combats bacterial penetration of this tissue layer with humoral and cellular defenses. The former consist of nonspecific and specific antibacterial substances in plasma and tissue fluid and the latter of fixed and wandering phagocytes. These two systems are complementary under natural conditions.

Antibacterial activity occurs continually at the site of bacterial contamination and also in the interstitial fluid, in the blood stream, and in the various segments of the reticuloendothelial system, as needed. The basic defect in antibacterial defense permitting burn-septicemia is the inability of the patient to prevent bacterial invasion from the wound site. Bacterial pathogens usually cause serious disease in otherwise normal individuals when they are permitted to enter the blood stream continually; a similar effect is to be expected in burned patients.

The present paper reports the results of bactericidal studies on freshly drawn blood and observations on the cellular-bacterial relationship in the peripheral tissues. We have found blood bactericidal capacity to be greater than normal in acutely burned patients but the peripheral cellular response to injury is impaired.

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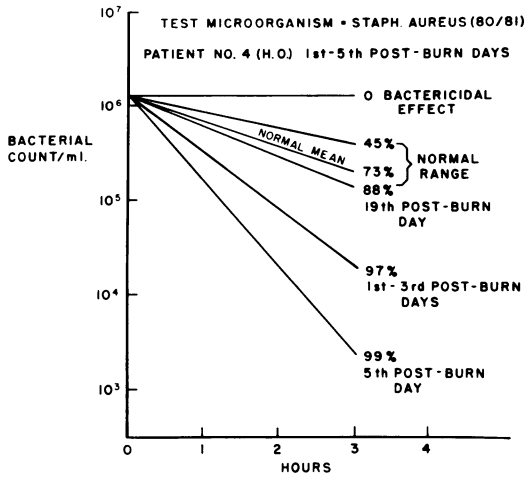

FIG. 1. Leucocyte-plasma bactericidal effect.

Methods

Evaluation of Antibacterial Defense Systems. Four components of antibacterial defense have been measured in individual patients:

1. *Leukocyte-plasma bactericidal effect.* This is the ability of leukocytes suspended in autologous plasma to destroy *Staphylococcus albus* (coagulase negative), *Staphylococcus aureus* (hemolytic, coagulase positive, mannitol positive, phagetype 80/81), *E. coli,* and *Pseudomonas aeruginosa.*

2. *Plasma bactericidal effect.* This is as above except that leukocytes are not included in the system. The methods used in the blood bactericidal studies have been standardized so as to work with a constant bacterial inoculum and with a constant ratio of bacteria to leukocytes. The use of the roller tube technic ensured continuous contact between micro-organisms and white cells. The experiments were terminated after three hours incubation at 37° C. All tests were done in triplicate. The results have been recorded as the percentage destruction of the original inoculum. A 10 per cent decrease or increase in bactericidal

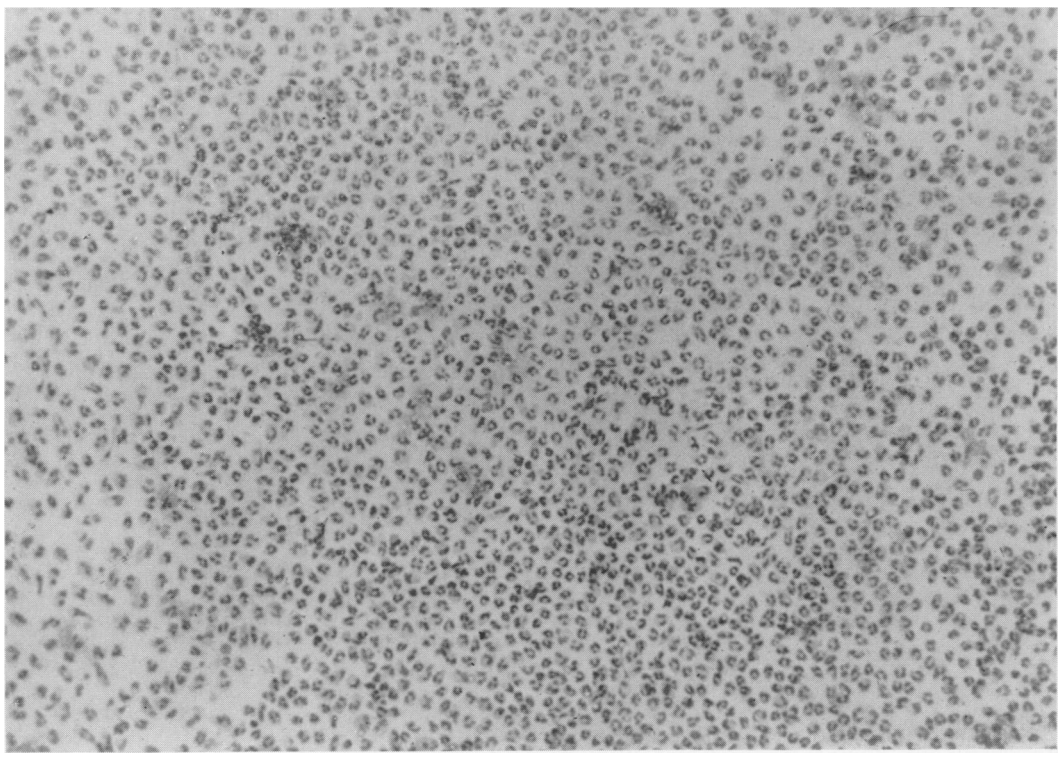

FIG. 2. Cover-slip impression smear. Normal subject. Degree cell response = 100%

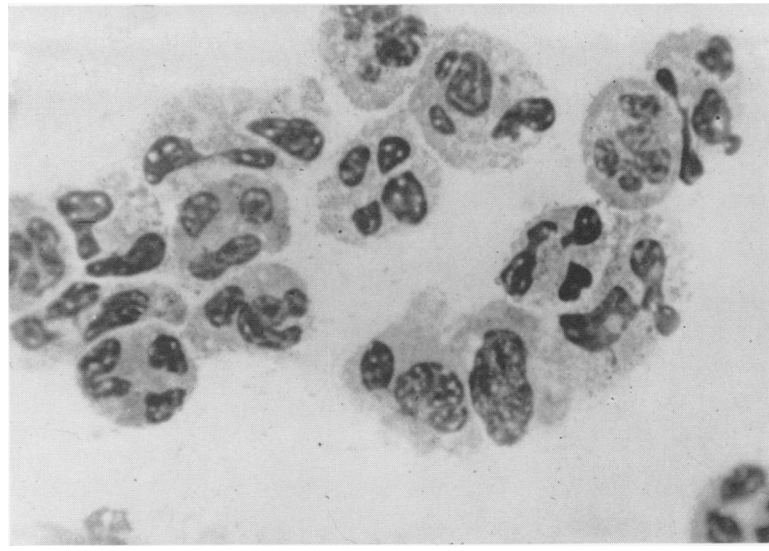

FIG. 3. Cover-slip impression smear. Normal subject 5th hour post-skin abrasion. Neutrophils (phagocytosis not tested).

effect in our test system, when the results are in the 90 to 100 per cent range, is statistically significant and this is more readily appreciated when presented graphically (Fig. 1). The bactericidal methods have been reported in detail in another paper.[1] All patients received antibiotics during the period of study but our test micro-organisms were resistant to the antibiotics used.

3. *Serum hemolytic complement.* This refers to the capacity of human serum to hemolyse a suspension of sheep red blood cells sensitized with its specific antibody. The end-point has been read as 50 per cent hemolysis. Complement participates in bacterial antigen-antibody reactions, especially those involving gram-negative bacilli.

4. *The degree and type of leukocyte and tissue macrophage migation in response to superficial skin trauma at a site away from the burned area.* The method used was a modification of that described by Rebuck [8] and consists in scraping a 0.5 × 0.5 cm. segment of epidermis with a scalpel until dermis is exposed. Sterile cover slips are successively applied to the site at hourly intervals, thus obtaining an impression smear of the changing cellular exudate. This is stained by Wright's method and examined microscopically. A killed suspension of the *Staph. albus* used in the bactericidal tests is dropped on the wound before applying the first cover slip. In this way an estimate can be made of the phagocytic capacity of the migrating cells (Fig. 2, 3, 4).

Evaluation of Blood Constituents. White blood cell count, hematocrit, blood sugar, serum acetone, total protein, albumin, globulin, serum sodium, serum potassium and pH were determined on blood samples used in the bactericidal studies. Standard methods were used for these determinations.

Evaluation of Bacterial Infection. The total bacterial count per mg. of wound exudate from representative areas of the burn wound was determined. Bacterial species were identified. Blood culture data were obtained.

All of the above observations were carried out simultaneously and serially, when possible, so as to obtain a continuing comprehensive analysis of the infectious process.

Data are presented in this paper from eight burned patients; three were severely burned and died, the others survived. Two

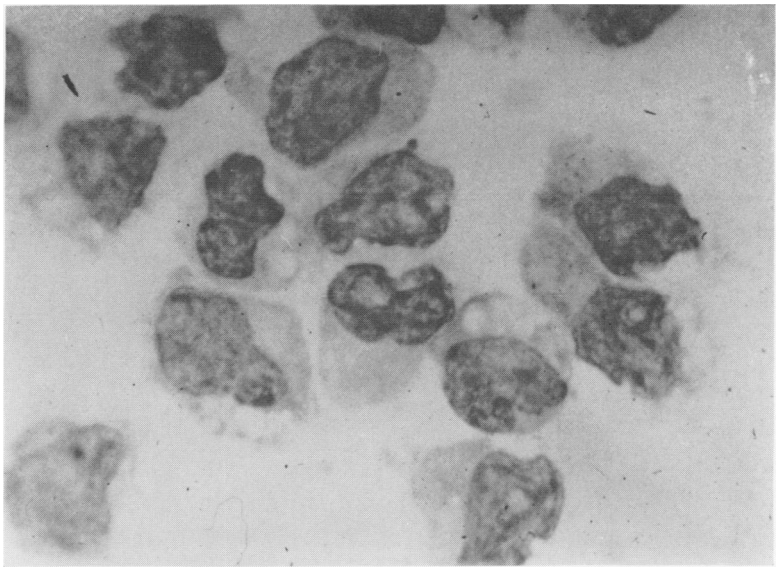

FIG. 4. Cover-slip Impression smear. Normal subject. 11th hour post-skin abrasion. Lymphocytes.

of the latter were studied within the first two weeks of injury, a third during the first four weeks, and a fourth during the late postburn phase of nutritional deficiency. A fifth moderately burned surviving patient has been studied through both early and late phases.

Results

Clinical Observations on Fatally Burned Patients

Patient 1 (O. M.). A 23-year-old woman admitted with a 75 per cent full-thickness flame burn and also pulmonary injury. The patient survived the phase of burn shock. The white blood cell count was 21,000 per cu. mm. on the first postburn day and 1,950 per cu. mm. on the day of death. Penicillin and streptomycin were administered therapeutically. The patient died on the fourth postburn day. The *Aerobacter aerogenes* isolated from the blood stream was resistant to the antibiotics used.

Patient 2 (D. C.). A 35-year-old man who sustained a 75 per cent full-thickness burn following an acetylene torch explosion. Blood sugar, serum acetone, serum proteins and serum electrolyte concentrations were relatively normal at the time of study. Hematocrits were 57 and 63 on the first and second postburn days, respectively (at the time of study). The patient died at the end of the third postburn day.

Patient 3 (R. B.). A 32-year-old woman who sustained a 65 per cent full-thickness flame burn. The patient developed chills and fever on the eighth postburn day. Initial debridement was performed on the fifthteenth postburn day. The patient expired on the nineteenth postburn day with terminal hyperpyrexia. Blood culture on the day before death grew *Pseudomonas aeruginosa.*

Laboratory Observations on Fatally Burned Patients

Bactericidal data and serum complement determinations from three fatally burned patients are presented in Table 1. Leukocyte-plasma bactericidal effect against *Staph. albus* was normal in all three except on day of death (4th postburn) in Patient 1 when a significant drop was noted. Plasma bactericidal effect against *Staph. albus* was within normal range in all three and was probably elevated in Patient 1 on third and fourth postburn days. Leukocyte-plasma bactericidal effect against *Staph. aureus* (phagetype 80/81) was statistically better than normal in all three patients, and plasma bactericidal effect against the coagulase positive staphylococcus was markedly increased in two patients (No. 1, 2). The latter observations were from the early postburn (but

TABLE 1. *Serum Complement and Bactericidal Effect of Leukocyte-Plasma Suspensions and of Plasma on Staphylococci*

Fatally Burned Patients

Patient	Postburn Day	*Staph. albus* Plasma B.E.*%	*Staph. albus* Leukocyte-Plasma B.E.%	*Staph. aureus* (80/81) Plasma B.E.%	*Staph. aureus* (80/81) Leukocyte-Plasma B.E.%	Serum Complement C'H50 units/ml.	Blood Cultures
1 O.M. 75% Burn	1	2	98	—	—	45	—
	2	—	99	—	96	—	—
	3	80	99	96	99	—	—
	4	62	92	87	91	—	Positive for *Ae. aerogenes*
2 D.C. 75% Burn	1	20	98	82	93	263	—
	2	16	97	78	90	—	—
3 R.B. 60% Burn	18	0	99	0	89	—	Positive for *Pseudomonas ae.*
Normal Mean		47±9	98±0.3	7±5	73±4	175	—
Normal Range		0–95	95–99	0–53	45–87	146–206	—

* B.E.—Bactericidal Effect.

immediate predeath) period. In Patient 3, studied on the eighteenth postburn day (the day before death) plasma alone did not inhibit growth of *Staph. aureus*. Serum complement on the first postburn day was below normal in Patient 1 and elevated in Patient 2.

A postmortem heart blood culture was postitive for *A. aerogenes* in Patient 1. Blood culture on the day of death was

TABLE 2. *Leukocyte Migration in Response to Superficial Skin Trauma*

Fatally Burned Patients

Patient	Postburn Day	Degree Cell Migration 1–14 Hrs. Post Skin Trauma % Normal	Degree Cell Migration 14–22 Hrs. Post Skin Trauma % Normal	% Neutrophils 5–7 Hrs. Post Skin Trauma	% Neutrophils 11–14 Hrs. Post Skin Trauma	Eosinophils	Phagocytosis (*Staph. albus*)
1 O.M. 75% Burn	1	25	50	100	90	Absent	Yes
	2	<25	50	100	80	Absent	Yes
	3	35	75	100	100	Absent	Yes
	4	5	0	100	—	Occasional	Yes
3 R.B. 60% Burn	18	5	—	100	—	Absent	Yes
Normal Control		100	100	70	30	Present	Yes

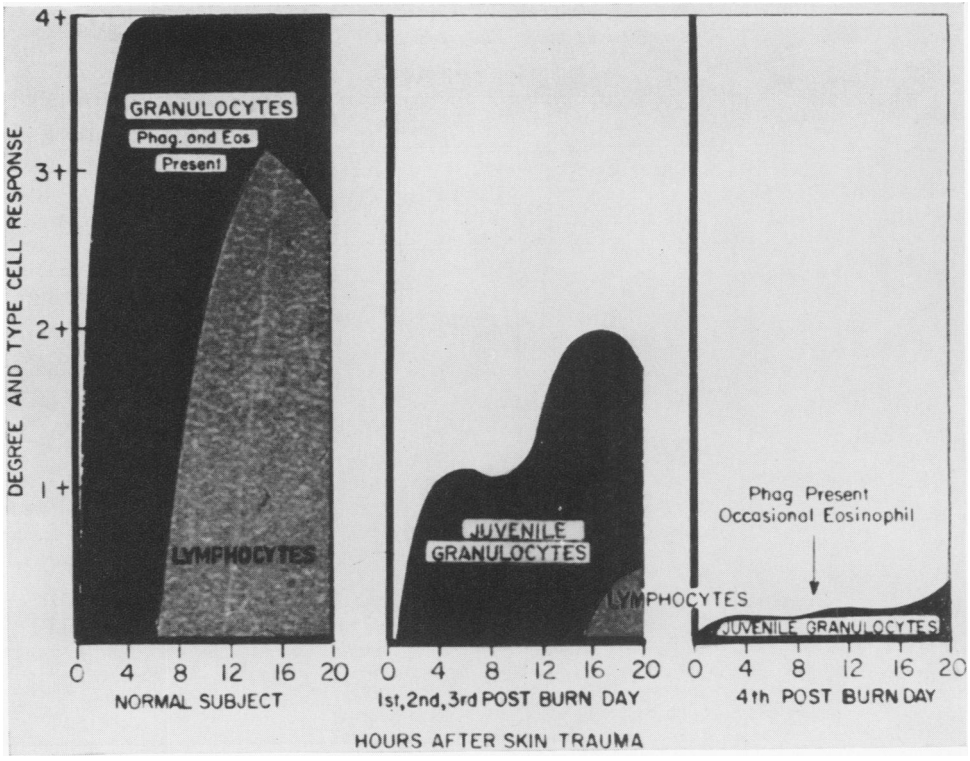

FIG. 5. Patient 1. Leukocytes Migration in response to superficial skin trauma (O. M. 75% full thickness burn. Death—4th postburn day—septicemia).

positive for *Pseudomonas aeruginosa* in Patient 3. Blood cultures were not done on Patient 2.

Data on the cellular response to superficial skin trauma from two of the fatally burned patients are presented in Table 2. The degree of cell migration was markedly reduced until death occurred in Patient 1 (Fig. 5) and almost absent in the preterminal period in Patient 3. The majority of the cells were neutrophil polymorphonuclear leukocytes (juvenile forms) and were actively phagocytic (Fig. 6, 7). There was a paucity of lymphocytes and monocytes in the smears; eosinophils were absent. It is of interest that urine output was maintained at between 20 and 40 ml./hr. in Patient 1 until death indicating an adequate central blood pressure.

Surviving Patients

Patient 4 (H. O.). A 17-year-old girl who sustained a 40 per cent deep dermal and full-thickness scald. Body temperature was maintained at approximately 35° C. by external cooling from the third to the fortieth postburn day. This was initiated because of continued high spiking temperature and associated mental confusion which suggested the presence of septicemia. The patient was given penicillin and streptomycin initially; Chloromycetin was given for a short period when *Aerobacter aerogenes* was found in the blood stream, but subsequently it was discontinued; thereafter, tetracycline and penicillin were given.

Bactericidal studies and serum complement determinations are presented in Table 3. Leukocyte-plasma bactericidal effect against *Staph. albus* was normal throughout the period of study. The data on plasma bactericidal effect were also within the normal range, but the findings from the 1st through the fifth postburn days may represent an increased effect, or the findings from

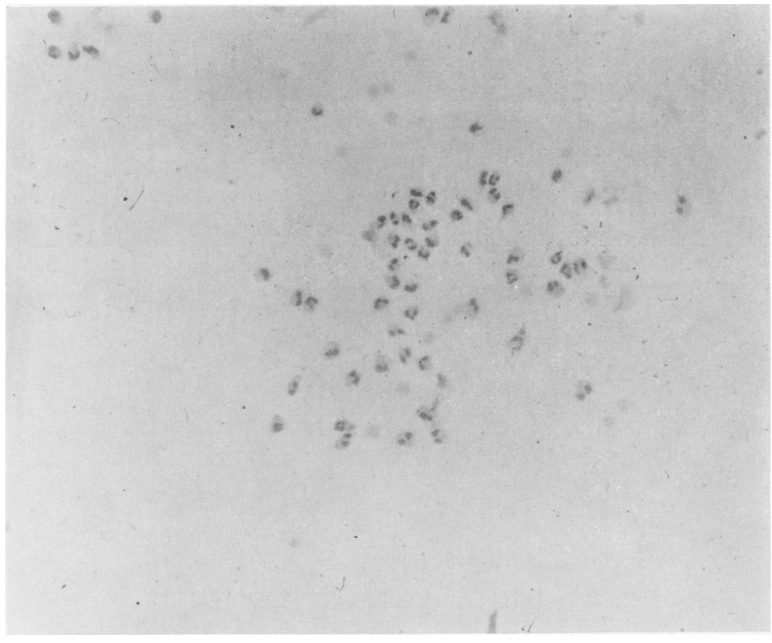

FIG. 6. Cover-slip impression smear. Patient 1 (O. M.). Cell response = < 25%. All neutrophils.

the eighth through the thirty-second day may represent a decrease. Leukocyte-plasma bactericidal effect against *Staph. aureus* (phagetype 80/81) was markedly increased from the first through the eighth postburn days (Fig. 1.) and thereafter fell within the normal range. The values on plasma bactericidal effect against *Staph. aureus* fell within the normal range and are similar to those obtained against *Staph. albus*. These data may also be interpreted as 1) uninfluenced by the burn state; 2) increased in the early postburn period; or 3) decreased from the eighth postburn day onward. The leukocyte-plasma bactericidal effect against *E. coli* was normal throughout, and the plasma bactericidal effect maximal. Serum complement was continually in the normal range.

Blood sugar, serum acetone, serum proteins, serum electrolytes, hematocrit and blood pH were determined on blood samples used for the bac-

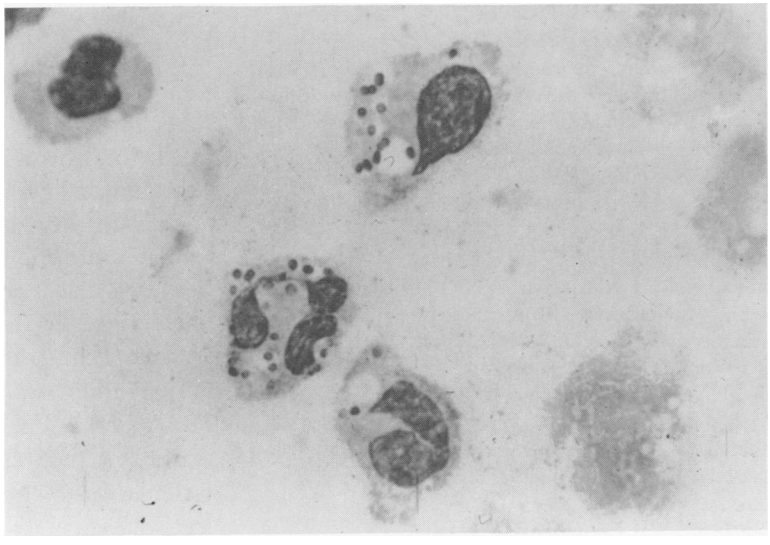

FIG. 7. Cover-slip impression smear. Patient 1 (O. M.). Phagocytosis by neutrophils. 1st postburn day.

TABLE 3. *Serum Complement and Bactericidal Effect of Leukocyte-Plasma Suspensions and of Plasma on Staphylococci and* E. coli

Patient 4 (H.O.)

Postburn Day	*Staph. albus*		*Staph. aureus* 80/81		*E. coli*		Serum Complement C'H50 units/ml.
	Plasma B.E.*%	Leukocyte-Plasma B.E.%	Plasma B.E.%	Leukocyte-Plasma B.E.%	Plasma B.E.%	Leukocyte-Plasma B.E.%	
1	66	99	54	97	98	99	131
2	53	99	34	97	99	99	161
3	59	99	30	97	97	99	173
5	64	99	31	99	93	99	197
8	0	98	7	95	98	99	205
11	—	—	—	—	99	99	125
16	—	—	—	—	71	99	192
19	0	98	0	88	91	99	141
26	0	99	0	82	95	99	171
32	0	96	0	68	98	99	186
39	57	99	0	59	90	99	192
60	0	99	0	87	—	—	127
89	62	98	17	74	—	—	222
Normal Mean	47±9	98±0.3	7±5	73±4	32±14	98±0.3	175
Normal Range	0–95	95–99	0–53	45–87	0–98	97–99	146–206

* B.E.—Bactericidal Effect.

On the 16th postburn day, the plasma bactericidal effect against the *Pseudomonas aeruginosa* previously isolated from the patients blood stream was zero, and the leukocyte-plasma bactericidal effect was 99%.

tericidal studies. There were some isolated deviations from expected normals but, in general, these findings were compatible with the postburn state. No correlation was found between the changing blood bactericidal results and the chemical data.

Data on the cellular response to superficial skin trauma from Patient 4 are presented in Table 5. The degree of cell migration was markedly reduced through the first five postburn days and probably until after the eighth day; thereafter, the response improved towards normal. Neutrophils (usually juvenile forms) predominated with a significant reduction in the number of lymphocytes and monocytes; eosinophils were absent. Leukocyte counts from peripheral blood during this period showed the expected elevation with an increase in neutrophils and decrease in lymphocytes (Fig. 8, 9).

Wound and blood culture data from Patient 4 are presented in Table 5. There was a persistent polybacterial flora, but the largest bacterial counts per milligram wound exudate were obtained on the fifth, eighth and eleventh postburn days. During that time the clinical signs of septicemia appeared and *Aerobacter aerogenes* was cultured from the blood stream. Later, *Staphylococcus aureus* (coagulase positive) was cultured from the blood stream.

A review of the findings in Tables 3, 4 and 5 reveals that, in the early postburn period, blood cultures were negative when the blood bactericidal capacity was high, even though the cell response to peripheral injury was markedly depressed. At that time, the wound bacterial count was relatively low. Gram-negative bacilli then appeared in the blood stream and this was associated with a rise in the wound bacterial count. The cell response to superficial injury was still subnormal and the plasma bactericidal capacity (but not the leukocyte plasma bactericidal capacity) against *Staph. albus, Staph. aureus,* and possibly *Pseudomonas aeruginosa,* had fallen to zero. However the leukocyte-plasma bactericidal capacity, and the plasma bactericidal capacity against *E. coli,* remained highly effective. *Staph. aureus* (coagulase

positive) appeared in the blood stream when the plasma bactericidal capacity against that species had fallen to zero, and the leukocyte-plasma capacity had dropped markedly to the normal range. At that time, the cell response to injury had improved toward normal.

Patient 5 (M. C.). A 50-year-old woman who sustained a 28 per cent full-thickness burn. The patient was febrile through the first 21 postburn days but blood cultures remained sterile. Debridement and grafting were carried out four times. Leukocyte-plasma bactericidal effect against *Staph. albus* was normal or better through the first 12 postburn days and so too was the plasma bactericidal effect; however, it must be noted that the plasma bactericidal effect on the first postburn day was zero (Table 6). The failure to find any plasma bactericidal effect on the first postburn day may represent an early fall from normal, or a normal value before rise. Serum complement values were normal or better. There was a polybacterial wound flora and a heavy bacterial count per mg. wound exudate on the fourth and seventh postburn days (Table 6). The cell response to skin trauma was approximately 75 per cent of normal by the eleventh postburn day with some delay in the appearance of lymphocytes; eosinophils were absent and neutrophils and lymphocytes were phagocytic. A review of the findings on patient No. 5 (M. C.) shows that all antibacterial defense systems were operating close to or better than normal and this is borne out by the satisfactory clinical course.

TABLE 4. *Leukocyte Migration in Response to Superficial Skin Trauma*

Patient Number 4 (H.O.)

	Degree Cell Migration						
Postburn Day	1–14 Hrs. Post Skin Trauma % Normal	14–22 Hrs. Post Skin Trauma % Normal	% Neutrophils 5–7 Hrs. Post Skin Trauma	% Neutrophils 11–14 Hrs. Post Skin Trauma	Eosinophils	Phagocytosis (*Staph. albus*)	WBC % Neutrophils % Lymphocytes
1	25	75	90	60	Absent	Yes	22,500/cu. mm.
2	25	50	95	65	Absent	—	13,900 N = 86 L = 13
3	—	50	100	75	Absent	Yes	8,700 N = 83 L = 12
4	50	50	95	80	Absent	Yes	
5	<25	25	100	95	Absent	—	13,700 N = 87 L = 8
8	—	50	—	80	Absent	—	20,200 N = 88 L = 11
11	75	—	95	50	A few	Yes	20,000 N = 89 L = 9
16	75	100	95	40	Absent	Yes	16,500 N = 83 L = 14
32	75	75	70	90	Absent	Yes	11,500 N = 70 L = 26
Normal Control	100	100	70	30	Present	Yes	

TABLE 5. *Serial Quantitative and Diagnostic Wound Culture Data and Blood Culture Findings*

Patient 4 (H.O.)

Postburn Day	Wt. of Cultured Wound Exudate, mg.	No. Organisms per mg. Exudate	Bacterial Species Identified in Wound Exudate	Blood Culture
1	66	6,150	*A. aerogenes, Pseudomonas sp., Staph. aureus* (Coag. +), *Staph. albus*	Negative
2	92	91,500	*A. aerogenes, Staph. aureus* (Coag. +)	Negative
3	123	<72	*Proteus sp., A. aerogenes, Staph. aureus* (Coag. +)	Negative
5	12	>100,000	—	Negative
8	50	44×10^6	*Pseudomonas sp., A. aerogenes, Staph. aureus* (Coag. +)	Positive—*A. aerogenes*
11	120	750,000	*Proteus sp., A. aerogenes, Pseudomonas sp., Staph. albus, Staph. aureus* (Coag. +)	1 of 2 bottles positive for *Pseudomonas sp.*
16	5	<200	*Proteus sp., Pseudomonas sp., Staph. albus*	Negative
19	—	—	—	Positive—*Staph. aureus* (Coag. +)
26	—	—	—	Positive—*Staph. aureus* (Coag. +)
32	15	32,500	*Pseudomonas sp., A. aerogenes, Proteus sp., Staph. aureus* (Coag. +)	Negative
39	32	9,450	*Proteus sp., Pseudomonas sp., Staph. aureus*	Negative

TABLE 6. *Serum Complement, Wound Culture Data and Bactericidal Effect of Leukocyte-Plasma Suspensions and of Plasma on Staphylococci*

Patient 5 (M.C.)

	Staphylococcus albus				
Postburn Day	Plasma B.E.*%	Leukocyte-Plasma B.E.%	Serum Complement C'H50 units/ml.	No. Bacteria per mg. Wound Exudate	Bacterial Species Identified in the Wound Exudate
1	0	99	242	3	*Staph. albus* (hemolytic) *E. coli*
4	72	99	164	1.5×10^6	*Staph. albus* (hemolytic)—4+ *E. coli* (a few)
7	57	99	—	5.2×10^4	*Staph. aureus* (Coag. + and Mann. +) *E. coli*
10	95	99	—	100	*Staph. aureus*—(Coag. +)
12	68	99	—	—	*Staph. aureus*—(Coag. +) *E. coli*
Normal Mean	47 ± 9	98 ± 0.3	175	—	—
Normal Range	0–95	95–99	146–206	—	—

* B.E.—Bactericidal Effect.

TABLE 7. *Leukocyte Migration in Response to Superficial Skin Trauma*

Patient Numbers 5 and 8

Patient	Postburn Day	Degree Cell Migration: 1–14 Hrs. Post Skin Trauma % Normal	Degree Cell Migration: 14–22 Hrs. Post Skin Trauma % Normal	% Neutrophils 5–7 Hrs. Post Skin Trauma	% Neutrophils 11–14 Hrs. Post Skin Trauma	Eosinophils	Phagocytosis (*Staph. albus*)
5							
M.C.	11	75	90	85	30	Absent	Yes
28% burn							
Survived	12	60	—	80	30	Absent	—
8							
O.C.	64	90	90	98	30	Numerous	Yes
25% burn							
Survived	108	70	100	80	20	Numerous	Yes
Normal							
Control		100	100	70	30	Present	Yes

Patient 6 (C. G.). A 19-year-old man sustained a 25 per cent second and third degree flame burn. His blood sugar, serum acetone, serum protein and serum electrolyte concentrations were normal each day of study (i.e., 10 days). The hematocrit was 57 on the second postburn day but normal thereafter. There was no significant clinical infection. The patient was discharged from the hospital on the thirtieth postburn day. Leukocyte-plasma and plasma bactericidal effect against *Staph. albus* was normal. Leukocyte-plasma bactericidal effect against *Staph. aureus* was normal or better and the plasma bactericidal effect against that species was increased. Blood bactericidal data against a *Streptococcus fecalis* strain isolated from the blood stream of one of our diabetic patients with fatal septicemia are also presented in Table 8. We do not have control data using this strain, but there was a maximal leukocyte-plasma bactericidal effect against it. Serum complement levels were normal early and subsequently increased. The above

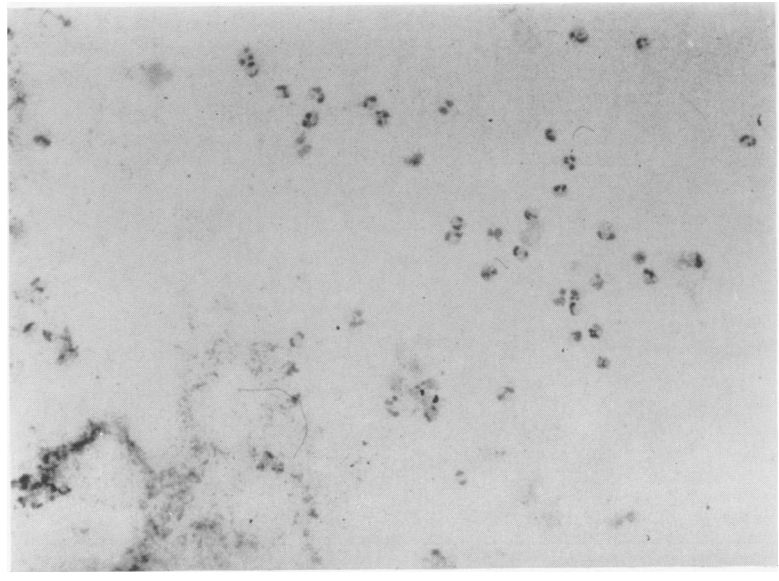

FIG. 8. Cover-slip impression smear. Patient 4 (H. O.). Cell response = < 25%. All neutrophils.

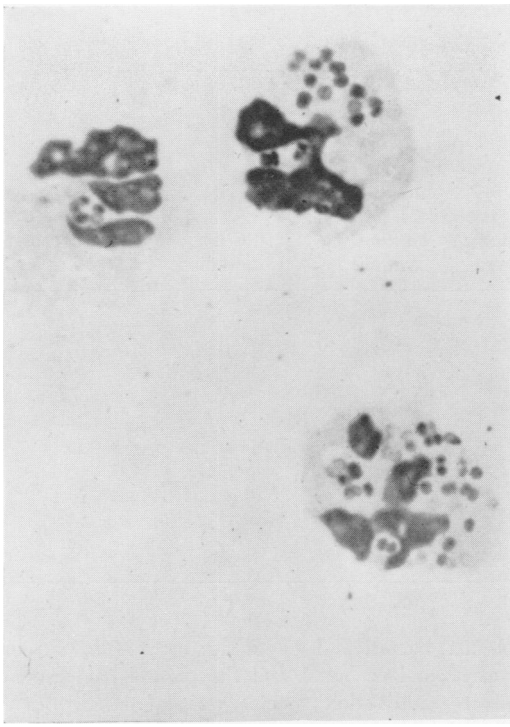

FIG. 9. Cover-slip impression smear. Patient 4 (H. O.). Phagocytosis by neutrophils. 1st postburn day.

modalities of antibacterial defense were normal or better and these are consistent with the relatively benign clinical course.

Patient 7 (M. M.). A 42-year-old man who sustained a 23 per cent second and third degree flame burn. The patient was treated with penicillin and streptomycin and later with tetracycline. External body cooling was used from the third to the fifteenth postburn days to keep the temperature at about 37.7° C. Septicemia did not develop and the patient recovered.

Bactericidal studies are presented in Table 9. Leukocyte-plasma bactericidal effect against *Staph. albus* was normal throughout the period of study. The data on plasma bactericidal effect were also within the normal range, but the findings from the first through the eleventh postburn days may represent an increased effect, or the findings from the eighteenth through the thirty-first postburn days may represent a fall. Leukocyte-plasma bactericidal effect against *Staph. aureus* (phagetype 80/81) was increased on the third, ninth, eleventh, eighteenth and twenty-fifth postburn days; plasma bactericidal effect, however, was elevated only on the ninth postburn day. The leukocyte-plasma bactericidal effect against *E. coli* was normal throughout, and the plasma bactericidal effect maximal. The leukocyte-plasma bactericidal effect against *Pseudomonas aeruginosa* was maximal and the plasma bactericidal effect varied.

Blood determinations on samples used for the bactericidal studies showed deviations compatable with the continuing postburn state and they could not be related to the results of the bactericidal studies.

Data on the cellular response to superficial skin trauma from Patient 7 are presented in

TABLE 8. *Serum Complement and Bactericidal Effect of Leukocyte-Plasma Suspensions and of Plasma on Staphylococci and Streptococci*

Patient 6 (C.G.)

Postburn Day	*Staph. albus*		*Staph. aureus* 80/81		*Streptococcus*		Serum Complement C'H50 units/ml.
	Plasma B.E.*%	Leukocyte-Plasma B.E.%	Plasma B.E.%	Leukocyte-Plasma B.E.%	Plasma B.E.%	Leukocyte-Plasma B.E.%	
2	25	99	74	86	—	—	157
4	26	98	40	56	—	—	—
9	—	—	89	87	0	99	260
10	—	—	84	97	0	99	237
Normal Mean	47±9	98±0.3	7±5	73±4	—	—	175
Normal Range	0–95	95–99	0–53	45–87	—	—	146–206

* B.E.—Bactericidal Effect.

TABLE 9. *Bactericidal Effect of Leukocyte-Plasma Suspensions and of Plasma on Staphylococci*, E. coli *and* Pseudomonas ae.

Patient 7 (M.M.)

Postburn Day	*Staph. albus*		*Staph. aureus* 80/81		*E. coli*		*Pseudomonas ae.*	
	Plasma B.E.*%	Leukocyte-Plasma B.E.%	Plasma B.E.%	Leukocyte-Plasma B.E.%	Plasma B.E.%	Leukocyte-Plasma B.E.%	Plasma B.E.%	Leukocyte-Plasma B.E.%
1	78	94	0	76	—	—	—	—
3	55	99	0	94	99	99	—	—
6	69	99	0	67	98	99	—	—
9	58	99	75	96	99	99	—	—
11	54	98	0	91	—	—	0	99
18	0	99	0	95	—	—	0	99
25	0	97	0	95	98	99	—	—
31	0	97	0	84	—	—	92	99
Normal Mean	47±9	98±0.3	7±5	73±4	32±14	98±0.3	84±9.6	98±0.84
Normal Range	0–95	95–99	0–53	45–87	0–98	97–99	0–99	94–99

* B.E.—Bactericidal Effect.

Table 10. There was some decrease in the expected cell migration but this was not marked. Neutrophils were more prominent than normal in the first nine postburn days, and they actively phagocytosed the test staphylococci; eosinophils were virtually absent. There was some delay in the appearance of lymphocytes

Wound and blood culture data from Patient 7 are presented in Table 11. There was a persistent poly-bacterial flora, but except on the third postburn day, all blood cultures were negative. There were large numbers of bacteria per milligram wound exudate on five of the study days. Cultures on the eleventh and eighteenth postburn days (for quantitative count) were taken shortly after the patient had been immersed in a Hubbard tank, and this may have accounted for the relatively low bacterial counts on those days.

A review of findings in Tables 9–11 reveals that blood bactericidal capacity was normal or better and cell response to superficial skin trauma was reasonably good;

TABLE 10. *Leukocyte Migration in Response to Superficial Skin Trauma*

Patient 7 (M.M.)

Postburn Day	Degree Cell Migration		% Neutrophils 5–7 Hrs. Post Skin Trauma	% Neutrophils 11–14 Hrs. Post Skin Trauma	Eosinophils	Phagocytosis (*Staph. albus*)
	1–14 Hrs. Post Skin Trauma % Normal	14–22 Hrs. Post Skin Trauma % Normal				
1	75	90	90	45	Absent	Yes
3	90	100	80	30	Present (few)	Yes
6	80	90	80	45	Absent	Yes
9	50	100	95	50	Absent	Yes
11	75	75	70	30	Absent	Yes
27	75	75	70	50	Absent	Yes
Normal Control	100	100	70	30	Present	Yes

TABLE 11. *Serial Quantitative and Diagnostic Wound Culture Data and Blood Culture Findings*

Patient 7 (M.M.)

Postburn Day	Wt. of Cultured Wound Exudate, mg.	No. Organisms per mg. Exudate	Bacterial Species Identified in Wound Exudate	Blood Culture
1	8	Heavy growth	—	Negative
3	4	Heavy growth	*Coliform intermedius, E. coli, Strep. fecalis, Achromobacter sp.*	*E. coli, Achromobacter sp.*
6	2	650,000	—	Negative
9	12	9,300,000	—	Negative
11	25	1,200	—	Negative
18	63	6,100	—	Negative
25	18	960,000	—	Negative
31	70	5,800	—	Negative

cells were actively phagocytic. Transient bacteremia was noted only on third postburn day when measured modalities of host resistance were normal or better. Wound exudate was heavily contaminated at that time and possibly the bacteria-cell ratio was such that bacterial invasion was permitted for a time. Antibiotics may have been of prophylactic value in preventing invasive infection.

Patient 8 (O. C.). A 37-year-old man who presented with late postburn nutritional depletion and delay in wound healing following a 25 per cent full-thickness burn. Studies were commenced on the forty-third postburn day. The patient remained febrile until the sixtieth postburn day and had a polybacterial wound infection. Weight on the forty-third and seventieth postburn days was 105 and 89 pounds, respectively. Skin-grafting was attempted unsuccessfully several times. Topical neomycin was the only antibiotic used from the fifty-third to the eighty-fourth postburn days. Tetracycline was given for 3 to 4 days with each skin-graft operation. Septicemia did not develop and the patient recovered. The leukocyte-plasma bactericidal effect against *Staph. albus* was significantly decreased until the one-hundred and eighth postburn day after which it was normal. The plasma bactericidal effect against *Staph. albus* varied but could not be interpreted as decreased in view of the findings on the two-hundred and thirty-first postburn day when full clinical recovery was apparent. An experiment was done on the ninety-sixth postburn day in an attempt to show whether burn plasma or leukocytes were primarily at fault when the combined system was defective. Cells and plasma from a normal donor were interchanged with cells and plasma from the patient. Each system was tested against the *Staph. albus*. The results were inconclusive and unfortunately could not be repeated owing to the return of the blood bactericidal capacity to normal after the initial test.

Normal donor leukocyte + plasma
bactericidal effect = 98%
Normal donor plasma + burn leukocyte
bactericidal effect = 87%
Burn plasma + normal donor leukocyte
bactericidal effect = 93%
Burn plasma + burn leukocyte
bactericidal effect = 93%

Bactericidal studies against *E. coli* showed a progressive increase in plasma bactericidal effect and the findings on the forty-fourth, fifty-second and sixtieth postburn days may be below normal for the patient; there was a decreased leukocyte-plasma bactericidal effect on the forty-fourth and fifty-second postburn days with subsequent return to normal. Blood bactericidal studies against *Staph. aureus* were normal in the late phase. Serum complement levels rose steadily from a normal level on the forty-third postburn day to a high of 278 C'H50 units on the ninety-fourth postburn day and thereafter fell toward normal. There was no direct correlation between serum complement and blood bactericidual studies. Data on cell migration to superficial skin trauma are presented in Table 7. Except for some delay in the appearance of lymphocytes, no significant

abnormality is apparent. White blood cell counts were perhaps somewhat low throughout the study period (range 5,000–10,000 per cu. mm.) considering the continued presence of a clean granulating burn wound; the differential count was not remarkable. Blood chemical values fell within acceptable limits throughout the period of study and could not be related to blood bactericidal studies.

In summary, Patient 8 had a significantly decreased leukocyte-plasma bactericidal effect against *Staph. albus* and, for a shorter period against *E. coli*. These abnormalities occurred at a time of progressive weight loss until he appeared emaciated. However, during that period, serum complement rose progressively above normal levels. Cell response to skin trauma was also relatively normal at that time. We have no data on wound bacterial counts, but there was no apparent invasive infection and wounds were clinically noninfected. Blood cultures were sterile. Septicemia may not have developed because of antibiotic therapy, although systemic antibiotics were not administered from 53 through 84 postburn days. Individual cells in our cover-slip impression smears were actively phagocytic but, as indicated above, bactericidal capacity was probably not continuously normal.

Discussion

The observations presented in this paper document clearly that the blood bactericidal capacity in acute severely burned patients was elevated against *Staph. aureus* (coagulase-positive) and normal against *Staph. albus, E. coli* and *Pseudomonas aeruginosa*. The lethal capacity is probably elevated against those species also but the test system was not adjusted to show it. This is probably a nonspecific effect which would be lethal to other bacterial species also. The increased bactericidal effect persisted through the second postburn week and then with the *Staph. aureus* returned to normal. In two patients, studied con-

TABLE 12. *Serum Complement and Bactericidal Effect of Leukocyte-Plasma Suspensions and of Plasma on Staphylococci and* E. coli

Patient 8 (O.C.)

Postburn Day	*Staph. albus*		*Staph. aureus* 80/81		*E. coli*		Serum Complement C'H50 units/ml.
	Plasma B.E.*%	Leukocyte-Plasma B.E.%	Plasma B.E.%	Leukocyte-Plasma B.E.%	Plasma B.E.%	Leukocyte-Plasma B.E.%	
43	6	47	—	—	—	—	200
44	—	—	—	—	58	91	—
46	0	40	—	—	—	—	221
52	—	—	—	—	76	88	243
60	—	—	—	—	81	98	—
68	50	66	—	—	—	—	—
79	43	80	—	—	—	—	263
87	0	35	—	—	—	—	—
94	0	77	—	—	—	—	278
96	—	93	—	—	—	—	—
108	56	98	—	—	—	—	222
122	0	95	13	56	94	99	186
231	0	96	8	80	98	98	—
Normal Mean	47±9	98±0.3	7±5	73±4	32±14	98±0.3	175
Normal Range	0–95	95–99	0–53	45–87	0–98	97–99	146–206

* B.E.—Bactericidal Effect.

tinually until the completion of grafting, blood bactericidal studies were persistently normal against all test organisms, but in another, first studied in the seventh postburn week, a decrease from normal was found for a time with eventual recovery. Wright showed, in 1927, that *in vitro* bactericidal studies in rabbits correlated well with the disappearance of the same organisms from the blood stream after their intravenous injection.[12] Therefore, the *in vitro* bactericidal studies reported in this paper probably reflect the functional activity of the fixed phagocytic cells so long as adequate tissue perfusion is maintained. Our studies indicate that micro-organisms entering the blood stream of resuscitated patients in the early postburn period will be trapped and destroyed as effectively or better than in the normal. In a later phase of the disease the blood bactericidal capacity may be reduced below normal in some but not all patients. This may be of significance in the pathogenesis of septicemia but will require further documentation.

Hemolytic serum complement was normal or better in all determinations except on the first postburn day of one lethally burned patient and this was not associated with a low blood bactericidal capacity. A survey of serum complement in burn patients has not been reported previously and the significance of the elevated findings is not clear. However, our findings may not be important as regards infection since there is said to be no correlation between the hemolytic and bactericidal activity of complement.[7]

Our finding of a markedly diminished cell response to superficial skin trauma in the early days following burn injury but after restoration of urine output is significant. We have noted similar findings after severe hemorrhagic shock or at the site of the intradermal injection of adrenaline.[2] There is also a marked delay in the appearance of lymphocytes and of lymphocytogenous macrophages in the exudates and an absence of eosinophils. However, neutrophils and mononuclear cells in the exudates are actively phagocytic until death. Peripheral blood leukocyte counts are elevated during the period of diminished cell response in the skin but there is a neutrophilia and a lymphopenia. The cell response to superficial skin trauma gradually returns towards normal after the first postburn week but there is a persistent delay in the appearance of lymphocytes. The failure of total cell response in the test wounds is probably a manifestation of inadequate peripheral circulation and probably reflects a similar situation in the burn wounds. The absence of lymphocytes and eosinophils in the test wounds is probably a manifestation of the severe stress response. The increased plasma bactericidal effect found against *Staph. aureus* might be related to the dissolution of lymphocytes, a manifestation of the steroid stress reaction. The significance of the relative absence of lymphocytes in our test wounds, and probably also in the burn wound, is not clear, but lymphocytes in inflammation have several functions in addition to phagocytosis. They possess enzymes which participate in the breakdown of products of protein catabolism and in the dissolution of fibrin. They also probably manufacture and presumably release antibodies. They undoubtedly participate in the localization of the infectious process.

Correlation between the state of the antibacterial defense systems, and the finding of bacteria in the blood stream must be guarded, because all patients received antibiotics systemically or topically. Patient 8 did not develop septicemia even though blood bactericidal studies were subnormal for a prolonged period, during part of which time only topical neomycin was used. However, the cell response to skin trauma was relatively normal and the wound bacterial count was probably normal. Patient 1 developed a terminal septicemia at

the time 1) the cell response to skin trauma was markedly reduced and abnormal; 2) a leukopenia had developed; and 3) the blood bactericidal capacity had begun to fall from an earlier high. Patient 3 first developed positive blood cultures following debridement and at a time of decreased and abnormal cell response to skin trauma. The leukocyte-plasma bactericidal capacity against staphylococci was normal but there was no inhibition of bacterial growth by plasma alone. The correlative data on Patient 4 are perhaps the most revealing. Gram-negative bacilli appeared in the blood stream, despite high blood bactericidal findings, when the total wound bacterial count was high and the cell response to skin injury was reduced. Septicemia, however, did not persist (perhaps a blood bactericidal or an antibiotic effect). Subsequent wound bacterial counts showed lower values, and the cell response to skin injury progressively improved. Unfortunately, all of our studies were not done at the time *Staph. aureus* appeared in the blood stream. However, at that time, the blood bactericidal capacity against that species had fallen to normal levels. Transient bacteremia occurred in Patient 7 when the wound bacterial count was very high, even though the observed modalities of antibacterial defense were normal or better.

The various findings reported in this paper suggest that several factors determine the balance between bacterial invasion and host resistance and that these are continuously shifiting in the same or in opposite directions. Phagocytic and plasma bactericidal capacity remain remarkably effective, however, in massively burned patients until death. The appearance of bacteria in the blood stream of such resuscitated patients is not due to impaired central humoro-cellular antibacterial defenses, but rather to an unfavorable ratio between defensive cells and bacteria in peripheral tissues.

Studies in animals have shown that bacteria injected intravenously are rapidly removed from the circulation and that the trapping capacity of the reticulo-endothelial system is seldom, if ever, exceeded. This is true for a wide variety of pathologic states; for example, animals moribund with metastatic infection can still rapidly remove other bacterial species from the circulation after intravenous injection.[9] If liver and splenic blood flow is markedly impaired, as may be true in the phase of burn shock, bacterial blood stream clearance is probably less efficient but, fortunately for the treated patient, this would occur within the first 24 hours, when wound bacterial counts are often low. Relatively nonpathogenic bacteria are more apt to invade tissues as the bacterial count increases with the development of wound sepsis, especially if the peripheral cell response is depressed; and this, more particularly, when the immediate post-trauma high plasma bactericidal effect has begun to fall to the normal range. Invasion by true pathogens, such as *Staph. aureus, Streptococcus hemolyticus* or *Pseudomonas aeruginosa* is even more likely under such circumstances. The significance of the ratio of bacteria to leukocytes in determining bactericidal effect is readily shown experimentally. A hundred-fold increase in the number of *Staph. albus* in our test system reduces the mean leukocyte-plasma bactericidal effect of control subjects from 98 to 47 per cent! Miles has called the initial infecting inoculation the *primary lodgement,* and has shown experimentally, that for some microorganisms, the resulting local infected lesion is potentiated by hypovolemic shock, which reduces peripheral blood flow.[6]

A recent report of studies on guinea pigs 24 hours after producing a 10 per cent burn notes a decrease in the expected exudation of mononuclear cells in response to the intraperitoneal injection of *Pseudomonas pyocyanea;* an effect on plasma bactericidal capacity could not be shown. In that study, blood clearance of intracardiac-

ally injected bacteria was impaired three hours after burn injury.[5] The paucity of mononuclear cells found in the peritoneal exudate of guinea pigs is similar to our failure to find lymphocytes in superficial skin wounds. The significance of the finding as regards antibacterial defense in burns is not known. Blood clearance data from minimally burned guinea pigs injected intracardiacally shortly after injury should be interpreted with caution when considering the problem of bacterial invasion from burn wounds in the human host.

The persistence of bacteria in the blood stream of burned patients (usually in small numbers per ml. blood) is of grave prognostic significance; but this does not necessarily mean that the blood or reticuloendothelial system has become less bactericidal (which is unlikely); it indicates a lack of local control of bacterial growth in an area of infection, usually in the burn wound.

The emphasis of the present study has been on host factors. An unfavorable leukocyte-bacteria ratio might also result from local factors in the wound which encourage bacterial growth; for example, the presence of necrotic tissue; or of the antibiotic suppression of some bacterial species, allowing others to multiply more easily. Some bacterial species, such as *Staph. aureus* may survive within leukocytes (to a limited extent[11]) and therefore a smaller ratio may favor bacteremia. Some gram-negative bacilli isolated from burn wounds are naturally resistant to the bactericidal action of normal serum (but not necessarily resistant to leukocyte-serum bactericidal effect[10]) and if present would probably permit tissue invasion with a smaller ratio.

This study has not dealt with the problem of specific antibacterial immunity. In another study we have shown that fatally injured patients could rapidly synthesize new antibody protein; there is no reason to believe that burn patients would react differently.[3] Tetanus is an uncommon complication of burn patients who have been previously immunized and who have received a booster injection of tetanus toxoid at the time of injury. This is indirect evidence of the satisfactory activity of the specific immune mechanism in burn patients. Langohr, Owen and Cope noted a fall in antistaphylococcal serum agglutinins and serum euglobulins in the late postburn period in a few patients developing staphylococcal infection.[4] They also noted regression of invasive staphylococcal infection in patients as the serum agglutinin titer against the offending staphylococcus and the serum euglobulin level increased in response to staphylococcal immunization with an autogenous vaccine. This also suggests that severely burned patients can synthesize antibody during the late postburn period.

The therapeutic implications of the present study reassert the importance of long established principles of meticulous wound management. Aseptic and isolation technics are mandatory to keep bacterial inoculation as low as possible. Bacterial growth in the wound should be kept to a minimum so as to preserve a favorable cell-bacteria ratio. The importance of debridement and early skin coverage is universally recognized but ill-timed and excessive surgical debridement may also upset a favorable cell-bacteria ratio. The importance of preserving optimal tissue perfusion for cellular and organ function and to allow maximal cell and plasma exudation to the area of injury is evident. Hypothermia should be used with caution because of its adverse effect on peripheral circulation. Prophylatic antibiotic therapy, both systemic and topical, should be used during the period of delayed cell response to injury and particularly when the early high plasma bactericidal effect begins to fall to normal. It is mandatory at the time of surgical debridement.

Summary

1. Leukocyte-plasma and plasma bactericidal effect against *Staphylococcus aureus* (phagetype 80/81) is significantly increased in severely burned patients. Values return to normal after several days. Bactericidal capacity against *Staphylococcus albus, Escherichia coli* and *Pseudomonas aeruginosa* is also normal or better.

2. The cellular reaction to peripheral injury is markedly depressed for several days in severely burned patients. There is also a significant delay in the appearance of lymphocytes and an absence of eosinophils. However, many of the migrating cells actively phagocytose staphylococci.

3. Terminal bacteremia was detected in an extensively burned patient at a time when 1) the blood bactericidal capacity had begun to decrease; 2) there was a marked leukopenia; and 3) the cell response to peripheral injury was depressed. Transient bacteremia was detected in a surviving patient with a decreased cell response to peripheral injury when the wound bacterial count was high despite an excellent blood bactericidal capacity. Transient bacteremia was also detected in another surviving patient in the early postburn period when the wound bacterial count was very high, even though the measured modalities of antibacterial defense were normal or better.

4. A decreased blood bactericidal capacity with eventual recovery was found in one burned patient studied during a later period of nutritional depletion. No such defect was found in another comparably burned patient.

5. Several factors determine the balance between bacterial invasion and host resistance and these are continuously shifting. With *Staph. aureus* (phagetype 80/81), tissue and blood stream invasion is probably more likely when the initial high posttrauma bactericidal effect has returned to normal levels.

6. The primary cause of bacteremia in burned patients probably is the presence of a high bacteria-cell ratio in the wound, especially if the micro-organisms are pathogenic. However, continual bacteremia by pathogenic bacteria can cause septicemia in burned and nonburned patients.

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