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The Effect of Severe Battle Injury and of Post-Traumatic Renal Failure on Resistance to Infection*

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INTRODUCTION

ACUTE INFECTION REMAINS one of the serious complications of severely wounded soldiers. A statistical analysis has been reported of 1273 battle casualties who died in forward surgical hospitals near the end of World War II.[34] Fourteen per cent of these died of complicating surgical infections. In the Korean War, five of 20 deaths occurring in 138 severely wounded soldiers at a forward level were the result of complicating infection.[1] Wounds are usually contaminated with many aerobic and anaerobic pathogenic micro-organisms.[35] Furthermore, devitalized tissue in wounds presents a suitable environment for bacterial multiplication, spread, or toxin production. Wound infection can usually be prevented by early and adequate surgical débridement which removes most of the non-viable tissue and perhaps the majority of contaminating bacteria; such adequately débrided wounds can often be closed on the fifth postwound day regardless of their bacterial flora at that time, provided the classical signs of infection are absent.[9]

Infection has been reported to be more common in patients with renal failure.[26] Sixty-nine per cent of a small series of fatal cases of post-traumatic renal insufficiency in World War II had severe complicating infection.[34] Likewise, a high incidence of infection was noted in similar cases under study in Korea.[3]

Several physiological and biochemical changes have been described following severe injury.[29, 39] The subsequent development of post-traumatic renal insufficiency causes further variations.[8, 39] Therefore, it is possible that body defense mechanisms may also be altered in these conditions, and so contribute to the development of infection.

There are several mechanisms of body defense following bacterial invasion. The micro-organisms may be trapped and eliminated by lymph nodes draining the area. Cells of the reticulo-endothelial system and wandering phagocytes may ingest and destroy pathogens, a process which is much more effective if specific antibody is present. In addition, natural and specific antibody (globulins) participates in the lysis of some bacterial species, and also neutralizes the toxic products of others. The term complement designates a group of proteins found in normal serum which possess several properties of importance in antibacterial defense. These include a capacity to render micro-organisms more susceptible to phagocytosis, and also to kill bacteria coated with antibody. Therefore, if a defect in globulin synthesis or a depression of reticulo-endothelial cell function or leucocyte activity follows

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TABLE I. *Phagocytosis Related to Injury.*

Post Wound Day	No. Subjects	% Neutrophils Showing Ingestion	Standard Error of Means
	Non-Wounded		
0 - 1	6	94.4	± 2.18
	Slightly Wounded		
0 - 1	12	88.2	± 2.5
	Severely Wounded without Clinical Post-Traumatic Renal Insufficiency		
0 - 1	6	68.4	± 5.5
1 - 2	5	61.2	± 12.1
2 - 3	4	81	± 4.2
3 - 4	5	82	± 5.4
	Severely Wounded with Post-Traumatic Renal Insufficiency		
1 - 2	5	83.6	± 3.7
2 - 3	8	82	± 1.7
3 - 4	6	83	± 2.1

severe injury, the defense effort might be seriously impeded.

The purpose of the present investigation was to study antibacterial defense systems in a group of severely wounded casualties with and without post-traumatic renal insufficiency. This was accomplished by measuring (a) the phagocytic activity of circulating leucocytes, (b) the body's capacity to synthesize specific antibody, and (c) the plasma complement level in patients. The findings were then related to other clinical data, and an attempt made to assess their significance in the development of infection.

The study was undertaken during the months of June, July and August, 1953, as one facet of the Army Medical Service Graduate School's research activities in Korea. Patients were studied at the 46th Mobile Army Surgical Hospital, and at the 11th Evacuation Hospital.

METHODS

Measurement of Phagocytosis. The method used has been described in detail in a previous communication.[4] The capacity of neutrophilic polymorphonuclear leucocytes (neutrophils) to ingest coagulase positive staphylococci (Strain I) was measured. One-tenth of a milliliter of a standardized suspension of micro-organisms was added to 1.0 ml. heparinized blood. The mixtures were placed in glass tubes 12 x 0.8 cm. in size, which were then stoppered and rotated in a 37° C. incubator at 11 r.p.m. for 60 minutes. Blood smears were then prepared and ingestion recorded as the percentage of neutrophils containing staphylococci.[20] Blood samples were taken from patients one or more times each day. Fourteen seriously wounded patients without oliguria and 12 seriously wounded casualties with posttraumatic renal insufficiency were studied. Twelve slightly wounded soldiers served as control subjects. All casualties had received chemotherapeutic drugs.

Complement Titrations. The level of complement was measured by determining the

TABLE II. *Complement Titer Leucocyte Count and Mean Ingestion of Staphylococci by Neutrophils Immediately Following Large Blood Transfusions.*

Patient Number	Post Wound Day	Volume Blood Used in Resuscitation	Volume Dextran Used in Resuscitation	WBC per cu. mm.	% Neutrophils	% Neutrophils Containing Staphylococci	Complement† Titer
		L	L				
3	1-2	5	1.9	15,400	73	56	16
4	1-2	5	—	14,000	81	18	32
15	0-1	7	0.5	13,250	72	66	16
16	0-1	7.5	—	14,650	70	56	8
21	0-1	8.5	1.0	16,800	79	62	16
26	0-1	5.5*	1.0	15,800	76	56	4
	"	10	—	13,400	66	62	4
	"	12	—	16,600	72	74	8
	"	13	—	15,300	74	70	8
27	0-1	12*	—	9,850	80	88	16
	"	23	—	11,850	77	90	16
28	0-1	26	—	5,500	70	82	16

*Volumes of blood are reported on a cumulative basis
†Reciprocal of highest dilution giving 100% hemolysis

highest dilution of plasma which would lyse 0.5 ml. of a standardized suspension of sensitized sheep red cells. All dilutions were made with Kolmer's saline (0.85 per cent NaCl + 0.01 per cent $MgSO_4$), the double-dilution technic being used. Sensitized sheep red cells preserved not longer than 21 days in dextran-gelatin-veronal solution[38] were used. The mixtures of plasma and cells were incubated in a water bath at 37° C. for 30 minutes, and the end point was read as the highest dilution of plasma causing 100 per cent hemolysis. Blood samples were taken from patients at least once a day. Plasma was separated immediately, and the titrations done without delay. Fourteen seriously wounded patients without oliguria and 12 seriously wounded casualties with posttraumatic renal insufficiency were studied. Seventeen slightly wounded soldiers served as control subjects.

Antibody Synthesis. The capacity to synthesize specific antibody was determined by measuring at intervals the level of circulating tetanus antitoxin following the subcutaneous administration of 0.5 ml. alum-precipitated formalized tetanus toxoid to previously immunized casualties. The toxoid was injected within a few hours of wounding. Blood was drawn for antitoxin titration at various intervals, depending in part on the patient's survival, or his evacuation from the combat zone. Plasma was separated from cells and frozen. At at later date the samples were carried to the United States for antitoxin assay. The antitoxin level was titrated

TABLE III. *Relationship of Daily Plasma Calcium to Phagocytosis.*

Patient No. 126		Patient No. 129	
Calcium mg. %	% Neutrophils containing staphylococci	Calcium mg. %	% Neutrophils containing staphylococci
5.8	78	7.0	82
6.4	84	7.1	80
7.1	86	7.9	90
7.5	88	9.0	88
7.8	80	9.1	86
8.0	72	11.0	80
8.0	88	11.5	84
8.1	92	13.2	78
8.4	82		
11.6	84		

TABLE IV. *Blood Chemistry, Phagocytic Index, and Complement Titer Before and After Hemodialysis.*

Patient No.	Ht	Na mEq/L	K mEq/L	Cl mEq/L	CO_2 mEq/L	NPN mg. %	Ca mg. %	P mg. %	WBC per cu. mm.	% Neutrophils	% Neut. Containing Staphylococci	Complement§ Titer
125												
0 Hours	31	151	6.0	104.5	21.2	255	8.8	9.7	17,200	91	88	8
6 Hours	29	138	3.5	99	25.8	91.4	9.0	5.1	18,000	91	84	8
126*												
0 Hours		125	6.8	81	27.5	—	5.8	15.7	11,050	54	78	16
6 Hours		150	2.8	92.7	24.3	—	8.1	6.9	10,500	59	92	16
127												
0 Hours	26.5	133	6.4	91.7	20	170	8.1	8.6	11,430	77	82	8
6 Hours	27	140	4.6	95.8	24.3	105	—	5.6	14,600	74	90	<1
128												
0 Hours	45	146	8.2	95	22.5	113	—	5.5	12,300	92	84	<1
4 Hours	44	145	5.4	96	26	—	10	3.6	13,200	90	92	<1
129†												
0 Hours	38	143	6.5	97.6	26	261	9.1	18.7	13,400	74	86	16
6 Hours	36	140	4.1	99.6	25.3	65	11.5	7.6	14,200	80	84	32
129‡												
0 Hours	29.5	147	8.5	89	13.3	320	11	20	14,300	68	80	32
6 Hours	35	144	4.1	97.4	17.1	100	13.2	10.8	17,200	76	78	16
133												
0 Hours	21	133	8.5	91.8	12.6	224	—	—	17,100	81	80	32
6 Hours	36	145	4.9	96.6	18.5	95	—	—	15,300	74	78	16

* Patient with acute hemorrhagic fever.
† Third post-wound day.
‡ Sixth post-wound day.
§ Reciprocal of highest dilution yielding 100% hemolysis.

TABLE V. *Relation of Early Post-Wound Phagocytosis to Development of Infection.*

Patient Number	% Neutrophils Showing Ingestion on 1st or 2nd Post-Wound Day	INFECTION Serious	Moderate	Minor
3	56	−	+	−
4	18	+	−	−
15	66	−	−	+
26	56	−	+	−
27	88	−	−	−

in mice, using a tetanus toxin of known potency obtained from the National Institutes of Health. In general, the method of assay was that used by Pillemer.[31] All dilutions of plasma or toxin were made with a solution of 0.9 per cent sodium chloride which contained 1 per cent peptone. Three mice were used with each dilution. Each titration was controlled by a parallel titration using standard tetanus antitoxin. Data from seven casualties are reported in this study.

Chemical data* reported in this study were determined as follows: Non-protein nitrogen, by the method of Folin and Wu;[17] sodium and potassium by the flame photometric method of Hald;[19] inorganic phosphate by the method of Fiske and Subbarow;[16] chloride by the method of Schales and Schales;[36] carbon dioxide capacity by the method of van Slyke and Cullen;[41] and calcium by Clarke and Collip's[10] modification of Kramer and Tisdall's method.

Clinical Record. Patients included in this study were carefully followed either at a forward surgical hospital or at a special center for the treatment of posttraumatic renal insufficiency. Evacuation or death of the patient sometimes prevented prolonged observation.

Patients were considered severely wounded if they had sustained multiple injuries, were in severe shock, and required a large volume of blood for resuscitation.

* I am indebted to Major Wm. H. Meroney, MC, Chief, Renal Insufficiency Center, Korea, for permission to publish the chemical findings from patients studied at that center.

TABLE VI. *Complement Activity in Plasma of Battle Casualties.*

End Point = 100% Hemolysis

	Slightly Wounded 17 Patients	Seriously Wounded without Oliguria 14 Patients					
		Post-Wound Day					
	0-1	0	1	2	3	4	5
Complement* Titer	No. Patients	No. Patients					
64					1		
32	12	1	2		2	1	1
16	5	5	4		2	1	
8		1	2	3	1		1
4		1					

*Reciprocal of highest dilution yielding 100% hemolysis

Posttraumatic renal insufficiency was diagnosed clinically when a wounded soldier excreted less than 500 ml. urine per 24 hours in the presence of an adequate blood pressure and a reasonable state of hydration. The diagnosis was confirmed by chemical data and, in the patients who died, by the autopsy findings.

RESULTS

PHAGOCYTOSIS BY NEUTROPHILS FOLLOWING INJURY

Table I records phagocytosis of coagulase positive staphylococci by neutrophils from different groups of subjects. The bacterial suspensions used were always adjusted to the same turbidity and the neutrophil count per cubic millimeter determined for

TABLE VII. *Complement Activity in Plasma of Battle Casualties.*

End Point = 100% Hemolysis

	Seriously Wounded with Oliguria 12 Patients Post-Wound Day						
Complement* Titer	1	2	3	4	5	6	7
	No Patients						
32		1	1	1	2	1	
16	1	1	3	3		1	
8	1	1	1		2		1
4							
<1	2						2

*Reciprocal of highest dilution yielding 100% hemolysis.

TABLE VIII. *Complement Titer in Wounded Casualties on Day of Death. Patients without Post-Traumatic Renal Insufficiency.*

Patient Number	Complement Titer	Most Prominent Cause of Death
4	64	Multiple lung abscess
16	8	Traumatic shock
21	16	Extensive head injury
28	16	Traumatic shock and hemorrhage
29	8	Clostridial myositis
Patients with Post-Traumatic Renal Insufficiency		
115	16	Clostridial myositis
120	32	Hyperkalemia
129	16	Clostridial myositis
132	32	Undetermined
133	8	Para-colon septicemia
135	8	Fat embolus
136	32	Undetermined
137	16	Undetermined

*Reciprocal of highest dilution yielding 100% hemolysis

each blood preparation. The over-all ratio averaged one neutrophil to approximately 30 bacteria. Since bacteria were always present in considerable excess, the difference in ratio of bacteria to neutrophils in different experiments is probably not an important factor. In any event, under the conditions of our experiments, the degree of phagocytosis appeared to be independent of the neutrophil count.

The differences shown between the non-wounded and slightly wounded subjects could have been due to chance. The latter group were all ambulatory patients, and were studied within 12 hours of injury. So, any general stress effect which might have resulted from exposure to enemy action did not interfere with staphylococcal ingestion under the experimental conditions of the study.

Phagocytosis by neutrophils from severely wounded subjects without posttraumatic renal insufficiency was significantly diminished within the first 24 hours of injury. The reduction was still present on the second postwound day, but the findings were not so definite. On subsequent days, the findings were within the range of those in the slightly wounded control group. The difference in mean phagocytosis by neutrophils from patients with posttraumatic renal insufficiency and those from the slightly wounded control group could have been due to chance.

Therefore, the only group of patients showing significantly diminished phagocytosis were the severely wounded who were studied on the day of injury. In all these instances, blood for study was drawn at the end of resuscitation from shock.

Table II relates the white blood count per cubic millimeter and neutrophil activity to the volume of blood and dextran used in the resuscitation of individual casualties. In six of the cases the data were obtained within 24 hours of injury and immediately following the administration of the volume of bank blood recorded in the table. The elevated white blood counts were probably not the result of hemoconcentration, since in the majority of such wounded soldiers hematocrits were normal or low. Even after the administration of three or four times a normal blood volume of bank blood, optimal numbers of leucocytes were present in the circulation. Furthermore, the per cent of neutrophils was higher than normal. But, as already reported, in several instances the percentage of active neutrophils was diminished. The findings did not appear to be related to the magnitude of injury as judged by the volume of blood required for resuscitation, nor to the amount of bank blood used. All the patients who received dextran showed a depression of phagocytosis, but so did others not receiving this colloid. White blood cells found in a patient's blood immediately following large transfusions were probably autogenous and not contributed by the transfusions. The latter were all of bank blood flown from the United States. Most of the bottles of blood were more than ten days old but all less than 21 days. Examination of several such samples invariably revealed very low white blood counts, neutrophils being absent.

These data do not show why phagocytosis is depressed. The return toward normal by

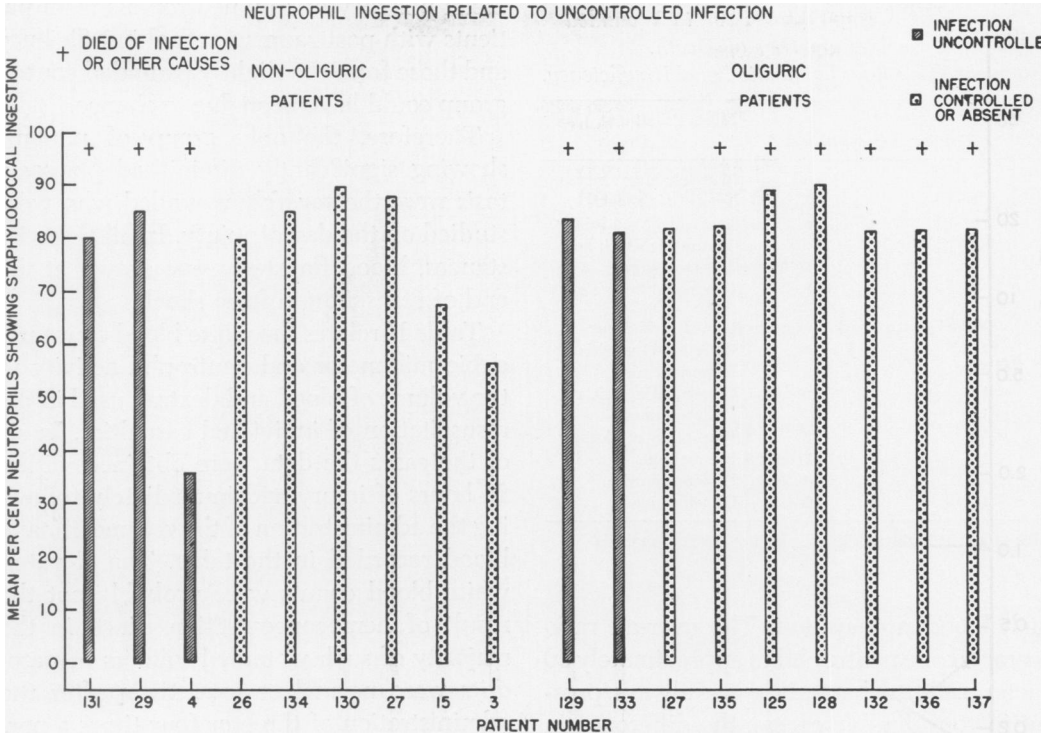


FIG. 1. Capacity of neutrophils to ingest staphylococci related to the presence of uncontrolled infection.

the second postwound day could have been due to the output of new neutrophils by the bone marrow, although there is no evidence to prove this. The initial depression in phagocytosis could also have been due to humoral factors, with restoration towards normal by the second day. Citrate has been reported to interfere with phagocytosis,[20] but it probably was not a factor in these patients, since patients numbers 27 and 28 showed no significant depression in phagocytosis; yet they received the largest quantities of citrate blood.

Calcium ions are reported to be essential for optimal phagocytosis.[20, 23, 25] Table III shows the relationship between daily total plasma calcium and the ability of neutrophils to ingest staphylococci. These data, taken from two patients with acute renal failure, show no consistent pattern. Therefore, under these experimental conditions, variations of plasma calcium within a wide physiologic range did not affect ingestion. Furthermore, other observations showed that the administration of sufficient calcium gluconate to elevate the blood pressure temporarily in hypotensive patients did not alter phagocytosis.

POSTTRAUMATIC RENAL INSUFFICIENCY AND PHAGOCYTOSIS

Posttraumatic renal failure leads to reasonably predictable changes in the level of many of the blood constituents. The hematocrit and the sodium, chloride, carbon dioxide and calcium levels in plasma are often depressed. But potassium, phosphorus and non-protein nitrogen are usually elevated. Successful dialysis of a patient's blood through an artificial kidney for six hours restores all of these, except the hematocrit, towards normal. Table IV records such data from patients with acute renal failure. The determinations were obtained at the begin-

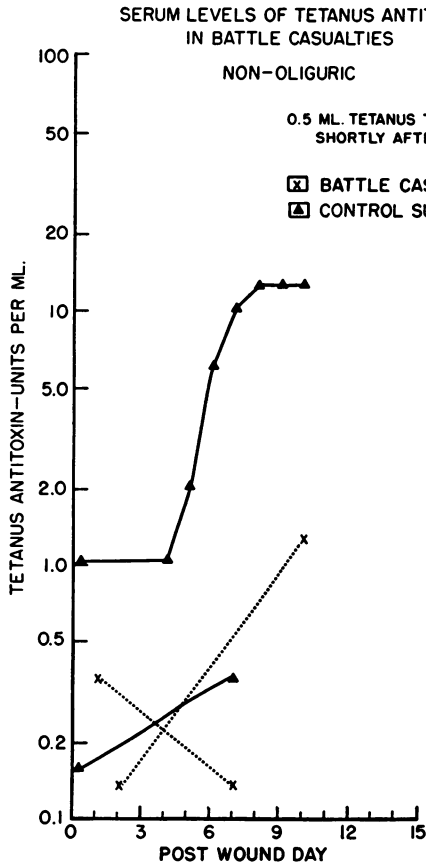


FIG. 2. Synthesis of tetanus antitoxin by severely injured battle casualties without complicating acute renal failure. Controls are from published observations of others.[29, 30]

ning of hemodialysis, and at its completion six hours later. The criteria employed for the use of artificial hemodialysis were either potassium intoxication or severe clinical uremia. Phagocytic activity did not appear to be related to any of the changes in plasma chemistry, and was not depressed even in the blood of patients with severe clinical uremia.

PHAGOCYTOSIS AND DEVELOPMENT OF INFECTION

Because multiple factors probably are concerned in the development of infection following injury, it may be unwise to attempt to relate phagocytosis and the development of infection in individual cases. With this reservation, a listing has been made in Table V showing whether or not infection developed in five cases with early postwound depression of neutrophil activity. In no instance can it be stated that the onset of infection was aided by the lowered neutrophil activity. The following case is an example:

Patient number 4 was first admitted to a Mobile Army Surgical Hospital 1½ hours after injury. Preliminary resuscitation was accomplished, and then because of a heavy patient load he was transferred by air to another surgical hospital. Here the patient load was also heavy, and further transfusion was required while awaiting operating room space, which became available 15 hours after injury. The patient's injuries included a large, penetrating flank wound with transection of the sigmoid colon, a compound fracture of the left tibia involving the knee joint, large perforating wounds of the thighs, traumatic amputation of the right hand, and compound fracture of the left humerus. At operation no definite peritonitis was noted and the colon was exteriorized; the extremity wounds were thoroughly débrided. At that time 18 per cent of the test neutrophils ingested staphylococci. The patient's postoperative course was marked by extreme lethargy and failure to cough or ventilate adequately. Atelectasis developed, which was not controlled by bronchoscopy or tracheotomy and frequent intratracheal aspiration. The abdomen remained soft, and the extremity wounds were not clinically infected. The patient died on the fourth postwound day, at which time approximately 62 per cent of circulating neutrophils were active. From the time of initial operation until death the temperature persisted at 102 to 103° F., the pulse at 120 to 130 per minute, and the respirations fluctuated between 30 and 50 per minute. Urine output was greater than 1000 ml. per day. The patient was given penicillin, streptomycin and oxytetracycline. The outstanding finding at autopsy was bilateral lung atelectasis with multiple small abscesses.

Because the wounds remained clean and significant peritonitis was absent, it seems unlikely that a general depression in neutrophil activity was of primary importance in the development of lung abscesses. It is more probable that some local factor, such as unrecognized aspiration of foreign material into the lung during anesthesia, was the precipitating factor.

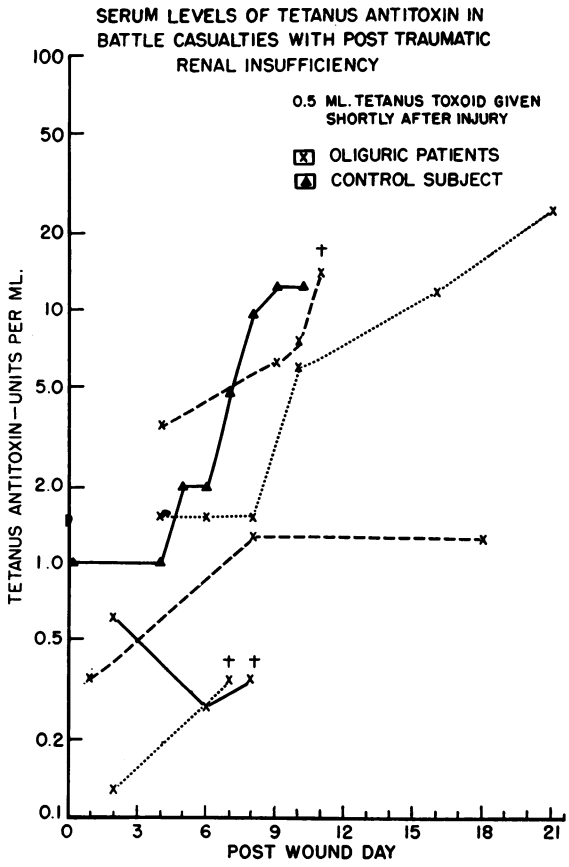


FIG. 3. Synthesis of tetanus antitoxin by severely injured battle casualties with complicating acute renal failure. Control is from published observations of Mueller and his associates.[30]

Figure 1 shows mean neutrophil activity determined from daily estimations in seriously wounded patients with and without acute renal failure. They are further grouped into those who died of uncontrolled infection (myositis, peritonitis, or septicemia), and those not clinically infected or in whom infection was controlled. Except for patients No. 3 and 4, the differences shown between individuals in each group did not seem of importance. Furthermore, there was no significant difference between the groups. These data appear to show that neutrophil activity was well maintained during the course of serious illness following severe battle trauma. The development of acute renal failure did not depress the ability of neutrophils to ingest staphylococci. The presence of normal numbers of active neutrophils did not prevent death from uncontrolled infection.

COMPLEMENT TITER FOLLOWING INJURY

Complement titers were determined in three categories of patients: a series with minor injuries which includes the patients used as controls in the phagocytosis studies; another series of severely wounded soldiers without clinical renal insufficiency; and a third group of severely wounded with acute renal failure. Freshly drawn plasma was used in these titrations because preliminary tests showed that the amount of heparin used to prevent coagulation did not alter the titer under the experimental conditions used. The original intent was to evaluate whole complement activity following severe injury. If any significant change was found, a more comprehensive study of the effect of injury on the several fractions of complement would be attempted. For this reason, and because the studies were done under field conditions, plasma was titrated by the double-dilution method, reading 100 per cent hemolysis as the end point. Because of the large blood volume shifts and *also* the hemodilution which accompanies hemorrhagic or traumatic shock, it seemed probable that the significance of any small variation in complement detected by more refined technic would be difficult to interpret.

Table VI records the dilution of plasma from different subjects required to hemolyse completely 0.5 ml. sensitized sheep red cells. The findings for the slightly wounded patients are comparable with those found by others in normal individuals.[15, 33, 40] The table also shows complement titers obtained from severely wounded patients without clinical evidence of acute renal failure. Because of the wide range in plasma dilution, the possible error between observations of successive tubes is at least 100 per cent. For

this reason the range of levels of complement activity shown by some severely wounded patients cannot be considered significant. However, the table shows that most of the specimens examined were within the control range.

Table II also records complement titers in the plasma of a group of patients immediately following large transfusions of bank blood. Complement levels were in the normal range even after the administration of bank blood in an amount equivalent to three or four times a normal blood volume. These patients had been in severe shock, which in patients No. 26 and 27, was continuing as the successive estimations of complement were made. In the former case there appeared to be some depression in complement titer until 12 liters of blood had been given, but the complement then changed towards normal. The administration of such large quantities of blood to Korean War casualties has been discussed by others.[32] The evidence suggests that despite such large transfusions, residual blood volumes were usually subnormal. In other words, continuing hemorrhage was probably occurring, and some of these patients may have received the equivalent of a complete exchange transfusion. It is possible that the complement found in these patients was contributed by the bank blood. Titration of complement activity in the plasma from several samples of bank blood (10 to 21 days old) showed titers of 1:32 to 1:16. The presence of magnesium in the diluent probably replaced the citrate-bound magnesium in the bank plasma and probably explains the finding of normal levels of complement in the bank-blood samples. Our observations do not include complement titers in severely injured casualties before resuscitation was commenced. If complement was depressed, it apparently returned to normal in the majority of cases following resuscitation.

Table VII records complement titers in patients with posttraumatic renal insufficiency. The results were similar to those found in the non-oliguric group, and most of the findings were within our normal range. Possibly there was some decreased activity in individual titrations of plasma from different patients, but the observations cannot be considered significant. Complement was apparently absent in single samples of plasma taken from four patients. One of these (patient 128) was suffering from hemorrhagic shock and had received 21 liters of blood before the specimen was drawn for assay. Twelve hours later the titer was 1:8. There was no apparent explanation for the failure to detect complement activity in these samples of plasma, and in each instance complement was present on the next day. Table IV records the complement activity of plasma from several patients with acute clinical uremia (125, 126, 127, 129, 133). These were within the control range, and the complement determinations before and after hemodialysis (with the exception of patient 127) were not significantly different in spite of considerable changes in the blood levels of non-protein nitrogen, potassium, or phosphorus following hemodialysis. Complement was also normal where plasma calcium was low, and in patients with relatively severe acidosis.

COMPLEMENT ACTIVITY AND PHAGOCYTOSIS

A reduction in complement activity may be accompanied by a depression in phagocytosis of micro-organisms if the complement fractions which have been identified with the normal opsonins of the plasma are absent.[13] In Tables II and IV, data on phagocytosis and complement activity from the same specimen of blood may be compared. There was no consistent relation between complement and phagocytosis under the experimental conditions studied. This is not necessarily significant because we have no data on the distribution of the separate fractions of complement. For example, if C'3 was lowered out of proportion to the other components, the plasma might fail to

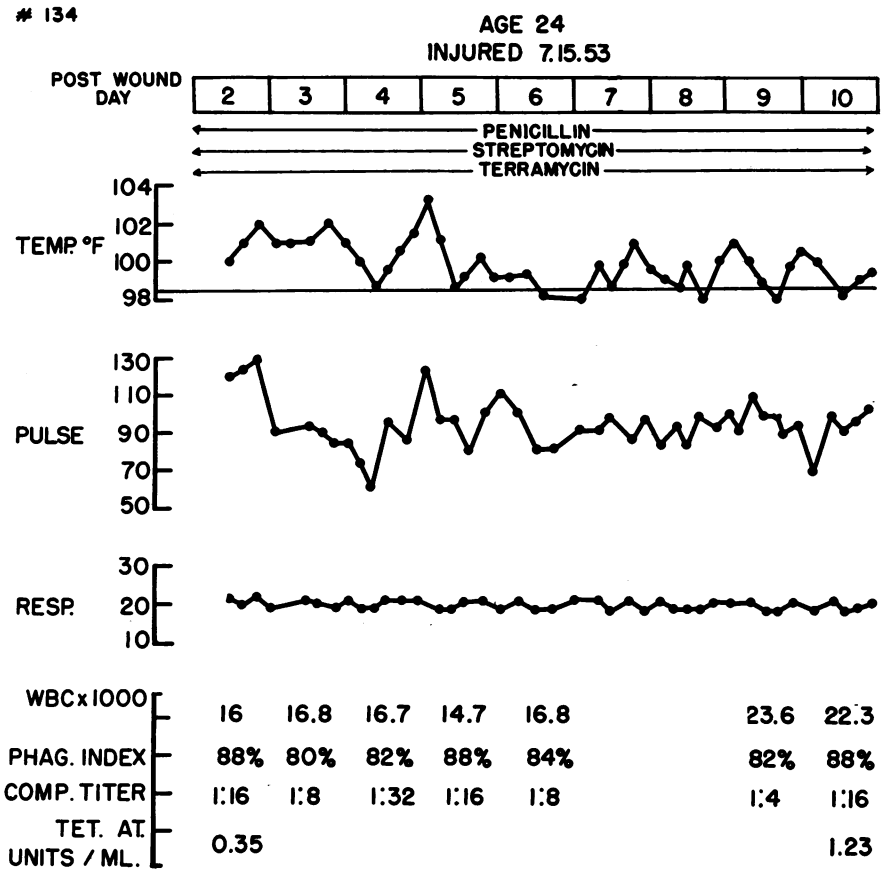


FIG. 4. Phagocytosis, complement titer and synthesis of tetanus antitoxin related to other clinical data from patient number 134.

hemolyse sensitized cells but still opsonize micro-organisms.[13]

The suggestion has been made that a low complement titer might predispose a patient to terminal infection,[24] but others have failed to find complement titers to be of any general prognostic value.[15] In Table VIII are listed the complement titers found in the plasma of several patients on the day of death. All were severely wounded, and patients numbers 115 through 137 had complicating acute renal failure. The values were all within our normal range. It is difficult to establish absolute cause of death in a complex disease, but the table also lists what was considered to be the most prominent cause of death in each case. The observed complement titers were not of any prognostic value, and were not significantly lower in patients dying of infection or of secondary irreversible traumatic shock.

ANTIBODY SYNTHESIS

Figure 2 shows the tetanus antitoxin response of two severely wounded patients who did not develop clinical evidence of posttraumatic renal insufficiency. They were given a booster dose (0.5 ml.) of alum-precipitated tetanus toxoid within a few hours of injury. Similar data are recorded in Figure 3 from five severely wounded patients with complicating acute renal failure. All of the patients had received a previous booster injection of tetanus toxoid within six months

of injury, and all the samples of plasma contained at least 0.1 units of tetanus antitoxin per ml. within one to four days after injury. These antitoxin levels were probably present at the time of injury, because the response to the booster injections in normal individuals does not usually appear before four days.[7, 18] Two of the patients studied failed to show any response during the period of observation. One of these was oliguric and died on the eighth postwound day from clostridial myositis. The other did not have renal failure but was evacuated from the combat zone, and so observation after the seventh day was not feasible. Antitoxin titers in all of the other patients showed a progressive rise. In one of these patients the rise did not appear until the eighth postwound day. Normal subjects show considerable variation in antitoxin response following the administration of a booster dose of tetanus toxoid.[7, 30] Furthermore, an increase in the circulating antitoxin may not appear until at least the sixth day after immunization, and perhaps longer.[28] The findings reported in this study fell within previously observed variations in the response of normal subjects to a booster injection of tetanus toxoid. Therefore, the data showed no evidence of any defect in the capacity of seriously wounded soldiers with or without acute renal failure to synthesize tetanus antitoxin. There might possibly have been some delay in the appearance of circulating antitoxin in this category of patient. This, however, does not necessarily reflect a defect in the capacity to synthesize antitoxin, but might have been due to a delay in the absorption of antigen following prolonged shock after injury. Both of the patients who appeared not to have synthesized antitoxin by the seventh and eighth postwound days suffered severe shock, as did one patient in whom we had daily determinations, and in whom the titer failed to rise until the eighth postwound day. On the other hand, antitoxin titers in two of the oliguric patients were rising despite clinical deterioration until death of the patients. This confirms previous observations[2] that the capacity to synthesize antitoxin may be retained by patients in spite of a progressively deteriorating illlness.

CLINICAL EXAMPLES

The following clinical examples are included to demonstrate the type and degree of injury studied. Daily observations of leucocyte and complement activity are recorded, and also tetanus antitoxin levels when determined. An attempt has been made to relate the laboratory data to the clinical course.

Severely Injured without posttraumatic renal insufficiency.

Patient 29, a 20-year-old white soldier, who was injured at approximately 1700 hours on July 19, 1953. He was admitted to a Mobile Army Surgical Hospital at 0100 hours, July 20, 1953. Injuries included a penetrating wound of the abdomen; a large avulsion-type wound of the posterior aspects of the left thigh and leg with extensive muscle destruction; soft tissue wounds of the left shoulder, left forearm, right foot and hand.

The patient received 1000 ml. blood before admission to hospital, was given another 2000 ml. before operation, and a further 3500 ml. blood during operation. No intra-abdominal lesion was found at exploratory laparotomy. An extensive débridement of wounds of the left leg was done, where the popliteal vein was found to be severed and the sciatic nerve severely damaged. The main arterial supply to the leg appeared intact. All other soft tissue wounds were débrided. A padded by-valved long-leg cast was applied to the leg. The following chemotherapeutic agents were administered: crystalline penicillin, 600,000 units b.i.d.; streptomycin, 0.5 Gm. b.i.d.; and terramycin (oxytetracycline), 0.5 Gm. b.i.d.

Sixteen hours after completion of the operation, the patient's temperature was 103.4° F., pulse, 120 per minute; and blood pressure, 105/70. The left leg was cold and moist, and the surgeon stated that he could not feel the *dorsalis pedis* pulse; the patient was given 500 ml. of blood. During the following 24 hours his condition was recorded as improved, the leg and foot were warmer, and the temperature subsided to 99° F. The pulse persisted between 120 to 140 per minute, and the respirations between 24 and 32 per minute. The blood pressures continued at about 100/70, and urinary output was 600 ml. during the first 24 hours after operation.

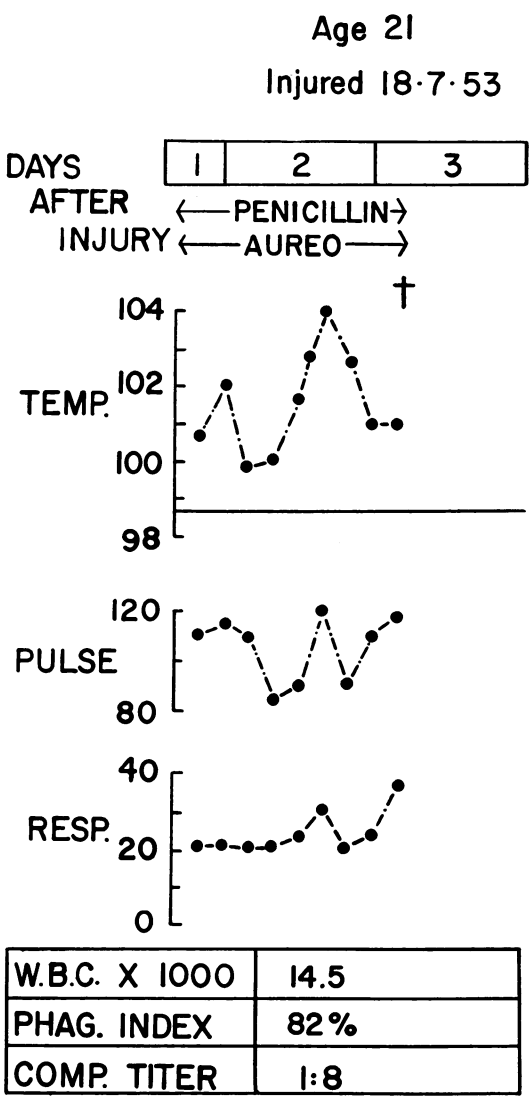


FIG. 5. Phagocytosis and complement titer related to other clinical data from patient number 135.

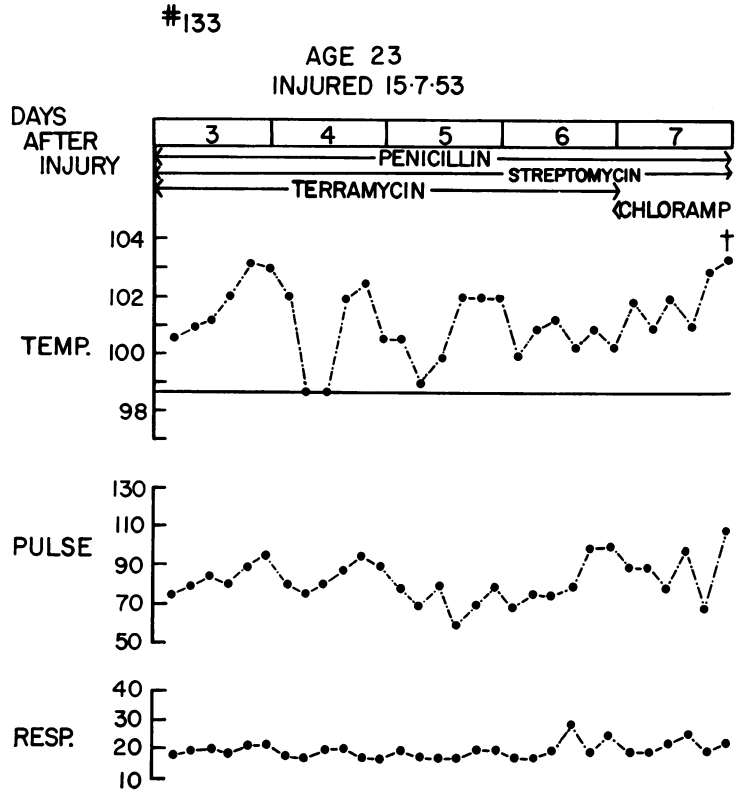
The patient was evacuated approximately 48 hours after operation because of the heavy patient load. He was held at a clearing station for 24 hours, where he stated that he complained of pain in the left leg but the wound was not examined.

On admission to an evacuation hospital 4 days after injury, the left foot and lower leg were gangrenous. There was also extensive infection and necrosis of the posterior thigh muscles, which were covered with maggots. An amputation was done below the knee joint and extensive secondary débridement done in the thigh. Two and one-half liters of blood and also noradrenalin were required during the operation because of hypotension. The thigh wounds continued to bleed following this procedure, and the patient was returned to the operating room for a further attempt to control oozing. This could not be accomplished with hemostats, so that a very tight padded compression dressing was applied. The patient's subsequent postoperative course was one of continuing hypotension, requiring blood and noradrenalin. Pulmonary edema developed but did not respond to treatment. An attempt to decrease metabolic activity in the infected left thigh by local hypothermia and the application of a tourniquet was unsuccessful. The patient died 5 days and 8 hours after injury. During the entire period at the evacuation hospital urinary output was between 50 to 100 ml. per hour so long as the systolic blood pressure was maintained by transfusion and/or vaso-pressor drugs.

Plasma chemical determinations on the day of death were: sodium, 145 mEq/L.; chloride, 97.8 mEq/L.; potassium, 3.3 mEq/L.; carbon dioxide capacity, 25.2 mEq/L.; nonprotein nitrogen, 54 mg. per cent; and inorganic phosphate, 2.8 mg. per cent. On the day before death the white blood count was 17,300 per cu. mm., of which 81 per cent were neutrophils. Eighty-four per cent of these neutrophils were active by our test of phagocytic function. The complement titer was 1:8.

The positive autopsy findings were severe pulmonary edema and extensive superficial necrosis and infection of muscle bundles in the posterior thigh.

Comment. Data reported prior to the patient's arrival at the evacuation hospital were taken from the records so that speculation about early treatment must be guarded. It is probable that an initial error in judgment was made in not amputating the extremity at that time. Furthermore, developing myositis was not recognized at the time of evacuation, yet the rapid pulse and respirations suggested its presence. The subsequent loss in continuity of care owing to patient evacuation during a time of heavy casualties allowed necrosis and infection to progress unobserved until the cast was removed at the evacuation hospital. In retrospect, only a hip disarticulation might have saved this patient at that time, but the surgeon thought the patient would not tolerate the procedure. There are no data on the state of the patient's natural antibacterial defense shortly after injury, but at the evacuation hospital no



WBC. X 1000	16.2	16.6	15.4	17.1	19.1	18.8
PHAG. INDEX	86%	78%	78%	80%	78%	78%
COMP. TITER	1:16	1:8	1:16	1:32	1:16	1.8
TET. AT. UNITS/ML.	0.13					0.35
BLOOD CULTURE	NEG.	NEG.	POS.	POS.	POS.	

FIG. 6. Phagocytosis, complement titer and synthesis of tetanus antitoxin related to other clinical data from patient number 133.

abnormality was apparent in complement level or phagocytosis. It seems more probable that the fatal infection was primarily the result of errors in surgical management.

Patient 134. This was a 24-year-old white soldier, who was wounded by a land mine at 0530 hours July 15, 1953. The patient was admitted to a forward surgical hospital shortly after injury, having been given 1250 ml. of albumin for shock. The wounds included extensive destruction of the right anterior thigh and knee joint, a compound fracture of left tibia and fibula, and a supracondylar fracture of the right humerus.

At operation the following procedures were done: a below-knee amputation of the left leg, radical débridement of the right anterior thigh and knee joint with ligation of the femoral vein, and débridement of the right arm. Eight liters of blood was given before and during operation. The operation lasted 2 hours, and the postoperative condition was reported as good. During the subsequent 24 hours the patient's condition was reported to be poor, with profuse sweating, hiccoughing and vomiting. The systolic blood pressure fluctuated between 90 and 100, the pulse was about 130 per minute, and urinary output was diminished. Twenty-four hours after injury an attempt was made to transport the patient to the posttraumatic Renal Insufficiency Center by air, but owing to fog the helicopter was unable to reach its destination. The patient was returned to the forward hospital where

his blood pressure fluctuated between 85/60 and 104/70 for several hours. This subsequently stabilized and transfer was effected to the Renal Center about 60 hours after injury. At that time the blood pressure was 110/80; pulse, 120 per minute; and temperature, 100.4° F. The urinary output was good. Examination of the wounds revealed extensive necrosis of muscles in the left lower leg amputation stump, together with necrosis of muscle groups in the right thigh. Bilateral supracondylar amputations were done, and also secondary débridement of the right arm. Urinary output remained satisfactory and the subsequent clinical course was uneventful. The patient was transferred to Japan 10 days after injury, where the wounds were successfully closed.

Chemotherapy at the Renal Center consisted of 300,000 units of crystalline penicillin every 3 hours, and 2 Gm. of oxytetracycline daily.

Figure 4 summarizes some of the clinical and laboratory observations. The daily white blood counts showed considerable elevation, which was also associated with an increase in the percentage of neutrophils. Phagocytic activity was within the normal range, as were the complement titers except for the observation on the ninth postwound day. During the period of observation the level of circulating tetanus antitoxin per milliliter rose about 251 per cent.

Comment. It is often difficult to decide whether all devitalized muscle has been excised at initial débridement with land mine injury of the extremities. It seems likely that inadequate débridement was the primary factor in the development of muscle necrosis in this patient. There was never any evidence of invasive infection, and following secondary débridement the problem was controlled. The data showed no defect in the defense mechanisms studied.

Severely injured with posttraumatic renal insufficiency.

Patient 135. This was a 21-year-old white soldier, who was wounded by mortar fire at approximately 0400 hours on July 18, 1953. The injuries were as follows: comminuted compound fracture of the right femur, tibia and fibula, with extensive soft tissue destruction; penetrating wound of left anterior thigh; and penetrating wounds of the right arm and hand. This patient was given 500 ml. dextran shortly after injury, followed by 1500 ml. of blood. A tourniquet was placed around the right thigh. He was admitted to a Mobile Army Surgical Hospital at 0850 hours, with a blood pressure of 80/50. Three liters of blood was given, and also 1,200,000 units procaine penicillin intra-muscularly. The tourniquet was released at 1100 hours. The patient was then transferred by air to another surgical hospital, owing to the heavy patient load and lack of operating space. Further transfusion was required and operation commenced at 1500 hours. The right leg was amputated through the femoral fracture site, and other wounds were débrided. Skin traction was applied to the stump. Two and one-half liters of blood was given at that time. Crystalline penicillin, 600,000 units b.i.d., and streptomycin, 0.5 Gm. b.i.d., were ordered. Diminished urinary output appeared on the first postoperative day, and the patient was transferred by air to the Renal Insufficiency Center. On arrival at the center, the patient appeared acutely ill, was perspiring, pale, but alert and well oriented. Blood pressure was 126/70; pulse, 110; and temperature, 100.8° F. Examination of the right thigh stump revealed a foul-smelling crepitant myositis. A right hip disarticulation was done immediately, and it was believed that all involved muscle had been excised. This second amputation was undertaken approximately 30 hours after the original amputation and 41 hours after injury. The patient's postoperative course was complicated by a high temperature, fast pulse rate and persistent oozing of blood. This could not be controlled with hemostats, and required tight compression dressings. On the day after hip disarticulation the left thigh wound was secondarily débrided. The patient remained euphoric and obviously ill, although there was no further evidence of clostridial myositis. He died suddenly approximately 28 hours after admission to the Renal Center. At autopsy the wounds appeared clean, without any evidence of myositis or of obvious infection. The pathologists reported fat embolism to be the primary cause of death.

Figure 5 records some of the data in connection with this patient. There was a leucocytosis of which 79 per cent were neutrophils. Of these, 82 per cent showed phagocytic activity. The complement titer was within normal limits. The plasma chemical determinations shortly before death were as follows: sodium, 162 mEq/L.; potassium, 8.0 mEq/L.; chloride, 83 mEq/L.; carbon dioxide capacity, 23 mEq/L; non-protein nitrogen, 151 mg. per cent; calcium, 20.8 mg. per cent; and inorganic phosphate, 10.9 mg. per cent. The following micro-organisms were reported from culture of the necrotic muscle: *Clostridium perfringens, Proteus vulgaris,* beta hemolytic streptococcus, non-hemolytic streptococcus, and *E. Coli.*

Comment. This case represented an example of *Clostridium perfringens* myositis fol-

lowing compound comminuted fracture of the femur. Important factors in assessing therapy were, a time lag of 11 hours between injury and initial débridement, and the presence of a tourniquet around the right thigh for about seven hours. Other important factors were that the surgical procedures were carried out by a surgeon who was not experienced in the handling of this type of traumatic injury. Furthermore, skin traction was applied to a stump which was probably inadequately débrided, and the wound was not inspected for 30 hours. Nevertheless, by radical amputation at the Renal Center, all of the affected muscle was excised. There was no evidence of any defect in body defense systems predisposing this patient to gas gangrene. On the other hand, on the basis of the autopsy findings it is probable that serious infection had been overcome by a combination of surgery and chemotherapy.

Patient 133. This was a 23-year-old colored male, wounded by mortar fire at approximately 1145 hours on July 15, 1953. The injuries were as follows: a penetrating wound of the left buttock, penetrating wounds of left thigh and both legs, comminuted fracture of the left ischium, and left pubic ramus. Resuscitation was commenced at 1230 hours, and the patient arrived at a Mobile Army Surgical Hospital at 1730 hours. He was reported to have been in severe shock and had been given albumin, but the quantity was not recorded. At the Mobile Surgical Hospital his initial blood pressure was 80/0, and 2,500 ml. of blood was given. At operation the following procedures were reportedly done: a left sigmoid colostomy because of a laceration of the lower sigmoid colon, a supra-pubic cystotomy because of complete destruction of the prostatic urethra, and débridement of all other wounds. The patient was placed on 600,000 units of procaine penicillin b.i.d.; oxytetracycline, 2 Gm. daily; and streptomycin, 0.5 Gm. b.i.d. The patient was transferred to the Renal Insufficiency Center on July 17, because of diminished urinary output, where he arrived approximately 50 hours after injury. At that time he appeared acutely ill but alert and well oriented. Examination of the wounds revealed obviously inadequately débrided gluteal muscles; the muscles of the left lower leg were foul-smelling, prolapsed, and non-viable; the left foot was cold and without detectable arterial pulsation. An extensive re-débridement was done of the entire left buttock, removing the gluteus maximus and medius muscles. the piriformis muscle, and part of the obturator internus muscle. The rectum, prostate, and inferior portion of the bladder were thus exposed in the wound. The left leg was amputated above the knee. During this time the blood pressure was unstable, requiring the support of blood plus noradrenalin. The patient's subsequent course was one of severe illness with episodes of unstable blood pressure, and continuing renal insufficiency. Artificial hemodialysis was carried out on three occasions, with the result that deviations in the normal levels of plasma electrolytes were corrected for relatively short periods of time. On the fifth day after injury a paracolon septicemia developed. The wounds were examined and re-débrided on several occasions, without any further evidence of progressive wound sepsis. The patient died on the seventh postwound day.

The ultimate cause of death was considered to be septicemia, possibly secondary to septic thrombophlebitis of pelvic veins. At autopsy no focus of infection was found other than that which had been observed clinically on the surface of the extensive buttock wound. An unexpected finding was a large contusion of the opposite buttock muscle which, however, was not infected.

Figure 6 records some of the data pertinent to this patient. The white blood count was elevated, with an accompanying increase in the percentage of neutrophils. The majority of these neutrophils appeared active, and complement levels were within the normal range. Circulating tetanus antitoxin was rising in response to the booster dose during this deteriorating illness. The patient had received penicillin, streptomycin and oxytetracycline. Chloramphenicol was finally used in an attempt to control the septicemia because the micro-organism was sensitive to this agent *in vitro*. Wound culture revealed the presence of an unclassified clostridium, *Aerobacter aerogenes, Proteus mirabilis,* beta hemolytic streptococcus, gamma streptococcus, and a micro-aerophilic streptococcus.

Comment. The data do not indicate a decrease in resistance to infection in this patient. It was quite apparent that the left buttock wound had not been properly débrided, not only because of the appearance of the muscle when first examined at the Renal Center but also because during secondary débridement, portions of the patient's identification card were removed from the wound. Furthermore, our finding of a cold lower leg with a wound showing prolapsed non-viable muscle indicates that this lesion had been

present for many hours, and should have been treated before the patient was evacuated by air. Although sepsis was an outstanding complication in this patient, it is probable that inadequate initial surgical therapy and postoperative care were predominant factors in its development.

DISCUSSION

The observations reported in this study are from a small but carefully-studied series of patients with battle injury. A significant depression in neutrophil polymorphonuclear leucocyte activity within the first 24 hours or so of severe wounding in several patients was the only abnormality noted in the antibacterial defense mechanisms studied. This observation was found shortly after the administration of relatively large volumes of stored blood, and may be directly related thereto. Unfortunately, there are no data on neutrophil activity from severely injured patients before resuscitation was commenced. But it is interesting that with two patients, neutrophil activity improved despite the continuing administration of bank blood. The depression in neutrophil activity shortly after severe trauma might have been a manifestation of adrenal cortical hyperactivity, although no observations were made in this study on endocrine function. Others have reported finding *in vitro* a depression in the phagocytosis of opsonized Type I pneumococci by leucocytes from nine patients receiving ACTH or cortisone therapy.[12] We did not find any significant fall in staphylococcal phagocytosis in a few patients receiving ACTH therapy.[6] Phagocytosis of *Streptococcus viridans* by the reticulo-endothelial system of rats treated with cortisone has also been reported normal, although the evidence suggested a possible delay in the subsequent destruction of the micro-organisms.[11] Also, macrophage activity in tissue culture exposed to Kendall's Compounds E and A has been reported normal.[22]

Another interesting observation from the present study is the finding of elevated total white blood counts in patients who have received the equivalent of two or three total exchange transfusions within a few hours of injury. Therefore, it must be concluded that either these cells were not lost during hemorrhage, or more likely that their replacement from the bone marrow or elsewhere was rapid and continuous.

The normal complement activity found in most cases following acute hemorrhage in this study agrees with observations of others on the effect of hemorrhage in animals on complement levels. The removal of 50 to 83 per cent of circulating complement in dogs by repeated plasma-phoresis was usually followed by a return to normal serum titers within 24 hours.[42] In guinea pigs restoration of complement occurred within four to six hours after its removal by severe hemorrhage.[14] It is probable, therefore, that complement continually enters the intravascular compartment by diffusion from tissues or by the lymphatics.[37] Isolated observations of depressed complement activity such as were found in this study must be interpreted with caution because of unpredictable fluctuations in complement in a variety of diseases, and even in the same disease.[15]

The finding of normal antibody synthesis reported in this paper is not surprising. The advantages in the use of the anamnestic response as a measure of the capacity to synthesize antibody have been discussed in a previous communication; quantitative studies showed that moribund patients retained a normal capacity to synthesize specific antitoxic globulin.[2] Furthermore, observations on guinea pigs showed that the secondary immune response to the administration of diphtheria toxoid occurred after protracted exposure to cold, or after the production of severe *Clostridium welchii* myositis, or after reticulo-endothelial blockage with large amounts of India ink.[21] The absence of a detectable increase of circulating antitoxin until the seventh postwound day in three of the patients studied is of interest. There has

been considerable discussion about the desirability of administering prophylactic tetanus antitoxin to previously immunized casualties instead of toxoid at the time of injury. This was the practice of the British Army in World War II because of the possibility that the response to a booster dose of tetanus toxoid might not be sufficiently rapid to protect in cases of tetanus with a short incubation period. Miller and Ryan[28] have recently advised the injection in opposite extremities of both prophylactic antitoxin and toxoid in previously immunized patients who sustain shock or have massively contaminated wounds. The data recorded in the present study lend support to this proposal.

No evidence has been found that patients with posttraumatic renal insufficiency are more susceptible to bacterial infection. Evidence is presented showing that such patients may synthesize antibody as well as normal control subjects. Furthermore, complement activity, white blood count, and the capacity of neutrophils to ingest staphylococci may be within the normal range.

The occurrence of infection following severe trauma may be the result of many factors. The case examples presented show the importance of delay in treatment, or of inadequate surgical débridement in initiating this complication. A deficient caloric intake has been said to predispose to infection. Many of the patients with renal failure had low caloric intakes, but the present studies failed to show that antibacterial defense was affected. This confirms previous observations of the effect of malnutrition on antibacterial defense in humans.[2, 5] Miles and Niven[27] have suggested, on the basis of observations in the guinea pig skin, that local tissue ischemia resulting from shock may promote the initiation of bacterial infection. This seems reasonable, but should not be an important factor if initial surgical treatment of the wound is adequate.

The present studies do not prove conclusively that severely traumatized patients possess normally functioning antibacterial defense mechanisms. However, it seems likely that other factors, such as the degree of tissue damage, the length of time between injury and initial operation, or the adequacy of débridement, are the most important factors in the initiation of infection.

SUMMARY

1. The capacity of the body to resist bacterial infection after severe battle injury has been studied. Phagocytosis, the level of circulating complement and the body's capacity to synthesize tetanus antitoxin have been compared in casualties with and without complicating acute renal failure. The above data have been related to the clinical course of the patients, with particular emphasis on the development of infection.

2. A significant depression in neutrophil polymorphonuclear leucocyte activity was found within the first 24 hours or so of wounding in several patients. This was the only abnormality noted in the antibacterial defense mechanisms studied. Phagocytosis returned towards normal within 48 hours.

3. No evidence was found that battle casualties with or without complicating acute renal failure are more prone to develop infection because of a possible deficiency in the antibacterial defense mechanisms studied.

4. Factors such as the degree of tissue damage, the amount and nature of bacterial contamination, the length of time between injury and initial operation, and the adequacy of wound débridement and of postoperative care are probably of prime importance in the initiation and development of infection after injury.

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