1965

Infection in burns

Philip Brian Olson
University of Nebraska Medical Center

INFECTION IN BURNS

by

Philip B. Olson

Submitted in Partial Fulfillment for the Degree of

Doctor of Medicine

College of Medicine

University of Nebraska

February 1, 1965

Omaha, Nebraska

TABLE OF CONTENTS

INTRODUCTION

The subject of burns involves a large variety of problems which challenge many aspects of a clinicians knowledge and training. The severely burned patient must have constant, diligent attention and requires a broad range of medical, surgical, and psychiatric skills.

Since the subject material is truly large, this paper is a review of the literature on the single large problem of infection which complicates, threatens, and often kills the burned individual. Infection is now the single largest threat to the patient's survival and is one of the main problems to be conquered if there is to be much further improvement in the survival statistics.

Infection in burns has many ramifications which branch into problems with grafting, pain, anemia, metabolic disturbances, septicemia, endotoxemia, shock, antibiotic chemotherapy, and immunology. Each of these categories could serve as a large area to review, but most have been consolidated in this paper and their pertinent roles discussed under the total infection problem.

THE MAGNITUDE OF THE INFECTION PROBLEM

Patient-Bacteria-Therapy: Three Integral Factors Increasing The Infection Problem

Review of the literature on the subject of burns reveals that primary and secondary infection continues to be one of the major problems threatening recovery of the severely burned person. As succinctly stated by Artz and Reiss (6):

> It is the cause of pain, nutritional disturbances, conversion of second to third degree burns, failure of skin grafts to take, and death.

At the conclusion of the first international congress on burns in 1960 Artz (4) stated:

> Burn therapy has improved, it is improving and will continue to improve. We have done fairly well with shock. We must now focus our attention on the major killers--respiratory tract damage and wound sepsis.

From 1943 to 1956 Clark and Hansen (3) at the San Francisco City and County Hospital noted the survival time in fatal cases involving 20% of the body surface. From 1943 to 1947, 69% of deaths occurred within 48 hours, the majority from shock. From 1952 to 1956 only 19% died during the first 48 hours, but 69% of those surviving this early period later died, with infection being the chief cause of death.

At the Massachusetts General Hospital, shock accounted for 20% of all burn deaths from 1939 to 1947, but it is now a relatively insignificant cause of burn deaths. During 1948 to 1957,

FIGURE I

MECHANISMS OF DEATH AT VARIOUS TIME INTERVALS

	WEEKS				
	1	2	3-9	10-12	
FINDINGS AT DEATH					TOTAL
NUMBER PATIENTS	16	12	9	4	41
IMMEDIATE	1				1
RESPIRATORY	5				5
SHOCK	8	5			13
SEPSIS	2	5	8	1	16
ULCER		1	1	1	3
HEPATITIS				3	3

From Wartmann
(65) p.7

one patient died from shock at this hospital. In a study of 106 consecutive burn deaths from the records of 1,140 burned patients between 1939 and 1958, Phillips and Cope (52) found that 42% of the fatally injured in the series died from respiratory tract damage with or without complicating respiratory tract sepsis. As a rule, respiratory tract infections become evident if such patients survived for 72 hours.

The improvements in burn therapy with consequent increased survival time has augmented the span of time over which bacteria can invade and multiply (1939-1947 = 4.6 days, 1948-1957 = 15.7 days). From this study, as well as others, it has been concluded that until we learn to control sepsis itself, the improvements in other areas of burn therapy will only increase the probability of septic involvement.

Along with increased patient survival time, a second factor contributing to the infection problem is the emergence of resistant bacteria through the processes of adaption and selection. The resistance through adaption implies that a specific metabolic change has occurred which allows the altered organisms to grow and reproduce in the presence of antibiotic. Resistance also implies that this metabolic change is permanent and is transmissable to following generations. With this phenomenon, only a small proportion of the flora become resistant, but in the continuing presence of the antibiotic, these resistant forms grow selectively, outnumber and replace the sensitive ones (8).

On the other hand, selection means that small numbers of resistant bacteria were present in the flora before the antibiotic was present, or that these bacteria arose before through chance mutation independent of the antibiotic. In such a case, the antibiotic merely selects the resistant organisms at the expense of sensitive ones. (15)

A third factor contributing to the infection problem in burns is the alarming development of the emergence of saprophytes as pathogens due to disturbances of equilibrium with antimicrobial drugs. Such organisms as Serratia marcescens and Herrelia vaginicola have been noted in burn septicemias. Pseudomonas aeruginosa is an organism of low virulence and invasiveness frequently noted as a local inhabitant of burns, surgical wounds and the genitourinary tract. Reports of Pseudomonas infections before the advent of antibiotics were quite rare and there is no doubt that the incidence of serious Pseudomonas infection has risen since 1940. Almost all of these cases have been noted in association with debilitating illnesses, such as severe burns, leukemias, cystic fibrosis, i.e., any problems requiring prolonged and widespread antibiotic coverage. (57)

The sustained increase in the incidence of gram negative and saprophyte infections of burn wounds and the blood stream indicates that antibiotic therapy has been of little or no value in their prevention and that these bacteria have assumed a major role in the etiology of burn infections.

THE CATEGORIES OF INFECTION

Several terms come under the heading of infection and require definition before proceeding on with further discussion. Artz and Reiss (6) have divided infection into two broad types, these being local and invasive. They then subdivide invasive infection into locally and generally invasive. Examples of local invasion are cellulitis and lymphangitis, whereas septicemia would represent a generally invasive infection.

Sepsis is a term used occasionally and is synonymous with infection since the word is entirely nonspecific. Suppuration refers to the presence of pus on the wound and is indicative of local tissue reaction. Sevitt (61) states that local infection is usually manifest by pain, spreading cellulitis, or a prolonged inflammatory edema. The local infection is sometimes obvious and at other times difficult to distinguish from the normal effects of acute burn inflammation with later sloughing and granulation tissue formation.

> Adverse changes may be manifest only by delayed healing, a failure of grafts to take, or by excessive scarring of the skin. Interference by bacterial infection in the healing of partial skin loss burns from which the hemolytic Staphylococcus and Streptococcus were isolated was noted histologically by Gordon. Apposition of newly spread epithelium over the dermis was sometimes focally unsuccessful and the epithelial cells were found lying detached in or on a purulent exudate.(61)

Bacteremia and septicemia must be differentiated since the two have separate clinical features and have entirely different clinical meanings.

Bacteremia is a transient infection of the blood

stream and a positive blood culture can be obtained if this is done at the proper time. Therefore, bacteremia is generally diagnosed by clinical impression rather than by bacteriologic confirmation since natural body defenses clear the blood stream invasion before blood culture is positive. It is suspected when there is a sudden transient rise in temperature after a dressing change.

Septicemia is a major problem threatening survival and is usually seen in patients who have had extensive full thickness burns. In Liedburg's (35) series of 35 consecutive burn deaths the total extent of burn was more than 50% of the body surface in all but 6 patients, and the extent of full thickness burn was 50% or more in 7 patients. The mean total extent was 58% and mean full thickness skin loss was 45%.

Meleny (47) correlates the depth of the burn and the amount of tissue damage with the severity of the infections encountered. Septicemia is rarely a formidable problem in properly treated partial thickness burns. With more severe burns it is difficult to maintain the patient in good nutritional balance, debilitation is greater, and intrinsic resistance to infection is altered, although this latter mechanism is poorly understood. Balch, et al (7) undertook a study of the burned patient's resistance to infection in 1963. They found that the leukocyte-plasma and plasma bactericidal effect against phage 80/81 Staphylococcus aureas is increased initially, but that values returned to normal

after several days. The bactericidal capacity against Escherichia coli and Pseudomonas aeruginosa was also normal or better. The cellular response to peripheral injury is markedly depressed for several days and there is a significant delay in the appearance of lymphocytes. In one extensively burned patient a terminal bacteremia was noted when the blood bactericidal capacity had begun to decrease, there was marked leukopenia, and the cellular response to peripheral injury was depressed. Of interest was that in one surviving patient a bacteremia was noted in the early postburn period when the wound bactericidal count was very high even though the measured modalities of antibacterial defense were normal or better.

Systemic factors will be given further consideration later in this review.

A principle objective in wound care is the prevention of septicemia. When does a bacteremia become a septicemia? There has been increased recognition of the early occurrence of septicemia, doubtlessly connected with factors already discussed. Pulaski and Altemeier (56) have demonstrated that organisms could be cultured from regional lymph nodes 12 hours post burn and this gives evidence that we can be dealing with invasive infection shortly after a burn has occurred.

Septicemia means clinical signs and symptoms of blood stream infection along with persistently positive blood culture. Even after antibiotic therapy with subsequent negative blood culture the continuation of clinical findings of blood stream infection

justifies the diagnosis of septicemia.

According to Liedburg, et al, (35) the clinical signs of septicemia are quite characteristic. The temperature is usually high, between 104 and 107 F., and it is rarely possible to reduce the fever to below 102 with antipyretics and frequent cold sponging. Marked fluctuations generally do not occur. Sinus tachycardia between 140 and 170 is nearly always noted and a rapid respiratory rate between 30 and 60 is also seen. Disorientation paralleling the fever and severe hypotension refratory to blood, electrolytes and vasopressors are usually present. Oliguria or anuria and paralytic ilius are common and the latter obviously contributes to debilitation. The blood picture shows leukocytosis and in severe cases bleeding tendencies are manifested by petechiae, ecchymoses, and wound oozing. Mild jaundice is also sometimes observed and is a poor prognostic sign.

Tumbusch, et al (64), make the point that the above pattern is characteristic of a gram positive septicemia and that patients with gram negative septicemia usually show hypothermia, paralytic ileus, leukopenia, mental alertness, and suddenly become hypotensive, anemic and die.

Why should a generally invasive bacterial infection cause death in a burned patient?

Through the years various theories have been advanced, the latest given by Stevens (63) which is based upon the competitive struggle for oxygen between patient and bacteria. If enough bacteria

FIGURE II

SIGNS OF SEPTICEMIA IN BURNED PATIENTS

Clinican Signs	Gram Positive	Gram Negative
Granulations	General Dissolution	Focal Hemorrhagic Necrosis
Course	Insidious 2-6 days	Rapid 12-36 hours
Ileus	Severe	Severe
Disorientation	Severe	Mild or Absent
Temperature	Hyperpyrexia	Hypothermia
Leukopenia	Absent	Marked
Hypertension	Insidious, Oliguria	Sudden with Anuria

From Tumbusch
(64) p.236

are present they will cause anoxia and death, and he feels that toxins alone cannot be responsible for the patient's demise. He claims that the oxygen consumption by gram negative bacteria is $9X10^{-12}$ ml. per bacterium per minute. The average man consumes 230 ml. of oxygen per minute which is equal to the amount consumed by 26 grams of Eschericia coli. Stevens (63) says that if the host loses 50% of the available oxygen it will be fatal, and, if this is correct, a concentration of $2X10^{8}$ bacteria per gram of tissue will cause death and this concentration is attainable in vivo.

Toxemia of burns is another term often seen. Specific "toxic" effects are burn scarlatina and tetanus. However, the word toxemia generally refers to the "nonspecific" form with malaise, pyrexia, tachycardia, and anemia; but, most important, "toxemia" is a bacterial septicemia. There is now no reasonable doubt that one of the main causes of "acute toxemia" in burns are bacteria and their products, and not unspecified "toxic substances" released from burned skin, (61). This is supported by a study of Newton, Fugii, and Moyer (51) in which they attempted to repeat experimental results of Rosenthal (60) and Malm (44) in this country, and Feodorov (20) in Russia with regard to a burn toxin.

Rosenthal (60) and Malm (44) claimed that the plasma from survivors of severe burns had a beneficial effect when infused into individuals suffering from fresh severe burns. This was based on the postulate that there was specific "burn toxin" to which the convalescent plasma made anti-bodies. Feodorov (20)

apparently demonstrated systemic anaphylaxis in guinea pigs sensitized by a single subcutaneous injection of serum or burned skin extract taken from animals burned two days earlier. Intravenous injection of the same type of preparation three weeks later led to systemic anaphylaxis. However, Newton (51) et al, were unable to repeat these experiments with a variety of sources of "burn toxin" and do not support the concept of a substance produced by burn injury that is antigenic to unburned animals.

THE SOURCES OF INFECTION

The first obvious source of infection is the environment of the burned patient. It was previously assumed that the fresh burn wound was sterile. However, Altemeier (1) states that the assumption is entirely unfounded and without supporting evidence. Even if the skin is sterilized at the instant of burning he found that it quickly became contaminated by a mixture of bacteria. These included the pyogenic cocci with coagulase positive hemolytic Staphylococcus in 31% of cases the coagulase negative, non-hemolytic Staphylococcus in 82%, the hemolytic Staphylococcus in 11%. The gram negative bacilli of intestinal origin, particularly Escherecia coli, Pseudomonas aeruginosa, Proteus, and Aerobacter aerogenes, were cultured in 84%. The anaerobic spore forming bacilli included the Clostridia of gas gangrene in 70% and tetanus in 3%. Anaerobic non-spore forming bacilli such as Bacteroides were found in 10% and anaerobic Streptococcus in 7%.

Lowbury (38) also conducted a study and noted that the bacteria isolated from burns varied with the time since injury, the area of the burn, the place where the patient was being treated and the type of treatment given. He states that skin bacteria are killed at the time of burning and swabs taken shortly afterward are sterile or sparcely contaminated. After 24 hours a variety of organisms will be found growing in all but the most superficial wounds. It was found that the gram negative bacilli, including

Pseudomonas and Proteus, are apt to predominate during the first two to three weeks. Streptococcus was sometimes common, and at other times absent from the wards for long periods. In later weeks after separation of the slough Staphyloccus and Streptococcus tended to predominate in unhealed burns. Lowsbury (38) concluded that:

> The bacterial colonization of burns in the hospital is an extreme example of what is generally called "hospital infection". The bacteria reflect the environment rather than the flora brought in by the patient on his skin and mucous membranes. The gram negative bacilli are also different in various ways from the alimentary flora and are clearly acquired by cross infection; Pseudomonas species, for example, is very common in burns, yet appears in only 3% or less of normal stools.

Williams (66) and his associates emphasized the importance of sputum as a reservoir for Pseudomonas aeruginosa on the general hospital ward, especially in patients with severe pulmonary disease. It was thought that the control of airborn spread may be of value in the prevention of Pseudomonas infection.

Colebrook (9) conducted an interesting investigation at the Birmingham Hospital on the role of the environment as a mode of infection. He utilized a specially constructed "slit sampler" which collected bacteria by suctioned air which was streamed over blood agar. The slit sampler was placed about 4 to 5 feet away from the patient before and during dressing changes.

> When I operate with only one slit open during the process of changing dressings, there are in the beginning only a few microbes in the air, about 1.5 cubic foot when we switch on the sampler. And when the patient comes in with a sterile sheet over his bed there are

> still very few, but as soon as the nurse starts removing bandages, you get great showers of microbes in the air. (9)

We must recall that Colebrook worked in the era of the Koch occlusive dressings which have been outmoded, due largely to their almost constant association with incidence of Pseudomonas infection.*

Colebrook (9) concluded that the air of hospitals was heavily contaminated and suggested several methods of purifying air in dressing rooms. The first was with aerosols and ultraviolet light which he felt were not suitable because such a method does not work well on dry particles and is very dependent on the degree of humidity. Other methods mentioned were electrostatic precipitation and heat stabilized air which were not discussed or evaluated.

In the Birmingham Hospital they have used the plenum system for 6 years. This provides for mechanical flushing of large streams of air.

> We bring into our dressing room which is 3000 cubic feet a volume of 1000 cubic feet of air per minute; so, theoretically, air is changed completely every 3to 5 minutes. Movement of air has not been troublesome to the patient and it also carries away smells. Experimentally we found a very high bacterial content in the air without ventilation. The majority of our wounds have been free from clinically significant infection. In many cases we have seen wounds from fairly extensive burns remain completely bacteriologically sterile so that we could not

* By personal communication with Robert M. Cochran, M.D.

> grow a single colony for two weeks. The stay in the hospital the past 3½ years has been cut by 50%. Pain has been, to a large extent, eliminated. Pain is almost always due to inflammation and this after the first two days is largely due to infection. I have never yet seen in over 1000 patients those striking personality changes which have so often been noticed in patients with bad burns. (9)

However, even with full thickness burns,,nonviable skin bacteria still survive in the crypts of the skin appendages and these are capable of rapid proliferation. (54) Altermeier (1) has shown that even the local application of strong antiseptics does not alter this source of infection.

In addition to the obvious sources of infection from the burn wound and environmental factors, many patients have been found to harbor other foci of infection such as necrotizing cystitis, prostatitis and necrotizing lesions of the larynx, tracheobronchial tree and pneumonic foci. Within the respiratory tract at least two mechanisms of infection exist. One is aspirative and usually occurs in association with damage to the epithelium of the tracheobronchial tree, but in other seriously ill patients it can occur on the absence of such lesions. The second mechanism is embolic with bacteria carried from another focus of infection. (4)

THE BACTERIAL FLORA
The Influence of Local and Systemic Variables

There has already been some previous mention of the bacteria which colonize burns soon after injury, but more important are the factors influencing their presence and pathogenicity and the local and systemic effects of the specific microorganisms.

There are indeed matters of variation affecting the burn flora of each individual patient, and these include the time since injury, the depth of burning, the area burned, the patient's environment and the type of treatment given. Consideration must also be given to the patient's innate resistance and changes produced by burn trauma.

Lowbury (38) stated that the gram negative bacilli were apt to predominate in the burn wound during the first two or three weeks and that with separation of the slough Staphylococci and Streptococci tended to predominate in the unhealed burn.

On the other hand, Colebrook (9) found coccal infections were most likely to predominate during the first week, this usually becoming a mixed cocco-bacillary infection in the second to fourth week when necrotic skin was separating, and later with cocdal infections of the granulating period. However, a graphic presentation of the natural history of a third degree burn with survival by Barnes (6) presents data just opposite to those of

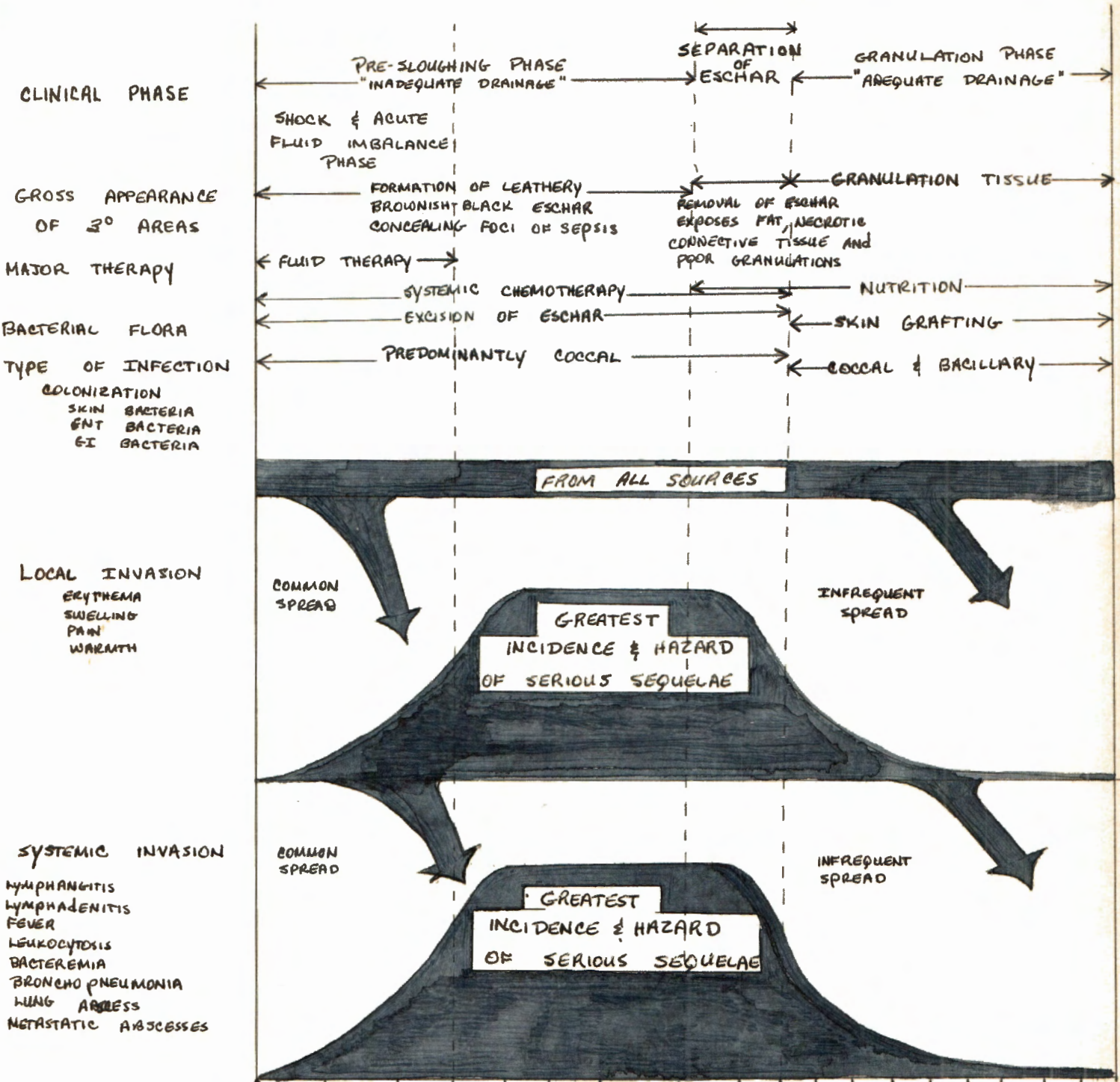


FIGURE III
From Barnes
Cited by Artz (6) p.147

FROM BARNES

Lowbury (33) where the first three weeks showed predominately coccal flora and a mixture of coccal-bacillary flora after three weeks.

Davis (14) also states that the burn flora is predominantly coccal during the first three weeks and this coincides with the phase of eschar formation through to the time it sloughs. Price (55) studied the flora of the burn wound in dogs which had undergone six different types of thermal injury. The bacterial counts were greatly reduced immediately after the burn but in two to four days had increased from 100 to 10,000 times the control level. This occurred irrespective of the type of dressing applied. As the wound began to break down, there was an enormous increase in the number of coagulase positive organisms.

Therefore, it is obvious from a brief look that various investigations achieve different and even opposite results in this regard. With this in mind, it is not wise to be too didactic about which organisms are most likely to predominate at any one time due to the variable factors listed. Examples of how these variables may influence bacterial problems can be shown in the following.

Elek (18) performed a small experiment to define the importance of the size of the innoculation of Staphylococcus aureus on human volunteers and found that an intracutaneous injection of 10^5 bacteria was necessary to produce suppuration. Thus, nearly a million cocci were defined as "the minimum pus forming dose".

When he left a silk suture tied in the wound at the time of innoculation he was able to enhance the infection 10,000 fold, i.e., 100 cocci on the stitch formed pus. This may be an indication of the correlation between the increased problems with infection as the depth of the burn increases.

The anatomical location alone may influence contamination in two ways. First, the normal bacteria of an area may contaminate the wound. Langohr, Owen and Cope (31) in 1947 found that burns of the lower extremity were contaminated by fecal organisms such as Clostridium welchii and enterococci more frequently than burns elsewhere. Secondly, the opportunities of contamination are reduced when the burn can be perfectly covered by a dressing and this depends on the anatomical area burned. Lowbury and Fox (40) in 1954 isolated Pseudomonas much less frequently from burned hands and feet which could be adequately covered by bandaging than from the trunk, face or scalp which were difficult to keep efficiently covered.

An additional theoretical factor was thought to influence bacterial flora of burns, this being a phenomenon of "mutual antagonism" among bacteria. In 1956 Karloff (29) concluded that fecal organisms were antagonistic to Staphylocci, but other workers have not supported this.

The influence of the patient's environment has been discussed and the effects of anti-microbial therapy will be given further consideration in a later chapter.

The other factors which influence the local wounds and the bacteria which colonize its surface are systemic in nature; thus, the problem of infection also overlaps into fluid and electrolyte alterations, nutritional status, and endocrine factors.

A negative nitrogen balance is characteristic of the severly burned patient due to increased excretion of urinary nitrogen, low protein intake, and protein losses in wound exudate. Urinary nitrogen as urea may be 2 to 3 times the normal 10 to 15 grams per day and wound exudates can lose 5 to 7 grams of nitrogen daily. (60b)

Davis (14) provides a succinct summary of these systemic factors in the following:

A. Fluid and Electrolyte Alterations
   1. Dehydration or overhydration--altered cellular response.
   2. Mg and Cu loss--low antihyaluronidase production.
   3. Co. loss--abnormal phagocytosis.

B. Vitamin Deficiencies
   1. Riboflavin--low complement titer, low bactericidal property of the blood for Staphylococci.
   2. Biotin--Coenzyme in formation of oxalo-acetic acid in the TAC cycle.
   3. Pantothenic Acid--Component of CoA, necessary in CHO metabolism, decreased in animals causes damage of adrenal cortex.
   4. Pyridoxine--essential for transamination, decarboxylation and antibody synthesis.
   5. Cyanocobalamin--necessary for synthesis of thymidine and other desoxyribosides.

C. Protein deficiency (starvation plus exudate losses.
   1. Inadequate amino acids for tissue replacement.
   2. Inadequate amino acids for antibody synthesis.
   3. Inadequate calories to permit protein utilization.

D. Pituitary-Adrenal Response (Stress)
   1. A state of mild diabetes is produced with altered host resistance.

2. Antibody is mobilized by rapid destruction of lymphocytes.
(We have recently shown that using the kidney as a target organ for staphylococcal infection introduced into the urinary bladder, the development of pyelonephritis can be increased from 10 to 60% when the animal is made diabetic by alloxan.)

THE MAJOR SPECIFIC MICROORGANISMS IN LOCAL AND SYSTEMIC INFECTION

Streptococcus pyogenes:

Sevitt (61) states in his review:

> This organism is the most feared of all. It can increase local damage to even superficial burns, delay healing, prevent skin grafts from taking and is sometimes responsible for a spreading cellulitis or septicemia which may endanger life.

Lowbury and Cason (39) studied the role of this organism in graft failure. One series of individuals with whole skin loss burns was cleared of Streptococci by local Aureomycin therapy and skin was grafted; other burns were skin grafted without Aureomycin therapy. Graft failure occurred in 25% of the former group and 89% of the latter.

Similar results were obtained by Liedberg (33) et al, using locally applied Chloramphenicol ointment or Furacin dressing.

It is important to recognize that only the group A, Beta hemolytic Streptococci cause a widespread destruction of grafts. Other Streptococci do not present this problem. The detrimental effects are thought to be due to streptokinase which increases the formation of the fibrinolytic enzyme plasmin. This destroys the fibrin which causes the grafts to adhere. (6)

Scarlatina does not offer much of a problem since only a minority of patients develop a generalized scarlatinaform rash. This rash spares the face and Dick antitoxin given intracutaneously

is followed by circumscribed disappearance of erythema. Probably more noteworthy is that complications are less frequent than in ordinary scarlatina.

Staphylococcus aureus:

It has been noted that Staphylococcus aureus is the most frequent contaminant of all burns and this organism continues to be prevalent because of its capacity to develop resistance to antibiotics.

Rivera (58) demonstrated a decrease in sensitivity to penicillin by the Staphylococcus aureus from 83% sensitive in 1950 to 10% sensitive in 1954. While resistance has not developed to an equal degree to all antibiotics, the general trend has been towards increased resistance to most all of the common antibiotics.

In partial skin loss burns the healing process is rarely affected. Langohr, Owen and Cope (30) found that the eventual healing of burns coincided with the rise in specific anti-staphylococcal agglutinin content of the patient's serum. From these results they concluded that the immune reaction was also quite important in staphylococcal infection.

Sevitt (61) finds that Staphylococcus is apparently not as big a threat in problems of graft failure as Streptococcus. In the early stages after burning, slight suppuration with little or no pyrexia might be found and only occasionally did the organism cause profuse suppuration of the wound.

Davis (14) concludes that:

1. Effective control of the organism by one or more antibiotics does not appear to be imminent.
2. Studies on Staphylococcus in vivo and in vitro have not provided information of help in treatment of staphylococcal infection.
3. We must consider the local and systemic factors which in turn affect bacterial growth in order to improve our therapy.
4. There is a great need for studies between the inter-relationships of metabolic changes in trauma and their effects on bacterial colonization, invasion, and multiplication.

Proteus and Other Gram Negative Coliform Bacilli:

Proteus and other gram negative bacilli such as Eschericia coli and the paracolon bacilli are relatively common invaders, particularly during the sloughing and granulating period of burns. These organisms are also more common in the gluteal area. (61) Various investigators have found different degrees of contamination by these organisms, ranging from .8% (11) to 84%. (1). Thus far it has been fairly difficult to assess the role of these organisms as burn pathogens. Lowbury and Topley (43) think that they can cause delayed healing and graft failure and certainly, bacteremia and fatal septicemia with these organisms has occurred.

Clostridium Tetani and Tetanus:

Sevitt (61) notes that this is a rare complication in most countries whether or not the patient receives an injection of antitoxin or toxoid. In the burns unit at Birmingham, only one

case occurred during the last eight years among 3000 patients. On this basis, neither toxoid nor antitoxin is used as a routine prophylactic measure:

> The rarity of tetanus does not warrant the risk of unfavorable reactions. Pertnup's case is given special interest because fatal tetanus developed in spite of giving antitoxin, toxoid and penicillin. (61)

A number of controversial opinions still exist concerning the use of tetanus toxoid and antitoxin. Eckmann (17) states that the status of actual immunity is acquired a few days after the second injection of toxoid. The actually immune person is protected against infection, the partially immune may acquire protection rapidly and the non-immune subject is not protected until after a full course of injections. Two injections of toxoid generally confer immunity upon 90% of patients and this is no better with absorbed or fluid toxoid. However, immunity with fluid toxoid lasts about one year and lasts two years for absorbed toxoid. Clinical tetanus has been reported in vaccinated persons after, but not before, these limits of time. The booster effect is underestimated and has never failed to develop and there is thought to be some protective effect even before there is an antibody response. In wounded individuals, a booster is indicated if the last toxoid injection was over 18 months previous to injury.

Antitoxin supposedly gives immediate protection, but if the patient was vaccinated incompletely or a long time previously it is still not indicated. Eckmann never gives antitoxin if the

patient has ever received toxoid since antitoxin does interfere with the secondary stimulus.

Clostridium welchii and Other Anaerobic Spore-forming Bacilli:

Altemeier (1) reported a very high frequency (70%) of Clostridium welchii in fresh burns and an incidence of 6% in burns during the sloughing period.

Lowbury (61) also found this at one time or another in 20% of burned patients, but the pathogenic role, if any, was not defined.

Poate and Metcalfe (53) reported two cases of gas gangrene following electrical burns. The condition of the two young patients had remained good until seven days after injury when both developed fevers of 102° and tachycardia. Onset of gas gangrene was then discovered. The patients were treated by debridement of necrotic tissue, penicillin, and were given intravenous doses of anti-gas gangrene serum ("Wellcome" Brand-Perfringens). Beginning with 40,000 units a total of 160,000 units was given over the next three days. However, they state that there is no theoretical advantage in giving more than one dose of antitoxin, as this alone will serve to neutralize all circulating toxin. Existing cellular damage cannot be undone by subsequent doses.

Pseudomonas aeruginosa:

This organism has now become the dominant threat in the

infection problems of the severely burned patient. As noted earlier, this is a frequent local inhabitant of low virulence and invasiveness which has assumed the role of a life threatening pathogen, especially in individuals in debilitated states who require prolonged and widespread antibiotic coverage.

As a local pathogen Pseudomonas has been shown to produce graft failure by Jackson (26) but this was not observed by Liedberg (34). It is generally recognized that this organism rarely affects the take of grafts and indeed the best method for eliminating Pseudomonas is to apply a graft. Graft failure has often arbitrarily been attributed to the blue pigment pyocyanin and although acetic acid decolorizes the organism it does not kill it and is not rational therapy.

Shires, et al (62), note that five years ago more than 65% of the septicemias in burns were caused by resistant staphylococci and that figures from major centers now show Pseudomonas aeruginosa is the cause in more than 50% of the total who die of septicemia. 85% of wound cultures showed Pseudomonas, but they added that the presence of bacteria on the wound surface had no correlation with the development of septicemias.

Rabin, et al, (57) conducted a study on fatal Pseudomonas infections and concluded that this organism was a contributing factor in the death of 14 patients from a group of 38 burn fatalities. This group consisted largely of infants, children and young adults in whom the total extent of burns was severe, over 40%

of body surface in all but one. Eleven of the 14 patients had 3d degree burns exceeding 20% of body surface. The burn surface was managed by the exposure method and every patient received multiple antibiotics. Penicillin-Streptomycin was used routinely in the post-burn period and other antibiotics were given as specific indications arose. The post-burn survival time varied between 12 and 50 days.

Before the terminal period every patient had between 15,000 and 40,000 WBC's. During the terminal period the white count was normal or showed leukopenia in all but one patient and the differential count showed a slight shift to immature granulocytes and relative lymphocytosis. A wide fluctuation of temperature from normal or above to hypothermic was noted in all but 3 patients.

Ten of the fourteen patients grew organisms in addition to Pseudomonas on blood culture and in five patients an initial Staphylococcal sepsis, partially or completely controlled by antibiotics, was followed shortly by fulminant Pseudomonas septicemia. Once Pseudomonas was grown from blood culture, it persisted until the time of the patient's death which ensued between one and sixteen days after the first positive blood culture. The median time to death was four days.

On postmortem examination six patients displayed extensive wound infections and burn surfaces were black, friable membranes. Foci of necrotic areas remained after the eschar separated

and these foci gradually enlarged and became covered by a black crust with intervening areas of green tinted fat necrosis. The transformation of the wound into a diffuse black membrane usually occurred between 24-48 hours before death and section through the involved burn wound showed nodular hemorrhagic necrosis extending for several centimeters into the underlying subcutaneous tissue.

Four patients developed circumscribed hemorrhagic nodules in non-burned skin, some being adjacent to the burn wound, but some remote. These nodules ranged from 1 to 4 centimeters. Early in development these lesions presented as vesicles surrounded by a border of erythema and at the later stage became diffusely hemorrhagic with central ulceration. When these nodules were cultured, all 4 patients revealed a pure growth of Pseudomonas.

The viscera also revealed hemorrhagic nodules including the heart, lung, kidneys, and meninges which grossly resembled those of both burned and unburned skin. Histologic examination showed a vast number of these organisms as the most characteristic feature with large areas of necrotic tissue, hemorrhage, and scattered inflammatory infiltrate. The bacteria were most noticeably packed into the walls of small arteries, often associated with necrosis of the vessel wall and thrombotic occlusion. (57)

Markley (45) et al, described the same lesions in fatal Pseudomonas septicemias which occurred during the course of the Peru Burn Project in which 172 children and 103 adults with burns involving 10% of the body surface, or more, were studied. In this

review it was demonstrated that Pseudomonas septicemias were the major cause of death occurring after the initial 48 hour post-burn period. Multiple characteristic vesicular or modular cutaneous lesions appeared in the non-burned areas in 78% of burned children with positive blood cultures for Pseudomonas. At autopsy, multiple hemorrhagic and necrotic lesions were found in the viscera which cultured Pseudomonas.

These authors noted that the lesions were of two types. The first occurred the 3d to 8th day following injury and was seen in 72% of patients. This lesion began as a vesicle filled with opalescent liquid surrounded by an erythematous base. This vesicle broke rapidly and developed a central area of necrosis while the erythematous base changed to a darker purple and became quite indurated. The initial vesicle was followed rapidly by multiple lesions similar to the first in areas not burned, although none of these lesions were found on the mucous membranes or palms. Death occurred 24-48 hours after the appearance of the first lesion. The second type of lesion noted was a subcutaneous nodule in the non-burned areas which appeared between the 5th and 28th day after burning. These nodules were painful to touch and accompanied by slight erythema. The patients who developed these lesions deteriorated less rapidly, but died 2 to 12 days after appearance of the first nodule. (45)

Since the lesions described are easily accessable and characteristic of Pseudomonas infection they constituted a good

FIGURE IV

PSEUDOMONAS SEPTICEMIA

SITE OF VISCERAL LESION	PER CENT OF CASES
LUNGS	97
KIDNEYS	74
HEART	24
INTESTINE	15
LIVER	9

From Markley
(45)p.175

diagnostic aid. Rabin (57) described four types of skin lesions, the most typical being eccthyma gangrenosum or pyoderma gangrenosum. This is the predominant lesion described above, usually measuring more than one centimeter, is purple-black, circumscribed, indurated and has an ulcerated center. A second type of lesion consists of clusters of discreet or confluent vesicles. A sharply demarcated cellulitis and maculopapular plaques distinguish the other two types. However, these lesions probably represent the same basic process, differing only in stage of development and all four types appear histologically.

There are many poorly understood alterations occurring in the general decreased resistance to infection in the severely burned patient, and it is only when host resistance is severely compromised and the growth of other organisms inhibited by antibiotics that Pseudomonas invades or proliferates. The extensive areas of necrotic skin may also be another factor predisposing the burned patient to Pseudomonas infection. This organism not only colonizes the eschar, but is capable of producing widespread infection in the subcutaneous tissue underlying the burn around.

The bacterial content increases progressively in the eschar so that when it separates in two to four weeks there is a large quantity of various species contained in it. This may be part of the reason why Pseudomonas infection occurs more commonly after the second week.

Even before the first positive blood culture, many of

the patients were suspected clinically of having Pseudomonas septicemia. This was based on a drop in WBC's to normal or leukopenic levels and development of hypothermia, rapid, shallow breathing and the appearance of black pockets of necrosis.

Since the average duration of life was four days from the first positive blood culture, the question arises as to whether the Pseudomonas septicemia was just an agonal event in an already moribund patient. Rabin and co-workers (57) do not feel this is so because their clinical observations of the burn wound indicated that Pseudomonas infection could advance extensively in the absence of the first positive blood culture.

Tumbusch (64) et al, also feel that this bacterial invasion is not an agonal phenomenon following host deterioration.

> The fact that certain patients do survive septicemia as a complication of burns suggests that bacterial invasion occurs before deterioration has progressed to irreversability. This leads us to believe that death due to septicemia is truly death due to septicemia. Elimination of all pathogens from the environment and the wounds of the burned patients would result in zero mortality.

Shires (62) discusses two types of systemic involvement caused by Pseudomonas, the first being the usual visceral type of septicemia as is seen with other organisms, that is, blood stream invasion with formation of multiple abscesses in various organs. In this form he found lungs and kidneys to be affected most often with infrequent involvement of liver and spleen.

The second type of systemic involvement with Pseudomonas

is what he calls the "vascular or muscular" infection in which the organism invades locally from the burn area into the vascular walls of the surrounding tissue where it is found in tremendous concentration. This type of Pseudomonas septicemia is characterized by negative blood cultures and is probably more of a "pure endotoxemia". (62)

The magnitude of the rising chemical importance of Pseudomonas is made readily apparent in a study by Tumbusch and coworkers. (64) Of 77 patients who died from septicemia following burns, 23 showed mixed cultures while pure cultures were present in 54. These 54 cases of pure culture septicemia showed 17 with Staphylococcus and 27 with Pseudomonas, therefore incriminating these organisms in 81% of patients. Pure and mixed blood stream invasion by one or both of these organisms then occurred in 66 of 77 patients (85.71%). Seven patients survived Staphylococcal septicemia (pure culture) and six survived septicemia due to Staphylococcus and other bacteria. There were no survivors of Pseudomonas septicemia even though Polymyxin B and Colistin sulfate were given soon after diagnosis was established.

Even though the organism may show in-vitro sensitivity to these antibiotics and large doses clear the blood stream, the continued poor clinical course and final demise of these patients leads to the concept that mere destruction of these organisms is not enough and therefore brings us up against the problem of endotoxemia and gram negative shock.

FIGURE V

YEAR	Total No. Septicemia Deaths	Staph aureus No.	%	Pseudomonas aeruginosa No.	%
1954	12	9	75.00	5	41.67
1955	15	10	66.67	8	53.33
1956	11	5	45.45	6	54.54
1957	12	6	50.00	7	58.33
1958	11	2	18.18	7	63.64
1959	16	5	31.25	10	62.50
TOTAL	77	37	40.05	43	55.84

From Tumbusch (64)
p.237

ENDOTOXINS, ENDOTOXIN SHOCK, AND A REAPPRAISAL OF ITS THERAPY

A table was presented previously which compared the clinical differences between gram positive and gram negative septicemia and the probable significant factor in these differences is due to the presence of endotoxins produced by the gram negative bacteria.

Bacterial toxins are generally classified as exotoxins or endotoxins and by definition exotoxins are those excreted by the organism into the media in which they are growing and endotoxins, being closely bound to the cell wall, are those liberated only after disintegration of the organism. Endotoxins are weakly toxic and produce nonspecific toxic effects such as local lesions at the sites of injection and generalized, nonspecific reactions such as fever. They are relatively resistant to heat (60°C) and are weakly antigenic. Specific neutralizing antitoxins are not formed to a significant extent in immunized animals. Furthermore, endotoxins are not converted into toxoids by treatment with Formalin. Endotoxins are chemically similar, but not identical and consist of a protein polysaccharide-lipoid complex. The carbohydrate complex is not toxic, but does determine the specific antigenicity. The toxicity is carried by the phospholipid-polysaccharide portion which is not antigenic. (67)

These endotoxins are produced by members of the family Enterobacteriaciae and therefore include Salmonella, Shigella,

Brucella, Neisseria, Vibrio coma, Eshericia coli, Serratia and Pseudomonas. The endotoxins each give rise to specific antibacterial antibodies, but these endotoxins all give rise to the same symptoms when injected into animals, these being pyrexia, shock, diarrhea, prostration, hyperglycemia which is followed by hypoglycemia, congestion, edema and hemorrhage into the lungs and abdominal viscera.

One of the mysteries is mans' inability to produce antitoxins to these endotoxins, but part of the problem must be based on the fact that the nontoxic carbohydrate hapten is the good antigen and the toxic moiety is a poor antigen. (67) There is no correlation between the virulence of the gram negative pathogen and its endotoxin content.

The mediators of the injurious effects of endotoxin are of paramount importance but very poorly understood. It is known that resistance is primarily in the reticuloendothelial system and involves certain humoral and endocrinologic factors. Small, nonlethal doses of endotoxin can bring about a state of extraordinary refractoriness to further increased doses of endotoxin and at the same time increase lost resistance to other seemingly unrelated phenomena. (59) This refractory state has been called tolerance and is altered reactivity in which there is no evidence of an antigen-antibody reaction. This tolerance phenomenon has been produced in children with agammaglobulinemia. It seems to depend on increased activity of the reticuloendothelial system

with more rapid disposal of foreign materials or upon inhibition of the reaction between the endotoxin and a cellular or plasma factor. (67) Reticulo-endothelial blockade with thorium dioxide, carbon or saccharated iron renders the animal exquisitely sensitive to the effects of endotoxin so that minute doses become lethal. (59)

This tolerance phenomenon is also related to the pyrogenic activity of endotoxin. The pyrogenic activity is very potent and Favorite and Morgan (19) produced marked febrile reactions in man with 0.1 microgram of a purified toxin from Salmonella typhosa.

Leukopenia also develops with the febrile response to intravenous endotoxin. When shock is induced by endotoxin injection the animal remains well for one or two hours, then becomes weak, followed by hypothermia or unabated hyperpyrexia, and becomes cyanotic with diffuse diarrhea and convulsions. At autopsy, gastro-intestinal hemorrhage is all that may be seen. (59)

Gram negative endotoxin shock is indeed a large and yet theoretical area in which there has been much excitement and investigation. Shock is the initial problem in the physician's effort to save the patient with severe burn and even with successful initial therapy the shock problem may again appear as the ominous and refractory terminal episode due to gram negative endotoxemia. Since this is such a large problem in infection in burns it would be well to review the new concepts in the altered physiology occurring in shock and its treatment.

Shock has had various definitions, e.g., that syndrome resulting from a deficit between actual volume load and volume capacity of the vascular bed; yet, ultimately we must accept the fact that in shock induced by hemorrhage or endotoxins the hemodynamics common to all is decreased venous return to the heart. There is a failure to get enough blood into the arterial side of the circulation to permit normal capillary perfusion.

When patients in shock fail to respond to volume replacements they are said to be in irreversible shock and this state is characterized by vasospasm of the arterioles and venules of the visceral organs. With the prolonged ischemia of these organs a state of stagnant anoxia is reached in the capillary beds and when the integrity of these beds is lost, plasma and red cells are lost from the circulation. (37) Thus, as proposed by Lillehei (36) ischemic shock and stagnant shock may be synonymous with and more descriptive than "reversible" and "irreversible" shock. In dogs, the net result of stagnant shock is hemorrhagic necrosis in the intestinal mucosa and other organs. Therefore, this concept of stagnant (or irreversible) shock implies a loss of fluid from the vascular space and it is not in agreement with Rosen's (59) statement that endotoxin shock is normovolemic.

Lillehei (37) disregards the causative stress, whether it be hemorrhage, endotoxin, staphylococcal septicemia or heart failure and considers the common hemodynamic disturbance. In the early stage of shock the sphincters on both arteriolar and venous

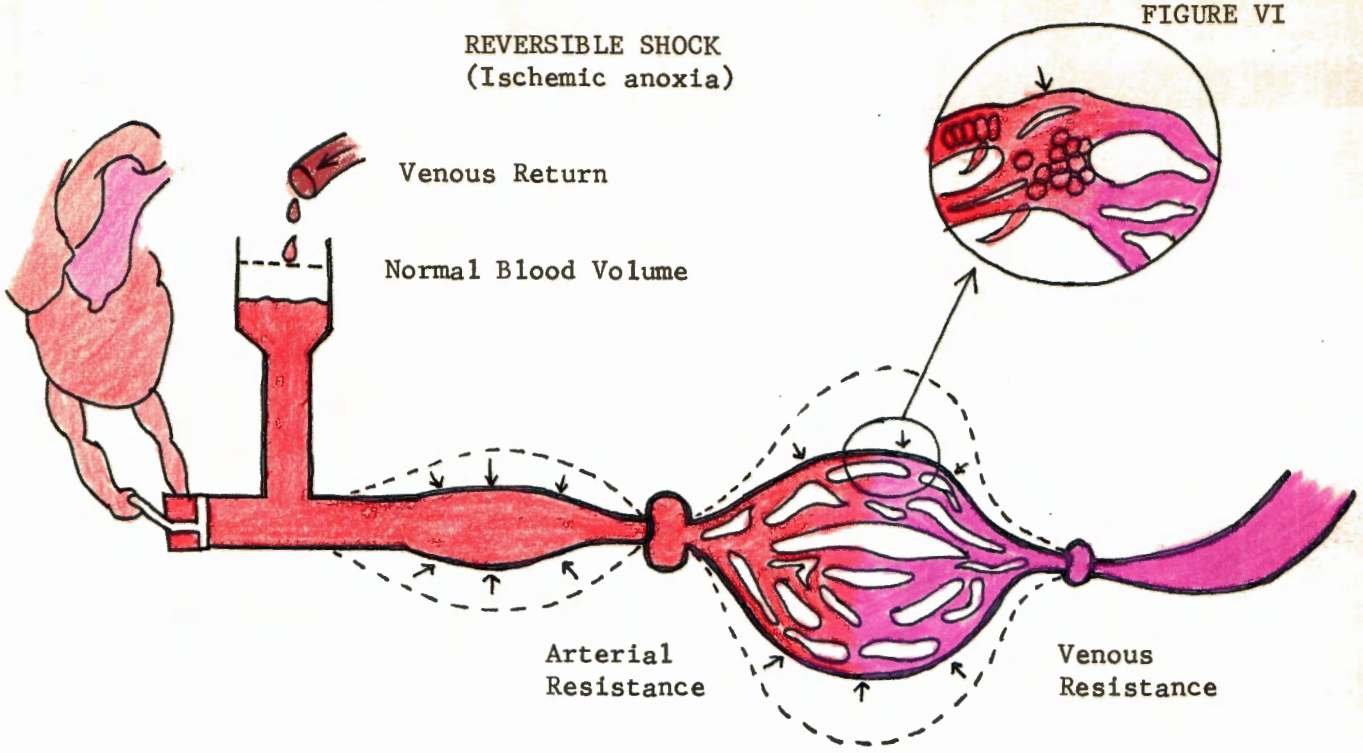
sides clamp down to decrease the size of the vascular space to the size of the circulating blood volume. According to the basic Starlings Law of the Capillary this results in decreased hydrostatic pressure so that the colloid osmotic pressure in the capillary tends to draw extravascular fluid into the capillary. The patient has the built in defense mechanism of transfusing himself with his own extravascular fluid. Within a reasonable period of time the lost volume will be replaced and the patient will survive; however, why is it that volume replacement after a certain time does not recusitate the patient?

Lillehei (37) continues that in time the arteriolar sphincters lose their tone, possibly because of acidosis, but because they also depend on circulation, become anoxic and relax. At the same time the venous tone is maintained and blood can get into the capillary beds but cannot get out--the stagnant stage of shock. At this point, the hydrostatic pressure rises above normal, overcomes the tendency of colloid osmotic pressure to pull fluid back into the capillary, and fluid is pushed out of the capillary into the extravascular space. It has been found that in many types of stagnant shock, there is a continued loss of blood volume due to fluid loss into tissues. The stagnation in the dilated capillary beds leads to an actual necrosis of tissues.

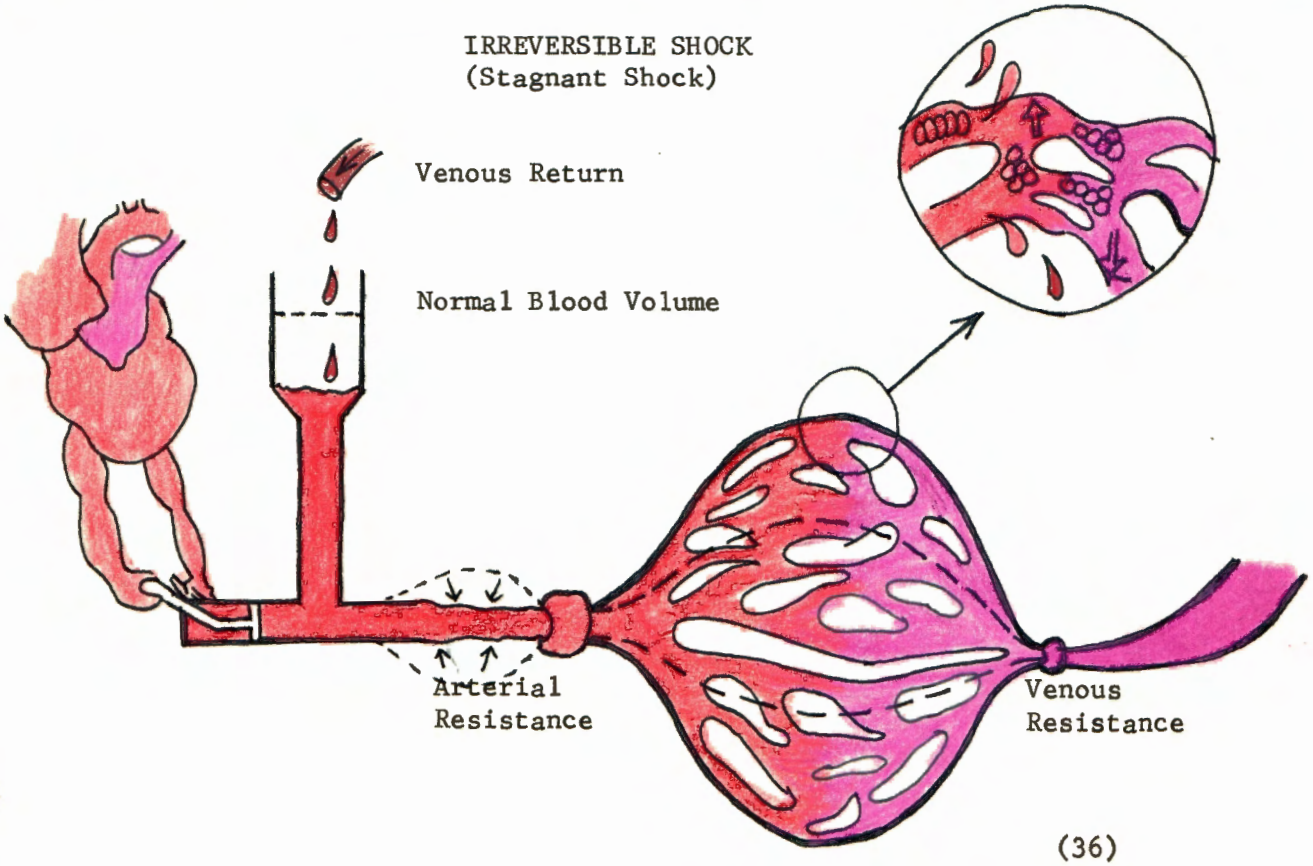
The shock due to gram negative endotoxemia has been called normovolemic shock in an attempt to classify it differently from hemorrhagic shock. Yet endotoxin shock bears many similarities

FIGURE VI

REVERSIBLE SHOCK
(Ischemic anoxia)



IRREVERSIBLE SHOCK
(Stagnant Shock)



to hemorrhagic shock and is probably the most common form of shock seen in the hospital today. It has a mortality rate of 50 to 75%, a figure which has remained unchanged over the last half century despite all other progress.

After endotoxin administration, the total peripheral resistance increases almost immediately since there is a rise in catecholamines and endotoxin (plus a plasma factor) is itself sympathomimetic. Peripheral resistance drops somewhat, then rises again just before death. There are profound decreases in cardiac output, urine output, and plasma volume. Visceral circulation is also greatly decreased. The survival rate in untreated dogs is 10%. (37)

With these facts in mind, does the administration of vasopressor make good sense? The blood pressure is raised artificially by means of increasing total peripheral resistance and this means acceleration of stagnation of the capillary beds and accelerated deterioration of the patient. One must remember that it is possible to have a normal blood pressure with almost no cardiac output and visceral blood flow. (36)

A more rational approach to the treatment would be to correct the peripheral vascular disturbance along with replacing the peripheral volume deficit. In order to block peripheral vasoconstriction Phenoxybenzamine (Dibenzylene) has been used as an adrenergic blocking agent. Hydrocortisone in a pharmacologic dose of 25 to 50 mg/kgm and chlorpromazine 12.5 mg also act in

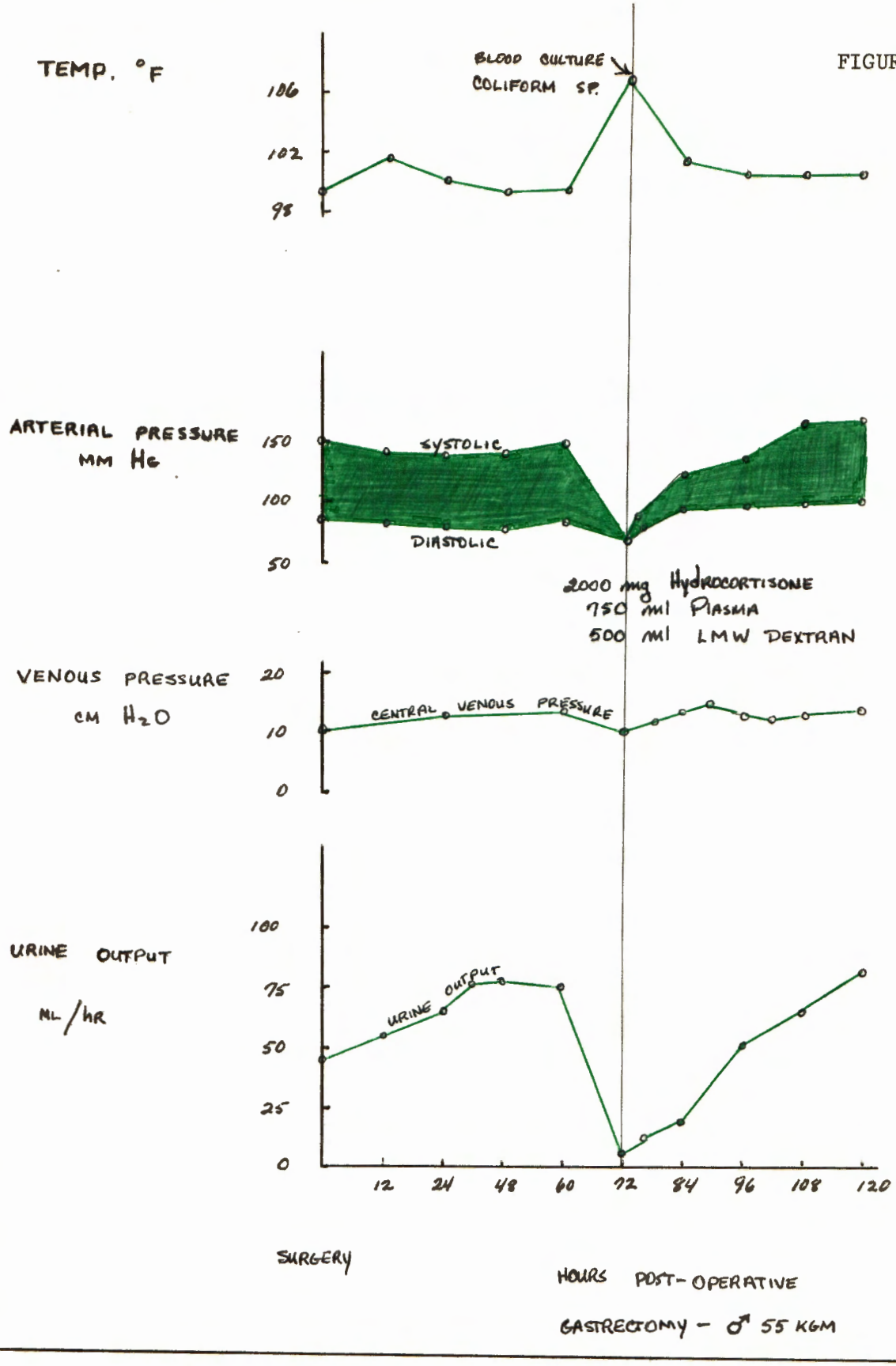
this way. It is very important that the blood volume deficit is replaced at the same time. The figure on the following page illustrates effects of this treatment in a patient who went into gram negative endotoxin shock. (36)

Suppose however, that one is not getting a good result even after volume replacement and vasodilator therapy. This brings consideration of a third factor which is sludging due to beginning agglutination of blood. The best deviscosity agent for this is low molecular weight dextran, and this therapeutic step must be considered when response is poor. (12)

> The use of hydrocortisone in dogs in doses of 50 mg/kgm IV alone decreases peripheral resistance and increases cardiac output and visceral blood flow so that the survival rate is 55%. If 25 mg/kgm of plasma is combined with 1 mg/kgm of phenoxybenzamine the survival rate is 50% and if the same volume of plasma is used with 50 mg/kgm of hydrocortisone the survival rate is 75%. Low molecular weight dextran (Rheomacrodex 40,000) in dose of 2 gm/kgm in 5% dextrose and water combined with 50 mg/kgm of hydrocortisone also gives a survival rate of 75%.

The role of low molecular weight dextran in endotoxin shock should be amplified here. When given alone to dogs in shock in a dose of 2.5 to 7.5 gm/kgm in 5% dextrose and water it is effective in reversing the disturbed chemistry and physiology of endotoxin shock. Nevertheless, almost all dogs so treated eventually die of a bleeding diathesis. This is apparently due to the fact that both endotoxins and low molecular weight dextran decrease fibrinogen and plasminogen and increase plasminogen activators.

FIGURE VII



From Lillehei (36)
p.425

When used together, the result is profound alteration in the concentrations of these substances. If the dose of low molecular weight dextran is kept at 2 gm/kgm and combined with hydrocortisone, these bleeding problems do not occur in the dog. (37)

Rosen (57) states that blocking agents which act as vasodilators protect animals against the lethal effects of endotoxin only when such agents are given before endotoxin.

> Presently, in treating patients we prefer a regimen of hydrocortisone in pharmacologic doses; plasma to replace volume loss; specific antibiotic therapy and surgical intervention when necessary to eliminate the source of infection. Hydrocortisone is used in preference to phenoxybenzamine because it has a more subtle effect on vascular tone and is less likely to cause further hypotension. Plasma is preferred to low molecular weight dextran until more is learned about the combined effects of endotoxin shock and dextran on basic blood clotting mechanisms in man. (37)

THE TREATMENT OF BURN INFECTION

PHYSICAL PROPHYLAXIS

Physical methods of prophylaxis include matters of the patient's environment which were given some consideration earlier in this paper. However, an assessment yet remains to be given to the advantages and disadvantages of the open and closed methods of local wound care.

The technique of the exposure method is a definite pattern of handling and does not mean mere exposure and neglect. The "open method" is a poor term since improper exposure leads to an open wound and is conducive to invasive infection.

Using sterile technique the burns are thoroughly cleansed with soap and all blisters are broken except those on the palms and soles. Electric lights and heat lamps are contra-indicated since this increases capillary oozing, bacterial multiplication and body metabolism and thereby delays eschar formation. Dusting of the wound with aluminum or other powders is not beneficial and prevents adequate inspection. With regard to local applications it is known that tannic acid has been proven very poor just on the basis of hepato-toxicity, but other similarly bad practices include such preparations as iodoform, picric acid, and butysin picrate. The wound should be inspected frequently and whenever a crack is found in the crust the edges should be cut away and covered with a piece of fine mesh gauze. When suppuration is

present the crust should be removed, and if eschar is too tight it should be insised and dressed. In partial thickness burns exposure provides free drainage so that only a thin layer of exudate can cover at any time. A crust formation will occur without infection in spite of bacteria on the surface. Bacterial reproduction is inhibited by the new crust, drying and the cooler surface. With full thickness burns the wound is infected regardless of the type of local care. Because of the dead tissue above and the impaired blood supply beneath due to thrombosis, easy bacterial multiplication enhances the autolysis that occurs beneath the third degree eschar on the tenth to fourteenth day. As long as the eschar is dry and intact it is an effective barrier against the environment, but the exposure method must be discontinued when antolysis becomes evident beneath the eschar. Then the wound should be cleared as soon as possible to prepare for skin grafts. Removal of adherent eschar should not be too energetic because granulation tissue has not formed and if bleeding occurs a ready avenue has been opened for the ingress of bacteria.

One of the chief advantages of this method is the effective control of infection especially in partial thickness burns. Deep dermal burns treated by exposure usually become dry and heal uneventfully while those treated with dressings usually become infected and are converted to full thickness burns.

The exposure method can be abused and the chief problem here is prolonged exposure during the sloughing phase which always delays preparation of the base for grafting and skin coverage. If

areas are permitted to remain open as the eschar begins to slough one allows for rapid onset of problems with infection. (5)

In 1962 Dupont,et al (16) treated 58 severely burned patients with the closed method and 11 by exposure. Hospitalization time was reduced from 107.6 days for patients treated with the closed method to 76.1 days for survivors treated with exposure, even though the average burn area was greater in the latter group.

In summary, the tendency now is to treat burned parts by exposure. After eschar removal is achieved all full thickness burns should be treated with bulky, absorptive, occlusive dressings and split thickness skin grafts are used as soon as granulation tissue is ready; for grafting. It should be recognized that the closed and exposure methods are not antagonistic but complimentary. (32)

DEBRIDEMENT AND GRAFTING

Since the burn wound itself is the chief source of infection, early excision of dead tissue and grafting must be used to afford early establishment of a tissue barrier against invasive infection.

Normally, in full thickness burns the eschar begins to slough between the 14th and 21st day and granulation tissue forms beneath the slough. The sooner the eschar is removed and the less the suppuration the earlier a graft can be applied. The three methods of removing eschar are (1) by wet soaks, (2) repeated change of dry dressings and, (3) surgical excision, The latter method is generally acceptable in burns involving less than 15% of the body surface, but has the disadvantages of entailing general anesthesia and considerable blood loss which may be detrimental to the body's defense mechanisms against infection. "Immediate excision" is recommended by some and indicates excision within 2 or 3 days following injury. The difficulty here is that it is hard to estimate the extent of full thickness burns and the initial injury is compounded by the surgical procedure. (6)

Liedburg (35) et al, felt that since the injured subcutaneous tissue offered such a ready avenue for invasive infection it would be best to apply the grafts immediately after the excision of the eschars rather than wait for the development of granulations.

> It would seem that a logical approach to the management of patients with massive

> burns would be prevention of septicemia by removal of the eschars in the immediate postburn period and the application of homografts.

However, Artz (6) later published the opinion that it is usually better to apply a dressing and delay skin grafting for 2 to 4 days.

Autografts are best since they provide permanent wound closure; however, in an extensively burned patient there may not be sufficient donor sites for autografts and the additional trauma of skin removal may be detrimental. In such a case homografts are a valuable adjunct and may be lifesaving. Haynes (24) showed that there was marked improvement in the clinical condition following application of homografts on a clean granulating wound in severely burned children with debility or sepsis. He had a "take" in 85% and with subsequent improvement in surgical risk autografting could be achieved after homograft rejection in 3 to 5 weeks. The homograft effectiveness was directly related to the relative extent of clean granulating wound available for homograft growth; therefore, homografts were not beneficial to the child with extensive third degree burns, severe sepsis, and a sloughing wound. There was no difference between the effectiveness of cadaver and relative homografts, but fetal homografts lasted significantly longer. It should be stressed that grafts should not be applied when the group A beta hemolytic Streptococcus is present.

Some research has been done with synthetic skin (Ivalon(R)) instead of autografts or homografts. Martin and

Boyer (46) make a pattern of Ivalon which is a polyvinyl alcohol sponge at the time of debridement. The sheets are about 4 millimeters thick, sterilized, wrung out in antibiotic solution and sewn on. In two of their burn cases there was "far less fever, infection and pain with more comfort, cheerfulness, and better nutrition". They stated that there was almost perfect graft site preparation as the polygrafts were fractionally removed and a reduction of exudate at the conjunction of graft and unautografted areas. However, Montgrief (46), in the same reports stated he had used Ivalon on four patients and did not notice any change in clinical course.

At present, it seems that one cannot make any definite commitment on the use of synthetic skin substitutes.

ANTIBIOTIC CHEMOTHERAPY

The role of antibiotics in the treatment of the infection problems in burns must be evaluated since undue reliance should not be placed upon this therapy. Monasterio (49) et al, concluded a study with the statement:

> The evidence produced by this study does not show any definite advantage in the treatment of burns by the use of antibiotics and we believe that they are not a substitute for meticulous local and general care of the patient in the management of infection in burns.

Nevertheless, in the same study Monasterio (49) did note a decrease in percentage of graft takes in the non-antibiotic group and total hospitalization time was higher in this group. In spite

of negative cultures they believed that infection was responsible for many of the deaths in both the antibiotic and nonantibiotic groups and they apparently were not preventable with antibiotics.

In the Peru Burn Project, Kafalides (28) concluded that routine antibiotic prophylaxis did not prevent the occurence of septicemias or the infection of burned areas. It favored the appearance of resistant strains of Staphylococcus and Pseudomonas not only to the antibiotics used, but to others also.

> We can state categorically that systemic antibiotic prophylaxis has no place in the prevention of infections in burns and if prophylaxis is desired, penicillin is the drug of choice, principally to prevent septicemia from hemolytic streptococci.

Although localized infection does not threaten survival as does a generally invasive infection, the consequences can still be detrimental from the standpoint of pain and increased healing time due to conversion of partial thickness to full thickness skin loss and graft failures.

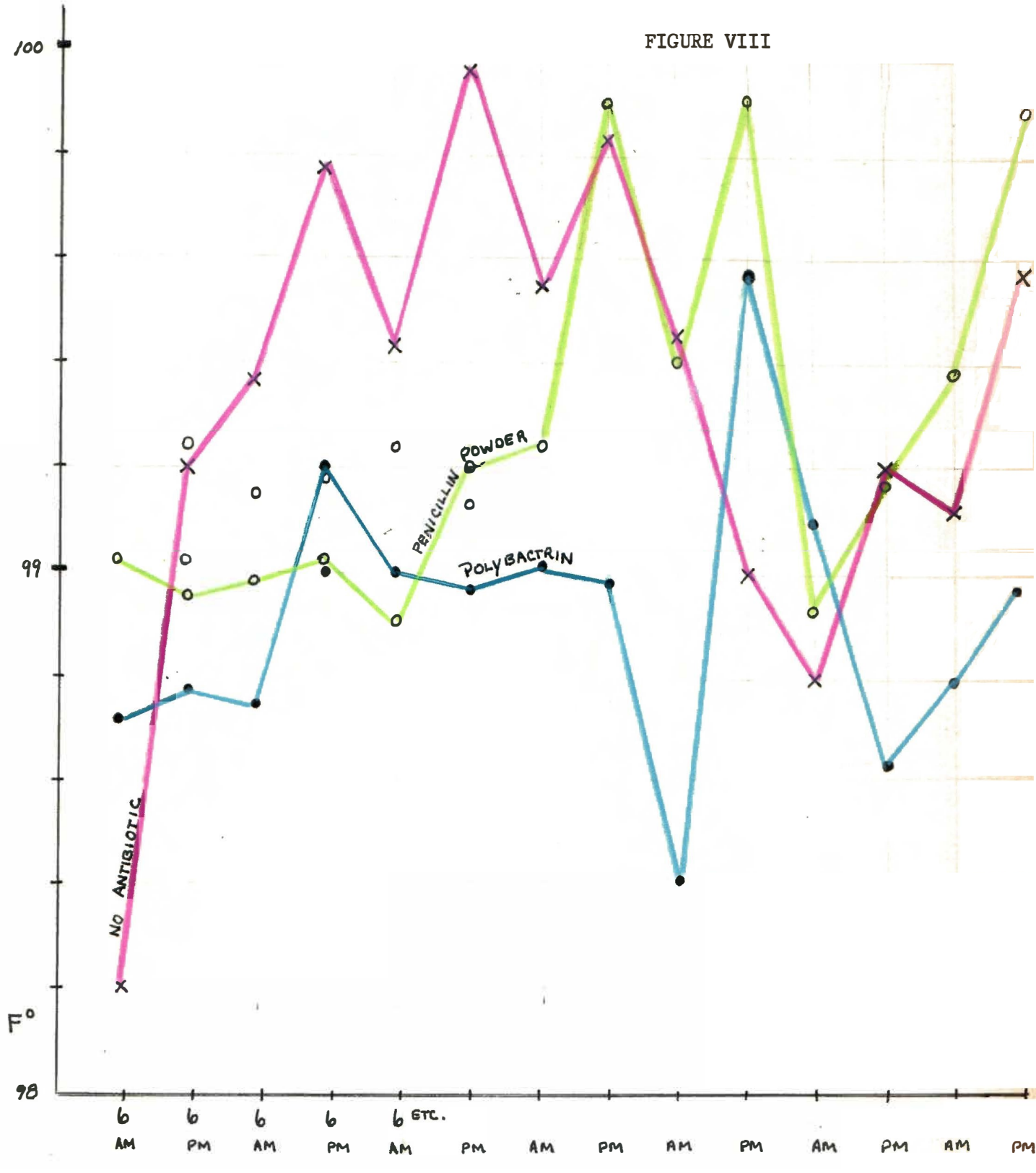
Local infection with Streptococcus can destroy epithilium before there is any gross evidence of its presence and this is a reason for obtaining a culture before grafting. When suppuration occurs in the wound there is danger of full thickness skin loss and is an indication for application of local antibiotics. All organisms may cause this problem, but the group A beta hemolytic Streptococcus is especially dangerous in this regard and systemic penicillin should be routine prophylaxis here. (6)

Schumer(60a) notes that many forms of antibiotics, antiseptics, sprays and powders have been tried without apparent benefit. Yet, Moore(49a)feels that the use of local antibiotics is underemphasized and that wounds can be treated with high local concentrations of such drugs as bacitracin, neomycin, polymyxin and tyrothrycin with profitable results.

Lowbury, et al, (42) have tried treatment of the wounds with neomycin-chlorhexidine tullegras (prepared by impregnating gauze strips with paraffin containing neomycin sulfate 5 mg per gram and chlorhexidine diacetate 5 mg per gram.) and showed significantly less infection with Staphylococcus, Pseudomonas, Proteus and Coliform bacilli than in a control series treated with penicillin cream. However, they had fewer infections with Streptococcus in both groups. They also found that prophylactic use of polymyxin, neomycin and bacitracin sprays were associated with less bacterial infection than in burns treated with penicillin lactose powder or no antibiotic. The objective of using neomycin-chlorhexidine tulle gras was to combine the advantages of antibacterial agents against gram positive cocci and of a dry surface directed against gram negative bacilli. The clinical advantage of this prophylaxis was seen in the lower temperature and respiration rate. However, despite the less frequent infection with this treatment, the separation of the slough was sometimes considerably delayed and skin grafts were slightly less good than those dressed with penicillin cream.

TEMPERATURE AND LOCAL CHEMOTHERAPY

FIGURE VIII



From Lowbury
(42)

If the local infection becomes locally invasive it is usually the result of the beta hemolytic Streptococcus and is most often seen in the partial thickness burn. Furthermore, this is very unlikely if penicillin has been used prophylactically. Cellulitis, lymphangitis and regional lymphadenopathy are the usual findings. A second degree burn is sometimes difficult to evaluate because it is often surrounded by a halo of erythema which may look like cellulitis. In all cases, IM penicillin should clear the difficulty. (6)

During grafting systemic antibiotics are valuable only as prophylaxis against the Streptococcus. For this, IM procaine penicillin (600,000 units) once per day for two days prior to grafting or dressing change and for two days thereafter is effective, but one gram tetracycline can also be used. The most effective method against Streptococci is a combination of local and systemic therapy using IM penicillin and chloramphenicol or tetracycline ointment, and a negative culture for Streptococci should be obtained before grafting. Most of the commonly used antibiotics have been used for local therapy and Furacin is the one which does not give complete protection against Streptococcus. Also, local antibiotic ointment cannot be expected to be effective beyond 48 hours. (6)

Altemeier (1) summarizes the role of antibiotics with the observation that parenteral antibiotics do not prevent local bacterial growth in areas of deep cutaneous slough. Chemotherapy

should be used primarily to protect the patient from invasive infection as a temporary measure until it is possible to remove the slough.

Septicemia is the life threatening situation and is the chief worry in full thickness burns during the first several weeks, although it may occur at any time. One must use blood cultures and sensitivity tests, and even then treatment is often difficult and without favorable outcome.

With the emphasis on rational use of antibiotics, their purpose in this instance is to reduce the blood stream load of bacteria and prevent the formation of abscesses in the internal organs until the wound itself can be treated to eliminate it as a source of blood stream invasion. (6)

The antibiotic treatment of septicemias is totally unsatisfactory at present. Liedburg, Artz and Reiss (35) reported on the cause of death in 35 consecutive burn cases in which the mean total extent was 58% and mean full thickness skin loss was 45%. In all instances the organisms from blood culture were sensitive to one or several antibiotics in vitro and sterilization of the blood stream was achieved, yet patients died of septicemia.

Altemeier and Macmillan (3) noted in one series that the Beta hemolytic Streptococcus has not been the cause of a single case of septicemia since introduction of the use of penicillin. Kafalides (28) found an increased incidence of Staphylococcal septicemia with use of broad spectrum antibiotics. The relative

sustained increase in gram negative bacillary infections indicates that antibiotic therapy has been of little or no use in their prevention and that better specific agents are needed to meet this threat. (3)

However, examples of such specific agents are to be found in Polymyxin B and Colistin sulfate. Rabin, et al (57) noted every strain of Pseudomonas from blood culture was sensitive to Colistin at an easily achieved blood level and most were sensitive to Polymyxin. Nevertheless, there was no improvement in the clinical status of these patients and they died.

Rabin's statement that treatment was with easily achieved blood levels of Colistin deserves some appraisal, because Petersdorf (51a) noted in 1963 that there are two salt forms of Colistin, one the sulfate and the other a methane sulfonate. The latter, (Colistimethate), is used for parenternal therapy and since it possesses only one third the inhibitory activity of the sulfate, its minimum inhibitory concentration must be a serum level of 9 micrograms, a level not attainable with the usual doses. The drug has been effective in eradicating bacteriuria since urine concentrations can be 10 to 20 times the serum concentration. In treatment of deep seated systemic infections it is much less impressive. That the blood is not cleared of bacteria under these conditions is not surprising. Therefore, it is recommended that in deep seated Pseudomonas infections Colistimethate must be given in high concentration. For adults, no less than 300 mg per day should be

given even at the risk of toxicity. Children should receive 5 mg/kgm, although they can usually tolerate 7 to 10 mg/kgm per day.

With regard to endotoxemia, even though these drugs caused sterilization of the blood one may also be raising the endotoxin level to a lethal range by accelerating the destruction of the organisms.

In discussing the supportive therapy of septicemias, Artz and Reiss (6) state that cortisone and hydrocortisone are contraindicated.

> The known deliterious effects of the hormones in decreasing the body's resistance to infection far outweigh the possible beneficial effects that might be expected from hormone therapy.

They also defend norepinephrine infusions as definitely useful in the treatment of hypotension of septicemia since "this is caused by intense vasodilation and is not the same as the hypovolemic hypotension in the first 48 hours after a burn.

These two recommendations are in direct contrast to the opinions of Lillehei which were discussed previously. We should observe Art's (6) statement that "all too frequently, septicemia progresses; the norepinephrine requirement increases progressively; eventually, even enormous doses fail to maintain the blood pressure and death ensues".

IMMUNOLOGY

Another approach to septicemia is immunologic. Fox and Lowbury (21) found an immune response in one-third of thirty nine patients who had acquired Pseudomonas in burns. Blood sera showed a high agglutinin titer which increased with passage of time.

Turnbusch (64) reported a generalized Schwartzman-like reaction in two patients with gram negative septicemia. Both showed intracapillary fibrinoid degeneration in the glomerulae. Graber (23) also reported a case following septicemia due to Serratia marcescens.

Some research has been done on the use of gamma globulin in the prevention of Pseudomonas and Staphylococcal septicemias. Kafalides, et al (28), compared the results of gamma globulin prophylaxis on late mortality in 106 burned children and 10 adults to 102 burned children and 19 adults who did not receive gamma globulin. Both groups were given similar antibiotic prophylaxis. Children with burns over 10% and surviving the first 48 hours received 1 cc/kgm on admission and on the 3d and 6th days after burning. Adults with burns of over 25% received 50 cc of gamma globulin at admission and 25 cc on the 5th day. Gamma globulin prophylaxis significantly reduced deaths with septicemias from 40.5% to 22.4%. Their results suggest that in young burned children and those with a surface area burn of less than 20% gamma globulin prophylaxis may be life saving.

Millican and Rust (48) found that serum from rabbits

immunized with Pseudomonas is very effective in protecting mice against death from infection and that it is several hundred times more effective than human gamma globulin. In infections 94% fatal to control mice treatment with normal serum .01 to 1.0 ml produced survivals of 0-15% while treatment with antiserum (10^{-7} to 10^{-4} ml) produced survivals of 30 to 100%.

SUMMARY

This paper is a review of the recent literature concerning problems associated with infectious complications of burns.

The first section begins with a consideration of the factors contributing to the appearance of these problems and is followed by a clarification of terms applying to infection in burns. The sources of infection are then discussed since these carry therapeutic implications and the variable factors such as host resistance which influence the course of infection are presented.

The second section is concerned with the separate problems associated with specific organisms and leads into the particular problem of endotoxemia. Since gram negative endotoxemia has been the subject of recent investigation and debate, this is given some special discussion because it is concerned with a re-evaluation of shock and its therapy.

The third section presents the treatment of infection in the burned patient, including physical methods of prophylaxis, the role of chemotherapy, and ends with a brief consideration of immunologic aspects in burn infections.

CONCLUSION

Infection is the single largest threat to the survival of the severely burned patient. This has arisen because of increased patient survival time due to better treatment of early shock, emergence of resistant bacteria and antibiotic chemotherapy itself.

Antibiotic chemotherapy has thus far failed to solve all of the infectious difficulties in burns and is leading to a more diligent search for solutions in immunology and therapy of endotoxin shock. As time goes on, proof of the efficacy of these controversial approaches will be strengthened or disproven.

REFERENCES

1. Altemeier, W. A., Antibiotic Treatment of Severe Cutaneous Burns. (In: Symposium on Burns. National Research Council, Washington, D. C., 1951, p. 132).

2. _____, Summary of Infections. (In: Research in Burns. The Proceedings of the First International Congress on Research in Burns Held at the National Naval Medical Center, Bethesda, Maryland. Co-published by The American Institute of Biological Sciences, Washington, D. C., and the F. A. Davis Company, Philadelphia, (Sept. 19-22) 1960, p. 411).

3. _____ and Macmillan, The Dynamics of Infection in Burns. (In: Research in Burns. Co-published by The American Institute of Biological Sciences, Washington, D. C., and the F. A. Davis Company, Philadelphia, (Sept. 19-22) 1960, p. 203.

4. Artz, C. P., Research in Burns. The Proceedings of the First International Congress on Research in Burns Held at the National Naval Medical Center, Bethesda, Maryland. Co-published by The American Institute of Biological Sciences, Washington, D.C., and the F. A. Davis Company, Philadelphia, (Sept. 19-22) 1960, p. 5).

5. _____ and Gaston, B. H., A Reappraisal of the Exposure Method in the Treatment of Burns, Donor Sites and Skin Grafts, Annals of Surgery, 151:939, 1960.

6. _____ and Reiss, R., The Problem of Infection. (In: Treatment of Burns, W. B. Saunders Company, Philadelphia and London, 1957, p. 144-162).

7. Balch, H. H. and others, Resistance to Infection in Burned Patients, Annals of Surgery, 157: 1-19, (Jan.) 1963.

8. Barber, M., Penicillinase Producing Staphylococci, Journal of General Microbiology, 8:111, 1953. Cited by: Sevitt, S., Burns: Pathology and Therapeutic Implications, London, 1957, p. 97.

9. Colbrook, L., Infections in Severe Burns: Mode of Infection and Its Control, (In: Symposium on Burns, National Research Council, Washington, D.C., p. 234).

10. _____ and others, Studies of Burns and Scalds, MRC Special Report Series Number 249, Part II, 1945. Cited by: Sevitt, S., Burns: Pathology and Therapeutic Implications, London, 1957, p. 90.

11. _____ and others, Control of Infection in Burns, Lancet, 1:893.

12. Collins, V. J., Shock Symposium. The New Physician, 13:433, (Nov.) 1964.

13. Collentine, G. E. and others, Two Years Experience in the Treatment of Burns in a Private General Hospital, Wisconsin Medical Journal, 61:511-516, (Oct.) 1962.

14. Davis, J. H., Staphylococcal Infections in Burns, (In: Research in Burns. Co-published by American Institute of Biological Sciences, Washington, D.C., and F. A. Davis Company, Philadelphia).

15. Demerec, M., Proceedings of the National Academy of Science, Washington, 31:16, 1945. Cited by: Sevitt, I., Burns: Pathology and Therapeutic Implications, London 1957, p. 97).

16. Dupont, C., and others, An Appraisal of the Exposure Method in the Management of Severely Burned Patients, Canadian Journal of Surgery, 5:265, 1962.

17. Eckmann, L., Active and Passive Tetanus Immunization, New England Journal of Medicine, 271:1087-1090, (Nov. 19) 1964.

18. Eleck, S. D., Experimental Staphylococcal Infections in the Skin of Man, Annals of the New York Academy of Science, 66:356, 1956.

19. Favorite, G. O., and Morgan, H. R., Journal of Clinical Investigation, 21:589, 1942.

20. Feodorov, N. A., and others, Pathological Physiology and Experimental Therapy, 6:53, 1959. Cited by: Newton, W. T., Immune Specificity of Burn Toxin, Archives of Surgery, 85:912, (Dec.) 1962.

21. Fox, J., and Lowbury, E. J. C., The Epidemiology of Infection with Pseudomonas pyocyanea in a Burns Unit, Journal of Hygiene, 52:403.

22. Graber, C., and others, Increasing Incidence of Nosocomial Herellia vaginicola Infections in Burned Patients, Surgery, Gynecology, Obstetrics, 114:109, 1962.

23. _____ and others, Generalized Schwartzman-like Reaction Following Serratia Marcescens Septicemia in Fatal Burn, Surgery, Gynecology, Obstetrics, 110:443-450, 1960.

24. Haynes, B. W., Skin Homografts - A Life Saving Measure in

Severely Burned Children, Journal of Trauma, 3:217-224, (May) 1963.

25. Horn, L.,and Converse, J. M., Chlorpromazine in Protection from Acute Effects of Extensive High Temperature Skin Injury in Rats, Proceedings of the Society of Experimental Biology and Medicine, 113-194-197, (May) 1963.

26. Jackson, D. M., and others, Pseudomonas pyocyanes in Burns: Its Role as a Pathogen and the Value of Local Polymyxin Therapy, Lancet, 2:137-147, 1951.

27. _____, and others, Chemotherapy of Streptococcus pyogenes Infection of Burns, Lancet, 2:705, 1951.

28. Kafalides, N. A., and others, Clinical Evaluation of Antibiotics and Gamma Globulin in Septicemias following Burns, (In: Research in Burns, co-published by American Institute of Biological Sciences, Washington, D. C., and F. A. Davis Company, Philadelphia, (Sept. 19-22) 1960).

29. Korlof, B., Infection of Burns, Acta Chirugi Scandiva Supplement 209, 1956, p. 1-70.

30. Langohr, J. L., and others, Bacteriologic Study of Burn Wounds, Annals of Surgery, 125:452, 1947. Cited by: Sevitt, I., Burns; Pathology and Therapeutic Implications, London, 1957, p. 88.

31. _____, and others, Bacteriologic Study of Burn Wounds, Annals of Surgery, 125:452, 1947.

32. Liedburg, N. C. F., and others, Infection in Burns, I: The Problem and Evaluation of Therapy, Surgery, Gynecology, Obstetrics, 98:535, 1954.

33. _____, and others, Infection in Burns, II: The Pathogenicity of Streptococci, Surgery, Gynecology and Obstetrics, 98:693-699, 1954.

34. _____, and others, Infection in Burns, IV: Evaluation of the Local Use of Chloramphenicol Ointment and Furacin Soluble Dressings on Granulating Surfaces Following Extensive Full Thickness Burns, Surgery, Gynecology and Obstetrics, 100: 219-231, 1955.

35. _____, and others, Infection in Burns, III: Septicemia, A Common Cause of Death. Surgery, Gynecology, Ob-

stetrics, 99:151, 1954.

36. Lillehei, R. C., Experimental Aspects of Shock Due to Hemorrhage, Infection and Myocardial Failure, The New Physician, 13:419 (Nov.) 1964.

37. Longerbeam, J. K., and others, The Treatment of Irreversable Shock, from the Department of Surgery, University of Minnesota Medical School, Minneapolis, Minnesota. An Exhibit Prepared For the 113 Annual Meeting of the American Medical Association, San Francisco, California,(June 21-25) 1964.

38. Lowbury, E. J. L., Infection of Burns, British Medical Journal, 1:994-1001, 1960.

39. _____,and Cason, J. S., Aureomycin and Erythromycin Therapy for Streptococcus pyogenes in Burns, British Medical Journal, 2:914, 1954.

40. _____, and Fox, J., The Epidermiology of Infection with Pseudomonas pyocyania in a Burns Unit, Journal of Hygiene 52:403.

41. _____, and Miller, R. W., Treatment of Infected Burns with BRL 1621, Lancet, 2:640-641, (Sept. 29) 1962.

42. _____, and others, Local Prophylactic Chemotherapy for Burns Treated with Tulle Gras and by the Exposure Method. Lancet, 2:958-963, (Nov. 10) 1962.

43. _____, and others, Chemotherapy for Staphylococcus aureus in Burns, Lancet, 1:1036.

44. Malm, O. M., and Slawikowski, G. J. M., Surgery Forum 12:36, 1961. Cited by Newton and others, Immune Specificity of Burn Toxin, Archives of Surgery, 85:912, (Dec.) 1962.

45. Markley, K., and others, Fatal Pseudomonas Septicemias in Burned Patients. Annals of Surgery, 145:175, 1957.

46. Martin, M. M., and others, The Use of Synthetic Skin in lieu of Homografts in Treatment of Extensive Third Degree Burns, Journal of Trauma, 3:87-94, (Jan.) 1963.

47. Meleney, F. L., Statistical Analysis of a Study of the Prevention of Infection, Surgery, Gynecology, Obstetrics, 80:263, 1945.

48, Millican, R. C., and Rust, J. D., Efficacy of Rabbit Pseudomonas Antiscium in Experimental Pseudomonas aeruginosa Infection, Journal of Infectious Disease, 107:389-394, 1960.

49. Monasterio, F. O., and others, Comparative Study on Treatment of Burns with and without Antibiotics. (In: Research in Burns, Copublished by American Institute of Biological Sciences, Washington, D. C., and F. A. Davis Company, Philadelphia, (Sept. 19-22) 1960.

49a. Moore, F. D., Metabolic Care of The Surgical Patient, Philadelphia, W. B. Saunders Company, p. 891.

50. Moyer, C. A., An Assessment of the Therapy of Burns, Annals of Surgery, 137:628, 1953.

51. Newton, W. T., and others, Immune Specificity of Burn Toxin, Archives of Surgery, 85:912 (Dec.) 1962.

51a. Petersdorf, Robert G., Piorde, James J., Colistin - A Reappraisal, Journal of the American Medical Association, 183:123-125, (Jan.12) 1963.

52. Phillips, A. N., and Cope, O., Burn Therapy II, Annals of Surgery, 155:1, 1962.

53. Poate, W. J., and Metcalfe, M. B., Gas Gangrene Following Electrical Burns, British Journal of Plastic Surgery, 15:17, 1962.

54. Price, P. B., and others, Histologic Changes in Experimental Thermal Wounds, Surgery 36:664-674, 1954.

55. _____, and others, Bacterial Invasion in Experimental Burns, Surgical Forum 6:64, 1955: Cited by: Davis, J., in Research in Burns, Co-published by American Institute of Biological Sciences, Washington, D. C., and F. A. Davis Company, Philadelphia, (Sept.) 1960, p. 214.

56. Pulaski, E. J., and Altemeier, W. A., Evaluation of the Exposure Method in Treatment of Burns, Surgical Forum, 1952, p. 518.

57. Rabin, M. C., and others, Fatal Pseudomonas Infections in Burned Patients: A Clinical, Bacteriologic and Anatomical Study. New England Journal of Medicine, 265:1225, (Dec.) 1961.

58. Rivera, J. A., and others, The Role of Antibiotics in Thermal Injury I. Evaluation of the Results of Antibiotic Sensitivity Tests During a Five Year Period, Antibiotics Annual 1955-1956.

59. Rosen, F. S., Endotoxins of Gram negative Bacteria and Host Resistance. New England Journal of Medicine, 264:919-923 and 980-985, 1961.

60. Rosenthal, S. R., and others, Journal of American Medical Asso-

ciation, 174:957, 1961. Cited by: Newton, W. T., and others, Immune Specificity of Burn Toxin, Archives of Surgery, 85:912, (Dec.) 1962.

60a. Schumer, William, Recent Advancements in the Management of Burns, in Surgical Clinics of North America, 43:236, (Feb.) 1963.

60b. Schneewind, John H., Surgical Physiology of Trauma, (In: Surgery Clinics of North America, 42:82, (Feb.) 1962.

61. Sevitt, I., Burns: Pathology and Therapeutic Implications, London, 1957, p. 88.

62. Shires, G. T., and others, Severe Burn, Texas Journal of Medicine, 58:828, (Oct.) 1962.

63. Stevens, K. M. Cause of Death in Acute Systemic Bacterial Infection and Cancer. Lancet, 1:1254, (June 6) 1964.

64. Tumbusch, W. C., and others, The Rising Incidence of Pseudomonas Septicemia Following Burn Injury (In: Research in Burns, Co-published by the American Institute of Biological Sciences, Washington, D. C., and F. A. Davis Company, Philadelphia, (Sept.) 1960, p. 236.

65. Wartmann, W., (In: Research in Burns, Co-published by the American Institute of Biological Sciences, Washington, D. C., and F. A. Davis Company, Philadelphia, (Sept.) 1960, p. 7).

66. Williams, R., and others, Cross Infedtion with Pseudomonas pyocyanea, Lancet, 1:376-379, 1960.

67. Zinssers Microbiology, edited by Smith and Conant, Appleton-Century-Crofts, Inc., New York, N.Y., 1960, p. 184.