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Pathogenesis of pseudomonas aeruginosa infection in burns

Wayne L. Jackson
University of Nebraska Medical Center

THE PATHOGENESIS OF PSEUDOMONAS AERUGINOSA INFECTION IN BURNS

Wayne L. Jackson

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PREFACE

It is fairly well known that of all of the accidental injurie, none are more terrible than that of a burn. None are more painful, more disabling, more prolonged in their convalescence, more time consuming for medical personnel, or probably more expensive in terms of actual medical cost as well as working time lost and other associated factors. Equally important, in my own mind at least, is the fact that it has been widely stated that overall mortality figures in burned patients are approximately the same now as they were a hundred years ago or more. This is in spite of recent advances in electrolyte therapy, acid-base balance, transfusions, and antibiotics. It is generally held that infection is responsible for this lag in progress. It is with this latter problem that this paper will be chiefly concerned, particularly the problem of infection with Pseudomonas aeruginosa, an organism widely distributed in nature, generally of low virulence, but with the capability of attacking the burned patient with deadly effectiveness. It is the aim of this paper to present the means and mechanisms of such infection rather than a general treatise on burn care or on antibiotic and other care of such infection when it occurs. Although it will be somewhat deviant from the scope of the study some statistics of interest to anyone concerned with burns will be presented which will deal with prevailing causes of death and other factors.

CHAPTER I

INTRODUCTION

Over the past several years literally thousands of journal articles, books, monographs, and other forms of communication have concerned themselves with the problem of the burned patient. Overall, these represent a rather complete albeit somewhat confusing discussion of the multifaceted problem involving all aspects of care, problems and complications, and rehabilitation. In some areas, however, particularly those dealing with controversial issues or in areas in which relatively little is known much of the available literature does little more than to state the problem exists, do a study, and quote some statistics. Each communication does to some degree in a small or greater way as the case may be, explore the problem in some depth and it is in this area that I propose to illustrate some of the theories on etiology, pathophysiology, pathogenesis, complications, and prevention (both natural and artificial) of Pseudomonas infection in the burned patient.

I THE PROBLEM

Purpose and limitations of the study

It was the purpose of this study to (1) show the incidence of infection in the burn injury with particular emphasis on infection with Pseudomonas aeruginosa; (2) the pathogenesis and pathophysiology of the infection; (3) the time in which such infection

may be expected; (4) the types of infection and pathology; (5) results of Pseudomonas infection; (6) minimal emphasis on treatment and prevention except on immunologic grounds in which the use of both natural and passive immunity will be discussed; (7) the relation of the burn toxin theory if any to the above; and (8) a very brief word on diagnosis.

This study was not intended to explore the entire field of burn injuries except as related theoretically to the specific topic of discussion. Of most importance are exclusions of generalized burn care such as the controversy of open versus closed treatment, treatment of shock phase (except when due to Pseudomonas), early or late excision of the eschar, or problems of aseptic care. This paper was likewise not intended to be a treatise on antibiotics although the problem of when to use such management and which antibiotic to use will be to a rather limited degree discussed when appropriate. Rehabilitation of the burned patient which is probably paramount in importance second only to the prevention of the burn in the first place is specifically excluded. As previously stated, statistics and other data of generalized interest to the student of the burn injury although admittedly beyond the intended scope of this paper will be included at the author's discretion.

Importance of the study

Without yet dwelling on the problem of specific instances of burn infections, it is generally held that infection is the major

cause of the lack of definite improvement in survival rates in burn injuries. The major difference seems to be that victims are now dying at a later time after their injury than was once the case. Progress in chemistry and immunology have eliminated many of the early deaths by use of transfusions of blood or its derivatives, correction of acid-base balance and fluid losses, and maintenance of the equilibrium once it is achieved. The problem of early Streptococcus sepsis was largely solved with the advent of the sulfa drugs and later with penicillin. More and more of burn victims are now surviving these once fatal complications only later to succumb to an infection which is resistant to any therapy in many cases. It appears that the solving of one problem has led to the development of another. The problem seems to dwell in the fact that the burned patient (as well as other chronically ill patients) is for some unknown degree more succeptible to infection by organisms which are usually known for their low virulence and lack of pathogenicity except when attacking these patients. In some degree, then, it is hoped that much of what can be learned about why this occurs can be usefully applied to others with chronic disease, for example the leukemias and cystic fibrosis.

II DEFINITIONS OF TERMS USED

Pseudomonas aeruginosa

This organism is lately being called simply Pseudomonas but earlier names were Bacillus pyocyaneus and Pseudomonas pyocyanea.

The specific species of aeruginosa is more important as a disease causing agent than that of pseudomallei. The organism was first isolated by Gessard in 1882 and was named Bacillus pyocaneus. It has been isolated from over 90% of sewage samples,[37] and from about eleven per cent of fecal samples. The bacterium is a small, slender gram negative rod which is usually straight (less often slightly curved) which measures one to two micra in length and about three tenths micra in width. It is motile, nonsporogenous, nonencapsulated, and stains readily with aniline dyes. Some strains will grow anerobically but best growth is obtained at normal atmospheric oxygen pressures. Optimum temperature is thirty seven degrees centigrade. Plain agar colonies are large, soft, smooth, and grayish. They are not pigmented but dye produced by the organisms diffused into the medium produces a bright green fluorescent color which becomes darker after several days and may impart a metallic sheen to the surface growth. Four per cent of the colonies produce no pigment. Gelatin is liquefied and a pellicle is produced when grown in liquid media. Hydrogen sulfide is produced as a by product of growth but urea is not split and indol not formed. In ordinary peptone base broth growth, acid without gas is formed from glucose but not from lactose or sucrose. A somewhat characteristic aromatic grapelike odor is produced by growing colonies. The organs affected by infection are many for a catalase, a proteinase and in some cases a creatinase are produced by growth. In addition several cytotoxic dyes are produced

by the growing organism. The genus is characterized by the production of a yellowish-green flourescent material which is water soluble. In addition, the genus aeruginosa produces a deep blue pigment, pyocyanine, which can be extracted from aqueous solutions by chloroform. Some strains produce an additional pigment, a red substance which is water but not chloroform soluble and is called pyorubin. Although the dyes are tissue toxic, it appears that the proteinase accounts for the liquification of body tissues in cases of infection.

Pseudomonas is killed by heating at a temperature of 55°C. for one hour but is somewhat more resistant to the usual chemical disinfectants than other gram-negative bacilli. It is likewise resistant to penicillin and the broad spectrum antibiotics but is often sensitive to the antibiotics colistin, Polymixin B, and sometimes neomycin. The various strains can be separated by their O and H antigens and there seems to be at least two serologic groups. There is a glucolipid complex which is toxic and antigenic.

Burn

In this paper a burn will be used as the expression of the reaction to and the production of an injury due to the undue exposure to heat, chemicals or electricity. Expressions of degrees of burns will be used according to standard definitions.

Initial or Primary Shock

Initial shock is that occurring in the immediate post burn period and up to two hours later. It is probably a generalized

vasomotor collapse of neurogenic origin and probably due to excitement and pain. The mortality rate is about 2.5%.

Secondary shock

Secondary shock is seen in the period of 2-48 hours after the injury. The cause is thought to be the result of a decreased blood volume because of the loss of "plasma" into the burned area. The result is an increased blood viscosity, hypovolemia, and reduction in blood flow. It is in this area that many of the recent advances in fluid and blood therapy have the most to offer. A s we will see later, some of the cases of secondary shock may in reality be septic shock in which the diagnosis was not made.

Toxemia

This term implies the absorption into the blood stream of poisonous substances from a variety of causes, whether it be from tissue necrosis, sepsis, or other reasons as the toxemias of pregnancy.

Acute Toxemia

This type of toxemia occurs roughly 6-100 hours after the burn injury and is probably due to the absorption into the circulation of the necrotic tissue which resulted from the burn. It is from this phenomena that the "burn toxin" theory developed.

Bacteremia

The term is broad and yet conservative and implies simply that viable bacteria are isolated from the blood. The term does not mean that any ill ness is produced, the organisms are pathogenic or that they are permanent.

Septicemia or Septic Shock

Septicemia implies that the organisms found in the blood are producing symptoms in the patient. The effect is often due to the toxins produced rather than the bacteria per se.

Pyemia or Septicopyemia

A condition which implies that the organisms in the blood are producing symptoms and are also initiating localized abscesses in the tissues of the host.

Infection

An infection is a pathologic presence of bacteria in a host with resultant inflamation and other reactions which differentiate the term and condition from only the mere presence of bacteria.

III SOURCES OF DATA AND TREATMENT OF FINDINGS

All data was secured by a rather exhaustive survey of the recent literature. In selected cases standard textbooks were used. Although in all cases the entire original study was surveyed, only that section which was germane to the purpose of this study is quoted. Every attempt was mad, however, to avoid inclusion of facts or figures which might be countradictory to the conclusions drawn by the original author except in a few cases to illustrate possible sources of error. All facts and figures will be credited to the original author unless it is believed that these represent common knowledge.

CHAPTER II

REPORT OF THE STUDY

A major portion of the following material concerning the present day concepts of burn infections will be dealt with in terms of progress in and concepts of host defense mechanisms and immunology and how these are altered by the burn injury and the treatment afforded. It is in this area that more and more work is being done and it appears that eventual success in prevention will be in a similar area.

HISTORICAL ASPECTS

Perhaps one of the most appropiate statements made in regard to burns was made by Lowbury[16] when he said that infection in burns presents a confused picture due to many factors. He felt that one of the most important of these was "our difficulty in telling which pathologic changes are caused by infection and which are caused by the initial trauma itself." Lowbury then sketches some of the early historical writings beginning with Fabricus (1607) who noted "ulcus profundum et putridum" after separation of the burn slough. In 1833 Dupuytren found suppuration, fever, wasting and death in patients with deep burns even when the injuries were not extensive. Bird in 1855 believed that these signs and symptoms were no different than those due to injuries of other types. Lister, who was one of the pioneers in antisepsis in surgery, apparently did not extend his new technique to burns perhaps

because he thought it was hopeless. Pack in 1926 noted that practically every burn becomes contaminated within a few hours, the commonest organisms being streptococci, staphylococci, and Pseudomonas aeruginosa. Aldrich (1933) and Cruickshank (1935) attributed the toxemia and fever often found on the second and third days after burning injuries to septic infection on the burned surface. Langohr (1947) reporting a detailed bacteriological study of the burned surface, considered hemolytic streptococci and staphylococci particularly damaging to deep burns, especially when present with the proteus group.

II INCIDENCE

As in all surveys of statistics the figures stating the incidence of infections in the burned patient and in particular, the relative incidence of Pseudomonas aeruginosa infections varies from source to source. The figures from different sources are, however, quite similar and a general trend can be predicted even if absolute values cannot.

Before a survey on morbidity is done, perhaps it would be germane to evaluate some mortality figures which seem to by their very nature be typical of many studies presently being conducted. Although many of the factors to be presented admittedly deviate from the topic of the present survey, it is felt that they should be included for the sake of completeness.

Some of the more recent work was done by Feller and Hendrix[6] in their clinical pathologic study done on sixty fatally burned

patients. Their study involved the selection of what they felt to be the cause of the death in these patients. The study involved a fifteen year time interval and was correlated with autopsy findings in sixty of the eighty five total deaths. It is interesting to note that 558 patients with burns were admitted to their center and that eighty five of them died, giving an overall mortality rate of 15.2%. This figure may deviate somewhat from others in that the study began in 1946 which is before some of the more advanced techniques in burn therapy had been developed.

Their study is felt to be typical, however, and is in part presented in Table I. From study of the table several factors immediately become apparent. We will first note that twenty of the sixty patients who succumbed did so because of or related to a burn involving less than forty per cent of their body area with a mean survival time of forty days and with a meanage of thirty nine years. The authors state that the average burned area was twenty one per cent of the body surface area. Likewise, forty or two thirds of the autopsy series but not of the total deaths died of injuries involving greater than forty per cent of the body area and with a mean area of sixty three per cent. In this series the mean age was thirty four years and the mean survival time was fourteen days. Of the total series the mean age was thirty six years and the mean survival time was twenty three days.

On closer examination of Table I several other factors become apparent. Although the series was small and the figures probably

not statixtically significant it is apparent that a greater percentage of the total deaths appeared in the 20-29%, 40-49%, and the 60-69% groups. This should not be misleading for no figures are given as to the case fatality ratio nor to the relative incidence of these groups. The table would obviously become more meaningful if these figures were included but since they were not we must assume that this is an artifact and be very careful in our evaluation. It would probably be safe to assume that a greater percentage of the mortality would be ascribed to burns involving greater than sixty per cent of the body surface but is not that already a commonly well known fact? These burns of this magnitude are less frequent than some of the others. Probably the greatest thing to be learned from the evaluation of the table is that deaths may occur irrespective of the area burned. Note that two of the deaths involved less than a ten per cent burned area! Further comment is not necessary.

The data concerning the patients ages and survival times must be likewise be viewed with a certain amount of caution. These figures are given as means or averages and perhaps would be more significant if the modal age and the range were given with similar treatment for the survival times. We can probably safely assume that the two patients who died with less than ten per cent surface burns were probably very young and that they died relatively quickly, for the greatest survival time possible with these figures is fourteen days for one patient and less than one day for the other.

TABLE I (After Feller and Hendrix[6])

MORTALITY IN BURNED PATIENTS

% Burn	No. Patients	Mean age, yrs.	Mean survival time, days
1-9	2	3	7
10-19	5	55	27
20-29	10	37	54
30-39	3	40	40
Subtotals	20	39	40
40-49	11	31	19
50-59	5	23	15
60-69	13	32	8
70-79	5	38	13
80-89	3	34	26
90-100	5	53	7
Subtotals	40	34	14
Totals	60	36	23

All of the data in Table I will assume greater significance when the material is presented again and its relation to the problem of infection is shown. Suffice it to say now that a definite correlation can be shown between whether infection occurs, type of infection, the organisms responsible, and the expected survival time as well as the timing of the infection. All relate to the percentage of burned area, the age of the patient, and the mean (or modal) survival time.

The study perhaps assumes a greater significance when the authors investigate the autopsies investigating the causes of death in these sixty patients. This data is given in Table II. Again no figures are given relating to the relative incidence of these complications to the total number of patients burned, only to causes of death. Likewise because of the smallness of the series many of the figures must be viewed with caution, but infection as a cause of death stands out. It is apparent that thirty seven or nearly two thirds of the sixty autopsied deaths died of this cause. Septicemia, with or without pneumonia, accounted for twenty eight or seventy five per cent of these deaths and represented forty seven per cent incidence in the total series.

Of the fourteen patients who died of septicemia alone, the diagnosis was made in the antemortem period by a high fever, leukocytosis, abdominal distention, tachypnea, terminal oliguria, and hypotension. Eight of eleven blood cultures drawn in this period were positive for the offending organism. It must be

remembered that in any autopsy series that a high incidence of positive blood cultures are obtained presumably because lack of integrety of the intestinal wall which occurs in the agonal period allows intestinal organisms to enter the blood stream. This is documented by the fact that gram negative organisms were found alone or in combination with other organisms in all of the fourteen latter series.

The findings of Feller and Hendrix were in a large degree substantiated by Kefalides.[12] He did not accept post mortem positive blood cultures as indicative of septicemia. The diagnosis of systemic infection was made in sixty seven per cent of their series of 454 patients. Pseudomonas aeruginosa was found to be the causative organism in seventy one per cent while Staphylococcus aureus was found in only eleven per cent. It must be pointed out that the patients were all children and the authors felt that the incidence of septicemia and in particular septicemia due to Pseudomonas was higher in this age group. Feller and Hendrix included patients of all age groups and their incidence of Pseudomonas infection was about forty per cent. In a subsequent article[13] Kefalides found the incidence of fatal Pseudomonas septicemia to be seventy five per cent of all septicemias. In an attempt to determine the source of the fatal infections he did cultures on skin wounds, blood, pharynx, stool, and urine on admission to the hospital and every other day for two weeks thereafter. His results are shown in Table III. It must not be construed that the results

TABLE II (After Feller and Hendrix[7])

CAUSES OF DEATH IN 60 BURNED PATIENTS

	Number	
Factors related to the initial trauma		5
Primary pulmonary irritants	4	
Hypovolemic shock	1	
Infection		37
Septicemia	14	
Septicemia with thrombosis of sigmoid sinus	1	
Septicemia with pneumonia	14	
Pneumonia with septicemia	1	
Pneumonia	7	
Pulmonary arterial emboli		6
Alone	2	
With septicemia	2	
With myocardial infarction	1	
With pneumonia	1	
Adrenal Insufficiency		1
Pre-existing disease		6
Cor pulmonale	1	
Arteriosclerotic heart disease	2	
Arteriosclerotic heart disease with septicemia	1	
Cerebral infarction	1	
Cerebral infarction with septicemia	1	
Iatrogenic factors		5
Transfusion reaction	1	
Transfusion reaction plus septicemia	1	
Sulfonamide sensitivity	1	
Congestive failure--excess fluid replacement	2	
Total		60

are indicative of infection but only of the presence of bacteria in or on these sources. The entire table is presented for the sake of completeness.

Several facts are brought into focus from observation of the table. The staphylococcus was overall the most frequent organism isolated and it appeared most often in the skin, pharynx, and stool. This is to be expected for this is the normal habitat of this organism. Pseudomonas, on the other hand, was found in approximately the same frequency in the stool and on the skin but less commonly in the partly enclosed pharynx. It was by far the most frequently encountered organism in the blood stream with about four times the incidence of the staphylococcus. The skin colonization by both organisms increased in frequency in direct proportion to the amount of skin burned. Lowbury[18] who noted a similar frequency of Pseudomonas infection attributed in part the predominance of this organism in the changing hospital environment. He felt that the large, open areas of injury as seen in a burn were succeptible to contamination from normal hospital organisms. As we shall see later, he was in all probability only partially correct.

Moreover, the problem of Pseudomonas sepsis is also seemingly increasing more rapidly than was once the case. Graber[11] in a survey done in a U.S. Army Surgical Research Unit pointed out that this organism was responsible for 47% of cases of septicemia in 1953-1954 but was responsible for about 65% of the cases in 1959. The staphylococcus declined from 58% to 14% over the same period of time.

TABLE III (Kefalides[14])

DISTRIBUTION OF MICROORGANISMS ISOLATED FROM 3940 CULTURES IN SEVERELY BURNED CHILDREN

	Urine	Blood	Skin	Pharynx	Stool	Total	
#	310	1600	833	557	640	No.	%
Organisms							
1.	17	33	671	311	223	1255	31.5%
2.	0	0	210	93	76	379	9.6%
3.	1	135	474	37	202	849	21.5%
4.	27	20	183	25	273	528	13.4%
5.	0	5	110	390	0	595	15.1%
6.	0	12	0	0	6	19	0.5%
7.	0	10	61	0	28	99	2.5%
8.	0	0	0	0	9	9	0.2%
9.	0	0	0	0	60	60	1.5%

KEY TO ORGANISMS

1. Staphylococcus aureus (coagulase positive)
2. Micrococcus species (coagulase negative)
3. Pseudomonas aeruginosa
4. Proteus
5. Streptococcus group
6. Salmonella group D
7. Providence group
8. Shigella group B
9. Escherichia freundii

III TYPES OF INFECTION AND PATHOLOGY

Although septicemia is given much discussion all surveyed literature it becomes apparent that this is not the only manifestation of Pseudomonas infection. In table III it was noted that this organism was isolated very frequently from the skin where it causes varying degrees of manifestations with resultant complications. Artz[1] believes that systemic infections begin with the localized skin infections. These are seen most commonly in second degree burns that become contaminated from the outside (the environment) and in third degree burns that have built up a good fibrin barrier against the invasion of bacteria. All granulating wounds are colonized by bacteria but the granulation itself usually prevents invasion into deeper structures. This type of infection is important in that it may interfere with the growth of remaining viable epithelium and thus cause a split thickness injury (second degree) to be converted to a full thickness or third degree loss.

An advanced degree of localized skin infection which is locally invasive is evidence by the same signs and symptoms as a similar infection in a non-burned patient. Cellulitis, lymphangitis, regional lymphadenopathy, fever, and leukocytosis are usually present. This infection may become a generalized septicemia by methods to be revealed later.

The pathology of such skin lesions takes various forms and results in varying degrees of complications. Rabin and his associates describe some of these at length and the study was limited to

those caused by Pseudomonas aeruginosa.[27] At autopsy all of their patients had varying degrees of infections in their burn wounds. Those of which that were extensive had their burned surfaces transformed into black, friable membranes. This involvement developed in stages and the progression could easily be followed. After the eschar separated foci of necrotic debris remained and these foci gradually enlarged and became covered by a black crust with intervening areas of green tinted fat necrosis. This complete transformation of the wound into a diffuse, black membrane often occurred between twenty four and forty eight hours before death. A section through the area would reveal nodular hemorrhagic necrosis extending several centimeters into the underlying subcutaneous tissue.

In some of the patients circumscribed hemorrhagic nodules in surrounding areas of non burned skin were found although some were found in remote areas. Their size varied from 1-4 centimeters. In their early stages they presented as vessicles surrounded by erythema. They later became hemorrhagic and demonstrated central ulceration. Pure culture of Pseudomonas aeruginosa was isolated from all of them.

Septicemia, although previously alluded to, has several complications in its own right, not the least of which is death in a high percentage of cases. The mechanisms of death are not known in some of the cases. In other cases the patients develop abscesses in vital organs following blood stream invasion by the organism.

Hemorrhagic nodules similar to those found on the skin may be found in the viscera, heart, lungs, kidneys, intestines, and meninges. All portray a similar microscopic picture in that they contain large areas of necrotic tissue with varying degrees of hemorrhage and scattered infiltrates of polymorphonuclear leukocytes and mononuclear cells. Intense numbers of gram negative bacilli were present throughout the lesions. They were most densely packed in the walls of small arteries with necrosis of the vessel wall and thrombotic occlusion. The significance of this latter fact is obvious in that the vessel necrosis will cause further tissue necrosis thus promoting the advance of the infection. Some of the patients also had destructive endocardial lesions involving the tricuspid and aortic heart valves. Pure cultures of Pseudomonas was grown from these lesions also.

CHAPTER III

ALTERATIONS IN HOST DEFENSE

It has been established that infection is a frequent complication of burn injury and is becoming increasingly more so. Several questions remain to be answered not the least of which is why this is so. Another relates to the increasing frequency of Pseudomonas. It is not known why this organism affects the burned, the chronically ill, the leukemic, or the patient with cystic fibrosis. The answer to these questions remains yet unsolved but there must be some disturbace in the defensive structure of such a patient. Other factors, of course, are the increasing use of prophylactic antibiotics which allows the emergence of Pseudomonas and other bacteria which are resistant to most of the commonly used agents. Some element of the former must be combined with the latter and it is with this former problem that therefore the following discussion will be centered. A section on pathogenesis and sequence will precede that on defense mechanisms so that the latter may explain and correlate the step-like sequence.

I PATHOGENESIS AND SEQUENCE OF INFECTION

If the preceding discussion on pathology is kept in mind, we may now digress to a discussion which will, in a small way, be repetitious, but will be founded on the alterations of the defense and their changes following the burn injury.

Much of the work in pathogenesis is being done in the Brooke Army Medical Center by Teplitz and his fellow workers including Moncrief.[32-34] They likewise find that Pseudomonas sepsis is a leading cause of death in their work. They point out that septicemia in burns is commonly thought of in terms of blood stream invasion (bacterial) with resultant hematogenously disseminated visceral and skin lesions---in association with leukopenia and clinical hypothermia---but that this is not the disease proper but is rather the end stage systemic manifestations of an overwhelming local or primary infection. Most cases, then, are cases of Pseudomonas burn wound sepsis resulting from the invasion of the nonburned hypodermal tissue subjacent to the colonized burn wound. To substantiate their theories, they performed experimental work on burned rats which in so far as they could predict paralleled the sequence in humans. The burn wounds were then contaminated with cultures of viable Pseudomonas organisms. Quantitative blood, heart, and tissue (burned and unburned) cultures were obtained at various intervals. Their data is summarized on Table IV. Of interest was the fact that soon after seeding in almost all instances staphylococci spontaneously colonized the wounds, similar timing was noted on nonseeded controls.

Their feeling from the results of this work clearly indicate that they believe that the pathogenic sequence of Pseudomonas sepsis in the rats was one of burn wound colonization followed by invasion into the subjacent viable tissue. Although the experiment

was admittedly somewhat artificial, they point out that their colony counts seem to be parallel to those found in similarly burned humans. Intrafollicular (hair) bacillary localization was felt to be an important aspect of the initial colonization stage and is again similar to that seen in humans. In contrast to other similar workers who make a point of bacteremia in early stages, these men feel that bacteremia occurring as early as day three when there was no evidence of wound invasion was a problem of sampling more than any other factor. At least in the early stages it was felt that the animals showing diseases were doing so because of primarily a toxemia rather than a septicemia. In later stages of the disease (days 7 & 8) the true nature of the septicemia was found and was found to correlate with the hematogenous findings and metastatic visceral findings. Some of the rats, however, died of the toxemia before septicemia occurred and thus lacked the characteristic visceral findings which were similar to those previously described.

Although not previously alluded to, there are certain findings present in rats and humans which are somewhat characteristic. Since in the early stages of the disease process there is no valid clinical finding or test for the detection of invasion of the tissue subjacent to the colonized eschar, the clinician is usually first confronted with signs and symptoms of the last stages of the disease, and thus frequently tends to think of the total disease entity in terms of bacteremia, hypothermia and leukopenia. Other findings included are the toxic arrest of the bone marrow which

TABLE IV

SEQUENCE OF PSEUDOMONAS BURN WOUND SEPSIS
(Summarized from data of Teplitz[32])

DAYS	FINDINGS
9 hours	Uniformity of bacterial count at 10^{4-7}, bacteria penetrated beneath the wound surface and found in hair follicles.
15 hours	Counts ranged from 10^{5-7} and all sections showed deep intrafollicular localization of bacilli.
24 hours	Surface counts showed 10^{6-8} organisms per cc. Intraeschar bacillary localization around and within hair follicles.
48 hours	Surface counts showed 10^{7-8} organisms per cc. No great change on microscopic exam. There were staphylococci in the unseeded controls.
3 days	Increased depth of intraeschar colonization was present and some of the animals had positive blood cultures.
4 days	Bacteria invaded to superficial muscle underlying the subcutaneous fat and bordering the viable tissue zone. All blood cultures were now positive.
5 days	Bacterial invasion was down to the zone of viable granulation tissue which was formed at 36 hours. Low grade bacteremia was present as on day four.
6 days	No great change in tissue pathology. Some animals now showed significant bacteremia and positive splenic cultures.
7 days	Invasion by bacteria through the granulation tissue layer was now present. All animals now had significant bacteremia and splenic trapping.
8 days	The findings were essentially the same but with a much greater degree of invasion of underlying normal tissue. Blood cultures were positive and positive cultures were obtained from liver, kidney, and lungs. Marked adrenal hyperplasia was now present.

explains the leukopenia, enlarged adrenals showing lipoid depletion in the absence of pseudotubular formation felt to be so characteristic of sepsis, and the absence of the significant pulmonary edema and congestion often found in staphylococcal septicemia. Likewise, Pseudomonas vasculitis was felt to be due to perivascular invasion rather than from direct intimal seeding as formerly thought.

In a subsequent article[33] the same workers again point out that signs of septicemia are often the first objective findings of the clinician. This coincided with the fact that the junction of the viable hypodermis and burn eschar is a circumscribed, congested, edematous area which might allow greater trapping and proliferation of hematogenously disseminated bacteria. There also frequently are a few large areas of third degree burn becoming infected with bacteria within a short period of time, shortly after the onset of bacteremia, and whole areas of partial-thickness burn sometimes appear to convert overnight into full thickness wounds owing to massive diffuse Pseudomonas invasion. All of these things piercingly point out that hematogenous dissemination to the burn wound may occur after the establishment of Pseudomonas bacteremia.

They found that 35 day old burn wounds and normal granulating skin were not succeptible to colonization by intravenous innoculated organisms. After innoculation of recently burned rats, they found that colonization of the burn wound did exist. It was then assumed that the edema and disrupted capillary bed of the hypodermal burn wound junction may contribute to increased trapping and proliferation

of hematogenously disseminated bacteria. There also frequently were found small, thrombosed vessels and small abscesses in the area. They felt that the variables included bacterial count in the blood stream, the amount of superimposed stress and the age of the burn wound. For several reasons they felt that hematogenous dissemination and infection of the burn wound seldom if ever occurred.

II MECHANICAL ALTERATIONS

Because of the nature of the wound there is an ideal situation for infection in third degree burns. Beneath the dead tissue and in the hair follicles are many viable bacteria. Thrombosis and edema in the subcutaneous tissue decrease the blood supply and defense mechanisms of the host have difficulty reaching the area where the bacteria persist. It seems natural, therefore, that all third degree burns would become infected because the abundance of nutritive material in the area provide ideal media for the bacteria.

At one time it was thought that full thickness burns were sterile initially and that they became colonized by bacteria only when inadvertant contamination occurred in the course of treatment. It is probably true, though, that immediately after the burn the wound is sterile but viable bacteria persist in the deeper tissues. They then grow rapidly in a manner to be described later and soon reach a concentration of 100,000,000 organisms per square centimeter. In all probability the most important protection against infection

that the body possesses, the skin, is the very thing that is destroyed by the injury. Once this important barrier is eliminated, a second source of infection is contamination of the wound from outside. This element is present in both partial and full thickness burns and is brought about by the access of the bacteria from unsterile dressings, unsterile instruments, or from the respiratory tract of the patient and of the attendants. The breakdown of the skin is the most important loss of the body defense mechanisms leading to increased susceptibility of infection.

This vital aspect is particularly important when there is full thickness necrosis of the skin. The effect of antibiotics when use here is only temporary and incomplete at best. The element of delay required for spontaneous separation of the eschar affords the opportunity for infection to advance under cover. It has been well emphasized by Langohr, Owen, and Cope[16] that such wounds become a quagmire of infection after the third week post burn. Therefore, with the passage of time the contaminating bacteria within the burn slough have a chance to multiply and the longer the delay after injury, the greater the bacterial proliferation. This period of delay also constitutes for many patients a dangerous interval of nutritional dependency and they may fare poorly in the hands of the disinterested or detached surgeon.

III METABOLIC ALTERATIONS.

Benson and his associates[14] describe certain metabolic alterations which occur in the burned patient (also in many other types)

or which seemingly too frequently are not only ignored by physicians in general but also by most authors who describe essentials of good care for this type of patient. Particular emphasis is given to the chronic burn patient who also may be chronically infected. They point out that many physicians become disinterested in their burn patients after the acute period is over and thus allow them to decline to the chronic state.

The metabolic alterations in this type of patient differ in many important aspects from the recently burned patient. In the acute post-burn period the degree of negative nitrogen balance is apparently roughly proportional to the severity of the burn. Although it may be altered somewhat by dietary pattern, reversal to positive balance is said to be rarely possible in less than a thirty day period. This is usually reflected by a steady loss of body weight which may be as much as a pound a day or more. The serum proteins are altered with decrease in serum albumin and elevation of the globulins, particularly the gamma fraction. These protein manifestations are consequences of extensive tissue catabolism, urinary losses, and wound exudate composition. As much as 49 grams of protein per day may be lost in exudate from early wounds.[4] This is decreased as the wound gradually closes. Among other metabolic changes are elevation of the BMR, electrolyte abnormalities with chronic urinary potassium loss, impaired hematopoesis, and anemia.

The author's concept of the metabolic alterations of the depleted post-burn state is that it represents adaption to chronic starvation.

As previously noted, the circulating plasma proteins reflect the hypovolemia of these starved state patients. Although the electrolytes are normal in the serum (often), the body stores are depleted. The factors leading to the debility are excessive undiminishing losses of protein, hemoglobin, fluids, and electrolytes from the wounds. Chronic sepsis augments these losses and obviously impairs the ability to fight the sepsis, the cycle occurring in a vicious circle.

IV MECHANICAL ALTERATIONS

There are many factors to be considered here, some of which are hypothetical, some of which are obvious, and some of which although well known are poorly understood. For the present we will exclude immunology per se from the discussion although indeed it is involved in the following mechanisms of defense.

Although it has been previously mentioned that the burned animal is more prone to develop infection in his wound than animals subjected to other types of injuries, no statement has yet been made about the ability to resist infection in general by these burned animals. Liedberg[17] is one of many authors to investigate this problem. In his experiments he subjected guinea pigs to the intraperitoneal injection of both killed and viable cultures of Pseudomonas aeruginosa organisms. One half of each group was then subjected to a 11-14 per cent burn while the remainder served as a control group. He found the difference in fatality rates between burned and unburned (control) animals to be highly significant.

The intraperitoneal injection of killed organisms affected the mortality rate adversely but to a much less degree than the injection of live bacteria. He also found that septicemia seemed to be found more often in burned than in unburned animals.

Liedberg felt that the bacteria invaded the blood stream prior to death as indicated by abscesses similar to those previously described which occurred as early as the fortieth hour. In some way the antibacterial barriers seemed to be more easily broken down as is evidenced by the increased incidence of septicemia. Liedgerg felt that one of the most important barriers was the reticulo-endothelial system and that this system was in some way made defective by the burn injury, possibly by the shock. It was also speculated that the bacterialcidal action of the blood was also interfered with. More about this latter statement will be discussed later.

McRipley and Garrison[20] performed experimental work similar to that of Liedberg. Their work was somewhat more complete in that the organisms were injected intraperitoneally as were Liedberg's, but also subcutaneously in both the burned and the non-burned areas. The results of the work was very similar. Rats injected two minutes after burning, intraperitoneally or subcutaneously in the burned or non-burned area, were extremely susceptible to Pseudomonas infection. It was found that the LD_{50} for such animals was less than ten organisms, compared to 2.0×10^9 for the subcutaneous injection in the non-burned controls and a similar amount per other routes.

The increased susceptibility to the subcutaneously injected organisms was still present at 24 hours but gradually returned to normal by the 15'th day. In the group of rats injected subcutaneously in a non-burned area the LD_{50} was found to be 10^6 greater than the LD_{50} for the burned area. Also of note was the fact that they found that within a twenty four hour period after the injury the intraperitoneal LD_{50} was about half that of the non-burned control.

A somewhat different conclusion than that of Liedberg was made, however. They felt that this increased susceptibility of the rats to Pseudomonas infection was probably secondary to the burn shock and trauma. They attached equal importance to the effect of the shock on the capillary permeability which has been shown to be increased in the burned and the non-burned areas as well. This effect would allow the bacteria to reach tissues which are normally protected by the impermeability of blood vessels to the passage of bacteria. Muscle and brain, which were shown to harbor the organism, along with the burned area were thought to be possible reservoirs of bacteria and that they could reproduce in these areas until sufficient in number to kill the animal.

Although surveying the problem in a somewhat different fashion and evaluating other factors involved in the resistance to infection, Balch[3] produced one of the more complete studies on the resistance to infection in burned patients. Its very completeness demands that full evaluation be given it. The purpose of his study was to determine whether burned humans were more susceptible to invasive

infection because of an associated impairment of the natural antibacterial defense system. Although he agrees that the basic defect is the destruction of the protective epithelial barrier, he attempts to evaluate the specific and non specific antibacterial substances in plasma and tissue fluid and the defenses of the cellular substances consisting of fixed and wandering phagocytes. In agreeing with the purpose of this paper, Balch points out that the basic defect in antibacterial defense is the inability of the patient to prevent bacterial invasion from the wound site. The blood bacteriocidal capacity was found to be greater than normal in the acutely burned patient but the peripheral cellular response to the injury is impaired.

Balch evaluated four components of antibacterial defense as measured in burned patients:[3]

> "1. Leukocyte-plasma bactericidal effect. This is the ability of leukocytes suspended in autologous plasma to destroy two species of Staphylococcus (including aureus) and E. coli, and Pseudomonas aeruginosa.
>
> 2. Plasma bactericidal effect. This is as above except that the leukocytes are not included into the system.
>
> 3. Serum hemolytic complement. This refers to the capacity of human serum to hemolyse a suspension of sheep red blood cells sensitized with its specific antibody. This is an indirect measurement of serum complement. Complement participates in bacterial antigen-antibody response and reaction especially those involving gram negative bacilli.
>
> 4. The degree andtype of leukocyte and tissue macrophage migration in response to superficial skin trauma at a site away from the burned area. The method used . . . consisted in scraping a 0.5 x 0.5 cm. segment of epidermis with a scalpel until the dermis was exposed. Sterile cover slips are sucessively applied to the site at hourly intervals, thus obtaining an impression smear of the changing cellular exudate.

> This is stained by Wright's method and examined microscopically. A killed suspension of the Staph. albus used in the bactericidal tests is dropped on the wound before applying the first cover slip. In this way an estimate can be made of the phagocytic capacity of the migrating cells."

Standardization of the testing procedure was accomplished in so far as possible by evaluating the white cell blood count, blood sugar and pH and other factors as well as the total bacterial count per milligram of wound exudate from prescribed representative areas of the burn wound.

Although not indicative of the total results of the experiment the results in one of the patients revealed that in the early post-burn period (roughly a week) blood cultures were negative when the blood bactericidal capacity was high even though the cell response to peripheral injury was low. Gram negative bacilli then appeared in the blood stream and this was associated with a rise in the wound bacterial count. The cell response to the superficial injury was below normal and the plasma bactericidal capacity (not the leukocyte-plasma combination) against Staph. species and possibly against Pseudomonas aeruginosa had fallen to zero although still effective against E. coli. On the other hand, Staphylococcus aureus appeared in the blood stream when the plasma bactericidal capacity against that species had fallen to zero and the leukocyte plasma capacity had dropped markedly to the normal range. At that time the cell response to injury had improved toward normal.

Balch felt that one of his most important findings was the markedly diminished cell response to superficial skin trauma in

the early days following burn injury. There was a marked delay in the appearance of lymphocytes and of lymphocytogenous macrophages in the exudates and an absence of eosinophils. The neutrophils and mononuclear cells were markedly phagocytic. The peripheral blood white cell counts are elevated during the period of diminished cell response in the skin but there is a neutrophilia and a lymphopenia which does not return to normal as rapidly as does the skin cell response. The failure of total cell response in the test wounds was felt to be possibly due to inadequate peripheral circulation and probably reflected a similar effect in the burn wound. The absence of the lymphocytes and eosinophils in the wound may have been due to a stress response and likewise the increased plasma bactericidal effect against the Staph. species might have been related to the disolution of the lymphocytes due to the special steroid stress response seen in a burn. Although the significance of all of these factors was uncertain, it was felt that in addition to theirphagocytic function, the lymphocytes had enzymes which assisted in the breakdown of proteins and in the dissolution of fibrin and participate in the localization of the infection. Lymphocytes also manufacture and probably release antibodies during an infectious episode.

In essence it seemed that the appearance of bacteria in the blood stream is not due to impaired central humero-cellular antibacterial defenses but rather to an unfavorable ratio between the defensive cells and bacteria in the peripheral tissue. The

trapping capacity of the reticulo-endothelial system was seldom if ever exceded, even in states of shock. (Note the contrast to McRipley, Garrison and Liedberg) In reference to the work of Liedberg, Balch notes the similar paucity of mononuclear cells in response to the injection of Pseudomonas but an effect on plasma bactericidal capacity could not be shown. The point was that caution should be used when correlating the findings of guinea pigs with that of humans. Nothing was suggested in the study to justify the theory that there is an impaired antibody response for no reason could be given to suggest that there is interference with antibody synthesis.

This problem of impaired body defenses at the peripheral level is presented in a somewhat hypothetical yet practical manner by Knisely.[15] He presented his arguments by elaborating on two major hypotheses or propositions:

> "1. A part of the increased susceptibility to the development of severe infections probably is a direct result of the enforced postponement of the selective ingestion of bacteria by phagocytic cells.
>
> 2. The fluids now necessarily given to the postburn patients to save their lives during the post burn circulatory shock may be postponing or preventing bacteria from entering the phagocytic cells."

Knisely apparently feels very strongly that this enforced postponement of phagocytosis is very definitely a part of the increased susceptibility to the development of severe infections following burn injuries.

The arguments to support these propositions are presented in a very logical sequential manner. It is pointed out as we all know that a single bacterium is produced as a member of a pair by the division of pre-existing organisms. Such an organism may then "end" its individual existence by dividing into two others, by being dissolved or lysed within the host's body fluids, or by being ingested by one or another of several types of phagocytic cells. It is obvious, then, that the length of life of such an organism is dependant upon the time involved before the organism either divides into two more organisms or is subsequently eliminated by one or more of the body defense mechanisms. This is a very important foundation for Knisely's arguments and should be kept in mind while evaluating his subsequential phrases. It is also the basis for criticism in that it implies that either one or the other occurrences takes place. There is no allowance made for those bacteria which remain "static" or those in which one member of the pair survives and the other does not.

Omitting such factors as "chemotaxis," it is than pointed out that in order that the one single bacterium enter one phagocyte, the bacterium must touch that phagocyte and the phagocyte must be in a physiological state to receive it. The transport problem is then introduced; either the bacterium has to go to the phagocyte, or vice versa, or each one moves and they meet by accident. Obviously, then, any thing which prolongs this transport time involved before the bacteria can be ingested increases the

time during which the organism can deplete the hosts nutrition, pour out endo or exotoxins or divide. Relating to the actual phagocytosis, it appears that optimum conditions must be met or there will be no effective phagocytosis including optimal pH, optimal histo-chemical conditions, and apparently the effective coating of the bacteria by opsonins.

As has already been made apparent, following the severe burn, bacteria rapidly multiply within or under the burned skin and the subcutaneous tissue. Sooner or later, they begin to invade the vascular system; and here the information being discussed becomes more useful. As we have already seen in the previously described guinea pig and rat experiments, the body effectively removes bacteria from the blood when it is healthy, hence the transport time must be relatively rapid if we are to accept Knisely's theories. In all of the experiments we have described to this point, the bacteria have been found in the phagocytes of the spleen, liver, and bone marrow. After suitable staining procedures a pink, clear staining zone can be seen surrounding each bacteria within the phagocyte and this is presumed to be the opsonizing material which reportedly aides in the phagocytic process.

Probably not all of the bacteria injected into the living vascular system of a healthy animal are transplanted without delay to the phagocytes of the spleen, liver and bone marrow. There are a number of places in which they could become lodged temporarily and later be released. Some of these places are terminal pulmonary

arterioles and small vessels in the striated muscles. It is pointed out that in the case of the latter, the small vessels are contracted during the times of relaxation of the muscle involved and if the bacteria should arrive to the muscle during such a time, they might be expected to remain there for varying lengths of time only to be released again whenever activity of the muscles is restored.

Several types of evidence indicate that following severe burns in animals and humans the rates of blood flow are sharply decreased and the transport times of bacteria in the blood are then correspondingly prolonged. It is pointed out that blood flowing through small vessels burned by radiant heat immediately begins to precipitate and agglutinate. In more severe burns the whole circulating blood agglutinates and thus slows its rate of flow. This is the "post-burn sludge" so frequently spoken of. As the body attempts to pump the stiff sludge through vessels which are often open and dilated from the very nature of the wound, the walls of small blood vessels do not get enough oxygen and dilate more and leak blood and plasma and thus become impacted with this sludge. This often becomes so severe that there is a prompt, severe and sustained fall in cardiac output of about 66% which may appear before there is a fall in blood volume. The mean circulation time may increase to 60% of the pre-burn value. These changes may occur by the end of the first or second hour. The effect on the patients well being is quite apparent.

It was subsequently found by other workers that a severe fall in cardiac output is accompanied by a marked decrease in the rate of blood flow through the liver which amounted to 44%. This is significant in that it is apparent that the Kupffer cells cannot remove bacteria from the blood at a rate faster than it is brought to them, thus prolong further the transport time. This is in agreement with the previously cited work by Artz who showed the rate of clearance of bacteria from the blood was prolonged by a factor of two to two and one half. In addition to the prolonged rate of return to the liver of the blood, temporary or permanent plugging of the small vessels within this phagocytic organ may occur by embolization of the sludge. In effect, part of these organs are amputated.

If this wasn't enough, the same mechanisms of "sludging" affect the peripheral phagocytes as well, as evidenced by Ball who was previously quoted. Normally some kinds of bacteria within the circulating blood can under some conditions be ingested by suspended circulating leukocytes. This must normally be somewhat erratic, however, for little is known about the forces of attraction or other things which cause this to occur. In the absence of any "special" forces the laws of probability state that such "chance" contact must be rare. Normally white cells do not stick to the linings of vessel walls, particularly venule walls. If they do, they will remove some of the foreign particulate matter from the blood passing them. It is not certain whether following

severe burns white cells attach to the linings of small venules and, if so, whether they remove bacteria from the circulating blood and what the transport and contact times may be.

When erythrocytes are agglutinated, the rates of blood flow become forcibly reduced. White cells have a lower physical density than red cells and they tend to float above the layer of red cells under such conditions. When a stream of thus horizontally layered blood cells is forced against a fork in a vessel, one branch of which proceeds mostly downward and the other mostly upward, a higher percentage of the white cells go upward, likewise, a higher percentage of the red cells go downward. There may be further separation of the cells at subsequent branching. It also appears that sometimes masses and clots of agglutinated blood cells settle to the lower sides of vessels and remain stationary for fairly long periods of time which may extend to hours. Sometimes they cement together, forming thrombi. It is plain that pathologic organisms nestled between masses of blood cells on the lower sides of such vessels are not being carried to the phagocytes of the spleen, bone marrow, and liver and are often quite isolated from even fairly high concentrations of white blood cells high in the horizontally layered flow system above the settled material. This has been documented in autopsy series but the frequency, location, time of occurrence or duration is not known. A similar occurrence apparently takes place in the lymphatics further complicating the problem.

Although previously mentioned, the factor of the rate of multiplication of the organism is now about to be emphasized. Depending upon whether they are inhibited in some way by the environment, bacterial colonies after the initial lag phase, grow at an almost uninhibited rate known as the logarithmic phase. This rate excluding environmental factors is different for each organism. Among the more rapidly growing bacteria are those of the coli-aerogenes group with a generation time of less than twenty minutes, Staphylococcus and Streptococcus 25-35 minutes, and Pseudomonas with 30-40 minutes. The slowest of all is the tubercle bacillus whose time is extended to 24 hours. This factor assumes rather astronomical proportions if we take, for example, an organism with a generation time of 30 minutes and prolong its transport time to three hours, the original organism could become 2^6 or 64 organisms. If we prolong the time to 24 hours and the organism were free to multiply ad lib, which is not really the case, the organism would become 2^{48} or 281,474,976,710,656!!! While it would be erroneous to take it for granted that such things of such great proportions happen unimpeded, the principle is nevertheless sound and is probably a sound basis for the problem of infection. Of equal importance, these theories of Knisely do not, for the most part, contradict those of most other workers and, in fact, is formed on principles established by some of them.

Knisely's theory that intravenous therapeutic fluids may interfere with, retard, postpone, or prevent phagocytosis of

of pathologic organisms is somewhat startling. Although the burned patient may have his life saved with the intravenous fluids such as saline, glucose, plasma expanders, plasma, or even whole blood and that after the prevention of the circulatory shock, the patient rapidly develops an overwhelming infection and bacteremia. Knisely feels that there may be a cause and effect relationship here. It is imaginable that the injection of saline, glucose, or other solutions may dilute the plasma of the patient to the point where the opsonizing proteins cannot precipitate from the plasma on to the surface of bacteria in amounts adequate to give the surface of one organism or each organism a complete opsonizing coating.

It may also be that the solutions given in the postburn period may alter the surfaces of phagocytes in such a fashion that the phagocyte surface cannot play its part in the ingestion process. This may be due to the alteration of surface tensions or other unknown factors thought to be present as part of the process of phagocytosis. Whether this in fact occurs or not is not at the present time known with any degree of certainty, but it makes for interesting discussion and is thought provoking. It will be remembered that Kefalides[14] noted a fall in gamma globulins in the first week after the burn which may be due to the accumulation of plasma components in the interstitial spaces around the burn and made worse by fluid therapy.

V IMMUNOLOGIC ASPECTS OF INFECTION

Up to this point the center of discussion has been concerned with what may be called chiefly the mechanical aspects of Pseudomonas infection in the burned patient. It has become apparent that there must be other elements involved, namely an alteration in the immunologic make up of the burned patient. Immunology is at least indirectly involved with any discussion of infection, mechanical or otherwise. Whether alterations of the body's immunology per se has any bearing on the aspect of infection whether to increase the chances or to decrease them, will now be discussed. If there are alterations in a particular aspect, for example gamma globulin, the therapeutic uses if any will be discussed whether in a preventative, curative, or combined approach. The sequence of the following discussion is somewhat arbitrarily arranged but such an approach is necessary for in this area much progress is being made and it is becoming apparent that much inter-relationship between factors exists. It may be such a thing that all of the following substances owe their activity to a common yet unknown substance.

A. THE BURN TOXIN THEORY

Much of the present day work being done on burn toxins is unfortunately being done in Russia and the results are not available for this study.

Historical Aspects

According to Matter[19] and Bailey[2] most of the early work done in this field was done in the immediate period following the Civil

War and was apparently done in the United States. Wertheim in 1868 and Avdakoff in 1876 studied the effects of injections of blood from burned into unburned animals and both observed that toxicity occurred. Brancati in 1923 advanced the theory of an antigenic toxin elaborated in burned tissue on the basis of anaphylactic shock in guinea pigs subjected to small experimental burns. Schutz in 1935 in Russia mentioned what appears to be the first therapeutic use of convalescent serum. In the United States, Rosenthal in 1937 described the presence of a toxin in the blood of burned experimental animals and human beings, and later in the same year described a corresponding antitoxin. He first described that a diffusate of burned skin was lethal for mice and rats and that precipitins were produced against this toxin after injection into a rabbit. During the 1950's the Soviets demonstrated in vivo evidence for specific antigens produced from skin injured by thermal trauma and for the development of burn toxin antibodies as an autoimmunization phenomenon. The antigen was described as being a thrombin-like material, heat labile, incapable of passing through a Seitz filter, and not species specific. According to these workers, in the presence of antigen from burned skin, serum of burned dogs showed an ability to fix complement, using the prolonged cold complement fixation test, whereas tests with normal skin extracts from the same animals were weak or negative. The complement fixation tests became positive on the seventh post burn day and reached a maximum titer in about 20-40 days. It was

found during dog experiments that improvement of toxemic symptoms and in early mortality was obtained by the use of convalescent burn serum.

Rosenthal in 1962 reported that serum from patients up to sixteen days after burning inhibited HeLa cells in fourteen of fifteen cases, while, following transfusion with healed burn donor blood the toxicity to HeLa cells disappeared. Others were unable to duplicate his results. Rosenthal used convalescent burn serum after the 1958 Chicago school fire and claimed dramatic results. Others have been unable to duplicate these results although certain Russian workers are in agreement. Many of the studies on the protective qualities of the convalescent burn serum were characterized by early degrees of enthusiam only later to be dampened by failure when the mortality figures were compared and statistical significance computed.

Report of the Study

In a study Bailey[2] (who set out to confirm or refute the value of convalescent serum in burned animals, to determine the specificity, and to find out under what circumstances immunity could be produced,) did a series of controlled rat experiments involving controlled degree of burns, active and passive immunity, and other factors. Under the controlled conditions of his experiment he found that the rat is capable of developing active immunity to scalding and that convalescent serum from previously scalded rats exerts a marked protective influence on other scalded rats.

After a severe scald this immunity reached a surprisingly useful level after about three months. This response was not produced by other forms of trauma. He also found that toxic but not lethal effects were produced by injection of scalded skin extract and serum from severely scalded rats. After a month these injections produce a degree of immunity which enables their serum to protect other scalded rats. The effective period after a major scald is about three months. Only when the rat showed obvious signs of survival from his burns did Bailey find the titer to be of any value. He did not state whether he thought that those who didn't survive died because they didn't develop an immunity.

Bailey states, however, that although "subjective" well-being and prolongation of life were obtained, immune serum did not prevent eventual mortality. He felt that many of the deaths of his subjects may have been due to the obvious problems of giving fluids to rats. In projecting his results to humans, Bailey feels that since most burn centers have no great problem with early death, the prevention of early deaths by his burn serum might not be of any great value.

In a later study done by Matter and others including Bailey[21] something happened which complicated many factors of the previous experiments and gave results which were somewhat less clear. In this study, the format of which was similar to the previous one, many of the rats survived the trauma irrespective of the form of previous therapy. Matter and the others felt that there was no

or at least insufficient evidence to demonstrate any protective effect of the convalescent serum. Since most of the late deaths were associated with local infection or with immobility from the constricting effect of the burn eschar which resulted in inadequate food and fluid intake, it was believed that the test was not suited for estimation of effects of the antisera on ultimate survival. These men furthermore felt that if a specific burn toxin does exist, it must be related to morbid rather than lethal factors.

They conclude by admitting that a consensus of workers in this country is that "there are indications . . . that specific antimicrobial antibodies may be present in the blood of organisms which have recovered from infected burn wounds. It is within the realm of possibility that beneficial effects might result from transfusions of blood, plasma, or serum from a donor possessing antibodies which react specifically against microorganisms infecting a recently burned organism." It would appear from this statement that protection from infection is what is needed and gained from exposure to burns rather than protection against the toxicity of the burned skin.

Millican and Rosenthal[22] in comparing the effects of chloramphenicol, human gamma globulin and burn convalescent serum on the late deaths in burned mice found all three elements to be effective in increasing the survival over a three week period. Their burned serum was obtained from the animals three to eight weeks after injury and pooled preparations were used. The sera

thus obtained was found to be effective in about 80% of the experiments which were similar to those of Bailey and Matter. Gamma globulin was found to be effective in only about 50% of the cases and furthermore did not last for more than about a week, although there was no difference between the two substances during the first week.

As will be explained later in the discussion of gamma globulin, the protective effect of this substance is apparently related to the content of antibodies specific for the bacteria involved. With convalescent serum it is not clear whether its therapeutic effect is related to its content of gamma globulin or whether it is due to some other substance such as properdin and other non-specific factors. As has been shown, some claim that the therapeutic effects of convalescent serum is attributed to the content of antitoxins effective against toxins derived from the burn injury. These workers apparently feel the effect is secondary to the protection from bacterial infection and is thus contrary to the beliefs of Rosenthal and Matter

Present status

Thus the battle rages and seems still to be raging. There are claims, counter-claims, and even cold war politics involved in evaluations. Although many of the variables may be due to the method of detection and obtaining the toxin and anti-toxin, there is no agreement as to what they are when they are obtained and if they protect, it is not known against what.

It would appear that much further research and experience needs to be done before the value of the use of convalescent antisera can be definitely established. The history of burns and therapy is marked by many forms of treatment which were at first acclaimed but in the end proved to be disappointing. By note of contrast, the cold water method treatment, which is probably the oldest form of therapy known, was abandoned many years ago but only within the last few years has been in common use again in anyplace except the home where its use probably began.

B. GAMMA GLOBULIN

Historical aspects

It follows in sequence that if burn anti-sera, plasma, and blood are effective in the treatment of burns there must be some element common to each which is the effective agent. There are those who believe that it is the gamma globulin fraction.

Compared to some of the other studies on blood and burns, gamma globulin is a relatively recent addition. According to Fisher and Manning[8] it was first demonstrated in 1947 by Cameron that gamma globulin isolated from various animal species exhibited therapeutic activity in mice infected with animal pathogens. More specifically, he found that the gamma globulin from each species was active against the organisms pathogenic for that species. Later, Sonea demonstrated the effectiveness of human gamma globulin in a staphylococcal mouse infection. Rosenthal later found similar activity in human globulin against Pseudomonas

aeruginosa and other organisms. Fisher in his work on mice found the substance to be effective against this organism and various staphylococcal and streptococcal organisms as well. He also demonstrated and described the synergizing activity of gamma globulin and chloramphenicol in these infections. Millican found that human gamma globulin protected mice against species of Pseudomonas, Escherichia, Proteus, Aerobacter, and Micrococcus. Finally, it was shown by Fisher and Manning that the therapeutic action of gamma globulin could be ascribed to its content of specific bacterial antibodies.

Report of study

Fisher and Manning[8], who are among the early pioneers of the use of gamma globulin, found this substance to be extremely useful in the treatment of bacterial infections in mic which were experi-mentally infected with various organisms. including Pseudomonas, Staphylococcus aureus, Proteus, and Klebsiella and Escherichia coli. Of interest here, they found the 50% curative dose to be three to twelve milligrams of gamma globulin per kilogram of body weight for the staphylococcal species but the dose was raised to 40-50 mg/kg for the Pseudomonas aeruginosa strains. By way of contrast some of the Proteus species needed up to 2,000 mg./kg. for the 50% curative dose.

Absorption experiments have indicated that the activity of gamma globulin is largely or entirely due to the presence of specific antibodies, the potency of the substance used in the experiment reflects a lifetime of contact of the donors with the

bacteria in question. This is substantiated by the fact that the organisms most sensitive to the use of gamma globulin were those which were commonly found in or on the body.

Other important pioneers who have continued their work and applied their findings to the burned patients are Rosenthal, Millican, and Rust. In some of their work[29] they use cortisone to make mice more succeptible to infection from Pseudomonas innoculated intraperitoneally. Human gamma globulin was then injected intraperitoneally, intramuscularly or intravenously. They found that relatively small doses of gamma globulin (0.4 ml.) gave complete protection against the infection. This dose gave complete protection against ten fold increases in doses of organisms over that of the controls. Citrated plasma gave a lesser degree of protection consistent with its gamma globulin content. Human serum albumin gave little or no protection against fatal infection. These men also found that burns increased the control groups susceptibility to the infection but that the gamma globulin gave a protection which was almost equal to that of the control group. They point out that the work of some people who feel the effect is due to the properdin system are in error, for this substance is not found in gamma globulin.

The same workers later extended their work to other bacterial species.[25] They used mucin to enhance the infection in this series which made the infection more rapidly fatal than the previous use of cortisone. They confirmed the effectiveness of human gamma

globulin against Pseudomonas and also found that protection was enhanced with the use of tetracycline but that the latter substance enhanced the protection afforded by plasma more than it did gamma globulin. Their basic conclusion was that gamma globulin was effective against gram negative and positive organisms, a feeling which is not universal among workers in the field. It is again pointed out that the degree of protection is proportional to the degree of exposure of the animal or human donating the gamma globulin or the substance from which it is extracted.

Chloramphenicol, gamma globulin and convalescent serum were later compared.[23] In this study, the chloramphenicol was found to be the most effective in lowering mortality. Similar, but less marked, effects were found using the other two substances. As previously stated in the section on the use of convalescent sera it was found that gamma globulin was effective in the first week only but that the convalescent serum protection extended to three weeks. The gamma globulin used was human and thus foreign to the animals used in the experiment and thus represented a foreign protein whose rapid disappearance might be expected. This must also be a factor in their previously described experiment.[22] It will be remembered that they found gamma globulin effective in prolonging three week survival in only 50% of the animals compared to 80% for chloramphenicol and convalescent serum.

A large amount of work is also being done by Kefalides and his co-workers including Rosenthal on the use of plasma, gamma

globulin, albumin, and saline therapy in human burns.[13] In their first study they point out that the work of previous men has shown that large amounts of saline were as effective as plasma in the treatment of burn shock in adults. In children, plasma in addition to the saline solution appeared to be superior to saline solutions alone, as far as both mortality from shock and delayed deaths were concerned. They feel furthermore, as we have tried to illuminate in this paper, that modern treatment of shock has reduced the acute mortality from burns but that the late mortality remains larely unaffected. Prophylactic and therapeutic use of antibiotics has shown little value in the septicemias that follow burn trauma. In their study they attempted to point out that infection plays an important part in the actue as well as the delayed deaths in children. Because Pseudomonas infection plays such an important part in the cause of death in their study, they had the chance to study this organism very closely.

Evaluation was carried out in burned children using plasma, gamma globulin, or both combined with saline solution therapy, as compared with serum albumin and saline solution, or with saline alone. All were administered during the early period after the trauma. Their intention was to attempt to determine if the superiority of plasma was due to the colloids or to the antibodies contained in the plasma. Similar prophylaxis with antibiotics was used in all cases.

The control groups (saline solution alone and combined with albumin) were compared with those receiving antibodies, as either plasma or gamma globulin or both. The addition of albumin to saline solution did not significantly influence the shock mortality. All groups receiving plasma or gamma globulin, except those receiving saline solution combined with gamma globulin, showed a significant decrease in mortality when compared with the combination control groups; the lowest value was in the group receiving saline solution and plasma and gamma globulin. It was noted that the decrease was obtained not only in relatively minor burns (10-30%) but also in much more extensive cases. The inability of the saline combined with gamma globulin to reduce the mortality was attributed to the small number of cases involved, however it may have been due to the fact that the combination of gamma globulin and albumin can reduce shock mortality whereas either alone is ineffective.

It was also shown that in burns greater than 50% gamma globulin is somewhat less effective than plasma but not significantly more so. This, besides to differences in sampling, may have been due to the slow rate of absorption of IM gamma globulin. It was also noted that the most beneficial effects of plasma and gamma globulin was to be noted on the second day, with little or no difference from the controls on the first 24 hours. They then assume, rightly I think, that some beneficial effect must have been achieved as soon as the second day and that there must be at least some element of infection present at that time.

Somewhat different results were noted in the effects on delayed deaths or those occurring at least 60 hours after the trauma. It was found that in burns of 10-30% a significant protection was achieved but that this effect diminished as the severity of the burn increased. Some of the late deaths were due to other factors besides infection, however. There were diminished numbers of septicemias, again most marked in the 10-30% burn group. It was also found that children under six and particularly those under three benefited the most from all gamma globulin containing therapy, whatever the form.

The young child with extensive burns appears to behave like a premature child, immunologically speaking. He becomes more susceptible to invasion by organisms of relatively low virulence. This was felt to be the basis for the good results obtained with the globulin containing solutions. This phenomenon seems to be peculiar to burn trauma, for pseudomonas septicemia has not been observed on the children on a neighboring ward with other types of traumatic injuries. The reduction in delayed mortality from the use of plasma or gamma globulin or both was due to primarily a decrease in pseudomonas septicemia and that this effect was due to specific antibodies present against this organism in the therapy given. This was not seen in similar work done on adults.[4]

In their later work these same authors attempt to show the relationship between age, serum gamma globulin levels and the frequency of fatal septicemias in a series of patients who

received saline alone or combinations of it with albumin, plasma and/or gamma globulin on an alternate case basis. The gamma globulin was given in a dosage of the standard prepartion of 1 ml. per kilogram body weight IM on admission and repeated on the third and the fifth day. Gamma globulin determinations were made on serum drawn within three hours after burning and on the third and fifth day. Gamma globulin determinations were also done on these patients on the seventh, ninth, eleventh, and thirteenth day after admission. Hemagglutinating antibodies to pseudomonas were determined on the day of admission in 41 normal and burned children. It will be recalled from our previous discussion that the most frequently cultured organism was the staphylococcus (31.5%) and that pseudomonas was next with 21.6%. These organisms rapidly colonized the skin in over 65% of the patients as early as the third day in spite of continued prophylactic antibiotics, and in the presence of this fact, resistant strains of these two organisms rapidly developed. Pseudomonas septicemias were significantly reduced in all therapy groups that received plasma and/or gamma globulin. Again, the effect was most marked in burns of up to 30%. No effect was noted on septicemias due to pseudomonas when gamma globulin was added to the plasma, but their mortality rate was reduced in all groups receiving the latter.

The gamma globulin levels in these patients was found to be decreased during the first week regardless of the type of therapy or age group of the patient. The lowest levels were seen between

the third and the fifth day. It was noted that in the 0-4 age group that therapy with saline solution in combination with gamma globulin and plasma maintained the highest serum gamma globulin titers. In children of the 4-12 age group, low gamma globulin levels were maintained up to the seventh day, with a rise to admission levels by the ninth day. The early drop may have been due to hemodilution secondary to fluid therapy. The highest numbers of septicemias were likewise seen between the third and fifth day when the gamma globulin level was at its lowest ebb. Of all septicemias, 85% were observed between the third and the sixth day. The frequency of septicemia deaths was also found to be inversely proportional to the pseudomonas antibody titer. Although the lowest gamma globulin levels were found in those less than six years of age, it would appear that it is not the absolute gamma globulin concentration that is the important factor but that the antibody against Pseudomonas is. Similar reports on the use of gamma globulin either alone or to potentiate antibiotics is to be found in the work of other researchers in this field.[5-12-35-36]

Present status

It is fairly apparent that the use of gamma globulin in the burned patient contributes to the lessening of the mortality although a similar effect may be found with plasma or blood. If used, gamma globulin should be used before the third to the sixth day when the levels are lowest after the burn injury. Since gamma globulin owes its activity to specific antibody which increases with age, it should be used mostly in children.

C. PSEUDOMONAS ANTISERA

Historical aspects

It follows that if the beneficial effects of gamma globulin are due to the pseudomonas antibody titer present, why not use the specific substance? In the past certain investigators have described sera which, as indicated by the results of protection tests, apparently contain significant amounts of antibody but which fail to show the presence of specific agglutinins or precipitans in vitro, Chow, 1936 and Boyd, 1947. Jackson and Hartman in 1927 were apparently the first to use Pseudomonas antisera in their treatment of Pseudomonas keratitis. Mention has already been made of those workers who in 1957 felt that certain factors in human gamma globulin made it useful in the protection from Pseudomonas infection. These men were Rosenthall, Fisher, Manning, Millican and Rust. Perez-Alva and Bazan (1957) in preliminary studies have used antiserum therapy with encouraging results in a small number of burned patients with Pseudomonas septicemia.

Report of the Study

Although from the above material it is apparent that it has been long known that among the members of any given animal species many who are in good health have in their sera components which will react specifically with a variety of organisms or other antigens invitro. It remained for Gaines and Landy[9] to demonstrate that by means of a hemagglutination procedure, antibody against Pseudomonas aeruginosa is demonstrable in the sera of normal human

beings which was not demonstrable by the conventional bacterial agglutination tests.

In the study it was found that more than 90% of sera from individuals over two years of age showed antibody titers ranging from 1:7.5 to 1:960 while antibody could not be detected in the majority of infants under one year of age. Furthermore the antibody, by means of absorption tests, was found to be species and strain specific. Although the antibody reacted with the organisms demonstrable agglutination was not seen in the majority of cases for some unknown reason. No incomplete or blocking antibody was found by the Coombs or Coca and Kelley technique. The important aspect was that the antibody did exist almost universally in humans and suggests that man may come in contact with and develop immunological response to this organism to a greater degree than was previously thought.

Millican and Rust[24] found that serum from rabbits immunized with Pseudomonas aeruginosa is very effective in protecting mice against death from infection and that it is several hundred times more effective than human gamma globulin. Gaines and Landy[9] pointed out that rabbits were able to agglutinate this organism in vitro while humans were not. Millican and Rust found that in infections 94% fatal to control mice, treatment with normal serum (0.01-1 ml) produced survivals of 0-15%, while treatment with antiserum (10^{-7} 10^{-4} ml.) produced survivals of 30-100%. Moreover, when the organism dose was increased 10 to 100 fold, protection by the antiserum was also demonstrable. Maximal effect was observed

antiserum was given prophylactically. Under these conditions of administration, antiserum, given IM, IV, or Sub Q, was about one-tenth and one-hundredth as effective as antiserum given intraperitoneally. When given optimally, the comparitive effectiveness of the antisera was found to be 230 to 780 times greater than human gamma globulin. The effect was found to be present even when the mice were burned and the infection with the Pseudomonas organisms was innoculated intraperitoneally..

Graber and his fellow workers[10] attempted to determine if antibody in the sera of burned and unburned individuals was protective for mice against induced fatal pseudomonas infection. Two types of antibody were studied: a naturally occurring hemagglutin to Pseudomonas polysaccharide (PC-9) demonstrated to be present in all healthy adult sera, and an immune agglutinin which is present presumably only in the infected people. Single serum samples were obtained from 10 unburned humans while sequential sera were drawn from a total of 10 burned patients.

It was found that mice injected with a lethal dose of Pseudomonas aeruginosa and innoculated with sera obtained from ten normal individuals whose hemagglutin titer was 1:25 had a 50% mortality when the serum was used undiluted and a sharp decline was noted after dilution. Twenty two sera obtained from burned patients ranging from day of admission to the fifteenth postburn day having a hemagglutinin titer from 1:9 to 1:22 demonstrated equally poor results. Burn sera from the 8-15 day post burn generally

possesed the same qualities of protection as the nonburn sera. The best results were observed in those sera obtained immediately after death where although the undiluted sera from this group were lessened in their effectiveness than the nonburn sera, a 10^{-2} dilution still protected 23% of the animals.

These authors feel it is difficult to asses the significance of elevated anti-pseudomonas titers in the burn patient. In previous workers who noted an association of greatly elevated titers with greater graft failure and higher mortality, comment is made that the association may be nothing more than a reflection of the severity and extension of the burn area. In animal experiments it was noted by contrast that smaller intradermal lesions in rabbits occurred when the challenging organism was the immunizing strain.

Present Status

This is the area in which most of the present work is being done and hence some of the results may seem to be somewhat controversial. Perhaps the best that can be said is that Rosenthal is using some form of crude anti-sera in his work in Peru. A worker in the U.S. is at the present time preparing human pseudomonas antisera made by injecting the killed organisms into human subjects. It is presumed that he will in some way test its protective qualities. It should be extremely interesting to follow his work as well as that of Rosenthal. In so far as I am able to determine, his work has not been published and is not available for this paper.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Summary

The problem of the pathogenesis of Pseudomonas aeruginosa infection in the burned patient has been surveyed. Emphasis was made on the incidence of infection, relative incidence of Pseudomonas infection, mortality figures, causes of death, types of infection and pathology and a final chapter on alterations in the host defense mechanisms making infection possible. In this chapter, emphasis was made on the pathogenesis and sequence of infection, mechanical alterations in the host defense, metabolic alterations, cellular alterations and finally immunological alterations.

The theme of the paper was centered around the premise that Pseudomonas aeruginosa, an organism of usual low virulence, is becoming a much more frequent pathogen in the burned patient, to the point that burn mortality figures are much the same now as they were a hundred years ago.

It was found that in what seemed to be a typical study, that the mortality rate of hospitalized burned patients was 15.2%, one third of whom died of injuries to less than 40% of the body area. Two thirds of the series died of infection and of the deaths caused by infection septicemia was responsible for 75%. Pseudomonas was responsible for a large percentage of these deaths and in another series was found to cause from 71-75% of all septicemias.

Septicemia with metastatic spread of the infection seems to be the most common type of infection. A localized skin lesion is probably the prodromal stage which later becomes hemorrhagic and then spreads to the viscera. Most infections including septicemias begin with a localized skin infection, probably in the hair follicles, which spreads to the eschar and eventually into the blodd stream after invasion of surrounding viable tissue.

Although loss of skin integrity is the basic defect in the host defense mechanism, other factors are involved including prolonged negative nitrogen balance. It has been shown that burned animals are more susceptible to infection than the non-burned and the burn shock is probably responsible. This results in the decreased function of the R.E. system and loss of integrity of the blood vessels both due to the nature of the wound and to a fall in cardiac output thus impeding and slowing circulation. The result is a delayed skin response to infection (perhaps in part due to adrenal "stress" reaction) and slowing of phagocytosis at the skin level. Impairment of the blood flow causes or allows bacteria a greater period of time to multiply before they come into contact with the body phagocytes allowing them to multiply in great numbers. This is in part due to the post burn "sludge."

Immunologic features were also discussed. It appears that there may be a substance produced by the toxic effect of the burn which is antigenic and may protect against future burn toxicity both actively and passively. It may be due to a gamma globulin and may be the reason for the protection against infection afforded by that substance.

Gamma globulin has been shown to provide good protection against infection when used prophylactically, especially in the child. Its effect is probably due to a particularly specific anti-bacterial substance in it which increases in amounts with increased exposure to the particular organism. It seems likely that it would be most useful in the young, and most useful between the third and the sixth day when gamma globulin levels fall in the burned patient.

It appears that there appears in animals and man a specific antibody against many of the organisms of nature including Pseudomonas aeruginosa. This antibody has been shown to be nearly universal in humans. Its effect is somewhat controversial in that it is not universally accepted that passive immunity can be achieved. If it can, it may or may not be a potent protector against Pseudomonas. It is also questionable whether its level rises in the post burn state and if it does whether it is specific against the organism in question.

Conclusion

The increasing incidence of infection by Pseudomonas aeruginosa reflects some stress in the patient, of which alteration of his defense against infection is prominant. Loss of integrity of the skin is the most basis factor. There appears to be some disturbance in ability to over come infection when it occurs. This is probably due to the decreased circulation of blood caused

by fall in cardiac output which is further inhibited by decreased effective circulation caused in part by increased capillary permeability complicated by the burn "sludge." All contribute to delayed phagocytosis.

There also appears to be a disburbance in the immunologic structure of the burned patient. It is not clear whether this is caused by the burn per se or by the following infection. It is likewise unclear whether this increases or decreases the possibility of infection or whether it is a nonspecific response. In any case, it can be shown that there is a fall in the gamma globulin level during the first week and this substance is probably useful in the prevention of infection, especially in the young patient.

It is likely that the increasing incidence of infection by Pseudomonas is due to all of these factors but also probably reflects a changing flora on the wound which is in part caused by the increasing use of prophylactic antibiotics which suppress the "normal" burn flora and allow organisms of low virulence to emerge which are resistant to the antibiotics. Why this should be Pseudomonas aeruginosa is unclear at this time.

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