BACTERIOLOGIC STUDY OF BURN WOUNDS*

A COMPARISON OF THE BACTERIAL FLORA OF BURN WOUNDS OF PATIENTS TREATED WITH SULFONAMIDES OR PENICILLIN

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PART I

AS LONG AS the contaminating bacteria of a burn wound are able to flourish, the resulting infection will be *the* deterrent to the healing of the wound. These bacteria do not flourish in a shallow, partial-thickness wound and, therefore, infection and retarded healing are not to be countenanced. It is suspected that this shallow wound does not nourish them. Prompt healing, therefore, is anticipated and the claims for various methods for expediting healing of the partial-thickness burn wound are discounted. In a full-thickness burn wound the bacteria flourish and will unless something is done to starve, kill or remove them.

To do one or more of these has been attempted by many methods, both local and systemic, and none has been entirely successful. Agents in the form of solvents, such as Dakin's solution, corrosive and coagulating chemicals and the more selective vital dyes have been applied to the wound surface; judged to discourage the bacterial growth and the infectious process, in their toxicity they have proved damaging to viable cells. The sulfonamides applied locally do not damage viable cells but, limited to an antistreptococcal specificity, they have failed to block the generation in the wound of other bacterial species equally insidious. Every effort to control the less susceptible organisms by increasing the local concentration of a sulfonamide drug is thwarted by its rapid absorption from the wound and a rise in concentration to toxic levels of the drug in the body fluids and renal shut-down. So predictable is this absorption that only in burns of minor extent can sulfonamides be applied in a medium permitting high and broadly useful concentrations.† The drug,

* The work described in these papers was done under a contract, recommended by the committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

† The possibility that the rapid absorption of sulfonamides from burn wounds might result in toxic levels of the drug in the extracellular fluid was reported in 1941 by Hooker and Lam.[1] Renal shut-down, with sulfonamide poisoning, was observed in two patients treated at the Massachusetts General Hospital in 1942. An extensively burned patient whose wounds were sprayed with triethanolamine containing 3 per cent sulfadiazine (Pickrell's solution[2]) developed a blood level of 37 and 54 mg./100 cc. of sulfadiazine on the 3rd and 5th days after injury and the initial application. Complete urinary suppression occurred on the 2nd day, and was unrelieved. Death in uremia occurred on the 6th day. Sulfanilamide powder applied to a large full-thickness wound of the second patient (Case 76) on the 11th day after injury was followed 24 hours later by a blood level of 21 mg./100 cc. Renal damage contributed to the death on the 27th day. The failure of sulfonamides in certain ointments to rise to toxic levels in the body fluids[3, 4] is to be ascribed to a sluggish release of the drug from the ointment base. No more effective a concentration of the drug is to be expected to reach the fluid recesses of the wound from such ointments than from internal administration.[5, 6]

systemically administered, fares no better; it has imposed upon it the same limitation of toxicity as when locally applied and is only successful in controlling the invasive infection of the streptococcus.

All of these agents, even including the most promising—the sulfonamides—have been discarded because of their toxicity or failure, and without elucidating the rôle which the burn slough of the wound plays in the growth of the bacteria. The necrotic tissue coagulum, deprived of its blood supply, should be fertile soil. It is also conceivable that this coagulum is impervious to antibiotic agents while at the same time permitting penetration of the bacteria; or it might contain drug inhibitors similar to those of the sulfonamides encountered in pus.

Such were the tools and the understanding of their abilities for attempting the control of infection at the time of the advent of penicillin. It was tempting to hope that penicillin would succeed where the sulfonamides had failed since it was reported nontoxic at any dosage and had wider specific antibacterial properties. The absence of toxicity at any dosage level would be peculiarly valuable for the extensively burned patient threatened with dehydration. The initial experience with penicillin in patients with infections other than burns showed it to have a specificity against the streptococcus equal to that of the sulfonamides as well as a specificity against the staphylococcus. It was wise however in view of the disappointing performance of the sulfonamides to be wary of penicillin's power to control the infection of the full-thickness burn wound.*

In our wariness of penicillin and desire to achieve the earliest possible healing of the full-thickness burn wound it was decided to combine the use of penicillin with prompt surgical excision and grafting of the wound. Surgical excision of the full-thickness burn wound should reduce the number of contaminating bacteria and eliminate necrotic tissue. The base left after excision would undoubtedly be contaminated, but since it would be composed of viable cells, any graft laid upon it would not only prevent further contamination but should proceed to heal without being forestalled by infection. To have the ideal base upon which to place the graft, excision of the burn wound down to viable tissue should be done immediately after injury. With the passage of time the contaminating bacteria within the burn slough would have a chance to multiply and, therefore, the longer the delay after injury, the greater the number which would inevitably be transferred to the excised base during the operative procedure. Also, with the passage of time an inflammatory reaction would appear beneath the necrotic skin in the living

* It is not possible by quoting the literature available in 1943, the time when this project was initiated, to give proper credit for the ideas and help upon which we drew freely. Information available at that time and currently throughout the war was promptly disseminated by bulletin to each group of investigators by the Office of Scientific Research and Development. An adequate survey of the literature is not intended. In the setting-up and conduct of this project, we are particularly indebted for advice to Dr. Champ Lyons, Dr. Rene J. Dubos, Dr. Chester S. Keefer, and Dr. Frank L. Meleney.

tissues which form the base left after excision. Any inflammation should be unhealthy for the successful take of the graft.

The full-thickness burn wound of limited extent has been excised immediately after injury and the defect closed with a graft. The excision and grafting of extensive full-thickness wounds has had to be delayed because of the precarious physiologic state of the patient and because unburned skin for the grafting was lacking. The excision and grafting of both circumscribed and extensive wounds has also been delayed because this project was set-up with the needs of the Armed Services in view and because the postulated conditions of warfare indicated that it would not be possible to operate immediately upon a burn casualty. The delay facing the casualty suggested that an arbitrary interval of from four to seven days between injury and excision and grafting should be tried in some of the cases.[7]

Penicillin was administered systemically to all patients whether excision and grafting was immediate or delayed. Although, admittedly, two therapies were being tried at the same time, and it might prove difficult to ascribe to each its share of credit, the drug was given advisedly to give the operation all possible support and, in those patients in whom time was allowed to elapse between injury and surgical excision, to afford the opportunity to judge the efficacy of penicillin in holding infection in abeyance.

The study has included a comprehensive survey of the bacterial flora of burn wounds and its alteration under penicillin therapy and of certain changes in metabolism of the organisms, such as the development of a resistance to penicillin during the course of its administration. Dissemination of the organisms of the wound through the blood stream and urinary tract and certain aspects of the metabolic response of the host, including the development of a specific immunity, have been studied as well.

Penicillin was not withheld from alternate cases for purposes of control; it was believed that those patients with comparable wounds who had been treated with sulfadiazine systemically in the year previous would serve as acceptable controls. The majority of these patients were those studied under the Contaminated Wound Project of Doctor Meleney by Drs. Champ Lyons and F. W. Rhinelander at the Massachusetts General Hospital.[8] The bacteriologic technics of Doctor Lyons and this study were identical.

To achieve clarity of presentation the data of the study have been divided into four papers. In the first, the nature of the bacterial contamination and subsequent flora found in partial and full-thickness burn wounds in patients treated with either penicillin or sulfadiazine is described. In the second, the natural occurrence among bacteria of penicillin resistance and inhibition and the development of penicillin resistance in the flora of the burn wounds of patients treated with penicillin is recounted. Presented in the third and fourth papers are the aspects peculiar to the staphylococcus and streptococcus. Brief comments only are made in conclusion of the first three parts. Discussion of the observations, including the clinical significance, is delayed until the end of the fourth part.

METHODS

Immediately upon entry to the hospital, each patient was placed in an isolation room in the Emergency Ward where every precaution was taken to avoid further contamination. The burn wounds were inspected, their location and depth charted. They were then covered, without débridement or cleansing, with a firm protective dressing of petrolatum impregnated, fine-meshed gauze, thick dry cotton and an elastic bandage or a towel. Subsequent changes of dressing were made in an operating room under aseptic precautions.

The patients were accommodated in single rooms on an isolation floor where supervising nurses were in constant attendance. Occasional bacterial counts of the air in these rooms showed a number and variety of organisms comparable to those recovered from the air of single rooms on a floor caring only for uninfected surgical cases; from the air of rooms in another part of the hospital containing infected cases cared for without special precautions were recovered a wider variety of pathogenic organisms with more than ten times the number of colonies.*

The burns were arbitrarily divided into areas or "wounds." A culture was taken of each wound on admission and at each subsequent change of dressing until healing occurred. The cultures taken on the day of injury (O-day) in the majority of the sulfonamide-treated cases were of dead epidermis removed from the wounds. All other cultures, including those of O-day of the penicillin-treated, and the remainder of the sulfonamide-treated cases, were taken by rubbing the exposed surface of the wound with dry sterile cotton swabs.

The technics used for isolation and identification of the organisms were essentially those established by Meleney[8] for the various units of the Contaminated Wound Project under the Committee on Medical Research of the Office of Scientific Research and Development. Anaerobiasis was secured by the chromium-sulfuric acid method of Rosenthal,[11] as modified by Mueller and Miller.[12]

Isolation and identification of all morphologic types, seen microscopically, were attempted, selective media, especially those for the suppression of gram-negative bacilli,[13] being resorted to when necessary. The gram-negative bacilli

* Colebrook,[9] and Cruikshank[10] have repeatedly called attention to the frequency of the spread of organisms from one burned patient to another on an open ward. Strains of streptococci, recognizable by their Griffith-type and sulfonamide resistance, were traced in the wounds and also in the dust of the room. It was in order to reduce such cross and continued contamination that the patients included in the present study of wound flora were cared for in isolation rooms under special precautions and had their dressings changed, each wound separately, with aseptic technic only in the operating room. These precautions resulted in some patients in preventing an organism, for example a staphylococcus, from spreading from one wound to an adjacent one in the same patient. On the other hand, in spite of all precautions, evidence was occasionally obtained of the spread of a bacterial strain from one patient to another. A morphologically typical diphtheroid, causing a green discoloration of blood agar with a fruity odor on growth, first isolated from one patient, spread during the course of two weeks to three other patients.

were identified to the genus. The presence of aerobic gram-positive sporulating and nonsporulating (diphtheroid) bacilli was merely recorded. The staphylococci were also examined for color, production of hemolysis on five per cent horse blood agar, and the power to coagulate normal human plasma; the streptococci for type of hemolysis produced on five per cent horse blood agar, the Lancefield groups (A, B, and C, only) if *beta* hemolytic, and "dreft" (bile) solubility for differentiation from the pneumococcus if *alpha* hemolytic[14]; and the *Clostridia* for species, as far as possible according to Spray's "Tentative Key to the Sporulating Anaerobes."[15]

OBSERVATIONS

The data to be given in this study of bacterial flora of burn wounds are limited to those obtained from patients who were in the hospital, and under observation, from the time of onset of chemotherapy until the wounds were healed or the study ended. Fifty-four patients were observed under systemic penicillin therapy and 38 under systemic sulfadiazine; these latter are the controls for the penicillin therapy. No patients were observed for any length of time who did not receive either form of chemotherapy; there are, therefore, no controls for both penicillin and sulfadiazine.

The penicillin dosage was relatively low in the first cases (100,000 units or less per day) and high in the later cases (250,000 to 500,000 units per day); in a few cases nearly 1,000,000 units per day were given over short periods. Sodium penicillin only was used; it was injected intramuscularly usually, occasionally intravenously. The dosage schedule for the intramuscular injections varied from two to four hours.

In the patients receiving sulfadiazine, the dosage was regulated by the blood level; it was maintained between 6 and 12 mg./100 cc. If a patient showed sensitivity to the drug it was stopped and the patient was dropped from the bacteriologic study at that time.

Cultures were obtained once a week from nearly all of the wounds; culturing was abandoned only when the wound was healed.

In addition to the study of the flora of the wound, the effectiveness of the penicillin therapy was also judged in certain cases by study of those of the blood stream and urinary tract.

(1) FLORA OF FULL-THICKNESS BURN WOUNDS UNDER PENICILLIN THERAPY (CHARTS 1A-C)

Twenty-eight patients with 80 full-thickness burn wounds were observed in the hospital from the day of injury until healing. Penicillin was administered to the patients for at least 49 days unless all of the wounds of a patient were healed before this time. The wounds were of varying extent. Some wounds were excised and grafted promptly; the grafting of others was delayed until after spontaneous separation of the slough. All cultures were planted from swabs which had been rubbed firmly on the wounds.

On the day of injury (O-day) an average of almost three bacterial strains

was recovered from each wound; from the 69 wound cultures on O-day 199 strains were recovered (Chart 1A). In the first two weeks there was an abrupt increase in the number of strains recovered so that by the 14th day an average of five strains was recovered from each wound (72 wounds cultured, 368 strains recovered). From the 3rd to the 7th weeks there was a gradual decrease in the number of all bacterial strains recovered, reaching an average of 4.2 strains per culture on the 49th day.

The increase in number of strains recovered in the first two weeks is primarily a reflection of an increase in the number of the streptococci and gram-negative bacilli, and to a less extent of the diphtheroids and aerobic spore forming organisms (*B. subtilis*) (Charts 1B and 1C). The gram-negative bacilli, after an abrupt rise in incidence in the first two weeks, continued to increase very little. The streptococci, after an initial abrupt increase, remained stationary in incidence during the remaining period of observation. The number of strains of *Clostridia* recovered varied but little over a period of seven weeks after a slight early increase. The strains of the staphylococci showed a slow and steady decrease in incidence throughout the period of observation.

The flora of the full-thickness wounds of patients who were admitted later than the day of injury but who had received no chemotherapy previously were also studied throughout a course of penicillin therapy which was started on admission and lasted for 42 days. Thirty-nine wounds in 19 patients who were admitted from 1 to 30 days after injury (average 7.2 days) were studied (Chart 2). The wounds varied in extent as did the amount of infection clinically apparent in the wound on entry and before chemotherapy. There was usually present a severe localized infection and occasionally an invasive process with cellulitis and lymphangitis. One patient had a positive blood culture of a *Staph. albus*. With the subsidence of the clinical infection, some of the wounds were excised and grafted. The cultures were by means of the swab technic.

The number of all bacterial strains recovered on admission was slightly lower than that found on the 7th day in the patients who were admitted and treated with penicillin beginning on the day of injury. Following onset of therapy there was immediately a decline rather than a rise in number. It was not until after the 30th day of therapy that the number of strains rose to that encountered in the previous group.

The incidence of certain individual bacterial strains was significantly either different from or similar to that encountered in the patients receiving penicillin from the day of injury. Of the differences, the staphylococci were found more often on the day of entry than at any time in those patients treated from injury; following onset of penicillin there was a sharp decline to the number of the previous group. The streptococci were also more often recovered and at their maximum on the day of entry. The incidence, however, of both *Clostridia* and the gram-negative bacilli was similar in the two groups of cases. The *Clostridia* scarcely varied while the gram-negative bacilli starting

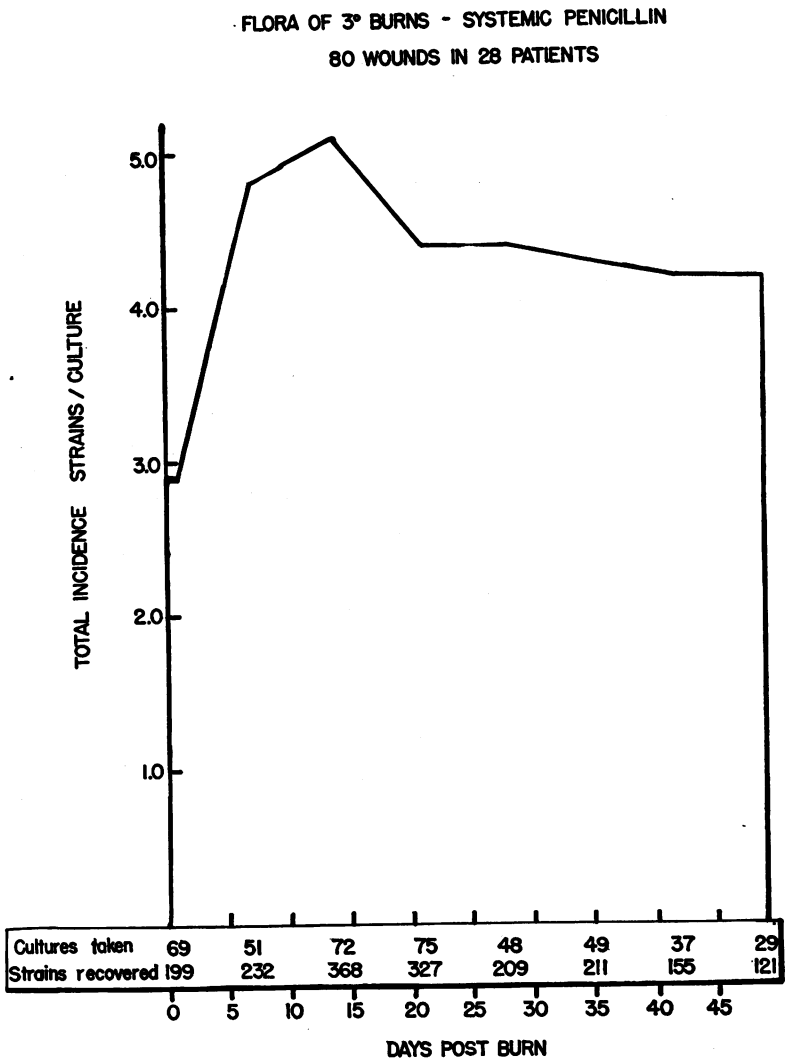

CHART 1A

CHART 1.—The Bacterial Flora of Full-thickness Burn Wounds in Patients Receiving Penicillin Therapy: Eighty wounds of 28 patients were followed from O-day through the 49th day unless healing had occurred previous to this time. Cultures were by the swab technic. The number of cultures planted on O-day, and within each subsequent seven-day period, and the number of strains recovered in these cultures are given at the bottom of each chart. Systemic penicillin was started on O-day and continued throughout the period of observation.

(A) Incidence of All Strains in All Cultures (Total Incidence: Strains per Culture): The abrupt increase in number of bacterial strains in the first two weeks and subsequent gradual decrease is evident.

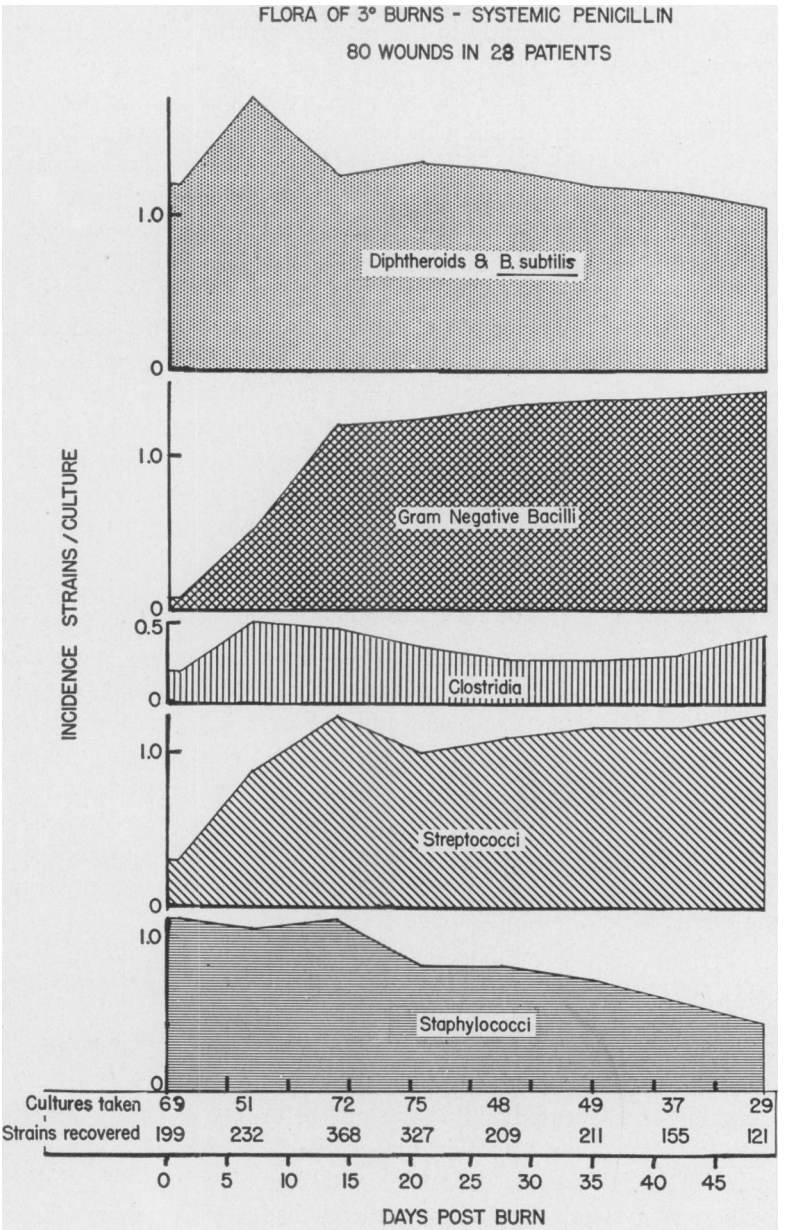

CHART 1B

CHART 1(B).—The Strains Recovered Plotted as Incidence or Number of Strains per Culture: Each strain is plotted in its appropriate bacterial group. The number of strains of staphylococci averaged slightly more than one per culture at entry and decreased gradually throughout the period of observation to less than one strain for every two cultures. The number of strains of streptococci, low on O-day, increased abruptly in the first two weeks and thereafter remained stationary in frequency. *Clostridia* were seldom recovered, and their incidence varied but little. Gram-negative bacilli, almost absent on O-day, increased abruptly in the first two weeks but only gradually thereafter. The incidence of diphtheroids and aerobic spore-formers (*B. subtilis*) increased slightly during the first week and gradually declined subsequently.

with but few increased abruptly in the second week of penicillin therapy and only slowly thereafter.

Bacteriuria.—A number of the patients with full-thickness wounds who were receiving systemic penicillin therapy developed signs and symptoms of lower urinary tract infection. The urine of several of these patients was cultured; various organisms, including gram-positive, were found but they were always of species present in the wounds of the same patient. All of the patients developing the infection had previously had in-dwelling catheters or been catheterized. The incidence of bacteriuria and urinary infection was not determined since the urine of the patients was not routinely cultured.

Bacteremia.—Blood cultures were made on 12 of the extensively burned patients, on 11 while they were receiving penicillin and on the 12th on the 2nd day after injury and before starting chemotherapy. This last patient and five of those on penicillin proved to have organisms in their blood. Four white staphylococci (2 coagulase-negative, 2 coagulase-positive), 2 *Clostridia* and 1 *B. proteus* were recovered. The same organisms were recovered from the wounds of the patient in each instance.

Most of the patients were sufficiently ill to arouse suspicion of invasive infection and the blood cultures were therefore taken. Two of the positive blood cultures found during the course of penicillin therapy were taken on the day after injury for chance information; both were positive, one showing *Clostridia* only, the other *Clostridia* and a *Staph. albus,* coagulase-negative. The cultures on the other eight were made at times of crisis; three were positive, two showing white staphylococci, coagulase-negative and positive, and the third *B. proteus.* The blood of the 11th case, cultured on the second day after injury, and before onset of penicillin therapy, contained a *Staph. albus,* coagulase-positive. A survey of the incidence of bacteremia in burns was not made.

(2) FLORA OF FULL-THICKNESS BURN WOUNDS UNDER SULFONAMIDE THERAPY (CHART 3)

The flora of 46 full-thickness burn wounds were studied in 25 patients who received sulfonamide therapy starting on the day of injury. Only 13 of the wounds were cultured on this day, and before the onset of chemotherapy, since 13 of the patients were victims of the Cocoanut Grove fire and the exigencies of their care discouraged the taking of cultures.[16] Sulfadiazine only was used systemically; a few patients had certain other sulfonamides applied locally at various dressings. The wounds were of varying extent. The first grafting was performed on the 24th day after injury when the slough had separated spontaneously. All wounds were followed as long as the patients were receiving systemic sulfonamides, or until healed. The 13 wound cultures accomplished on O-day were made from dead epidermis. All subsequent cultures were planted from swabs which were pressed firmly on the wounds in the manner used in the penicillin-treated patients.

The interpretation of the trend of the incidence of bacterial strains

recovered under sulfonamide therapy depends upon the significance ascribed to the numbers recovered in the cultures taken on O-day. A decrease in all bactrial strains in the first two weeks is shown (Chart 3). This is in contrast to the observations under penicillin therapy but its significance is to be discounted because of the discrepancies in the methods of culturing on O-day in

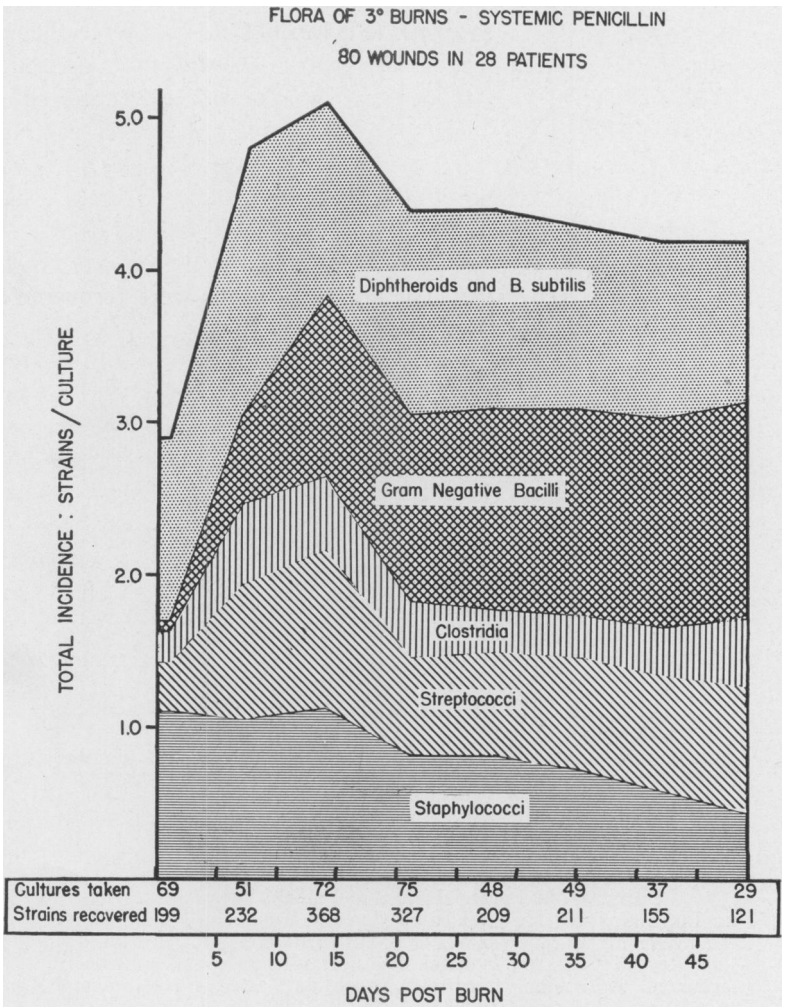

CHART 1C

CHART 1(C).—Composite of Charts 1A and B: Both the Incidence of Strains in the Individual Bacterial Group and the Part which each Plays in the Total Incidence: The top (heavy) line shows the incidence of all strains recovered and is in the identical position as in Chart 1A. The area occupied by each bacterial group is planometrically the same as that in Chart 1B. The zero line of each group other than that occupying the bottom position (staphylococci in this instance) is distorted by the top line of the group or groups beneath. (All of the subsequent charts of bacterial incidence are composed in this manner.)

the two groups and because the number of sulfonamide-treated patients cultured on O-day was small (only 12 patients with 13 wounds).

If one discounts the O-day findings then the incidence of bacterial strains shows a slow decrease in number in the first 35 days with an increased num-

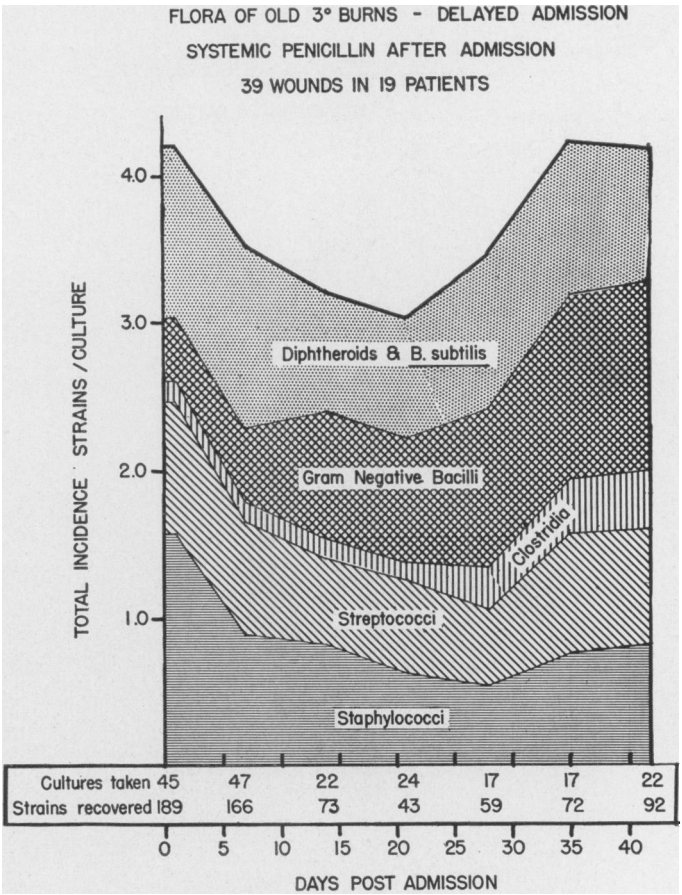

CHART 2

CHART 2.—The Bacterial Flora of Full-thickness Burn Wounds in Patients whose Hospital Admission and Penicillin Therapy were Delayed: Thirty-nine wounds of 19 patients were followed from day of entry to the 42nd hospital day unless healing had previously occurred. Admission varied from 1 to 30 days after injury (average 7.2 days). Cultures were by the swab technic. The number of cultures planted and strains recovered are given at the bottom of the chart, as in Chart 1, and all subsequent charts of bacterial incidence. Penicillin was started on the day of admission and continued throughout the period of observation.

ber later. The incidence of the staphylococci ran parallel to that of all bacterial strains. There is a consistent slow decline in the number of the streptococci with their complete disappearance on the 55th day. The *Clostridia* varied in incidence but little throughout the period of observation except for

disappearance on the 55th day. There is a gradual increase in the incidence of the gram-negative bacilli; it is slower than that encountered under penicillin therapy. The incidence of diphtheroids and aerobic spore-forming organisms (*B. subtilis*) fluctuated to some degree but the changes are probably not significant.

(3) FLORA OF PARTIAL-THICKNESS BURN WOUNDS UNDER SULFONAMIDE THERAPY (CHART 4)

The bacterial flora of 58 burn wounds of partial-thickness destruction were studied in 35 patients treated with sulfadiazine systemically. The

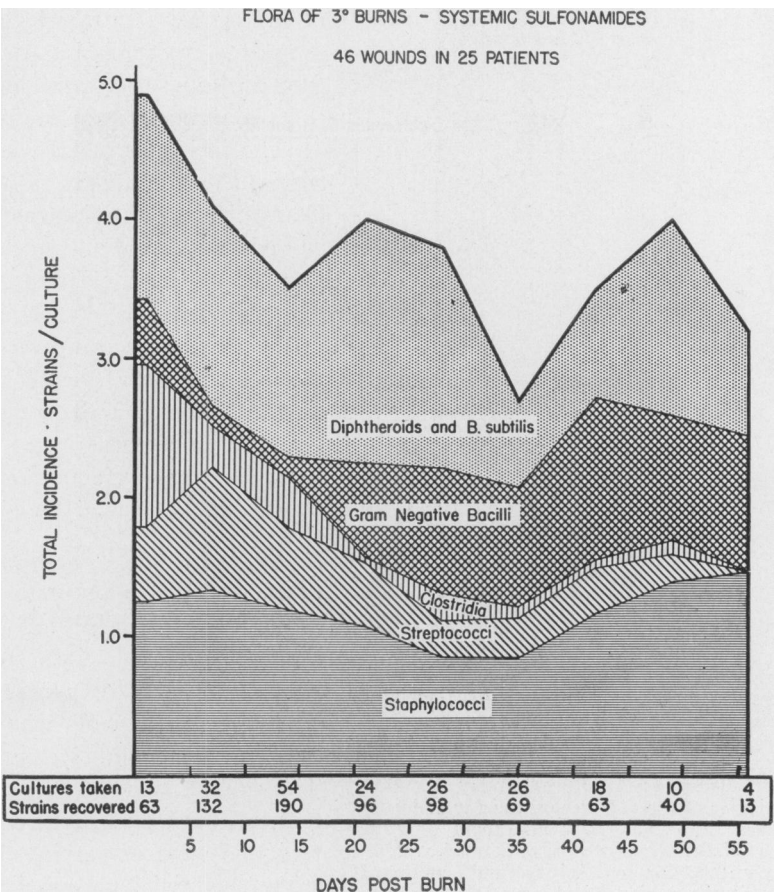

CHART 3

CHART 3.—The Bacterial Flora of Full-thickness Burn Wounds in Patients Receiving Sulfonamide Therapy: Forty-six wounds of 25 patients were followed from day of injury to the 56th day, or until healed; however, 33 wounds of 13 patients (all victims of the Cocoanut Grove fire) were not cultured on the day of injury. Most of the cultures on the day of injury were of dead epidermis; all others were by swab technic. Sulfadiazine was started systematically on the day of injury and continued throughout the period of observation.

patients entered the hospital on the day of injury and were followed for not longer than 21 days. But 29 of the wounds were cultured before the onset of chemotherapy because 16 of the patients (with 29 wounds) were victims of the Cocoanut Grove fire. The wounds were of varying extent; all were open with ruptured blisters. The depths varied from superficial to deep intermediate- or almost full-thickness. In addition to the systemic sulfadiazine, sulfonamides were applied locally to a few of the wounds at the initial and later dressings. The majority of the 29 wound cultures made on the day of injury were grown by dropping dead epidermis into the culture medium; thereafter all cultures were made from swabs.

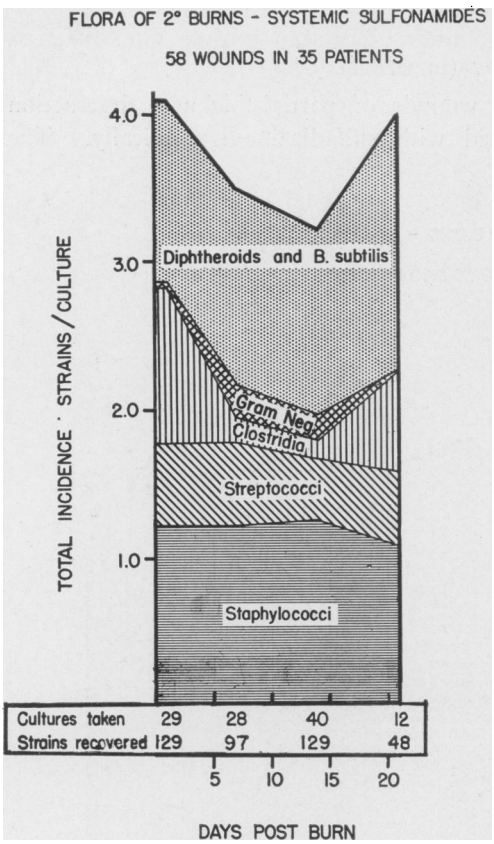

CHART 4

CHART 4.—The Bacterial Flora of Partial-thickness Burn Wounds in Patients Receiving Sulfonamide Therapy: Fifty-eight wounds of 35 patients were followed from day of injury to the 21st day, or until healed; however, 29 wounds of 16 patients were not cultured on day of injury. The majority of the cultures on the day of injury were from dead epidermis; all others were by swab technic. Sulfadiazine was started systemically on the day of injury and continued throughout the period of observation.

The incidence of all bacterial species encountered approximates that of the full-thickness wounds under sulfonamide therapy (Chart 4; cf. Chart 3). The only significant differences found in the partial- as compared with the full-thickness wounds are a slightly lower initial contamination and an increase in the third week in the incidence of the *Clostridia*.

(4) FLORA OF PARTIAL-THICKNESS BURN WOUNDS UNDER PENICILLIN THERAPY (CHART 5)

The bacterial flora of 35 wounds of partial-thickness were studied in 18 patients who were receiving penicillin systemically. The wounds were of varying extent, all open and ruptured blisters, and were of varying depths from superficial to nearly full-thickness. All were cultured on injury and were followed until the 21st day, or until healed. The cultures were all by swab technic.

The pattern of the bacterial strains recovered in the three weeks' period from injury to healing paralleled, in general, that of the first three weeks of the full-thickness burn wounds under penicillin therapy (Chart 5; cf. Chart 1).

The differences noted are that the number of strains recovered throughout were fewer, that the increase in number of strains in the first two weeks is numerically less and that neither the streptococci nor the *Clostridia* increased at all in incidence. What increase there was in bacterial strains, therefore, is accounted for by the increase in gram-negative bacilli and diphtheroids and *B. subtilis*.

It is to be noted that although there was no significant change in incidence of the gram-negative bacilli in the wounds of partial-thickness in the patients under the sulfonamides, the same rapid increase in numbers as in the full-thickness wounds took place in the partial-thickness wounds in the first 14 days under penicillin.

(5) DISTRIBUTION OF FLORA ACCORDING TO BODY AREA

In order to see whether the quality of the bacterial contamination differs in various body areas, influenced perhaps by proximity to the mouth or anus, the flora encountered in wounds of the head and neck, upper, and lower extremities have been separately listed (Table I). Using both partial- and full-thickness wounds of patients receiving no chemotherapy as well as of those receiving penicillin or sulfadiazine, there are available for this analysis 35 wounds of the head and neck, 105 of the upper extremities and 65 of the lower extremities. To permit differentiation of the initial contamination from that occurring while in the hospital the flora found on the day of injury and that from the 1st to 14th days after injury have been separately considered.

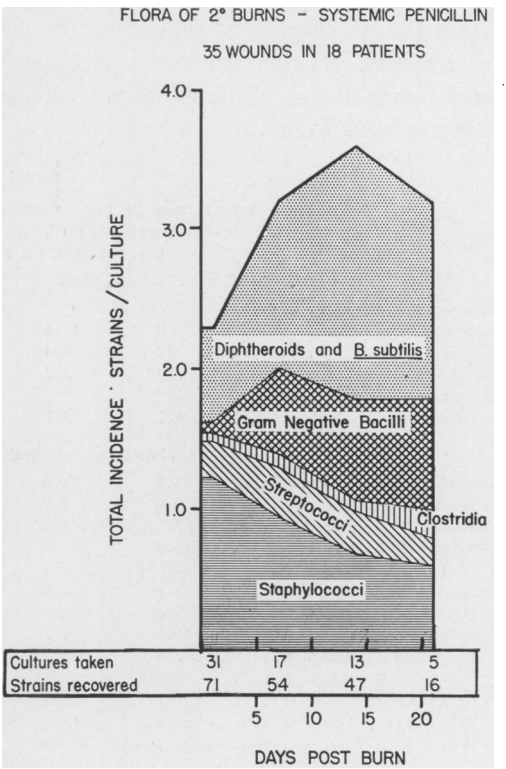

CHART 5

CHART 5.—The Bacterial Flora of Partial-thickness Burn Wounds in Patients Receiving Penicillin Therapy: Thirty-five wounds of 18 patients were followed from the day of injury to the 21st day or until healed. Cultures were by Swab technic. Systemic penicillin was started on the day of injury and continued throughout the period of observation.

From this cross-filling of the data it is apparent that wounds of the lower extremities were maximally contaminated at the time of entry to the hospital. Of all wounds these also showed the highest initial and subsequent *Clostridial* and *gamma* streptococcal infestation. Wounds of the head and neck showed the lowest initial contamination. Wounds both of the head and neck and of the upper extremities partook of the over-all increase in number and varieties

of bacterial strains in the first two weeks but the strains were throughout more abundant in the wounds of the upper extremities. In the wounds of the head and neck, *Clostridia* were least often encountered, but one-third as often as in the leg wounds, and, again in contrast to the legs, no *gamma* streptococci were recovered.

Of the other streptococci, not a single *beta* streptococcus was recovered from the 54 cultures taken on the day of injury from wounds of the upper extremities, yet the occurrence and distribution of the *alpha* streptococci in all wounds and at all times were remarkably even; it was the most common streptococcus found.

TABLE I

COMPARATIVE FLORAS OF BURN WOUNDS OF DIFFERENT BODY AREAS
PARTIAL AND FULL-THICKNESS BURN WOUNDS
SULFADIAZINE OR PENICILLIN

Body Area	Head and Neck		Upper Extremities		Lower Extremities	
Number of wounds	35		105		65	
Day postburn	0	1–14	0	1–14	0	1–14
Cultures taken	12	54	54	139	41	102
Strains recovered	39	202	182	563	167	423
Incidence of strains (strains per culture)	3.2	3.7	3:4	4:1	4.2	4.2
	Percentage of Strains Isolated					
Diphtheroids and *B. subtilis*	35.9	37.6	39.1	36.8	36.5	34.0
Gram-negative bacilli	2.6	9.9	3.3	6.9	3.0	9.6
Clostridia	5.1	3.0	13.7	6.2	15.6	9.6
Streptococci:	15.3	14.8	12.6	17.8	12.6	20.2
Beta streptococci	2.6	4.0	0	3.0	1.8	3.1
Alpha streptococci	12.7	10.8	12.6	12.3	10.2	12.8
Gamma streptococci	0	0	0	2.5	0.6	4.3
Staphylococci:	41.1	34.7	31.3	32.3	32.3	26.6
Staph. aureus, Coag. +	2.6	20.3	3.3	17.4	3.6	12.1
Staph. albus, Coag. +	2.6	1.5	0.6	2.1	1.8	4.1
Staph. aureus, Coag. −	7.7	1.0	2.2	2.1	6.6	0.9
Staph. albus, Coag. −	28.2	11.9	25.2	10.7	20.3	9.5

Contrary to the slow decline in number of strains of staphylococci found generally, including the wounds of head and neck and legs, there was no decrease in number in the wounds of the upper extremities. The increase in gram-negative bacilli is more prominent in wounds of the head and neck and lower extremities than in those of the arms.

CONCLUSIONS

In a study of the effectiveness of systemic chemotherapy in the control of the infection of burns, the appearance and subsidence of invasive infection has been observed and a comparison of the bacterial flora of burn wounds made in patients receiving either penicillin or sulfadiazine. These chemotherapeutic agents were found to exert a limited but different control of the organisms in the wound.

Statistical analysis of the influence of chemotherapy upon invasive infection has not been possible because of the difficulty in judging objectively its presence in the burn wound. The effectiveness of penicillin and sulfadiazine on invasive infection remains, therefore, a clinical impression. It will be discussed at the end of the fourth part.

The study of the bacterial flora of the burn wounds is objective, detailed and subject to analysis. Neither sulfadiazine nor penicillin obliterated bacteria in a burn wound. The lesser number of bacterial strains recovered in wounds of partial-thickness compared with those of full-thickness, under either penicillin or sulfadiazine, suggests that there are unknown influences in the full-thickness wound, presumably related to the amount of dead tissue, limiting the action of these chemotherapeutic agents.

In patients receiving penicillin the number of bacterial strains recovered from the wounds increased rapidly in the first two weeks after injury; from the wounds of full-thickness destruction the number of strains subsequently recovered was slightly higher than in the comparable wounds of patients receiving sulfadiazine.

Penicillin exerted a more decisive brake than sulfadiazine on the growth of staphylococci. In the wounds of patients treated with penicillin from the time of injury, the number of strains of staphylococci slowly declined, while in those of patients receiving sulfadiazine the number of strains did not vary. In patients in whom penicillin therapy was delayed the number of strains of staphylococci before therapy was the highest observed; following onset of penicillin there was a prompt fall in the number recoverable.

There was an abrupt rise in the number of strains of gram-negative bacilli recovered from the wounds of patients receiving penicillin in the 2nd week of therapy. That this rise did not appear before onset of therapy in patients in whom the penicillin was delayed and did appear in the same time sequence following administration of penicillin and that this early rise was not observed in the sulfadiazine-treated patients, suggests that the penicillin was responsible for this profusion of gram-negative organisms.

The number of strains of streptococci was held lower by sulfadiazine than by penicillin. The nature of this control is described in detail in the fourth part.

The wounds of certain body areas showed different bacterial contamination. Wounds of the lower extremity on the day of injury already showed the maximal number and variety of strains encountered. The *Clostridia* and the *gamma* streptococcus, organisms commonly found in the colon, were more often found in these wounds than in those of other areas.

From wounds of the head and neck the least number of bacterial strains was recovered on the day of injury. The number from the wounds of the upper extremity lay midway between that of the head and neck and of the lower extremity. No *gamma* streptococci were found initially in the wounds of either head and neck or upper extremities and no *beta* streptococci in the wounds of the latter area.

In seeking the source of the bacteria contaminating burn wounds it should be pointed out that the flora found in the three wound areas on the day of injury are reminiscent of the resident flora expected. Fecal organisms should be more prevalent on skin near the anus. Skin of the hands has been shown by Colebrook and Maxted,[17] to have the power to rid itself of the *beta* streptococcus. It is tempting, therefore, to assume that the organisms found

are the resident flora which survive the burn. If this were true the greater number of strains of organisms should have been found in the less deeply burned wounds rather than in those of full-thickness. This not being so, the resident flora may have been killed by the burn and the contaminating bacteria may have been transported from a location close to the wound, such as the clothing, or from the original source, as the anus itself.

Even if the quality of the flora found on the day of injury tends to exclude dust or other chance contamination as the principal source of the original bacteria, the inadvertent transfer of organisms from the wound of one patient to that of another, which has been observed in the hospital in spite of strict precautions, points-up the necessity of stringent regulations for the care of burn patients.

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(Continue with Part II)

PART II

THE DEVELOPMENT OF RESISTANCE TO PENICILLIN BY THE FLORA OF FULL-THICKNESS BURN WOUNDS IN PATIENTS TREATED WITH PENICILLIN SYSTEMICALLY

THE ENCOUNTERING of drug-resistant bacteria in patients during the course of sulfonamide therapy, the report of a comparable development of resistance of organisms to penicillin and of the power of bacteria to inhibit the action of penicillin, prompted the estimation, as an integral part of the study of the bacteriology of burn wounds, of the sensitivity and the development of resistance to penicillin and of the inhibition of penicillin by the bacterial flora. When this study was initiated in 1943 there was evidence that certain types of bacteria, normally sensitive to the sulfonamides, could develop an indifference or resistance to the drug.[1] Grown *in vitro* in media containing increasing concentrations of a sulfonamide, the metabolism of the organisms changed to the extent of becoming inured to the drug and growing without restraint. Such resistance was also believed to develop *in vivo*. Colebrook,[2] from the burn wounds of patients receiving sulfonamides, recovered first a sulfonamide-sensitive *beta* streptococcus, subsequently a drug-fast organism of the same strain, and reasonably suggested that the fastness had been induced.

When penicillin first became available to civilian Centers in this country, observations were available suggesting that similar development of drug resistance was to be expected during the course of penicillin therapy.[3, 4] Measurement of the development of resistance or loss of sensitivity should indicate not only the period during which the drug could be expected to be maximally effective but also whether the drug penetrated the burn slough, should the organisms in the slough retain their sensitivity.

Just as the bacteriostatic action of the sulfonamides had been found by several workers to be inhibited by breakdown products of bacteria and cells.[1] so that of penicillin had been reported thwarted by growth of nonsensitive organisms. Abraham and Chain[5] recorded, in 1940, that the gram-negative bacilli produced an enzyme-like substance which destroyed penicillin, and which they termed penicillinase. Because of the deterring influence which contaminating bacteria might exert through such inhibition, measurement of inhibition by those organisms commonly encountered in the flora of burn wounds was deemed prudent.

METHODS

A first part of the laboratory effort of this study was the development of methods for the measurement of penicillin sensitivity and inhibition. The first test of the bacterial sensitivity to penicillin (Introductory Test), useful as a step in the measurement of bacterial inhibition, proved unnecessarily cumbersome as a routine test and was supplanted by a simpler method (Routine Test).

Bacterial Sensitivity to Penicillin (Introductory Test).—The naturally-occurring penicillin sensitivity of a bacterium was determined by comparing its growth in a medium containing penicillin with that in a penicillin-free medium. Growth was judged by turbidity of the media. Five-cubic centimeter portions of nutrient broth containing 50, 25, 5, 1 and 0 units of penicillin per cubic centimeter were inoculated with 0.1 cc. of an 18-hour broth culture of the organism to be tested and incubated for 18 hours at 37° C. Any strain whose growth was not inhibited by penicillin in the concentrations above five units per cc. was considered to be resistant to penicillin. Any strain whose growth was inhibited by concentrations of five, or less, units per cc. was considered sensitive.

Bacterial Inhibition of Penicillin.—To decide whether a test organism had the power to inhibit the antibacterial action of penicillin, small disks of sterile filter paper which had been dipped into the inoculated broth tubes containing none or varying concentrations of penicillin were laid upon the surface of blood agar plates previously inoculated with a staphylococcus of known high sensitivity to penicillin. (No attempt was made to destroy the test strain growing in the broth tubes.) After incubation at 37° C. for 18 hours the plates were examined for zones of inhibition of the growth of the staphylococcus around the disks. An organism was considered to be a penicillin-inhibitor if the growth of the known staphylococcus on the plate was not inhibited and if the test fluid had come from a tube which had contained five units, or more, of penicillin per cubic centimeter.

Bacterial Sensitivity to Penicillin (Routine Test).—All gram-positive organisms isolated from the patients on penicillin, except diphtheroids and aerobic sporulating bacilli, were tested for sensitivity to penicillin. A five per cent horse blood agar "pour" plate was made with 0.05–0.1 cc. of an 18-hour liquid culture of the organism to be tested and allowed to harden. Filter paper disks, cut with an ordinary paper punch from thin, "rapid filter" type paper and sterilized by dry heat, were dipped into solutions of 50, 25, 5 and 1 units per cc. of penicillin in isotonic saline, drained on sterile gauze sponges, and laid on the surface of appropriately marked segments of the plate. After 18-hour incubation at 37° C., aerobically or anaerobically according to the requirements of the organism under test, the plates were examined for zones of inhibition of growth of the test organism by the penicillin. Strains not inhibited by concentrations of penicillin above five units per cc. were considered resistant and are classified as such in the charts.

OBSERVATIONS

1. *Natural Ability of Wound Bacteria to Resist and Inhibit Penicillin.*—The penicillin resistance and inhibitory power of 207 bacterial strains were studied. The strains were either isolated from patients with various surgical infections or found as contaminants in the laboratory media. Since none of the patients from whom the bacteria were isolated had received penicillin and, indeed, since no patient had been studied up to this time who had re-

ceived penicillin, it was deemed that none of the organisms could have an artificially-induced reaction to penicillin. The findings are summarized in Table II.

Of the 16 strains of streptococci tested, only one (6 per cent) was found to be resistant to penicillin. This was a *beta hemolytic Streptococcus,* Group A-Lancefield, which was not inhibited by concentrations of 50 units of penicillin per cc. of broth and which showed no evidence of inhibitory action against penicillin. (This native resistance of a *beta hemolytic Streptococcus* has not otherwise been encountered in our experience, nor have we found it mentioned in the literature.) One strain (6 per cent), a sensitive *gamma nonhemolytic Streptococcus,* was found to be inhibitory to penicillin.

Of the staphylococci, 8 of 118 strains (7 per cent) were resistant to penicillin; 8 (7 per cent), 6 of the 8 resistant and 2 sensitive strains, inhibited the drug. Five (16 per cent) of the 32 coagulase-positive and four (5 per cent) of the 86 coagulase-negative strains were resistant, a three-fold greater incidence of resistance among the more virulent staphylococci. Of the coagulase-positive strains, none of the 27 sensitive and three of the five resistant organisms (60 per cent) were inhibitory; of the coagulase-negative, two of the 82 sensitive (3 per cent) and 100 per cent of the five resistant strains were inhibitory. Of the 45 *Staph. aureus,* no sensitive and four of the six resistant (67 per cent), and of 73 *Staph. albus* the two resistant and two of the 71 sensitive strains (3 per cent) inhibited the penicillin. Thus, penicillin inhibition by sensitive strains of staphylococci was found only in 2 coagulase-negative *Staph. albus,* both recovered as contaminants on laboratory media; natively resistant strains do not invariably produce penicillin inhibitors detectable by this method.

Nine of 30 diphtheroids (30 per cent) were resistant to penicillin; all of these and three of the 31 sensitive strains (14 per cent) inhibited the drug's action.

Twenty-two of 34 aerobic spore-formers (*B. subtilis*) (65 per cent) were resistant to penicillin and 27 (80 per cent) inhibited its action. One resistant strain did not inhibit, but six (50 per cent) of the sensitive strains did so.

Only nine strains of gram-negative bacilli of the *coli-pyocyaneus-proteus* group were tested at this time. All were resistant to penicillin, and all but the two strains of *B. proteus* were inhibitory. Since that time we have tested four more strains of *B. proteus* and found them to be inhibitory; these were, however, isolated from patients who were receiving the drug.

In summary, there is no absolute correlation between the organisms resistant to the bacteriostatic action of penicillin and those capable of inactivating or inhibiting this agent.

2. Resistance to Penicillin of the Flora of Full-thickness Burn Wounds in Patients Treated with Penicillin.—The initial occurrence of resistance to penicillin and its subsequent development was followed in the gram-positive strains recovered from the 80 wounds of the 28 patients with full-thickness burn wounds treated with penicillin from the day of injury. (The wounds and

TABLE II

RESISTANCE TO, AND INHIBITION OF, PENICILLIN BY ORGANISMS NOT PREVIOUSLY EXPOSED TO THE DRUG

Organism	Number of Strains Tested	Penicillin-sensitive Per Cent	Penicillin-resistant Per Cent	Penicillin-inhibiting Per Cent	Penicillin-noninhibiting Per Cent	Penicillin-sensitive			Penicillin-resistant		
						Total Number	Inhibiting Per Cent	Non-inhibiting Per Cent	Total Number	Inhibiting Per Cent	Non-inhibiting Per Cent
Streptococci	16	94	6	6	94	15	7	93	1	0	100
Beta	8	88	12	0	100	7	0	100	1	0	100
Alpha	6	100	0	0	100	6	0	100	0	..	..
Gamma	2	100	0	50	50	2	50	50	0	..	..
Staphylococci	118	93	7	7	93	110	2	98	8	75	25
Coag +	32	84	16	9	91	27	0	100	5	60	40
Coag 0	86	95	5	6	94	82	3	97	5	100	0
Staph. aureus											
Coag +	30	83	17	10	90	25	0	100	5	60	40
Coag 0	15	93	7	7	93	14	0	100	1	100	0
Staph. albus											
Coag +	2	100	0	0	100	2	0	100	0	..	..
Coag 0	71	97	3	6	94	69	3	97	2	100	0
Diphtheroids	30	70	30	47	53	21	14	86	9	100	0
Aerobic spore-formers	34	35	65	79	21	12	50	50	22	96	4
Gram-negative bacilli	9	0	100	78	22	0	..	..	9	78	22
Total	207										

patients are the same as those studied under section one (Chart I) of Part I.) The sensitivity of a few strains, mainly *Clostridia,* which were difficult to grow and isolate, was not observed. The penicillin sensitivity of diphtheroids, *B. subtilis,* and gram-negative bacilli was, also, not observed. It was taken for granted from the previous studies that the diphtheroids and *B. subtilis* groups were 50 per cent penicillin-resistant, and the gram-negative

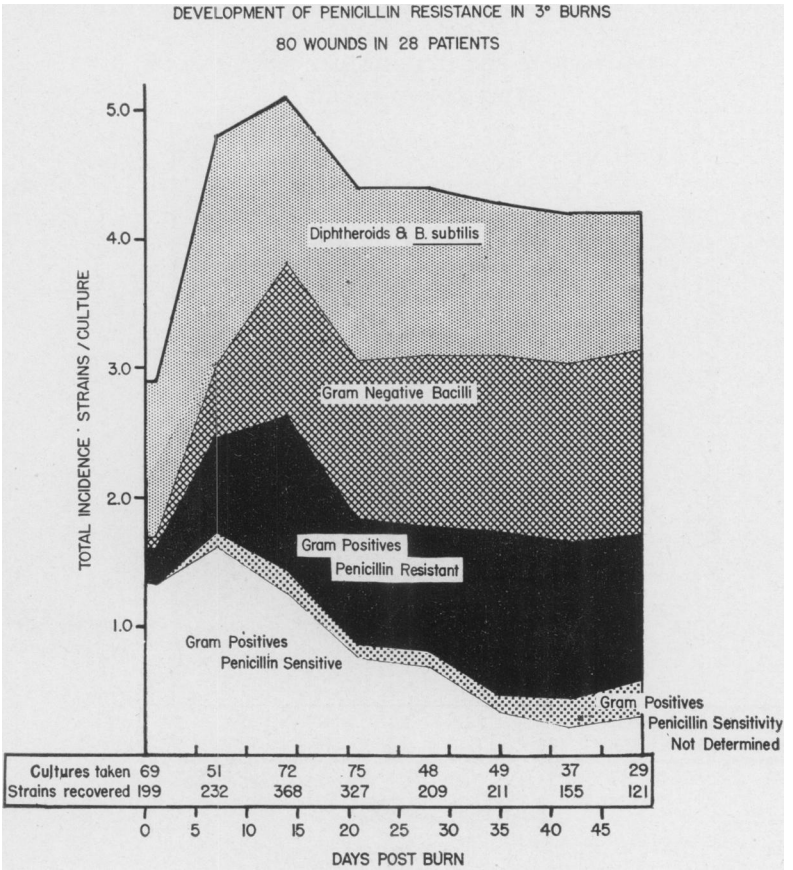

CHART 6

CHART 6.—The Occurrence of Penicillin Resistance in the Flora of Full-thickness Burn Wounds of Patients Receiving Penicillin Therapy: Eighty wounds of 28 patients (cf. Chart 1 of Part I) were followed from the day of injury throughout a course of penicillin therapy. All gram-positive organisms, except diphtheroids and aerobic spore-formers (*B. subtilis*), were tested for sensitivity to penicillin. Strains not inhibited by concentrations of penicillin of 25, or more, units per cubic centimeter were considered penicillin-resistant; strains inhibited by 5, or less, units per cubic centimeter were considered penicillin-sensitive.

bacilli 100 per cent resistant. No study of the inhibitory power of any of the organisms was undertaken although the experience with the four *B. proteus* strains quoted in the previous section suggests that this might have been interesting.

Approximately 30 per cent of the bacterial strains recovered on the day of injury by swab culture were found to be penicillin-resistant (Chart 6). This 30 per cent was made up of one-half of the diphtheroids and *subtilis*, all of the gram-negative bacilli and 20 per cent of the gram-positive organisms, including streptococci, staphylococci and *Clostridia*. During the course of penicillin therapy, after an increase of penicillin-sensitive strains in the first seven days, there was a progressive disappearance until the 42nd day when

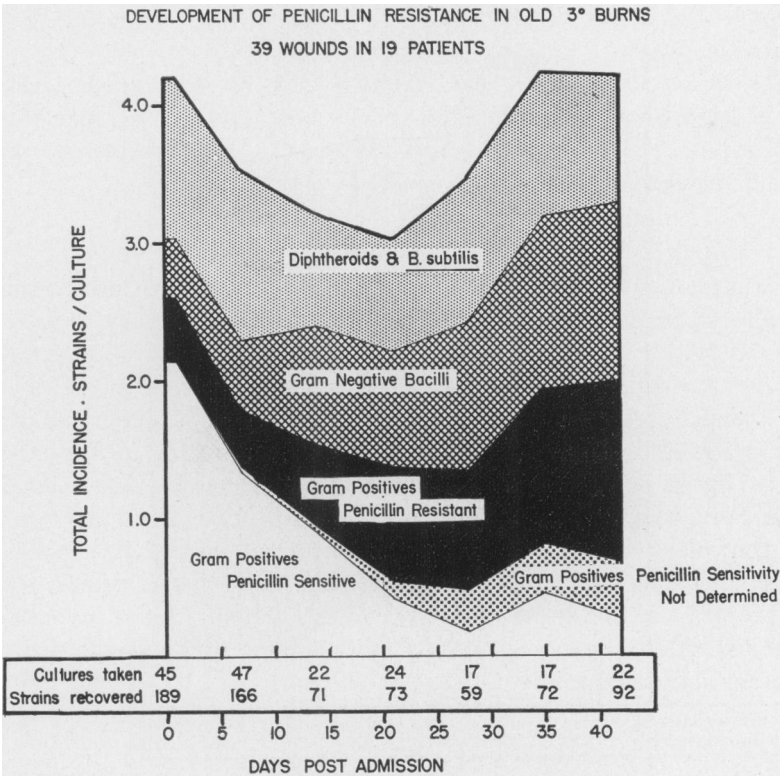

CHART 7

CHART 7.—The Occurrence of Penicillin Resistance in the Flora of Full-thickness Burn Wounds of Patients whose Hospital Admission and Penicillin Therapy were Delayed: Thirty-nine wounds of 19 patients (cf. Chart 2 of Part I) were followed from the day of admission throughout a course of penicillin therapy. Penicillin resistance of the flora was ganged in this group of patients as described in the legend of Chart 6.

the minimum number was reached. During the first seven days there was also an increase in number of resistant gram-positive organisms. After the 7th day, as the sensitive strains disappeared they were replaced by resistant strains in approximately the same proportion. By the 14th day after injury the number of resistant gram-positive strains was quadrupled, and by the 21st day there was only half the original number of gram-positive sensitive strains left. By the 42nd day only 10 per cent of all the gram-positive strains re-

covered were sensitive. In addition to these changes in the gram-positive flora, in the 42 days there had been an eleven-fold increase in the gram-negative strains. Thus, in contrast to the day of injury, when 70 per cent of all of the strains had been sensitive, on the 42nd day not more than 19 per cent were sensitive *in vitro* to penicillin.

The initial occurrence and subsequent development of resistance to penicillin was also studied in the bacteria recovered from the wounds of those patients with full-thickness burn wounds in whom the onset of penicillin therapy was delayed. The gram-positive flora of the 39 wounds of the 19 patients was studied (Chart 7; cf. Chart 2 of Part I).

The trends of decreasing sensitivity and increasing resistance are the same as in those wounds of the patients in whom penicillin was started on the day of injury. The minimum number of sensitive strains is reached earlier, however, at the 28th instead of the 42nd day.

CONCLUSIONS

The progressive increase in numbers of bacteria which are resistant to penicillin and which normally are predominantly sensitive, the concomitant decline in numbers of penicillin-sensitive bacteria and the early overgrowth of bacteria commonly inhibitory of penicillin action in the full-thickness burn wounds of patients receiving penicillin therapy, suggest that the most effective therapeutic period of the drug is sharply limited to the first three weeks. By the 5th week of penicillin therapy the wound is a quagmire of resistant and inhibiting bacterial organisms over which penicillin exerts but little control. Though both drug resistance and inhibition are measured *in vitro*, the parallelism of the observed resistance to, and inhibition of, penicillin by the bacteria recovered from the burn wound and the clinical impression of the limited effectiveness of penicillin in controlling infection in the later weeks of therapy indicate that the tests are significant.

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(*Continue with Part III*)

PART III

THE INCIDENCE, VIRULENCE AND RESISTANCE TO PENICILLIN OF THE STAPHYLOCOCCUS IN BURN WOUNDS OF PATIENTS TREATED WITH PENICILLIN OR SULFONAMIDES

THE SIGNIFICANCE OF STAPHYLOCOCCAL IMMUNITY TO THE HEALING OF THE WOUNDS INFECTED WITH THE STAPHYLOCOCCUS

THE POSSIBLY injurious rôle of the staphylococcus in burn wounds has received but scant attention, overshadowed, as it has been, by the flamboyant infection of the streptococcus. With the exception of the tetanus organism, known in a burn wound to give rise occasionally to clinical tetanus, students of infection in burns, traditionally, have been engrossed with the streptococcus. The vital dyes (gentian violet, brilliant green and acriflavine) were introduced by Aldrich,[1] with the objective of controlling the streptococcus. Investigating the effect of the sulfonamides in burns during 1942 and 1943, Colebrook, and his collaborators,[2] although recounting the presence of the staphylococcus and other organisms, have continued to center their thoughts on the streptococcus. They state: "Staphylococci have given rise to comparatively little acute or serious sepsis, and their influence upon the rate of healing in burns has been much less evident than that of hemolytic streptococci. Unlike the streptococci, too, they have seldom, if ever, been responsible for the failure of grafting operations."

The advent of the sulfonamides, the first relatively nontoxic agent achieving control over the streptococcus, and recent knowledge of the virulence and immunologic reactions of the staphylococci have set the stage for the study of the staphylococcal infection of burn wounds. Lyons,[3] in his bacteriologic study of the nine victims of the Cocoanut Grove disaster with full-thickness burns, who were treated with sulfadiazine, found a singular discrepancy in the incidence in the wounds of the streptococci and that of the staphylococci. The *beta* streptococcus was recovered from 30 per cent, or less, of the wounds and had disappeared by the 6th week. The *alpha* streptococcus, present in the 1st week in 60 per cent of the wounds, had disappeared by the 5th week. The *gamma* type was recovered even less often than the *beta*. The staphylococcus, found in every wound, persisted in the virulent form in more than 80 per cent of the wounds throughout the nine weeks of observation. Such a disparity in incidence between the streptococcus and staphylococcus, presumably due to the suppression of the streptococcus by the sulfadiazine, offered a means of judging the destructiveness of the staphylococcus in the wounds of patients to be treated with sulfadiazine. The disparity also offered the control needed to evaluate the effectiveness of penicillin against both streptococcus and staphylococcus.

Also, indicating a more extended study of the staphylococcus in burn wounds, was the recent recognition of the varying pathogenicity of its types and of an immunologic response to its presence. As the result of experience

with surgical infections other than burns, such as the carbuncle, in which the staphylococcus was the sole organism recovered, it was generally agreed[4-9] that the clinical virulence of the infection varied with the metabolic type of the staphylococcus. The yellow staphylococcus, *Staph. aureus,* is more virulent than the white, *Staph. albus;* the property of coagulating human plasma protein also denotes greater virulence. Lyons[10] has reported the presence in the blood serum of rabbits injected with heat-killed staphylococci of agglutinins specific for the strain of staphylococcus. By observing the color and coagulating property of the staphylococci found, by search for specific agglutinins and by quantitation of certain nonspecific globulins in the blood serum of the burned patients, it was believed objective data could be obtained regarding the virulence of the infection and the degree of immune response.

METHODS

In addition to the bacteriologic methods recounted in the first two parts and the usual chemical methods for estimating plasma protein, the following methods have been used:

The euglobulin and pseudoglobulin levels of the blood serum were determined by the sodium sulfate protein fractionation method of Howe.[11]

Specific agglutinins to the staphylococcus recovered from the wound were tested for in the blood serum of the patient by the macroscopic slide-agglutination technic of Lyons.[10]

Autogenous staphylococcal vaccine was prepared according to Lyons.[10]

OBSERVATIONS

Incidence.—In every full-thickness burn wound the presence of a staphylococcus is to be expected. From each culture taken on the day of injury, 1.1 strains of staphylococci were recovered from the wounds of the patients subsequently treated with penicillin (Chart 9), and 1.2 strains from those of the patients subsequently treated with sulfadiazine (Chart 8).* As pointed out in the first part of this study on bacteriology, during the course of systemic therapy with penicillin the over-all incidence of the staphylococcus in the full-thickness burn wound slowly decreased, while under sulfadiazine there was no change. When the individual wounds are examined, approximately one-third of the wounds under either drug lost their staphylococci before healing had taken place. Of the 46 wounds in patients treated with sulfadiazine, 33 per cent lost their staphylococci. Of the 80 wounds in patients receiving penicillin, 37 per cent lost theirs. This apparent discrepancy with the over-all incidence is explained by the fact that certain patients under

* A slightly higher incidence of the staphylococcus in the 13 cultures made by means of débrided tissue in the cases subsequently treated by sulfonamides might appear to be due to the method of culturing. In the second-degree burns, however, the different methods of culturing yielded identical numbers of staphylococcal strains per culture (Charts 3 and 4 of Part I. The slight difference in the full-thickness burn wound is probably not statistically significant since but 13 cultures were taken in the cases subsequently treated by sulfonamides.

sulfadiazine having multiple wounds lost all staphylococci, whereas other patients acquired two, and even three, different staphylococci per wound, and lost none.

A comparable disappearance of staphylococci from wounds of patients

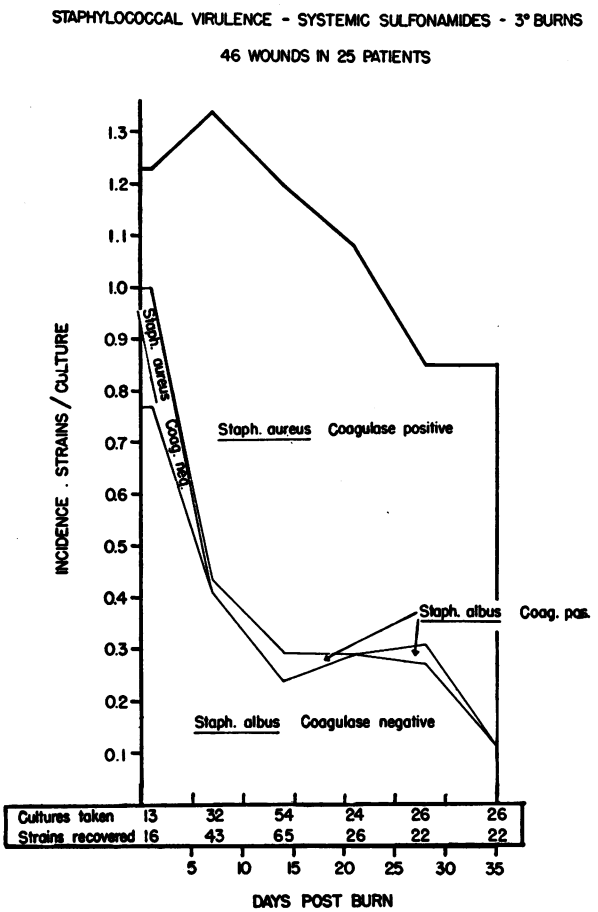

CHART 8

CHART 8.—The Incidence and Virulence of the Staphylococci in the Flora of Full-thickness Burn Wounds of Patients Receiving Sulfonamide Therapy: The staphylococci isolated from 46 wounds of 25 patients (cf. Chart 3 of Part I) were tested for chromogenesis and the power to coagulate human plasma protein. (Hemolysis was noted but is not recorded.) Virulence was graded on the basis of these two attributes; thus, the *Staph. aureus*, coagulase-positive, is fully virulent, the *Staph. albus*, coagulase-negative, avirulent.

under no chemotherapy was observed in the few patients available for study during the course of these observations who did not receive one, or another, chemotherapeutic agent. No common factor in the condition of the wounds

of the groups of patients, such as the presence of slough or the local treatment applied, were discerned which could account for the disappearance of the staphylococci.

Virulence.—The number and proportion of virulent strains of the staphylococci increased in full-thickness burn wounds with the passage of time under either sulfadiazine or penicillin therapy. Of the staphylococci recovered on the day of injury 75 per cent were of the least virulent form, *Staph. albus,* coagulase-negative, and but 15 per cent of the fully virulent, *Staph. aureus,* coagulase-positive. The two intermediary groups made up the remaining 10 per cent.

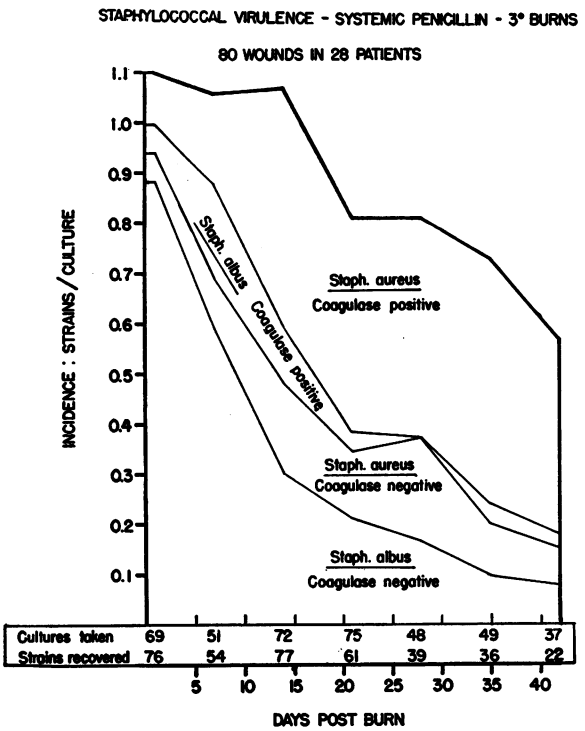

CHART 9

CHART 9.—The Incidence and Virulence of the Staphylococci in the Flora of Full-thickness Burn Wounds of Patients Receiving Penicillin Therapy: The staphylococci isolated from 80 wounds of 28 patients (cf. Charts 1 and 6 of Parts I and II) were tested for chromogenesis and the power to coagulate plasma and the virulence graded as recounted in legend of Chart 8.

During the course of the first six weeks there was a progressive loss of the avirulent and a steady increase of the virulent forms, until by the 6th week the relation of *"albus"* to *"aureus"* was reversed. At this time, in the penicillin-treated cases the *"albus,"* coagulase-negative, accounted for less than 20 per cent of the strains recovered, and the *"aureus"* for 70 per cent;

in the sulfadiazine-treated cases, 85 per cent of the staphylococci were of the *aureus* form. Even when the decrease in number of total staphylococcal strains under penicillin is taken into account, there was, after six weeks of therapy with either drug, a greater number of virulent strains recoverable per culture which were fully virulent than there had been on the day of injury.

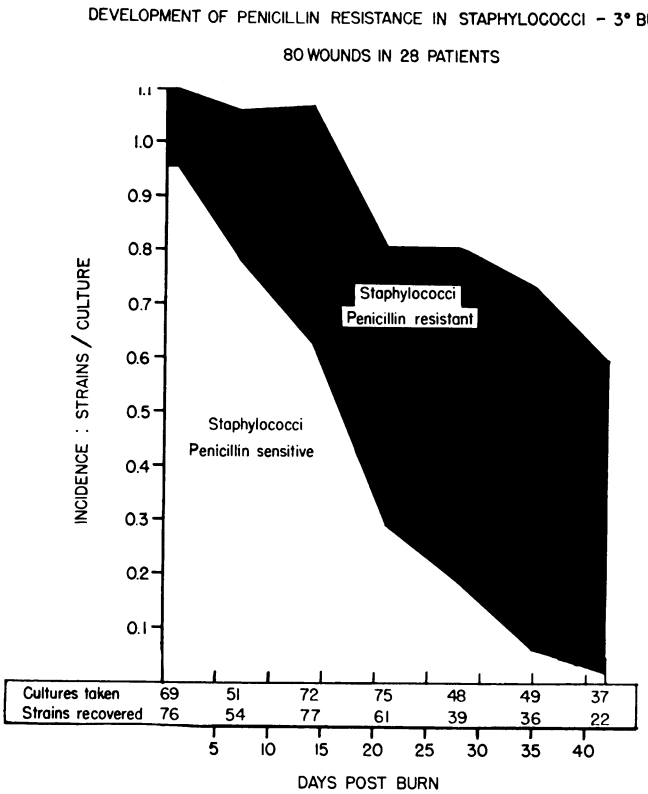

CHART 10

CHART 10.—The Penicillin Resistance of the Staphylococci in the Flora of Full-thickness Burn Wounds of Patients Receiving Penicillin Therapy: The staphylococci were isolated from 80 wounds of 28 patients (same group as in Chart 9; cf. Charts 1 and 6 of Parts I and II).

The *Staph. aureus,* coagulase-negative, and *Staph. albus,* coagulase-positive, the intermediary types, at any one time made up but a small proportion of the total staphylococcal strains; their number was almost negligible during the course of sulfonamide therapy, while their slightly greater incidence under penicillin is probably statistically significant.

Penicillin Resistance.—Even more striking than the changes in virulence of the staphylococcus recovered from full-thickness burn wounds, with the passage of time, is the development in these organisms of penicillin resistance. In the burn patients subsequently treated with penicillin but 14 per cent of the

strains of staphylococci were resistant on the day of injury and before onset of therapy. At the end of six weeks of penicillin treatment more than 90 per cent of the staphylococci recovered were resistant to the drug (Chart 10). All four types of the staphylococci developed resistance, but, apparently, not quite to the same degree (Chart 11). The fully virulent types by the 6th week were all resistant. Only the most avirulent form, *Staph. albus,* coagulase-negative, had

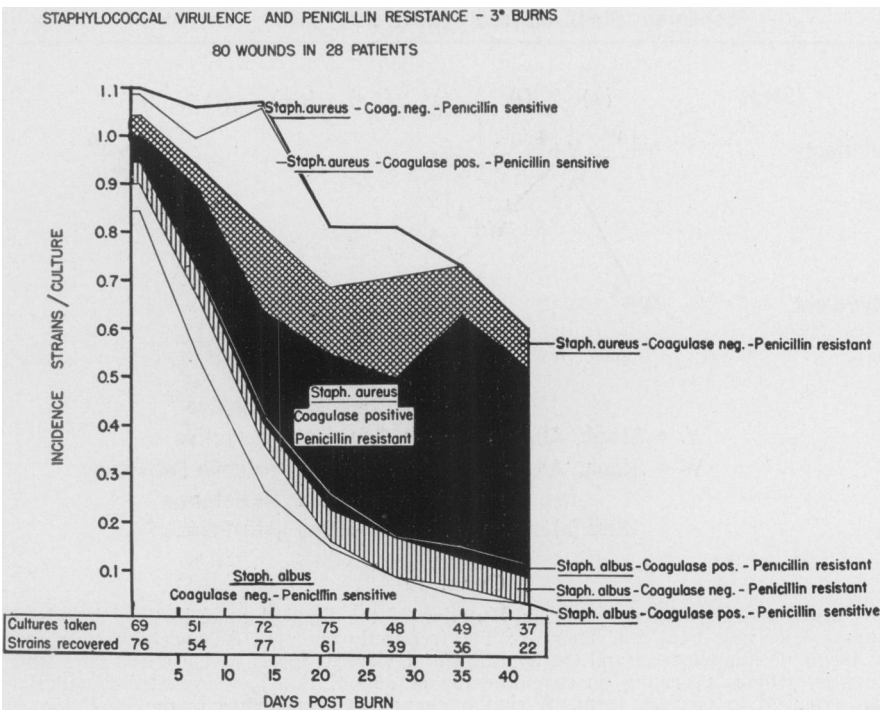

CHART 11

CHART 11.—The Virulence and Penicillin Resistance of Staphylococci in the Flora of Full-thickness Burn Wounds of Patients Receiving Penicillin Therapy: This is a composite of Charts 8 and 9.

a significant number of penicillin-sensitive organisms during the 5th and 6th weeks of treatment.

Evidence was obtained of two types of acquired resistance to penicillin. The *in vivo*-acquired resistance may be permanent. The burn wound of one patient (Case 188), and the donor site of another (Case 137), both of which were infected originally with a penicillin-sensitive staphylococcus, during the course of penicillin therapy, yielded a resistant organism in place of the sensitive one. After two and 15 weeks of therapy, no further penicillin was administered. Two and one-half and three months after cessation, the staphylococcus recovered from each was still resistant. From the burn wounds of a third patient (Case 168) a penicillin-resistant staphylococcus was recovered three months after cessation of therapy. It is possible in this 3rd

patient that the staphylococcus was resistant before onset of therapy, since she had been treated with penicillin in another hospital before coming here, and no culture had been taken prior to onset of the therapy. The staphylococcus recovered from all three patients was of the fully-virulent type.

The *in vitro*-acquired resistance, which we have succeeded in producing, on the other hand, has proved transient. Penicillin resistance was induced

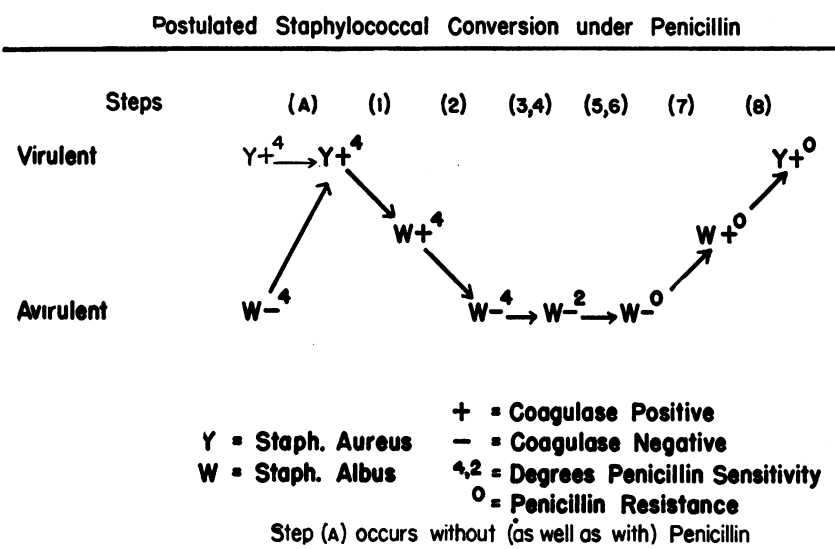

CHART 12

CHART 12.—Postulated Metabolic Mutation of the Staphylococci in the Wounds of Patients Receiving Penicillin Therapy: The frequently observed disappearance of avirulent forms of staphylococci and the appearance of virulent forms in the initial days after injury in patients receiving no chemotherapy or sulfadiazine has suggested a mutation from avirulent to virulent form. A similar change has been noted in the staphylococci recovered from patients treated with penicillin. During the succeeding two weeks of penicillin therapy avirulent forms are again found; such a reappearance has not been encountered in sulfonamide-treated patients or in those receiving no chemotherapy. After the 3rd week of penicillin therapy, when penicillin-resistant strains predominate, virtually all strains recovered are fully virulent. It is postulated that an initial effect of penicillin on staphylococci is a reduction of virulence but that if the staphylococcus survives by developing resistance, full virulence is regained.

in sensitive staphylococci by repeated cultures in media containing increasing concentrations of penicillin. This induced resistance was quickly dissipated by culturing the organisms in penicillin-free media. Only the *Staph. aureus,* coagulase-positive type, was used in these experiments. Spink, *et al.*,[12] have reported comparable findings.

Mutation Theory.—The gradual replacement of the avirulent by the virulent form of the staphylococcus in all burn wounds (Charts 8 and 9) and the frequency with which a change in form has been seen in wounds in which a single staphylococcal strain has been recovered at any one time, point strongly to a mutation of the staphylococcus. Such a mutation from the *Staph. albus*

to *Staph. aureus,* and from coagulase-negative to coagulase-positive has been previously postulated.[13-15] With the advent of penicillin, evidence has been obtained which carries the concept of the mutation two steps further (Chart 12). The first change of the penicillin-sensitive avirulent white staphylococcus is to a virulent yellow staphylococcus, still penicillin-sensitive. This step is the previously postulated mutation and, apparently, takes place whether the patient is on penicillin or not. In the patients under systemic penicillin therapy, the next change appears to be a reversion to a white form, with loss of coagulating power but still sensitive to penicillin. If the organism does not disappear and chemotherapy is continued, resistance to the drug develops. When the resistance is fully developed there is a second and final reversion to the virulent form.

Proteolysis.—During the course of penicillin therapy the wounds of nine of the patients with full-thickness burns developed a superficial dissolution which we had never previously seen and which was associated with a penicillin-resistant *Staph. aureus* and a *B. proteus.* The wound is characteristic in appearance, stubborn in therapy, and has been termed "proteolytic" because of its salient character. Such proteolytic wounds have not been encountered until after three weeks of penicillin therapy. There is a superficial liquefaction of epidermis and derma unaccompanied by any deep or subcutaneous inflammatory reaction. The margins of the wound, or of grafts previously placed upon the wound, melt away, leaving cadaveric-appearing, uninflamed yellow fat. The veins of the subcutaneous plexus may lie exposed on the surface of the fat, apparently intact, giving the wound its cadaveric look. There is little bleeding from the wound with change of dressing, and no granulations or fibrous tissue proliferation; little edema is visible. The wounds may lie, weeks long, in such an inactive state. After final epithelial closure, a late, and prolonged, deep inflammatory process is common with tenderness persisting as long as six months, and eventual profuse keloid formation.

In all of the proteolytic wounds of the nine patients, a penicillin-resistant *Staph. aureus* and a *B. proteus* was recovered during the proteolytic phase. This same combination of organisms has been encountered in nine of the wounds of other patients under penicillin therapy, without any evidence of proteolysis. Although there were no other organisms common to all the proteolytic wounds, an *alpha* streptococcus was found in several, and clostridial organisms were found in a few.

The late inflammatory reaction occurred in five of the seven patients in whom a proper follow-up was obtained. (Two of the nine patients were obliged to leave this region soon after discharge from the hospital.) One of the two patients not developing the late inflammation was injected with autogenous staphylococcal vaccine in an effort to prevent it (Case 168, see below under Immunity). None of the nine patients having wounds with the penicillin-resistant staphylococcus and *B. proteus* combination, but without proteolysis, had a late inflammatory reaction. Cultures were not obtained in the stage of the late inflammatory reaction, but it is believed that the staphy-

lococcus is involved (see below under Immunity). Up until the time of healing both organisms had been consistently present.

Because this type of proteolytic wound had never been observed by us before, and because all of the patients were on penicillin therapy, it is a temptation to ascribe this proteolytic process to the penicillin-resistant staphylococcus.* It is possible, of course, that chance prevented its occurrence in the sulfonamide-treated patients, or that it was not observed, when present, in these or in burn patients seen before the days of chemotherapy.

The wounds have proven stubborn in healing. All methods of local therapy, including gramicidin, have been tried, largely without success. Attempts to find another common factor have proved unsuccessful. Cachexia was present in one patient at onset, but the wound did not heal with improved nutrition. All of the patients received a high vitamin (including 1 Gm. of cevitamic acid a day), high caloric, high protein diet, comparable to that given to other patients. No evidence was obtained of any different pattern of the 17-ketosteroid excretion or creatinuria from those patients without comparable wounds.

That the staphylococcus was implicated, by more than mere presence, is suggested by the fact that eventual healing in the most stubborn of the wounds was achieved only after specific immunity with rise in agglutinin titer was obtained by injections of autogenous staphylococcal vaccine (Case 168, see below under Immunity).

Immunity.—The control of invasive staphylococcal infection and the healing of full-thickness burn wounds infected with the staphylococcus, may involve immune reactions or considerations beyond local surface treatment and specific antibacterial chemotherapy in the form of penicillin. The immune reactions presumably are both specific and nonspecific. They have been encountered during the study of patients with wounds showing the proteolysis of the staphylococcus and *B. proteus,* described in the preceding section, and with invasive staphylococcal infection not controlled by large doses of penicillin. The staphylococcus in both its penicillin-sensitive and resistant forms has been involved. The specific immune reaction measured was the blood serum's agglutinating power of the specific staphylococcus infecting the patient. The nonspecific tests included the blood serum globulin, euglobulin, pseudoglobulins I and II, albumin and total protein. The situation in which immune reactions are believed to be involved are illustrated by the following case reports:

CASE REPORTS

Immunity and the Healing of Proteolysis: Case 168 illustrates spectacular repeated wound healing and wound recession of proteolytic type, with concurrent change in the titer of agglutinins to the staphylococcus. Final healing was achieved, coincidentally, with vaccine therapy and maintenance of a high agglutinin titer.

* This same proteolytic process was incidentally seen at the Hartford Hospital, at the invitation of Dr. Donald Wells, in one of the victims of the circus fire, in the 4th week after injury. This patient had also been on penicillin therapy.

A 34-year-old housewife was transferred to this hospital 37 days after receiving extensive deep burns when her house burned down. She had been treated with both sulfonamide and penicillin therapy before coming to this hospital. On entry, she ran a high, spiking fever. Extensive full-thickness wounds of both arms, back, and front of the chest showed signs of deep infection. The patient had been on a "slimming" diet before her injury and had managed to lose 25 pounds. She was thin and cachectic on arrival here, but on a high vitamin, high caloric, high protein diet made a rapid weight gain in the first six-weeks' period. She was given a high dosage of penicillin therapy, with slow subsidence in the febrile reaction.

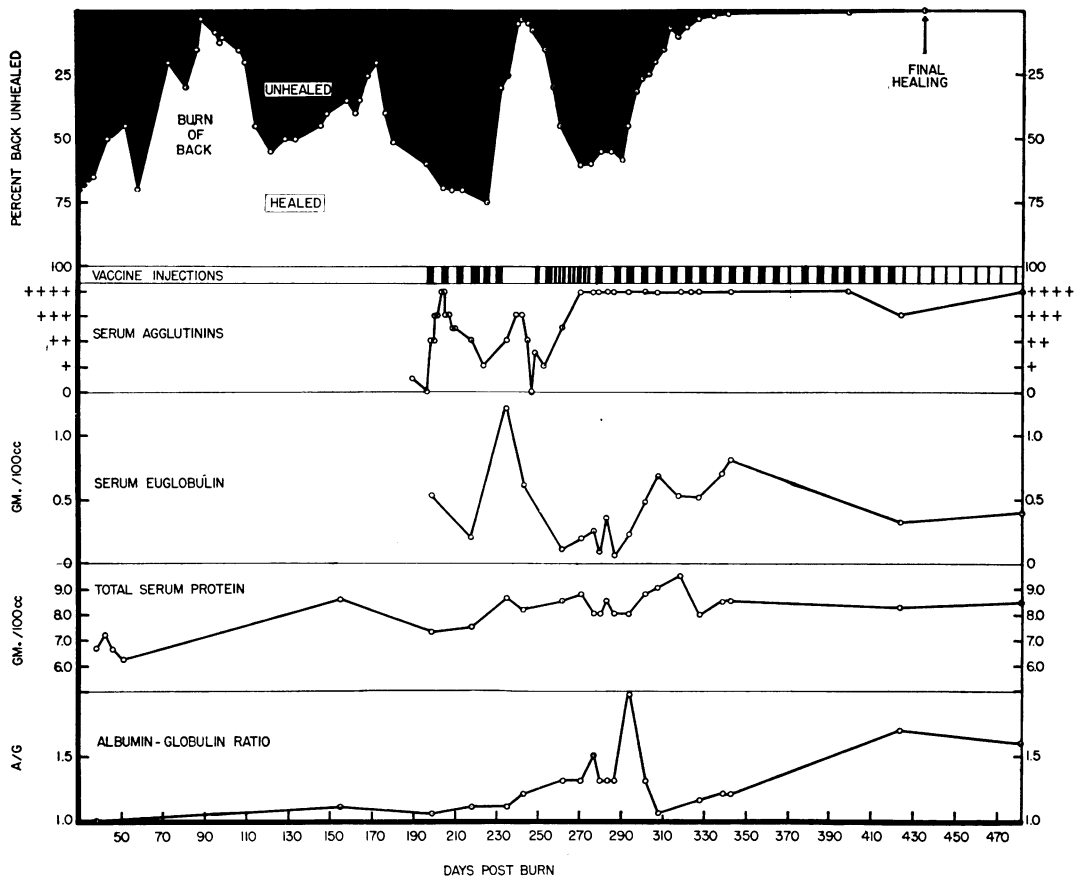

CHART 13

CHART 13.—Case 168: Proteolysis (Wound Dissolution) and Significance of Immunity to Healing: An extensive full-thickness burn wound of the back, 15 per cent of the body surface of a 34-year-old housewife. Thrice nearly healed and thrice dissolved with the fourth and final healing 14 months after injury, coincidentally with a rise in specific immunity. (The patient was transferred to this hospital 36 days after injury; five weeks later, closure of the full-thickness burns of the arms was achieved by grafting.) The back wound was infected with penicillin-resistant *Staph. aureus* and *alpha hemolytic Streptococcus* and *B. proteus*. Penicillin was administered until the 150th day after injury. Autogenous staphylococcus vaccine was started on the 197th day, stopped on the 235th day, resumed on the 250th day, and continued until after healing. The last two waves of healing followed rises of staphylococcal agglutinin titer and coincided with rises of serum euglobulin concentration.

The original burn slough was irregularly distributed over the full-thickness burns on entry. During the first four weeks of her stay in this hospital, concomitant with her nutritional improvement, spontaneous epithelization of the wounds started. Five weeks after admission the wounds of both arms and parts of the back were grafted with mosaic grafts. These took well, and there was rapid closure of the wounds of the arms. Subsequent graftings of the wounds of the back and the front of the chest did well initially, the wounds almost closing. On the 8th week after entry, however, the 95th day after injury, the proteolytic process appeared in the wounds of the back and anterior chest and loss of grafts began (Chart 13). By the 120th day after injury virtually all the wounds of the back and anterior chest had reopened. The wounds of the arms remained healed. Various local measures, including gramicidin, were tried in succession. Systemic penicillin was continued throughout. By the 150th day, wound healing was again apparent but the wave of healing was only temporary. By the 190th day proteolytic dissolution of the wounds was once more gaining. Since the patient had achieved an apparently good nutritional state and since the systemic penicillin and the variety of local measures tried had been without effect in ridding the wounds of the proteolytic process or the penicillin-resistant staphylococcus and *B. proteus,* stubborn bacterial inhabitors of the wounds, a trial of staphylococcal vaccine was considered. The agglutinin titer to the persistent *Staph. aureus* proved to be a trace, or 0. The vaccine was, therefore, started on the 197th day, by intramuscular injection, and within a week there was an apparent response, the agglutinin titer rising abruptly to ++++ (Chart 13). There was no immediate change in the condition of the wound; indeed, the dissolution had progressed and the vaccine was, therefore, continued.

In the 4th week of vaccine therapy, spontaneous closure of the wound had recommenced and proceeded rapidly. The vaccine injections were discontinued on the 235th day, there being in the serum a +++ titer of agglutinins and 1.2 Gm. of euglobulin. On the 245th day, proteolysis was again apparent in the wound, and the serum agglutinin titer had fallen to 0. The serum euglobulin level had also declined.

In spite of renewed injections of the vaccine on the 250th day, the proteolysis continued until the open wounds of the back were nearly as extensive as they had been before initiation of the vaccine therapy. Enlargement of the wound ceased simultaneously with the return of the agglutinin titer to ++++. Healing of the wound did not recommence, however, for another 20 days, occurring at a time when the euglobulin level once more was rising. This healing phase, the fourth to have occurred, was again from spontaneous regeneration of epithelium; it was prompt and complete. The agglutinin titer remained high throughout the healing period, as did the euglobulin level. During the period when the healing rate was at a maximum the serum total protein level reached the peak of 9.5 Gm. per 100 cc.

The patient was discharged on the 343rd day. Because of the previous tendency of her wounds to dissolve and because of the late inflammatory reaction observed previously in the proteolytic wounds of other patients, the vaccine therapy was continued for another five months. When seen for final follow-up on the 546th day, no inflammatory reaction had appeared. The serum agglutinin titer was ++++.

Immunity and Late Inflammation of Proteolysis: Case 210, illustrating subsidence of the late inflammation following the healing of proteolytic wounds coincidentally with a rise in the blood serum of specific agglutinins to the staphylococcus previously recovered from the patient's wounds.

A previously healthy housewife, age 34, received a flame burn of 31 per cent of her body, five-sixths of which was of full-thickness destruction. Although physiologic balance was never stable, a good part of the full-thickness wounds were excised on the 8th day. Small, thin skin grafts were irregularly distributed over the excised areas on the 13th day. The grafts took successfully but before the grafted wounds had closed by spread of epithelium, proteolysis developed in them and in the unexcised sloughing wounds. For two months the grafts retreated. Final closure of the wounds after further grafting was

achieved by the end of the 4th month. The patient was discharged home on the 128th day after injury.

The patient returned on the 295th day after injury complaining of pain and intense itching of her wounds. Those wounds which had previously harbored the proteolytic process, though firmly epithelized, were swollen, deeply indurated, red and tender, typical of the late inflammation. Fortunately, the virulent, penicillin-resistant staphylococcus recovered from the patient's wounds before healing had been preserved in the laboratory and the patient's blood serum on this day showed a plus two specific agglutinin titer to it. The serum was also found to contain:

Total protein	7.9 gm./100 cc.
Euglobulin	0.3 gm./100 cc.
Pseudoglobulin I	1.7 gm./100 cc.
Pseudoglobulin II	0.9 gm./100 cc.
Globulin	3.0 gm./100 cc.
Albumin	5.0 gm./100 cc.

The patient was not given autogenous staphylococcal vaccine because of the presence of some specific agglutinins. Vitamin C, 2 Gm. per day, was added to her diet.

By the 336th day the late inflammation had subsided. The serum agglutinins were found to have increased to ++++. The euglobulin level had more than doubled. The serum protein findings were:

Total protein	7.6 gm./100 cc.
Euglobulin	0.8 gm./100 cc.
Pseudoglobulin I	1.2 gm./100 cc.
Pseudoglobulin II	0.8 gm./100 cc.
Globulin	2.7 gm./100 cc.
Albumin	4.9 gm./100 cc.

The rise in titer of the specific staphylococcal agglutinin and in the level of the euglobulin concomitant with subsidence of the late inflammation suggests that the staphylococcus known previously to have infested the wounds was in part responsible for the inflammation and that specific immunity in the host plays a rôle in the conquering of the organism. Whether the vitamin C aided the host or whether the rise in immunity would not have occurred spontaneously cannot of course be determined from this case.

Immunity and Invasive Infection: Case 218 illustrates the exhaustion of the immune bodies during the phase of advancing cellulitis uncontrolled by penicillin and eventual healing of proteolytic wounds only with return of specific agglutinins. A 54-year-old heavy-set seaman entered the hospital with limited burns of head and face, hands and feet, considered initially to be of incomplete destruction. The wounds were covered; the patient was started on 240,000 units of penicillin intramuscularly per day. Because of advancing cellulitis in the right forearm a blood culture was taken at 48 hours which showed *Clostridia* and *Staph. albus*, coagulase-negative, penicillin-sensitive. Similar organisms were found in the wounds. Because of the continued advance of the cellulitis the penicillin dosage was doubled. The cellulitis was not effectively controlled until the 9th day, after which time it slowly subsided. After three weeks a proteolytic process was noted in the wound of the right hand and what should have been a burn of incomplete destruction turned into one of full-thickness. A similar process was also apparent in the wounds of the feet. Initial grafting tried on these areas was unsuccessful.

The blood serum agglutinins, measured routinely on entry, were +++. On the 2nd and 4th days during the advancing cellulitis they were ++ and +++ but by the 9th day they were 0. The euglobulin titer was also falling during this period. It appeared possible, therefore, that specific antibodies were being exhausted during the period of the advancing infection which was taking place in spite of the large dosage of penicillin. The euglobulin level fell further, reaching 0 during the phase of proteolytic infection.

Because of the proteolysis and absence of detectable antibodies, vaccine therapy was

initiated. The vaccine was injected on three successive days of each week for four weeks before a return and rise in the agglutinins was observed. The reappearance and rise in euglobulins was longer delayed. Wound healing occurred coincidentally with the euglobulin rise, the agglutinins remaining elevated. Although the latent period between onset of vaccine therapy and agglutinin rise was unusually long, bringing up the question of the efficacy of the vaccine, it is to be stressed that healing, as in Case 168, occurred simultaneously with the rise in the euglobulin level and at a time when the agglutinin titer remained high.

Immunity and Invasive Infection: Case 254 was an extensively burned healthy male, age 38, with scattered full-thickness, electric flash-burns who developed, in spite of continued penicillin therapy, an invasive staphylococcus infection in the week following an extensive grafting procedure. Coincidentally with the invasive infection and abscess formation there was a fall in the immune body titer and with its healing there was a rise.

In the first nine days after injury there was a good and immediate immune response to the staphylococci present in the wounds. The agglutinin titer on the 2nd day was 0 but by the 9th had risen to +++. The euglobulin in the same period rose from 0.1 to 0.6 Gm. per 100 cc. The same agglutinin titer was present on the 35th day, though there had been a drop in the euglobulin level to 0.3 Gm. per 100 cc. During these first 35 days there had been prompt healing of the second-degree burns and marginal healing of the full-thickness areas. On the 35th day a multiple grafting operation was performed. Five per cent of the body surface was grafted.

On the 40th day phlebothrombosis was recognized in one leg. Since there were burn wounds covering the usual area of the incision for femoral vein ligation, the phlebothrombosis was treated with intravenous heparin. This was injected into a superficial vein just above the ankle in the leg opposite that showing the signs of phlebothrombosis. This injection was followed 24 hours later by a subcutaneous thrombophlebitis along the calf above the site of the injection and accompanied by a rapidly progressive cellulitis. In order to identify the organism responsible for the cellulitis, on the 46th day an area of necrosis in the cellulitis was aspirated. Culture revealed a virulent *Staph. aureus* identical with that to be found in the wounds; both were penicillin-resistant.

The necrosis in the cellulitis increased and formed a small abscess which was treated successfully with two further aspirations rather than open drainage. The grafts healed satisfactorily during the period of cellulitis and abscess.

It is to be stressed that penicillin, 360,000 units per day intramuscularly, had been continued uninterruptedly throughout the course in the hospital. During the period of abscess formation the agglutinin titer had fallen to plus one on the 46th day. Gradual healing of the abscess occurred coincidentally with the rise in agglutinin titer to +++ on the 60th day, and ++++ on the 70th day. During this period of rise of agglutinin titer, the euglobulin level fell to 0.05 on the 60th day.

CONCLUSIONS

The staphylococcus is destructive and invasive in deep burn wounds of the skin. Its presence is to be anticipated in every wound. In the absence of chemotherapy the number of strains apparently increased, while under systemic sulfadiazine the number was held constant. Under systemic penicillin the number of strains slowly decreased.

The prominence which we have observed the staphylococcus to assume in the infection of the burn wound may conceivably be due to the suppression by chemotherapy of the *beta hemolytic Streptococcus.* The virtual elimination of the rampant streptococcal cellulitis may have destroyed a screen hiding the staphylococcal infection; it is also possible that the *beta* streptococcal infection formally restrained the staphylococcus. The presence of staphylococcal cellu-

litis and bacteremia and the recovery of a greater number of strains of the staphylococcus from the wounds of the burned patients in whom chemotherapy was delayed, however, suggests that staphylococcal infection was present in burn wounds before the days of sulfadiazine or penicillin. It merely failed to attract attention.

Under either sulfadiazine or penicillin the virulence of the staphylococci increased rapidly. On the day of injury three-quarters of the staphylococci were avirulent but by the 6th week this proportion was fully virulent. Even with the gradual reduction in total number of strains under penicillin, there were more fully virulent strains of the staphylococcus in the 6th week than on the day of injury.

Resistance to penicillin developed so rapidly in the staphylococcus and proved of such permanent nature that only within the first three weeks of therapy is the maximum effect of penicillin to be counted upon. On the day of injury but 10 per cent of the staphylococcal strains recovered were penicillin-resistant. By the 6th week of therapy, 90 per cent, including all the fully virulent forms, were resistant.

Proteolysis, or liquefaction of the superficial aspect of a burn wound without deep inflammation, has been encountered in nine patients three weeks, or more, after starting penicillin therapy. The wounds have proved stubborn in healing and after eventual epithelial closure have exhibited later a deep inflammation. The only common factor of these wounds has been the combination of a virulent, penicillin-resistant *Staph. aureus* and a *B. proteus*. Because of the coincidence of the healing of these wounds with a rise in the blood serum of agglutinins specific for the infecting staphylococcus and of the euglobulin level in the patients in whom these measurements were made, it is believed that the staphylococcus is the principal offender and that specific immunity to the staphylococcus is the deciding factor in overcoming the infection. The rôle of the *B. proteus* may be merely that of inhibiting the antistaphylococcal activity of the penicillin, a property which *in vitro* it has been demonstrated to possess.

Other examples of the importance of specific staphylococcal immunity to the control of infection by the staphylococcus in patients receiving penicillin have been found. Invasive infection by the staphylococcus, advancing cellulitis and lymphangitis, and bacteremia, has been repeatedly observed in patients with widespread burn wounds infected with virulent staphylococci and in spite of massive doses of penicillin. In the patients in whom the serum levels of agglutinins and euglobulins have been followed, subsidence of the infection has been accompanied by changes in their levels. The failure of penicillin to achieve control of the infection and the need, therefore, for an immune response by the host may of course have been due to an inadequate concentration of penicillin in spite of the huge dosage. The presence in burn wounds of bacteria inhibiting penicillin and the extensiveness of the infected area may have reduced the drug concentration to an ineffectual level.

Additional evidence for the mutation of staphylococci from one form to

another has been found. An initial action of penicillin appears to transform virulent forms to the avirulent. If the organisms survive, drug resistance develops and reversion to the virulent form follows.

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(*Continue with Part IV*)

PART IV

THE INCIDENCE, VIRULENCE AND RESISTANCE TO PENICILLIN OF THE STREPTOCOCCUS IN BURN WOUNDS OF PATIENTS TREATED WITH PENICILLIN OR SULFONAMIDES

IN SPITE of the attention bestowed upon the streptococcus as the most reprehensible contaminating organism of the burn wound, there has been a disparity in the reports of its incidence and disagreement regarding its relation to toxemia in the burned patient. Firor and Aldrich[1] were the first to test the concept that the toxemia of burns is a toxemia of wound infection. From a bacteriologic survey of the flora of burn wounds they reported that although but few contaminants, such as the staphylococcus and *B. coli,* were recovered immediately after injury, by 12 hours after injury the wounds of all the severely burned patients and the large majority of the mildly burned, harbored streptococci. Either the *beta* hemolytic or the *gamma* streptococcus was found. They believed that the subsequent toxemia of the patients was explained by streptococcal sepsis. Such a high streptococcal infestation has not been subsequently reported. Neither the number of patients examined nor the relative frequency of the two types of streptococci were recorded, and it is not known, therefore, whether a representative sample of burned patients was studied.

Cruikshank,[2] in 1935, also, recorded a high incidence of streptococci in burn wounds. Of 100 burns examined on admission, 11 were found to harbor hemolytic streptococci. Of 32 burns examined between the 3rd and 6th days, 21, or 66 per cent, revealed the hemolytic type of streptococcus. Ten of 490 burned patients developed scarlet fever. Cruikshank made the significant observation that the same kind of streptococcus was to be found in abundance in the air and dust of the wards housing burn patients, but not in those of other wards. He mentioned the presence of the *alpha* type, *Streptococcus viridans,* in some cultures, but he did not record its incidence in detail.

In spite of Cruikshank's collaboratory evidence, Wilson, MacGregor and Stewart,[3] in 1938, were loath to accept Firor and Aldrich's concept of early streptococcal toxemia. They found infection in superficial burns to be rare in spite of the presence of hemolytic streptococci and although signs of infection and hemolytic streptococci were found in deep burns they concluded that infection was unusual before the 5th day after injury and, as a rule, not obvious until after the 7th day. They, too, gave no details regarding their bacteriologic studies, mentioning only the hemolytic streptococcus as the usual cause of sepsis.

Seven years later than Cruikshank, and in the same Glasgow wards, Colebrook, and his collaborators,[4] found a rise in the incidence of the hemolytic streptococcus in the early hours after injury comparable to that reported by Cruikshank, but the actual incidence throughout was slightly lower. This organism was recovered from 4 per cent of 370 burns in the 1st 12 hours, from 23 per cent of 39 in the 2nd 12-hour period, and 42 per cent of 107 burns cultured after 24 hours. Colebrook records strong evidence for contamination subsequent to hospital admission as the source for the increasing number of hemolytic streptococci. Eleven Griffith-types were found among 37 strains recovered from patients cultured on admission, with no one type predominating.

Subsequently, one Griffith-type clearly predominated, nearly all new cases acquiring a strain of this type. Dust and blankets were incriminated as the carriers. Only passing mention is made of a streptococcus other than the hemolytic; two strains of the *gamma* streptococcus were reported as encountered.

In the Contaminated Wound Project of Meleney,[5, 6] of 1942–43, interest in the streptococcus was also centered in the *beta* hemolytic. No accounting was made of either the *alpha* or *gamma*. It remained for Lyons,[7] to record the first comprehensive account of the distribution of the *beta* hemolytic, *alpha* and *gamma* streptococci in burns. The flora of the full-thickness wounds of nine victims of the Cocoanut Grove fire were studied in detail subsequent to the 1st five days after injury. Streptococci of all three forms were found, and the *alpha* in the greatest profusion. Sixty per cent of the cultures taken at the end of the 1st week contained an *alpha* strain. *Beta* hemolytic strains were recovered from 30 per cent, or less, of the wounds. *Gamma* strains were found least often. The relatively low incidence of the *beta* strains was ascribable to the sulfadiazine which was being systemically administered to the patients, but the high incidence of the *alpha* type was without precedent and unexplained.

Such were the reports of the incidence of the *beta* hemolytic streptococcus in burn wounds at the time this study was undertaken. With each report, from 1933 to 1943, a lesser incidence of this streptococcus was recorded. In judging the effect of penicillin on the *beta* hemolytic streptococcus in burn wounds, an explanation for this decrease in incidence should be sought. Could it have been due to improvement in measures employed to prevent contamination; to a difference in numbers of this streptococcus in the various cities where the studies were made; or to a difference in season when made or to the recent introduction of the sulfonamides? The sulfonamides had been used both locally and systemically in the therapy of the burned patients reported by Meleney and Lyons.

Such were the meager reports of the occurrence of the *alpha* and *gamma* streptococci in burn wounds. Was Lyons' finding of a prominence of the *alpha* type a chance occurrence peculiar to the night club fire or the result of the sulfadiazine therapy? Are the *alpha* and *gamma* streptococci of burn wounds of any clinical significance?

OBSERVATIONS

The streptococci recovered from the burn wounds were subdivided into the *alpha, beta* and *gamma* types. (The *alpha* is the *Streptococcus viridans,* the *beta* is the hemolytic and the *gamma* the nonhemolytic streptococcus). The wounds were of both partial- and full-thickness destruction and of patients treated with either sulfonamides or penicillin. The streptococci recovered from the wounds of patients being treated with penicillin were tested for resistance to penicillin. The coagulase test was also applied to a number of the strains of the *alpha* type recovered from the wounds of the penicillin-treated patients. Search was made for evidence of mutation from one type to another.

Incidence of Types on Day of Injury.—The number and proportion of strains of streptococci per culture recovered on the day of injury differed in the groups of patients treated with either sulfadiazine or penicillin. The major-

ity of the patients treated with sulfadiazine were studied in the two years previous to the introduction of penicillin, and the initial cultures of the wounds of these patients were made from débrided tissue. Seven strains of streptococci were recovered from the 13 cultures of the full-thickness wounds (Chart 14), and 16 strains from the 29 cultures of the partial-thickness wounds (Chart 17), an incidence in each of 55 per cent. The *beta* hemolytic type accounted for three of the strains from the full-thickness, and but one from the partial-thickness wounds. The remaining strains were of the *alpha* type.

The patients treated with penicillin were studied from January, 1944 through December, 1945. The cultures from the wounds of these patients were by the swab technic on the day of injury, as well as later; a larger number of wounds was cultured and a lower incidence of streptococci was found. Streptococci were recovered from 30 per cent of the wounds. No *beta,* 19 *alpha,* and two *gamma* strains were recovered from the wounds of full-thickness, 69 wounds having been cultured (Chart 15). One *beta,* nine *alpha,* and no *gamma* strains were recovered from the 31 wounds of partial-thickness (Chart 18).

Incidence of Types after Day of Injury and without Chemotherapy.—The chance to observe possible changes in the incidence of the streptococci occurring in the absence of chemotherapy, was obtained in the wounds of the 19 patients whose admission to the hospital was delayed and who had received no chemotherapy, local or systemic, prior to entry. Admission of the patients varied from 1 to 30 days after injury, an average of 7.2 days. From the 45 cultures taken on entry and before chemotherapy, 40 strains of streptococci were recovered (Chart 16). Twenty-eight of the strains, or 70 per cent, were of the *alpha* type. Only eight of the strains were of the *beta* and four of the *gamma* type. These patients were studied throughout the same period as the groups receiving penicillin from the day of injury. Some had been treated at home, some in other hospitals prior to entry to this hospital.

Incidence of Types during Sulfadiazine.—In the 1st week after injury in the full-thickness wounds of the patients who were being treated systemically with sulfadiazine, there was an abrupt rise in incidence of the streptococci to a level 60 per cent higher than on the day of injury (Chart 14). During the 2nd and 3rd weeks there was an equally precipitous decline in the incidence to a level approximately half that of the day of injury. Both the rise and fall were due almost entirely to changes in the number of *alpha* strains. After the 3rd week the number of the *alpha* strains remained approximately constant until their final disappearance on the 56th day. The number of *beta* strains remained nearly constant from injury until the 6th week, when there was a decline, with disappearance on the 48th day. On this day, also, all the *gamma* streptococci had disappeared; at any one time but few *gamma* streptococci were recovered.

In the wounds of partial-thickness of the patients who were receiving sulfadiazine there was no increase in incidence of the *alpha* type; there was instead a decline in the 1st two weeks followed by a rise in the 3rd (Chart 17). The *beta* hemolytic type increased slightly in incidence in the 1st two weeks. There was a slow rise in the number of the *gamma* type in the three weeks during which the wounds remained open.

Incidence of Types during Penicillin.—There was a three-fold rise in the

incidence of the streptococci in the full-thickness wounds during the first week of penicillin therapy (Chart 15). The levels reached in the 1st week were maintained in the 2nd. Although there was a decline following this initial rise, it did not occur until the 3rd week, and in contrast to the findings in the sulfadiazine treated patients, it constituted only a partial return to the initial incidence. At the lowest level of this decline, at the end of the 3rd week, the number of strains was still twice as many as on the day of injury. Again in contrast to the sulfonamide cases from the 4th week on the number was again increasing. By the 55th day the number had surpassed the peak of the 1st and 2nd weeks.

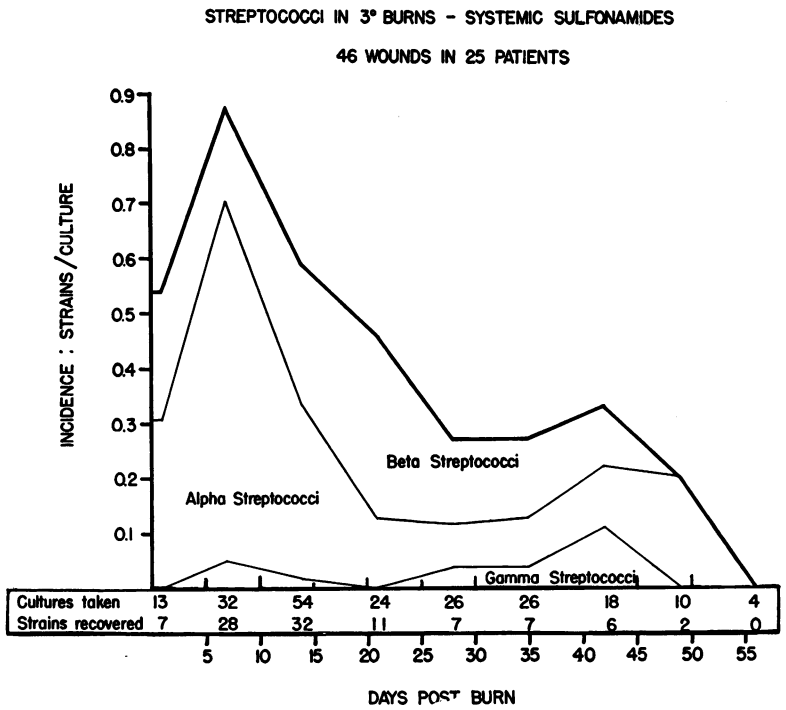

CHART 14

CHART 14.—The Incidence of Streptococci in the Flora of Full-thickness Burn Wounds in Patients Receiving Sulfonamide Therapy: The streptococci isolated from 46 wounds of 25 patients (same patients as in Charts 3 and 8 of Parts I and III) are classified according to the type of hemolysis produced on the blood-agar medium.

A few *beta* strains were recovered in the 1st week. During the succeeding three weeks there was little change in their number and by the end of the 5th week they had virtually disappeared. The number of *beta* strains encountered throughout the period of treatment in these patients was significantly less than in the sulfonamide-treated cases. Although the number of *gamma* strains increased in the 1st week, their number was never large and any fluctuation was not notable. The changes in incidence of the *alpha* type accounted for most of the changes in the over-all incidence of the streptococci. With but 30 per cent of the wounds containing an *alpha* or a *gamma* streptococcus on the day

of injury, by the 57th day 90 per cent of the wounds had an *alpha* streptococcus and a majority of the remaining wounds harbored a *gamma* strain, and a few wounds both. This incidence of the three types of streptococci is again in contrast to the wounds of the sulfonamide-treated cases, which by this time had lost all streptococci.

The changes in incidence of the three types of streptococci following the onset of penicillin therapy in the full-thickness wounds of the patients whose hospital admission and chemotherapy were delayed (Chart 16), were comparable to those encountered following the 2nd week of therapy in the patients

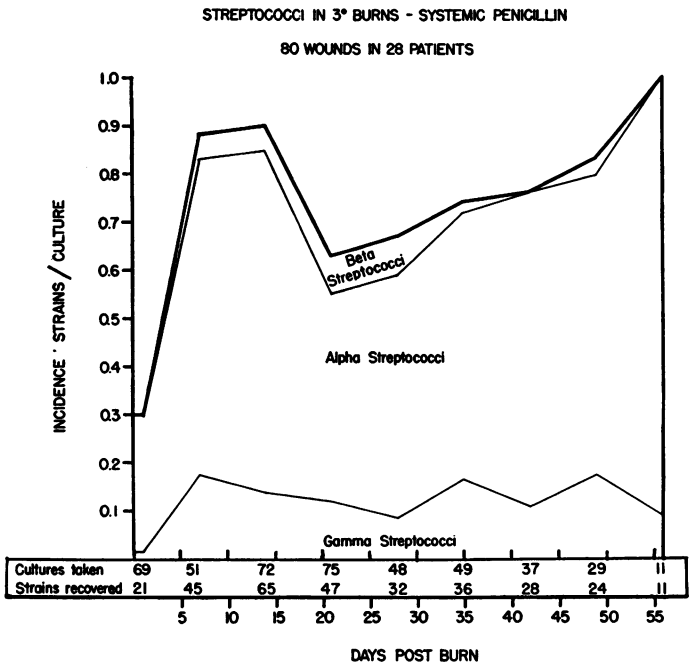

CHART 15

CHART 15.—The Incidence of Streptococci in the Flora of Full-thickness Burn Wounds of Patients Receiving Penicillin Therapy: The streptococci isolated from 80 wounds of 28 patients (same patients as in Charts 1, 6, 9 and 10 of Parts I, II and III) are classified according to the type of hemolysis produced.

whose penicillin was started on the day of injury. At entry, there were three times as many streptococci as on the day of injury in the other group of penicillin-treated patients and more than were ever found in the sulfonamide-treated patients. The *beta* strains had virtually disappeared at the end of the 3rd week of therapy. There was an initial decline in the number of the *alpha* strains followed by a continuous rise. In the 6th and 7th weeks of therapy the number of *alpha* streptococci exceeded that at entry. The number of the *gamma* type fluctuated somewhat more than in the previous group.

In the partial-thickness wounds of the patients treated with penicillin the single *beta* strain disappeared in the 1st week, the *alpha* strains remained constant in the 1st two weeks, declining slightly in the 3rd, and the one strain of the *gamma* type encountered was found at the end of the 1st week (Chart 18).

Penicillin Resistance.—The resistance to penicillin of all the streptococci recovered from the wounds of patients being treated systemically with penicillin was measured *in vitro*. No change in the penicillin resistance of the *beta* hemolytic streptococci was found to occur during the course of the penicillin therapy. This finding is in conformity with the earlier report of Rammelkamp and Maxon.[8] Considerable variation in the resistance of the various strains recovered, however, was observed; some were very sensitive, others

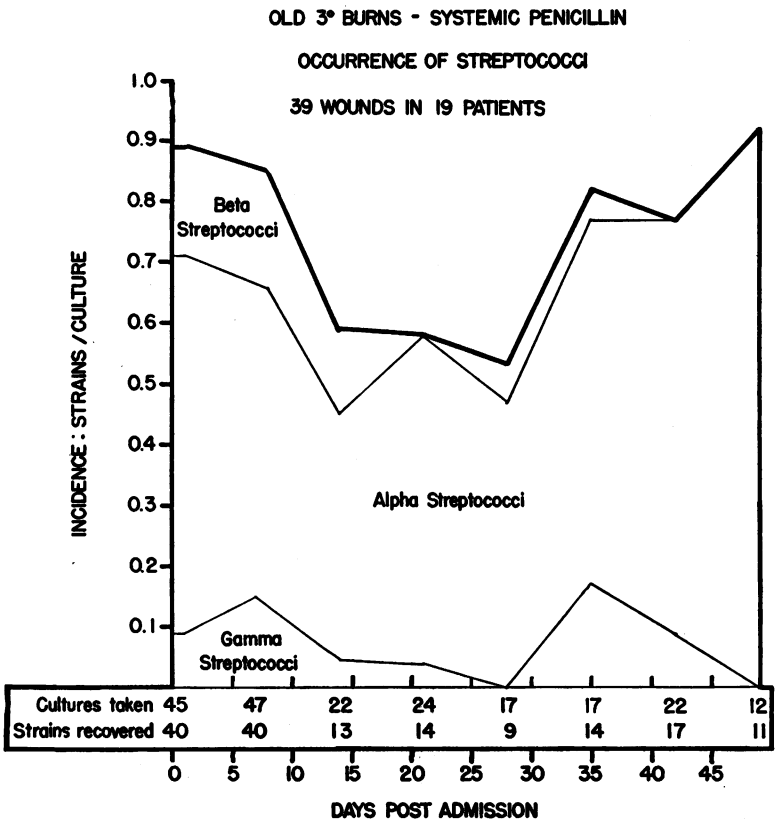

CHART 16

CHART 16.—The Incidence of Streptococci in the Flora of Full-thickness Burn Wounds of Patients Whose Hospitalization and Penicillin Therapy were Delayed: The streptococci isolated from 39 wounds of 19 patients (same patients as in Charts 2 and 7 of Parts I and II) are classified according to the type of hemolysis produced.

resistant. Because of the lack of change in resistance during therapy, the *beta* hemolytic streptococci are omitted from the charts in which the over-all change in resistance is depicted (Charts 19 and 20).

Both the *alpha* and *gamma* streptococci rapidly developed a resistance to penicillin. The resistance was proportionate in the two types to their incidence. In the wounds of the patients whose penicillin therapy started on the day

of injury, only 25 per cent of either organism were resistant on the day of injury, whereas by the 50th day 83 per cent were resistant (Chart 19). In the wounds of the patients whose penicillin therapy was delayed, slightly more, 30 per cent of the *alpha* and *gamma* streptococci, were resistant before onset of therapy (Chart 20). In the 4th week more than 80 per cent were resistant. In this group of patients, the number of sensitive strains increased after the 4th week, but during the same period that of the resistant strains was also increasing. By the 48th day, approximately two-thirds of the *alpha* and *gamma* types were resistant.

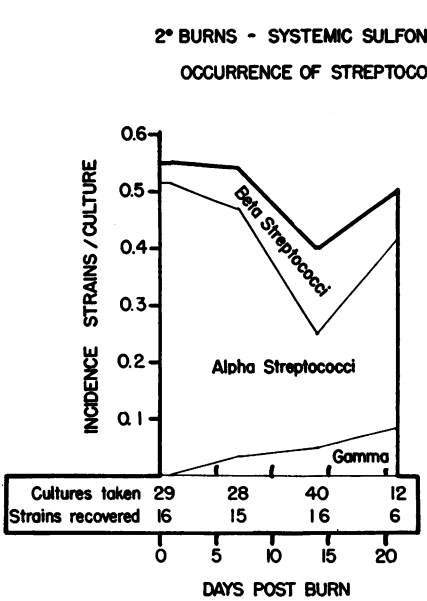

CHART 17

CHART 17.—The Incidence of Streptococci in the Flora of Partial-thickness Burn Wounds of Patients Receiving Sulfonamide Therapy: The streptococci isolated from 58 wounds of 35 patients (same patients as in Chart 4 of Part I) are classified according to the type of hemolysis produced.

Streptococcal Survival beneath the Closed Wound.—Four cases were encountered which indicated that the *beta* hemolytic streptococcus is able to survive the chemotherapy and superficial wound healing. After at least four weeks of continuous sulfonamide or penicillin therapy and complete epithelial closure of the burn wounds, four patients returned to the hospital one to four weeks after discharge with recurrent streptococcal infection deep in the wounds. One patient, who had had a partial-thickness burn, had been given sulfonamide therapy; the other three had had full-thickness burn wounds and had received prolonged penicillin therapy. On readmission, in each case the same strain of *beta* hemolytic streptococcus which had been found in the wounds before healing was recovered by needling the wound. There was prompt subsidence of the invasive inflammation after renewal of the chemotherapy. The cultures taken during the two-week period before healing in the sulfadiazine-treated and two of the penicillin-treated patients had not shown the streptococcus.

Virulence.—The coagulase test, similar to that used on the staphylococci, was applied to a number of the *alpha* streptococci recovered from the wounds of the penicillin-treated patients. A trend of increasing coagulase activity with the development of penicillin resistance was noted. No consistent change in the inflammation of the wound or in the clinical condition of the patient was observed which correlated with this change in coagulase activity. It was, therefore, not ascertained whether this test is an indication of virulence of

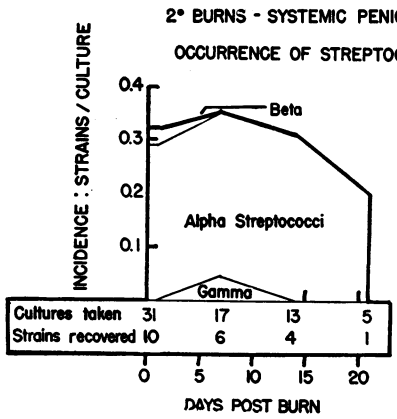

CHART 18

CHART 18.—The Incidence of Streptococci in the Flora of Partial-thickness Burn Wounds of Patients Receiving Penicillin Therapy: The streptococci isolated from 35 wounds of 18 patients (same patients as in Chart 5 of Part I) are classified according to the type of hemolysis produced.

the streptococcus as it is of the staphylococcus.

Mutation. — The rapid disappearance of the *beta* hemolytic streptococcus during penicillin therapy and the simultaneous increase in incidence of the *alpha* and *gamma* forms poses the question of a streptococcal mutation from *beta* to *alpha* or *gamma,* and *gamma* to *alpha.* No consistent sequence of change from one type to another has been noted in wounds from which but one streptococcal strain has been recovered, comparable to that encountered when but one staphylococcal strain was recovered. Evidence for a mutation of the streptococcus *in vivo* in burn wounds is, therefore, lacking.

CONCLUSIONS

It was expected, from the writings of Aldrich, Cruikshank, Colebrook and Meleney, that streptococci would abound in burn wounds but it was not expected that so few of the *beta* hemolytic type, so many of the *alpha* on the day of the injury, and such a profusion of the *alpha* and *gamma* types during the course of penicillin therapy would be found. The incidence of the *beta* hemolytic streptococcus in wounds both on the day of injury and of those of patients whose admission was delayed, was lower than had previously been reported. Could this be ascribed to an attention to prevention of contamination?

Colebrook has emphasized that the *beta* hemolytic streptococcus is not an usual inhabitant of the skin but that the dust of the hospital and ambulance and hospital blanket are likely to be heavily populated with this organism. He deduced that it is from such extraneous sources that burn wounds become contaminated with this type of streptococcus. Inspired by Colebrook, measures to prevent contamination were introduced during the course of this study. Formerly, and during the sulfonamide era, the community was not so burn conscious and not so scrupulous in the emergency handling of burn patients. After the Cocoanut Grove disaster, and at the time when penicillin became available, the physicians of several industries and the staffs of neighboring hospitals were collaborating and forwarding to this hospital their most severely burned patients. Their hospital and ambulance attendants were instructed to use sterile cotton sheets rather than blankets. On arrival at this hospital the patients were promptly placed in an operating room where

examination and initial care were carried out with precise aseptic technic.

The low incidence of the streptococcus found on the day of injury can probably be ascribed to a new emphasis on prevention of contamination. It is not to be ascribed to a seasonal variation since this study was continuous for two years. The low incidence of the *beta* hemolytic streptococcus found in these same wounds subsequently, during the penicillin therapy, as compared

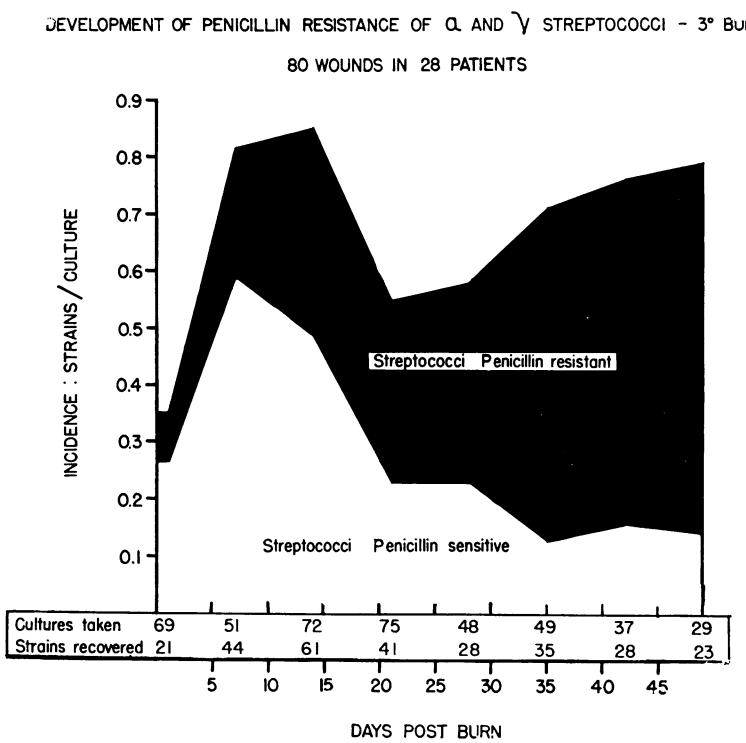

CHART 19

CHART 19.—The Penicillin Resistance of the *Alpha* and *Gamma* Streptococci in the Flora of Full-thickness Burn Wounds of Patients Receiving Penicillin Therapy: The *alpha* and *gamma* streptococci isolated from 80 wounds of 28 patients (cf. Chart 15) were tested for resistance to penicillin. Since the penicillin sensitivity of the *beta* hemolytic streptococcus remained *in vitro* unaltered during the course of penicillin therapy, the strains of this type are omitted from the chart.

with those of the patients treated with sulfadiazine, may be due as much to this emphasis on the prevention of contamination as to the penicillin. Only a few of the patients treated with sulfadiazine were cared for late in the study after penicillin had become available and at a time when the prevention of contamination was being stressed.

More *alpha* streptococci were found in the burn wounds of patients on entry and before chemotherapy was started than had been anticipated and only because the incidence of the *alpha* streptococcus (as well as of the *gamma*) had not been hitherto reported.

The profusion of both the *alpha* and *gamma* types during penicillin therapy was unexpected, particularly since their incidences were so out of proportion to that of the *beta* hemolytic type.

The reason for the profusion and disproportion of incidence of the three types of streptococci during penicillin therapy, however, is explainable when

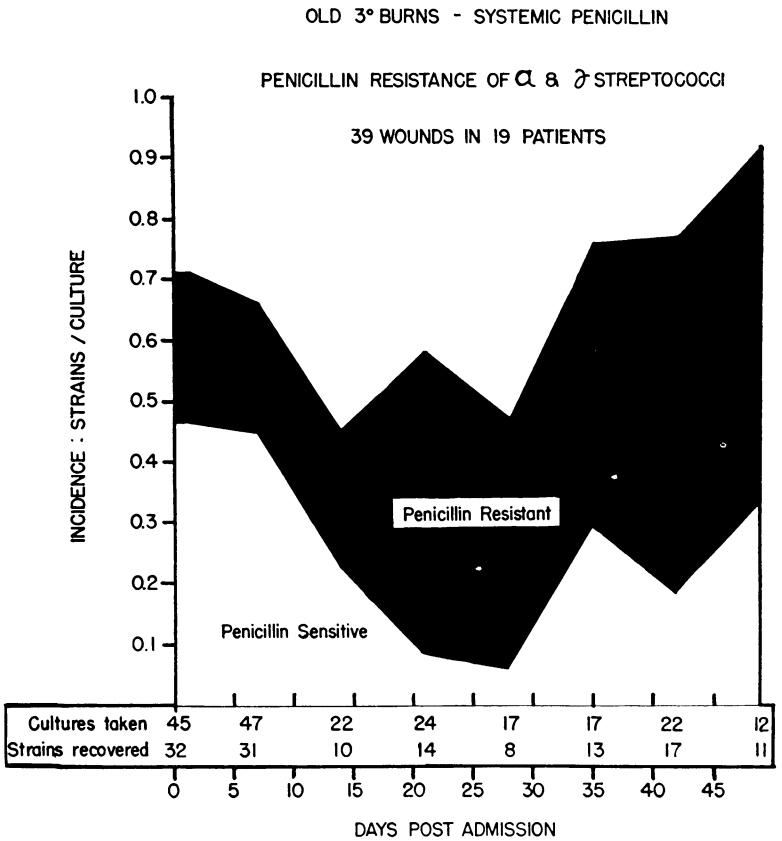

CHART 20

CHART 20.—The Penicillin Resistance of the *Alpha* and *Gamma* Streptococci in the Flora of Full-thickness Burn Wounds of Patients Whose Hospitalization and Penicillin Therapy were Delayed: The *alpha* and *gamma* streptococci isolated from 39 wounds of 19 patients (cf. Chart 16) were tested for sensitivity to penicillin. The *beta* hemolytic streptococci are omitted as from Chart 19.

the *in vitro* studies of the resistance to penicillin are taken into account. The *beta* hemolytic type is unable to alter its metabolism to resist penicillin; it is eliminated.* The *alpha* and *gamma* types, on the other hand, rapidly develop a resistance, survive, multiply and spread.

* It is possible that the slightly greater incidence of the *beta* hemolytic streptococcus in the sulfonamide-treated cases found during the course of therapy was due to its development of resistance to sulfadiazine rather than to the early lapses in preventing contamination. Such drug-fastness has been reported by Colebrook.[4]

Since the *alpha* and *gamma* streptococci were controlled better by sulfadiazine and the *beta* by penicillin, it is conceivable that penicillin and sulfadiazine should be combined in the therapy of patients with deep burns. Only a few of our patients were treated systemically with both of these drugs simultaneously, and too few observations were made for any proof of benefit from such combined therapy. The value of combined therapy depends upon how pernicious the *alpha* and *gamma* organisms are judged to be. The rôle of the *beta* hemolytic streptococcus is unquestioned; its presence in burn wounds is clinically associated with invasive infection, fever and delirium.

The rôle of the *alpha* and *gamma* streptococci in burn wounds is a matter of speculation. Both types are undoubtedly the cause of disease in other body regions. The *alpha* streptococcus recovered from these burn wounds is the same type as that associated with subacute bacterial endocarditis; certain other surgical infections;[9] and, occasionally, pneumonia in children and young adults.[10] The *gamma* streptococcus is sometimes the micro-aerophilic organism encountered in the undermining ulcer of Meleney and in nonbacillary gangrene. It is also an enterococcus found in the intestinal tract and surmised to be a salient pathogen of mixed infections of the peritoneal cavity. In the burn wound, the *alpha* streptococcus, penicillin-resistant, was commonly, but not always, present in those wounds showing proteolysis. Although no characteristic type of wound inflammation was typical of the presence of either the *alpha* or *gamma* streptococcus, it certainly cannot be concluded, in view of their pathogenicity elsewhere, that they can be dismissed as of no importance in a burn wound. The patients with these organisms in their wounds were sufficiently ill to be hospitalized.

Because of the postulated perniciousness of the *alpha* and *gamma* streptococci in the burn wound it is advised to combine sulfadiazine with penicillin in the therapy of the extensively and deeply burned patient. Since the sulfadiazine is toxic, particularly to the kidney of the dehydrated patient, it is wise to withhold it until an adequate fluid balance is assured.

COMMENT

Infection of the deep burn wound still exists—this study has not eliminated it. It is of mixed bacterial origin, dependent upon the presence of dead tissue, and inadequately controlled by the known chemotherapeutic agents. Its nature is such, and its toxins so mysterious, that there is still a goodly amount of "trial-and-error" involved in the healing of an extensively and deeply burned patient.

Measures designed to avoid bacterial contamination are tried so that there will be fewer bacteria to flourish in the wound.

Measures are chosen in the attempt to starve, remove or kill those bacteria which circumvent the efforts to reduce contamination. The quickest way to starve the bacteria is to remove their favorite food—the slough—by surgical excision. The way to remove the greatest number of bacteria is, also, by excision of the slough, where they have taken refuge.

To kill the bacteria in the wounds which are not excised and not grafted immediately following excision, a combination of penicillin, sulfadiazine and

streptomycin is to be tried. The penicillin, in massive doses, will be the most effective in reducing the number of *beta* hemolytic streptococci and in preventing invasion by the staphylococcus. Its use will be followed by resistant forms of the staphylococcus and of the *alpha* and *gamma* streptococci. The sulfadiazine should be tried in the hope that it will check the profuse growth of the *alpha* and *gamma* streptococci which have become penicillin-resistant. The streptomycin should be tried, also, in the hope that it will abolish the gram-negative bacilli which penicillin allows to flourish and which inhibit the action of the penicillin.

Neutralization of tetanus and clostridial toxins by administration of their respective antitoxins is indicated, particularly in patients with wounds of the buttocks, thighs and legs. (Diphtheria and scarlet fever antitoxins will rarely be needed.)

The patient is not passive as he watches us try to heal his wounds. He, too, can kill bacteria and neutralize their toxins. He will collaborate the better if we help him regain and maintain his physiologic balance.

The problem of the infection of burns will not be solved in the future by repeating a bacteriologic study with the introduction of a new chemotherapeutic agent. This study informs us of the character of the enemy. In the combat, chemotherapy will be a weapon but the replacement of trial-and-error will come only with a better understanding of the toxins of the bacteria and the immune processes of the host.

SUMMARY

A bacteriologic study of the floras of burn wounds has been made, and the effectiveness of penicillin in controlling infection has been judged. The floras of 80 full-thickness wounds of 28 patients treated with penicillin during 1944-45 have been compared with those of 46 similar wounds of 25 patients treated with sulfadiazine during 1942–43. Likewise, and during the respective periods for each drug, the floras of 35 partial-thickness wounds of 18 patients treated with penicillin were compared with those of 58 wounds of 35 patients treated with sulfadiazine. The floras of 39 full-thickness wounds of 19 patients whose admission to the hospital and onset of chemotherapy were delayed and who were subsequently treated with penicillin, were compared with the 80 wounds of the 28 patients whose penicillin therapy was started on the day of injury.

Neither sulfadiazine nor penicillin exerts more than a limited control over the growth of bacteria in the deep burn wound. The staphylococcus was found to be a contaminant of every wound, and the number of its strains to multiply in the absence of chemotherapy. The multiplication did not occur with sulfadiazine therapy and the number of strains recovered slowly decreased during penicillin therapy. Resistance to penicillin developed rapidly, however, and avirulent gave place to virulent forms. The staphylococcus was judged to be a destructive organism in burn wounds. Evidence for the need of immunity to it was obtained.

No *beta* hemolytic streptococci were recovered on the day of injury from

the full-thickness wounds of the patients subsequently treated with penicillin, and very few from the partial-thickness wounds, in contrast to those whose admission was delayed and to the sulfadiazine-treated patients of the previous two years. It is surmised that improved precautions against wound contamination were the cause of this low incidence. There was more prompt elimination of the *beta* hemolytic streptococcus by penicillin than by sulfadiazine from those wounds in which this organism was encountered. This is presumably related to the observed inability of this streptococcus to develop resistance to penicillin.

The *alpha* and *gamma* streptococci slowly disappeared from the wounds of patients treated with sulfadiazine. In the wounds of the penicillin-treated patients, in contrast, these streptococci grew and spread in profusion until by the 8th week, 90 per cent of all the unhealed wounds contained an *alpha* strain and a majority of the remainder, a *gamma* strain, and a few both. These two streptococci were found to develop rapidly a resistance to penicillin.

The number of strains of the gram-negative bacilli increases rapidly in the 2nd week of penicillin therapy. The consistency of this increase with penicillin and its absence in patients receiving sulfadiazine or no chemotherapy, suggests that it is in some manner induced by the penicillin. These organisms were found *in vitro* to inhibit the action of penicillin.

Wounds of the buttocks, thighs and legs showed the greatest contamination with the highest incidence of the *Clostridia* and the *gamma* or fecal type of streptococcus. Wounds of the head and neck revealed the least contamination with no *gamma* streptococci and but few *Clostridia.* The amount of contamination of the wounds of the upper extremities lay midway between the other two body areas. It is suggested that clostridial antitoxin be considered in addition to tetanus antitoxin and prompt excision of slough in the therapy of patients with full-thickness wounds of the lower extremity.

The rapid development of resistance to penicillin by the previously sensitive staphylococci and streptococci and the profusion of penicillin inhibiting and insensitive gram-negative bacilli indicate that the effective period of penicillin therapy is sharply limited to the 1st three weeks. By six weeks after injury the wounds have become a bacterial quagmire.

The infection of burns is a problem of deep burns. The fight against it combines prevention of contamination, starvation of the bacteria by prompt excision of slough, an attack by penicillin, streptomycin and sulfadiazine, and bolstering the immune processes of the host by restoration and maintenance of physiologic balance of the patient.

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