STUDIES ON THE PATHOGENESIS OF STAPHYLOCOCCAL INFECTION*

III. The Effect of Tissue Necrosis and Antitoxic Immunity

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Plates 41 to 43

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Staphylococci usually produce infection in man only after inoculation into areas of tissue injury, and in the preceding paper (1) it was shown that acute non-specific inflammation of rabbit skin is susceptible to infection with smaller numbers of the microorganism than is normal skin. It seems unlikely, however, that the number of staphylococci required to initiate infection in areas of experimental inflammation are commonly, if ever, involved in production of natural infection in human beings. In epidemiological studies it has been found that staphylococcal infection occurs more often in patients with severe burns or exfoliative dermatitis (2), and these are characterized by skin necrosis. The influence of a necrotic burn of rabbit skin upon the infectivity of staphylococci was, therefore, investigated and it was found, as might have been expected, that acute necrosis produced by thermal injury was associated with a marked increase in local susceptibility to staphylococcus infection, as described in this report.

The vast majority of staphylococcal infections in man heal spontaneously after a variable course, and similar healing occurs in infection of experimental animals. It seems clear, therefore, that a high level of natural resistance is present in man and experimental animals to invasion by staphylococci and that many severe infections can be contained and eventually overcome. In addition, it is evident that recovery from naturally occurring staphylococcal infection in man or animals provides little if any protection against subsequent infection. In fact, in a previous report (3), it was shown that repeated infection of the skin of rabbits could result in an increase in susceptibility to infection rather

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than an increase in resistance, and this was associated with the development of delayed hypersensitivity to the staphylococcus.

Many previous studies have been reported showing that vaccination of experimental animals with killed staphylococci or the extracellular toxins of the bacteria results in little protection against staphylococcus infection (4–10). The very high level of natural resistance to staphylococcus infection, however, leaves little opportunity for the assay of acquired immunity. The possibility that acquired immunity might have a demonstrable influence upon infection induced in tissues or in animals with reduced natural resistance, nevertheless, led us to examine the influence of immunization upon infection by staphylococci in necrotic burns. It was found that high levels of serum antibody to the alpha hemolysin or dermonecrotoxin of pathogenic staphylococci were associated with a striking increase in resistance to infection in necrotic tissue, and these studies are presented here.

Methods

Bacteria.—Staphylococcus aureus, bacteriophage type 80/81, as described previously (3), was used in these experiments unless mentioned otherwise. Three other strains of *Staphlyococcus aureus* were also used, including bacteriophage type VA4/77/75/42D, a coagulase-positive strain capable of producing enterotoxin, a coagulase positive non-typable strain, and a coagulase-negative non-typable strain.

Staphylococcus Alpha-Hemolysin.—Staphylococcus aureus type 80/81 was cultured in 3.7 per cent brain heart infusion broth (Baltimore Biological Laboratories) for 3 to 4 days at 37°C. 30 per cent CO_2 and 70 per cent O_2 was bubbled constantly through the broth. The culture was then centrifuged at 3,000 R.P.M. for 90 minutes. The supernatant was filtered through a Seitz filter. The filtered culture supernatant contained alpha hemolysin in high concentration, but no detectable beta or delta hemolysin (4). Neither fibrinolysin nor coagulase activity were detected in the culture supernatant.

Staphylococcus Alpha Hemolysin Toxoid.—The alpha hemolysin described above was made toxoid by adding formaldehyde in a final concentration of 1.0 per cent. This toxoid contained neither hemolytic activity nor the ability to produce dermonecrosis of rabbit skin.

Anti-Alpha Hemolysin.—The method of assay of anti-alpha hemolysin activity of rabbit serum was described in a preceding paper (3). Rabbit sera were also tested for precipitating antibody in the agar gel diffusion as described previously (3).

Blood Cultures.—Blood cultures were taken as described in the preceding papers (1, 3).

Histopathology.—Biopsies of rabbit skin were stained with hematoxylin and eosin and by Gram's method for detection of bacteria.

Thermal Burn of Skin.—The instrument used to produce thermal injury of skin was described in the preceding paper (1). With a temperature of 100°C., applied for 30 seconds, coagulation necrosis of the skin was regularly produced.

RESULTS

Infection of Necrotic Burns.—

A necrotic burn was produced in 5 areas on the side of groups of 3 rabbits as described in Methods. Four of the burns on each rabbit were injected immediately with 10^7, 10^6, 10^5, and 10^4 type 80/81 staphylococci, respectively, that had been washed once with 0.85 per cent

NaCl. In addition, the opposite side of the rabbit, where no burn had been induced, was similarly injected with the same concentrations of staphylococci. The lesions produced were photographed at 24, 48, 72, and 168 hours. Other groups of rabbits were treated in the same way but the staphylococci were inoculated at intervals varying from 2 to 8 days after production of the burns.

Inoculation of as few as 10^4 staphylococci into a necrotic burn of rabbit skin resulted in severe infection characterized by necrosis, hemorrhage, and inflammation extending considerably beyond the confines of the area of thermal injury (Fig. 1). These severe infections, however, did not lead to bacteriemia or death of the animals, and they healed spontaneously with eventual scarring. In contrast, inoculation of staphylococci into normal skin of the burned rabbits resulted only in small abscesses with doses of 10^7 bacteria, differing in no way from lesions with similar numbers of organisms inoculated into the skin of normal unburned rabbits. The increased infectivity of staphylococci in areas of necrotic burns, however, was demonstrable only when the bacteria were inoculated within 3 days after inducing the burn. After the 4th day, no increase in infectivity by the staphylococcus was observed.

Serum antibody assays were performed on the rabbits recovering from infection of necrotic burns. Thirty days after the infection the serum anti-alpha hemolysin titers were found to be 4 to 8 units (as defined in reference 3) per ml., and at 100 days the titers were 8 to 12 units. In addition, the serum of these rabbits possessed high titers of precipitating antibody for culture filtrate of the infecting staphylococcus. To determine the influence of high levels of serum antibody and prior severe infection with the staphylococcus upon reinfection, the following experiments were done.

Rabbits that had recovered from infection of necrotic burns initiated 30 to 100 days previously were burned again in exactly the same way in previously uninvolved skin and 10^7, 10^6, 10^5, and 10^4 type 80/81 staphylococci were injected immediately into the burned areas as described in the previous experiment. Similarly, staphylococci were injected again into unburned normal skin. A group of normal rabbits was treated in the same way, as a control.

The normal (control) animals burned and infected with staphylococci for the first time developed extensive lesions identical with those described in the preceding experiment. The animals burned and infected for the second time, however, failed to develop lesions comparable to those of the control rabbits. In fact, necrotic burns inoculated with staphylococci in the animal, burned and infected 100 days previously, failed to show an infection at all; and the infected burned skin was indistinguishable from the uninoculated burned skin, regardless of the dose of injected bacteria (Fig. 2). There was no difference, however, between the lesions induced by staphylococci inoculated into unburned skin of the control and of the previously infected animals, indicating that the acquired resistance to staphylococcus infection in the animals with high serum antibody

titers (previously burned and infected) could be demonstrated only when staphylococci were injected into necrotic burns.

Immunity to Alpha Hemolysin.—The spreading hemorrhagic necrosis produced in rabbit skin by the intracutaneous injection of staphylococcus culture filtrate containing high alpha hemolysin activity bears a resemblance to the lesion produced by inoculating staphylococci into necrotic burns (Fig. 3). It seemed possible, therefore, that the serum anti-alpha hemolysin of rabbits which had recovered from infection of a necrotic burn might protect against the dermonecrosis produced by staphylococcus filtrate and against the extensive lesions produced by staphylococci inoculated into necrotic burns. To evaluate these possibilities, the following experiments were done.

The dermonecrotic effects of culture filtrate of type 80/81 *Staphylococcus aureus* were assayed by inoculation into rabbit skin of 0.2 ml. of increasing dilutions of the filtrate. It was found that undiluted filtrate and filtrate diluted 1:10 and 1:100 produced necrosis of rabbit skin in decreasing severity, respectively. Dilutions greater than 1:100 produced erythema but no necrosis. The LH_{50} hemolytic activity of the filtrate was 0.3 ml.; and as indicated in Methods, the filtrates contained no coagulase, or fibrinolytic activity and by assay on rabbit, sheep, and human erythrocytes contained no beta or delta hemolysin (4). By agar-gel precipitin analysis with rabbit antiserum prepared against the culture filtrate one major and one minor precipitate band was observed. In studies now nearing completion it seems certain that the alpha hemolytic activity and dermonecrotic activity of culture filtrate are attributable to the same toxin and cannot be separated from each other. Hereafter, therefore, the culture filtrate will be referred to as alpha hemolysin.

Alpha hemolysin, undiluted and diluted 1:10 and 1:100, was injected intracutaneously in doses of 0.2 ml. into groups of normal rabbits and into animals that had recovered from infection by the staphylococcus in a necrotic burn and had high titers of serum anti-alpha hemolysin activity.

As mentioned above, the alpha hemolysin produced extensive dermonecrosis in normal rabbit skin, but the animals possessing serum antibody developed only mild erythema following injection of the undiluted hemolysin.

It has been found in the preceding experiments, therefore, that recovery from staphylococcus infection of a necrotic burn was associated with (*a*) development of high titers of serum antibody to the alpha hemolysin of the microorganism, (*b*) acquired resistance to subsequent infection in a necrotic burn, and (*c*) immunity to the dermonecrotic effects of the hemolysin. As mentioned above, furthermore, there was a resemblance between the extensive hemorrhagic necrosis produced by staphylococci inoculated into a burn and the hemorrhagic necrosis produced by the alpha hemolysin. The following experiments were done, therefore, to determine if active or passive immunization to the alpha hemolysin would similarly result in immunity to the dermonecrotic effect of the toxin and to infection by staphylococci in necrotic burns.

Rabbits were immunized to alpha hemolysin in two ways:

1. Formalinized alpha hemolysin (toxoid) was emulsified with an equal volume of Freund's adjuvant (Difco—incomplete Freund's adjuvant) and 2.0 ml. of this was injected subcutaneously into rabbits at weekly intervals for 5 weeks.

2. Undiluted culture filtrate (alpha hemolysin) was injected intracutaneously into different sites at weekly intervals for 5 weeks.

At the completion of immunization and a short rest period of 1 week the degree of immunity to the dermonecrotic effect of the toxin and to infection in necrotic burns with type 80/81 staphylococcus was examined, as described before. Concomitantly the serum of each immunized animal was assayed for anti-alpha hemolysin activity. In the experiments in which immunity to the toxin and to infection was evaluated, normal rabbits were tested simultaneously as a control.

At the completion of immunization with either toxin or toxoid, the rabbits were immune to the dermonecrotic effects of the toxin and their serum anti-alpha hemolysin titers varied from 4 to 24 units. The normal rabbits behaved in the same way as before. In addition, the immunized animals were resistant to infection by staphylococci in necrotic burns, as were the rabbits which had recovered from a previous burn infection, in contrast to the extensive hemorrhagic necrotic lesions produced in non-immune animals which had been burned and infected at the same time. In other experiments in which less intensive immunization was attempted the degree of acquired resistance to the toxin and to infection in necrotic burns was correspondingly less. Furthermore, the resistance to infection was not evident when the lesions produced by inoculation of staphylococci into normal skin were compared in toxin immune and non-immune rabbits, but was demonstrable only in the necrotic burned skin.

Serum obtained from the blood of rabbits immunized with toxoid, as described in the preceding experiment, was used for passive transfer to normal rabbits. A group of 3 rabbits was given 60 units of anti-alpha hemolysin (15 ml. of serum) intravenously. 24 hours later these animals and a group of rabbits that had been injected with 15 ml. normal rabbit serum were given injections in necrotic burns with type 80/81 staphylococci as described before.

The animals given normal rabbit serum developed severe extensive hemorrhagic necrotic infections in the burned areas injected with staphylococci. On the other hand, passive transfer of serum from animals immunized with toxoid protected two of the three animals from infection by the staphylococcus in necrotic burns, although the degree of protection was not as great as in the animals from whom the immune serum was obtained, probably attributed to differences in levels of serum antibody in the actively and passively immunized animals.

Histopathology of Staphylococcus Infection in Necrotic Burns of Normal and Toxin Immune Rabbits.—The impressive acquired resistance to infection by staphylococci in necrotic burns, induced by immunity to culture filtrate (or alpha hemolysin) of the organism necessitated analysis of the histopathological features of the lesions in immune and non-immune animals.

Immune and normal rabbits were burned as described previously and 10^7, 10^6, 10^5, and 10^4 saline-washed type 80/81 staphylococci were inoculated into burned areas immediately thereafter. Burned but uninfected skin as well as the infected burned areas were excised at 24 and 48 hours after killing the animals by intravenous inoculation of pentobarbital. The tissues were fixed in 10 per cent formalin, sectioned, and stained as described in Methods. Similar preparations were made from the skin of another group of rabbits injected with undiluted alpha hemolysin.

Burned but uninfected rabbit skin, whether from immune or non-immune animals, showed coagulation necrosis. Leukocytic infiltration was seen only at the margin of the necrotic tissue. The necrosis attributed to the burn extended through the entire skin and subcutaneous tissue but did not involve the underlying muscle to any significant degree. Normal skin inoculated with the varying concentrations of staphylococci showed small abscesses only at the site of inoculation of 10^7 organisms, as described previously (1). Burned skin of non-immune rabbits inoculated with staphylococci showed necrosis similar to that of the burned skin not injected with bacteria although the involved area was more extensive. There was evidence of hemorrhage, particularly near the margins, and there were clumps of Gram-positive cocci observed throughout the involved tissue, but leukocytic infiltration was observed only at the margins. Burned skin of toxin-immune animals injected with staphylococci was quite different from that in the non-immune rabbits in that there was considerable leukocytic infiltration throughout the area and few bacteria could be observed (Fig. 3).

From these observations, therefore, it appeared that the most striking histopathological differences in the burned infected animals was the absence of visible bacteria and the presence of extensive leukocytic infiltration into the area in immune rabbits in contrast to the absence of leukocytic infiltration and presence of bacteria in the non-immune animals.

The dermonecrosis produced by alpha hemolysin in normal rabbits resembled in all details that described by Thal (11) and was characterized by liquefaction necrosis and little leukocytic infiltration. In immune animals the reaction of the skin to alpha hemolysin was characterized only by acute inflammation.

Effect of Immunity to Alpha Hemolysin upon Infection by Strains of Staphylococci Other Than Type 80/81.—The multiplicity of strains of *Staphylococcus aureus* capable of causing infection in man has suggested that perhaps the lack of acquired resistance to repeated staphylococcus infection is explained by this variability of strains of the microorganism. Therefore, although there are no known immunological differences between the alpha hemolysin derived from strains of staphylococci, it was important to examine the effect of immunity to alpha hemolysin obtained from type 80/81 staphylococcus upon resistance to infection in necrotic burns by other strains and types of the bacteria. In addition, we wished to determine if strains other than the type 80/81 used in the previous experiments were also able to induce severe infection in necrotic burns.

Groups of rabbits were immunized to alpha hemolysin toxoid emulsified in Freund's adjuvant, as described before, by weekly subcutaneous injections for 3 weeks. One week after the last injection of toxoid the immunized and groups of unimmunized normal rabbits were burned in the same way as in the other experiments and the staphylococci in doses of 10^7, 10^6, 10^5, and 10^4 were injected as before into the burned areas as well as into normal skin. The serum anti-alpha hemolysin titers of the immunized rabbits varied between 1 and 2 units per ml. at the time of challenge. Groups of 3 immunized and 3 non-immunized animals were used in each of the experiments.

With *Staphylococcus aureus*, bacteriophage type VA4/77/75/42D, no visible infection developed in necrotic burns of immunized rabbits, even with an inoculum of 10^7 bacteria. In contrast, large, extensive, hemorrhagic and necrotic lesions developed with all doses of the bacteria when inoculated into necrotic burns of non-immunized normal animals. In both immune and normal rabbits the lesions induced with staphylococci inoculated into unburned skin were indistinguishable from each other.

Staphylococcus aureus that was coagulase- and mannitol-positive but ***non-typable*** with bacteriophage produced severe infection with hemorrhage and necrosis in burned skin of non-immune animals with all four concentrations of the microorganism. In the rabbits immunized with toxoid, infection also developed in the burned areas of skin but the lesions were localized and not as extensive as in the non-immune rabbits. Inocula into normal skin of burned, immune and non-immune animals resulted in small abscesses with the 10^7 dose of staphylococci and there was no difference between these lesions in the two groups of rabbits.

A ***coagulase negative***, non-typable staphylococcus that produced no ***alpha hemolysin in vitro*** produced no visible sign of infection in necrotic burns of normal rabbits or rabbits immunized with toxoid. This organism produced erythema and induration in normal skin of both groups of animals but no abscesses, even with the highest concentration of bacteria.

DISCUSSION

Although staphylococcus infection can occur in normal persons, it becomes most problematical and usually most severe in persons with reduced resistance to infection whether attributable to systemic or local disease. In recent studies of the occurrence of infection in hospitalized patients it has been found, for example, that individuals with severe burns or exfoliative dermatitis develop staphylococcus infections 10 to 25 times more commonly than do other hospitalized persons (2). This epidemiological observation of increased susceptibility to infection in patients with necrosis of the skin has been substantiated in the studies reported in this paper showing a striking increase in infectivity of staphylococci in necrotic burns of rabbit skin. It seems likely that any disease of the skin or other tissues associated with necrosis would be similarly more susceptible to infection by the staphylococcus, but this has not been thoroughly

studied. Certainly many bacteria seem to induce infection preferentially in necrotic tissues, and it is possible that the frequency of postoperative wound sepsis with staphylococci may be more attributable to necrosis than to inflammation unassociated with necrosis. That this is probably the case is suggested by the present study and the preceding one (1) in which a distinct difference in the influence of non-specific inflammation and necrosis of skin upon staphylococcus infection was evaluated. Even though non-necrotic inflammation does increase the local infectivity of staphylococci to some degree, it is of small order when contrasted with the effects observed in necrotic burns. The number of bacteria capable of inducing severe infection in necrotic skin more closely approximates the number of staphylococci probably responsible for naturally occurring infection in man. It is reasonable to assume, however, that other factors than necrosis can similarly increase susceptibility to infection, as indicated by the increased frequency of staphylococcal infection in patients with diabetes mellitus, neoplastic disease, and systemic lupus erythematosus (2), but these have not been adequately studied.

The mechanism by which necrosis enables staphylococci to initiate infection is not clear, but it seems likely that the presence of dead organic material, separated as it is from the mechanisms of natural host resistance, can provide an adequate site for unrestricted growth of bacteria. In the present experiments, to be sure, once the infection was begun it invariably extended beyond the limits of the burn, but bacteriemia and metastatic infection was never observed, showing the continued operation of host defenses. This is further illustrated by the fact that bacteria inoculated into normal skin of the burned rabbit are as incapable of producing a significant infection as in normal unburned animals. In addition, this observation indicates that the effect of the burn is localized to the site of injury and is not detected in other tissues of the animal.

It has been repeatedly said that *Staphylococcus aureus* has an extremely low level of pathogenicity for experimental animals and man, although occasionally able to induce severe infection. In a sense, this indicates that man and experimental animals have a high level of natural resistance to invasion by the staphylococcus. Certainly this has been shown experimentally in these studies (1, 3). Under most circumstances, therefore, assay of acquired resistance or immunity in normal animals is almost impossible, and, in fact, such studies have failed to reveal any significant additive protection of immunization in normal animals given staphylococci (4). It is true that immunization with extrabacterial toxins or killed bacterial vaccines has shown some slight prolongation of life of experimental animals given massive doses of bacteria by a variety of routes (4–10) but the degree of protection has been sufficiently small to discourage further studies. In the investigations reported here we have been dealing with infection in areas where the mechanisms of natural resistance are not operating or are of little effectiveness, and in this sense we are studying infection in such a way as to examine it in the absence of natural resistance. This is probably more like

the naturally occurring severe infection in man than experimental studies in normal animals. To detect, as we have done, a considerable influence of acquired resistance or immunity upon infection in necrotic burns illustrates that acquired immunity may be of greater significance in the control of many types of severe staphylococcal infection than was previously suspected. That this acquired resistance is not demonstrable in infection by masses of organisms inoculated into areas where mechanisms of non-specific resistance can operate was shown very well when the lesions induced in normal skin of toxin-immunized animals was found to be no different from the lesions in non-immune animals. In this instance, natural resistance so contained the infection that any added effect of acquired immunity could not be demonstrated.

An obvious question derived from these experiments is, what is the evidence that acquired immunity operates to influence naturally acquired staphylococcus infection of man? There is inadequate data acquired under the proper circumstances to answer adequately this question. Treatment of human infections with staphylococcus antitoxin, however, have been disappointing (12). It is probable that immunity may not significantly influence the course of furunculosis as this may, perhaps, be analogous to infection induced in normal rabbit skin. However, it is not clear whether or not immunity might significantly influence severe localized infections such as those in burns and necrotic skin disease of other types. It will be important, therefore, in view of our present observations, to re-examine the influence of antitoxin immunity in selected instances of staphylococcus infection. In addition, however, it is likely that acquired antitoxin immunity may have little or no influence upon localized abscesses due to staphylococci since toxin may play little if any role in the pathogenesis of lesions of this type. This is illustrated by the fact that non-toxin producing strains of staphylococci may produce infection with abscesses.

The relationship of the alpha hemolysin production of staphylococci to pathogenicity has been repeatedly suggested (13). Although this toxin is usually elaborated by pathogenic staphylococci, it is nevertheless true that infection in man has been shown to occur with strains of bacteria that may not produce the toxin (14). The variability of elaboration of alpha hemolysin, however, and the difficulty occasionally encountered in its detection does not allow a definitive statement about its *invariable* association with virulence of staphylococci (4). It is evident that *in vivo* production may differ significantly from that produced *in vitro*.

In the experiments reported here the importance of alpha hemolysin in the production of host injury during the course of infection of necrotic burns is indicated by (*a*) the similarity of the infected burn to the dermonecrosis produced by the isolated toxin, (*b*) the association of acquired immunity in infection of necrotic burns to the presence of serum anti-alpha hemolysin activity, (*c*) the demonstration of high titers of serum anti-alpha hemolysin after infection of necrotic burns, (*d*) the ability to provide protection against infection in necrotic

burns by active or passive immunization to the alpha hemolysin, and (*e*) the inability of a strain of bacteria that produces no hemolysin to cause infection in a necrotic burn. Furthermore, it was shown that acquired immunity to the alpha hemolysin of one strain of staphylococcus (80/81), provided protection against infection by different strains (VA4/77/75/42D and non-typable) of staphylococcus that also produced hemolysins. This observation amplifies previous suggestions that there is no or little immunological difference between the alpha hemolysins of varying strains.

Particularly important is the fact that immunity to the alpha hemolysin resulted not only in neutralization of the toxin and its dermonecrotic effects but also altered the host's response to infection in necrotic lesions. This was illustrated by the intense leukocytic response in the lesion of immune animals and the absence of a leukocytic response in the non-immune animal. That culture filtrates of staphylococci may possess a leukocidin has been previously described (15, 16) and it is possible that in the non-immune animal the absence of leukocytes in the lesion is attributable to this leukocidin effect. Neutralization of the toxin would therefore allow the leukocytes to survive in the lesion (17). The absence of bacteria in the infection of immune animals, furthermore, suggests that the presence of the leukocytes has enabled the host to resist bacterial multiplication, and it has been previously shown that leukocytes may kill most staphylococci during phagocytosis (18). Whether or not antitoxin immunity enhances phagocytosis or inhibits the leukocidin effect of culture filtrates of staphylococci, however, has not thus far been studied.

Many questions remain unanswered, and among them are: Will antitoxin immunity protect against infection by staphylococci inoculated into necrotic tissues other than that produced by thermal injury? What influence will antitoxin immunity have upon the development of metastatic staphylococcus abscesses in bacteriemia? Will antitoxin immunity protect patients against severe infection with staphylococci, particularly infection in patients with burns and exfoliative dermatitis?

As to the mechanism of action of alpha hemolysin in the production of host injury the most definitive study to date is the work of Thal (11) in which it is shown that the hemorrhagic necrosis produced by this toxin is attributable to its effect on vascular reactivity leading to ischemic necrosis. Whether or not drugs capable of inhibiting the vasospastic effect of the toxin would interfere with the development of infection in necrotic lesions is not known at this time.

From these studies one thing is certain, and that is the necessity for a reevaluation of the role of immunity in staphylococcal infection.

SUMMARY

Necrosis of rabbit skin produced by thermal injury was found to result in a striking increase in local infectivity of staphylococci that were coagulase-posi-

tive and hemolytic, but no local increase in the infectivity of non-pathogenic staphylococci. Infection produced in necrotic burns extended beyond the area of burn and was characterized by hemorrhage, edema, and necrosis of contiguous normal skin. Such infections, however, never resulted in bacteriemia or metastatic abscesses, and there was no effect of the necrotic burn upon the infectivity of staphylococci injected into normal skin of the burned animal. Recovery of rabbits from severe burn infections was associated with the development of high titers of serum antibody to the alpha hemolysin or dermonecrotoxin of the staphylococcus. Thirty to 100 days after the initial burn infection, it was found that rabbits could no longer be infected in a necrotic burn, although infection induced in normal skin of these resistant animals was no different from that in normal rabbits.

Immunity to infection by pathogenic staphylococci in necrotic burns could be induced by vaccination with potent alpha hemolysin toxoid, and this immunity was passively transferable with rabbit antiserum. No strain specificity was detected for this immunity in that immunization with toxoid prepared from bacteriophage type 52/42B/80/81 staphylococci protected animals against infection in a necrotic burn by other typable and non-typable staphylococci.

Histopathological study of infected necrotic burns in normal rabbits showed extensive necrosis, hemorrhage, edema, and many masses of bacteria but leucocytic infiltration was observed only at the margin of the infection. In contrast, the infected necrotic burns in animals immunized with alpha hemolysin toxoid showed few bacteria and marked leucocytic infiltration throughout the burn.

These experiments have, therefore, demonstrated a significant immunity to *infection* by pathogenic staphylococci in necrotic tissue but not in normal skin, associated with serum antibody to the alpha hemolysin or dermonecrotoxin of the bacteria. The implications of these findings are discussed.

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EXPLANATION OF PLATES

PLATE 41

FIG. 1. *Infection in Necrotic Burns.*—The upper two illustrations show the uninfected necrotic burn immediately after and 8 days after induction of the burns. The middle and lower illustrations show, a rabbit in which *Staphylococcus aureus* had been inoculated into the burned areas (right) and normal skin (left) in varying dosages (10^7, 10^6, 10^5, and 10^4 bacteria) immediately after induction of the burns, showing the extensive, spreading, suppurative, edematous, and necrotic infection in the burned areas but not in normal skin after 4 days and 7 days.

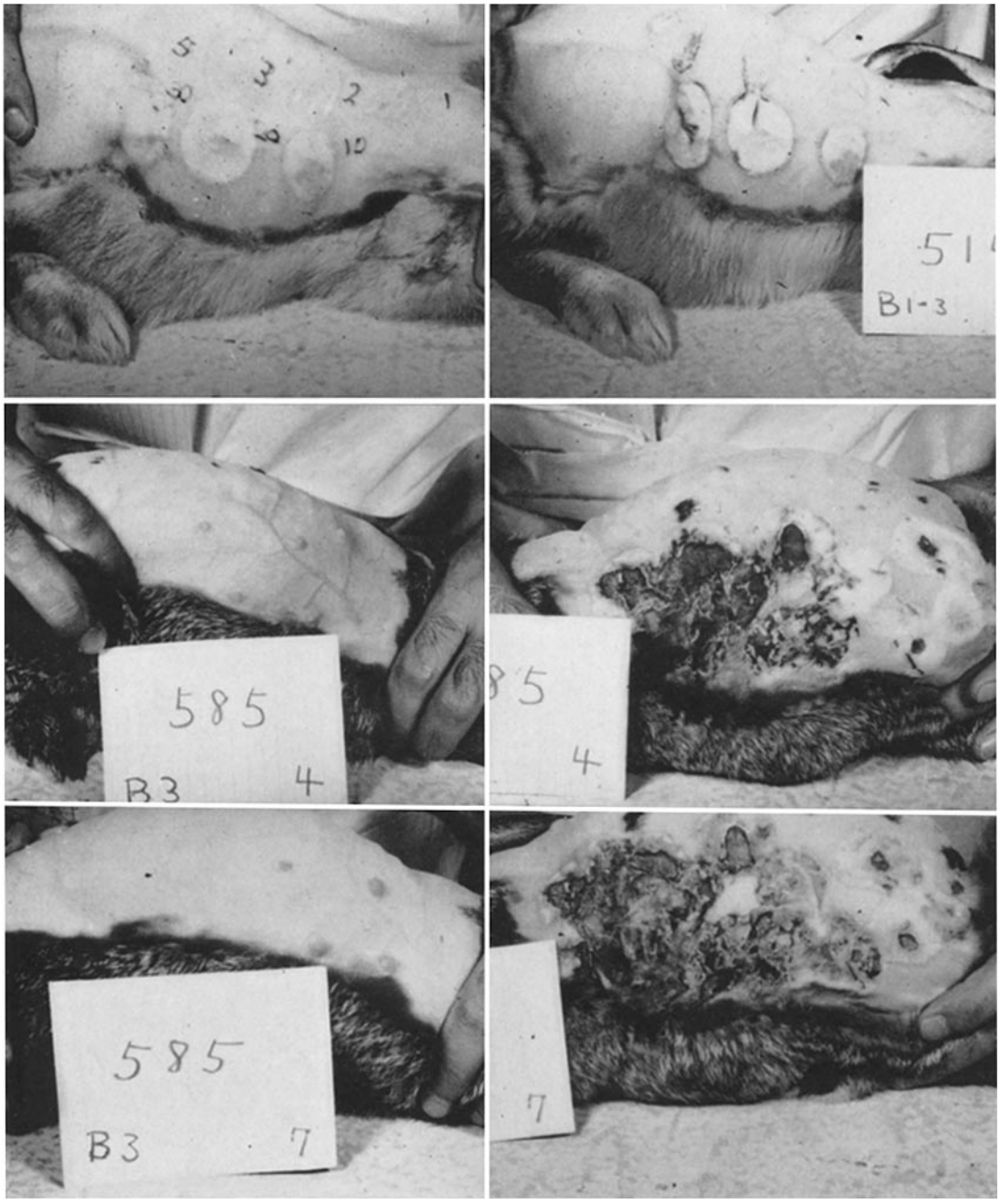

FIG. 1

(Goshi *et al*: Pathogenesis of staphylococcal infection. III)

PLATE 42

FIG. 2. *Infection in Necrotic Burns of Rabbits Infected 30 and 100 Days before in the Same Way.*—The lower two illustrations show the extensive infection in necrotic burns (left) and normal skin (right) induced with 10^7, 10^6, 10^5, and 10^4 staphylococci in normal, previously uninfected, animals. The upper and middle two illustrations show the lesions in rabbits infected 100 and 30 days previously in necrotic burns and normal skin showing the complete and partially acquired resistance to infection in necrotic burns in these animals.

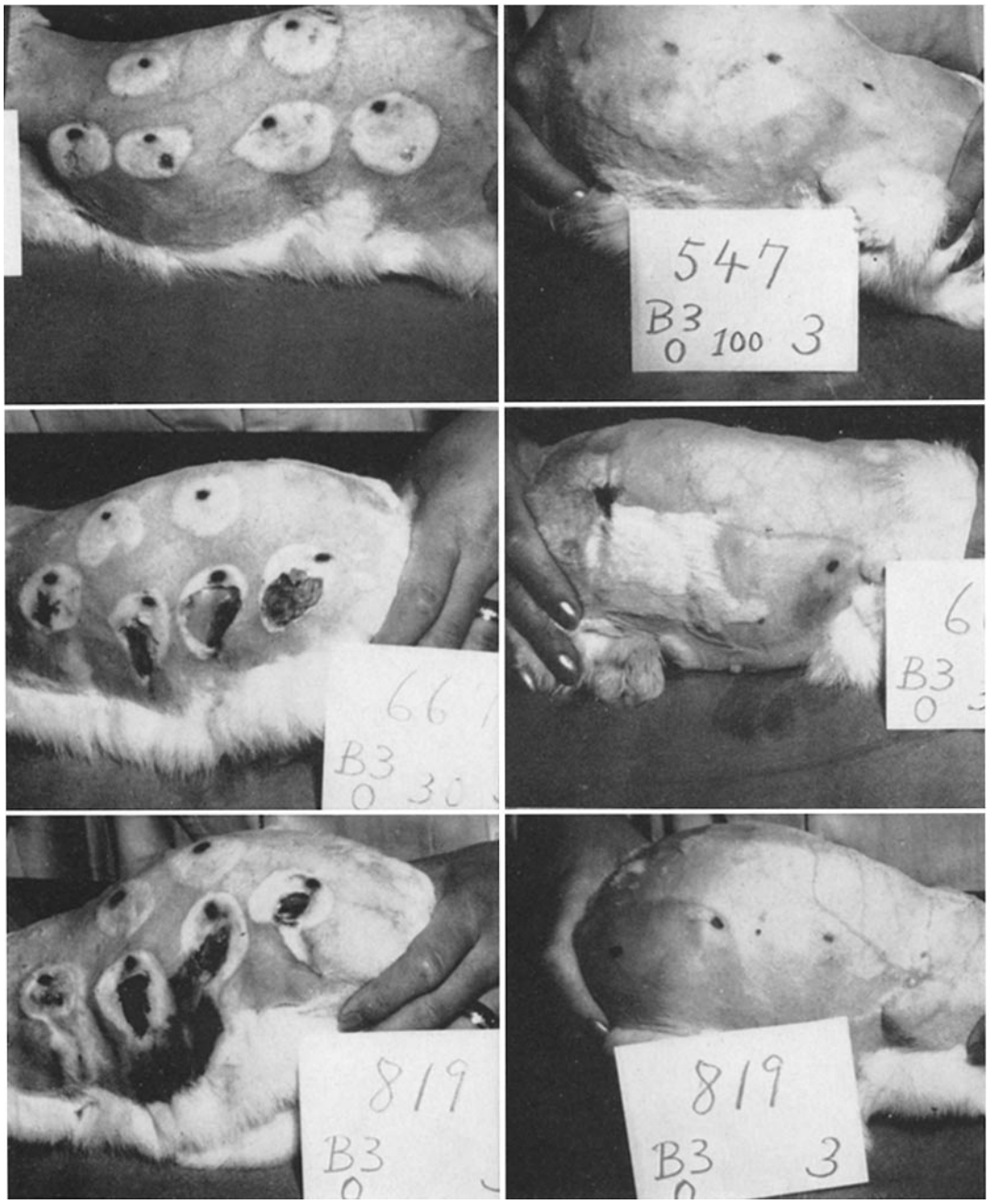

FIG. 2

(Goshi *et al*: Pathogenesis of staphylococcal infection. III)

PLATE 43

FIG. 3. Dermonecrosis in normal rabbit skin following intracutaneous inoculation of culture filtrate of *Staphylococcus aureus* (type 80/81) containing a high titer of alpha hemolysin.

FIG. 3