STUDIES ON THE PATHOGENESIS OF STAPHYLOCOCCAL INFECTION*

IV. The Effect of Bacterial Endotoxin

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Reduction in host resistance probably is necessary for staphylococcal infection to occur. For example, extraordinarily large numbers of pathogenic staphylococci must be injected into most normal tissues and animals to induce even an insignificant infection (2). On the other hand, inoculation of small numbers of staphylococci with a foreign body such as a silk suture (3), or into non-specifically inflamed or necrotic skin can lead to infection of varying severity. In addition, staphylococcal infection in man is most troublesome when it occurs in persons with severe systemic or cutaneous disease such as exfoliative dermatitis or diabetes mellitus (4).

Bacterial endotoxin has pronounced effects upon host resistance to infection, in addition to many other biological activities (5). Studies were done, therefore, to evaluate the influence of endotoxin upon staphylococcal infection and, as reported here, the toxin produced a striking increase in the infectivity of the microorganism in rabbit skin. The enhancement of staphylococcal infection was evident only for a brief period after injection of toxin and was attributed to the effect of endotoxin upon leukocytes. The lesion produced by staphylococci in endotoxin-prepared rabbits was attributed to elaboration by the organism of alpha hemolysin.

Materials and Methods

Endotoxins.—Two types of purified lipopolysaccharide endotoxin were used. One was derived from *Shigella flexneri*, type Z (6) and the other was derived from *Escherichia coli* and obtained commercially (Difco Laboratories, Detroit). Each endotoxin produced a monophasic fever of 0.5 to 1.0°C. when 0.01 μg. was injected intravenously into normal rabbits. The endotoxin

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solutions were prepared in 0.85 per cent NaCl and the volume of inoculum was maintained at 0.1 to 0.2 ml.

Staphylococcus.—A coagulase-positive, hemolytic *Staphylococcus aureus*, bacteriophage type 80/81 (Lafferty strain), described previously (7), was used throughout the experiments. The stock strain was cultured in trypticase soy broth overnight, and the concentration of staphylococci at the conclusion of its growth was about 10^9 organisms per ml. The staphylococcus culture was centrifuged at 4°C. for 30 minutes at 2500 R.P.M. The supernatant was discarded and the bacteria were resuspended in 0.85 per cent NaCl for inoculation. A coagulase-negative, non-hemolytic *Staphylococcus albus* was also used and prepared in exactly the same fashion.

Rabbits.—Albino rabbits, weighing approximately 3000 gm. were used. At the termination of each experiment the animals were sacrificed, and no rabbit was used for more than one experiment.

For purposes of intracutaneous inoculation the rabbits' backs and sides were shaved prior to the experiment.

Serum.—Rabbit blood was obtained by cardiac aspiration using sterile precautions and allowed to clot at room temperature. The serum was stored at 4°C. after separation from the clotted blood.

Preparation of Alpha Toxin.—The coagulase-positive hemolytic *Staphylococcus aureus* was grown in brain-heart infusion broth with increased CO_2 tension for 4 days as described before (7). The culture was filtered through a Seitz filter and the filtrate, containing high alpha hemolysin activity (7), was stored at 4°C.

Immunization with Staphylococcal Alpha Hemolysin.—*Staphylococcus aureus* culture filtrate obtained as described above, was toxoided with 1 per cent formalin (1). This was emulsified with Freund's adjuvant (Difco), and injected subcutaneously into rabbits at weekly intervals for 3 to 4 weeks. 1 week to 10 days after the last injection the rabbits were bled to obtain the antiserum, in the same way as described above, and the anti-alpha hemolysin titers were 6 to 8 units, when assayed in comparison with an NIH standard (3).

Peritoneal Exudate.—5 ml. of an overnight broth culture of *Staphylococcus aureus* was injected with 45 ml. of saline intraperitoneally. The rabbits died within 18 hours. Their abdomens were greatly distended with fluid and the entire bowel was dilated. No bowel perforations were noted. The peritoneal surface was relatively clean, but the peritoneal fluid was abundant and purulent.

The peritoneal exudate was Seitz-filtered and the filtrate was used almost immediately. *Staphylococcus aureus* was the only bacterium isolated from the exudate prior to filtration.

Endotoxin-Tolerant Rabbits.—Rabbits were injected intravenously with *E. coli* endotoxin for 9 consecutive days in increasing dosages ranging from 10 to 75 μg. Rectal temperatures were taken at 30 minute intervals for 6 hours on the 1st and 10th days after injection of 10 μg. of the toxin to demonstrate the development of tolerance to the pyrogenicity of the endotoxin as characterized by a lengthening of the lag period, decrease in pyrogenic response, and elimination of the secondary temperature elevation (5).

Kidney Inoculation.—Rabbits were anesthetized with intravenous sodium pentothal (3.5 ml. of a 10 per cent solution). Their backs were shaved and cleansed with merthiolate and alcohol. Sterile drapes and instruments were used. A 2 inch incision was made below the costovertebral angle and ¾ inch lateral to the vertebral column. The kidney was easily exposed by blunt dissection of muscle and fascia and could be inoculated directly. In some experiments, both kidneys were exposed and inoculated. Attempts were made to make the inoculation into the cortex of the kidney by inserting the needle parallel to the kidneys' longitudinal axis. The quantity of the inoculum was never greater than 0.2 ml. After 24 hours the animals were sacrificed and the kidneys removed aseptically. Each kidney was homogenized in 20 ml. 0.85 per cent NaCl in a Virtis "45" homogenizer at 50,000 R.P.M. at 0°C. for 10 minutes. Bacterial counts from the homogenates were then done by the agar pour plate method.

RESULTS

Effect of Intradermal Endotoxin upon Staphylococcal Skin Infection.—Endotoxin in relatively large dosage can produce inflammation when inoculated intradermally. In view of the effects of non-specific inflammation of rabbit skin upon infectivity of pathogenic staphylococci, described previously (2), experiments were done to investigate the effects of endotoxin upon infectivity of staphylococci.

10 μg. (0.1 ml.) of *E. coli* endotoxin was injected intradermally into rabbit skin and immediately, 2 hours, 4 hours, or 12 hours later 0.1 ml. of the phage type 80/81 staphylococcus was injected into the endotoxin-prepared skin. 5 separate sites of the rabbit skin were injected with endotoxin and into 4 of these 10^9, 10^8, 10^7, and 10^6 staphylococci were inoculated, respectively. The remaining endotoxin-prepared area was used as a control. Similarly, the same dosages of staphylococci were injected into normal skin on the opposite side of the rabbits as control. 3 animals were treated in the same way and the experiment was repeated several times. Similar experiments were performed using 500 μg. of *Shigella flexneri* endotoxin and the same dosages of staphylococci.

The rabbit skin inoculated with endotoxin only showed slight pallor at 24 hours but no other change. In most experiments the skin inoculated with staphylococci only showed small furuncles at the sites injected with 10^9 and 10^8 bacteria but there was slight erythema at the sites injected with fewer staphylococci. At the skin sites prepared with endotoxin and injected immediately or 2 hours later with staphylococci extensive hemorrhagic necrosis was observed at 24 hours but was apparent within 6 hours after injection of the bacteria (Figs. 1 and 2). In areas of skin prepared with endotoxin, but injected 4 or more hours later with staphylococci, there were no differences from the lesions in skin inoculated with staphylococci only.

In other experiments similar hemorrhagic and necrotic lesions were induced when staphylococci were inoculated into the "normal" skin of rabbits injected intradermally with endotoxin. Such lesions, however, were never seen in animals not inoculated with endotoxin.

The severity of the reaction produced with endotoxin and staphylococci was dependent to a degree upon the dosage of bacteria inoculated. Although hemorrhagic and necrotic lesions could be induced with as few as 10^6 bacteria these were less severe than with inocula containing larger numbers of staphylococci.

Additional experiments were performed to evaluate the effect of endotoxin dosage upon the skin infection with staphylococci. *E. coli* endotoxin, varying in dosage between 1 and 50 μg. was injected intradermally into different groups of rabbits, and these areas were inoculated immediately with staphylococci as described in the preceding experiment. Similar experiments were done using *Shigella* endotoxin in dosage varying from 10 to 500 μg. The hemorrhagic, necrotic reaction produced in endotoxin-prepared skin injected with staphylococci was marked with a dosage of *Shigella* endotoxin of 250 μg. or more. Less

endotoxin than this resulted in progressively less severe reactions, indicating dependency upon the amount of endotoxin injected.

Effect of Intravenous Endotoxin upon Intradermal Inoculation of Staphylococci.—As shown in the experiments described above intradermal injection of endotoxin frequently resulted in an effect upon staphylococcal infection induced by injection of bacteria at a site distant to the inoculation of endotoxin. This systemic effect of intradermal endotoxin suggested that a similar reaction to staphylococcal skin infection might be elicited by intravenous injection of endotoxin. To evaluate this possibility the following experiments were done.

Groups of rabbits were injected intravenously with 10 μg. *E. coli* endotoxin and were injected into the skin immediately thereafter with 10^9, 10^8, 10^7 and 10^6 *Staphylococcus aureus*. Similar groups of rabbits were injected intravenously with doses of *E. coli* endotoxin varying between 1 and 100 μg. before intradermal inoculation of staphylococci. With each group of rabbits studied, a comparable group of rabbits to serve as control was given 0.85 per cent NaCl intravenously prior to the injection of staphylococci. In previous experiments it had been found that within the dosage range of endotoxin used in these studies the animals always survived without effects other than development of fever and transient leukopenia.

In these studies, *E. coli* endotoxin given in dosages of 10 μg. or more produced severe hemorrhagic, necrotic reactions at the sites of injection of staphylococci in the same way as endotoxin injected intradermally. Below these dosages of endotoxin, however, no such reactions were observed and the lesions resulting from injection of staphylococci evolved in no way differently than lesions produced in rabbits not given endotoxin; small furuncles appeared at the site of inoculation of 10^9 and 10^8 bacteria. As few as 10^6 or 10^5 staphylococci injected intradermally into animals given endotoxin, however, produced hemorrhagic, necrotic reactions.

Mechanism by Which Staphylococci Produce Dermonecrosis in Endotoxin-Prepared Rabbits.—The mechanisms involved in the interaction between endotoxin and staphylococci producing severe hemorrhagic and necrotic lesions were investigated to determine whether or not the same reaction could be elicited with culture filtrates of staphylococci, with heat-killed bacteria, with subnecrotic doses of alpha hemolysin, with exudate from staphylococcal peritonitis, or with non-pathogenic staphylococci.

A group of 6 rabbits was injected intracutaneously in 5 sites, as before, with 10 μg. *E. coli* endotoxin, and immediately thereafter a non-hemolytic, coagulase-negative staphylococcus was inoculated into 4 of the endotoxin-prepared areas in doses of 10^8, 10^7, 10^6, and 10^5 bacteria, respectively. At the same time the same dosages of the staphylococci were inoculated into normal skin of the same animals. Another group of rabbits was injected with the same quantity of endotoxin but injected with the coagulase-positive staphylococcus used in previous experiments.

The animals injected with endotoxin and coagulase-positive, hemolytic staphylococci developed the same hemorrhagic, necrotic reactions seen before, whereas the non-pathogenic, coagulase-negative, non-hemolytic staphylococcus

produced no such reaction, and small areas of erythema only were seen with inocula of 10^8 and 10^7 bacteria. These areas did not differ from the lesions seen from injection of the staphylococci into normal rabbit skin.

From this experiment, therefore, it appeared clear that pathogenic staphylococci were required to produce the hemorrhagic, necrotic reaction in the skin of endotoxin-prepared rabbits.

The similarity of the hemorrhagic, necrotic lesions developing after inoculation of pathogenic staphylococci into the skin of endotoxin-prepared rabbits to the dermonecrosis resulting from the intracutaneous injection of alpha hemolysin of staphylococcal culture filtrate suggested that endotoxin may be potentiating the action of alpha hemolysin produced by the injected staphylococci. In order to evaluate this possibility the following experiment was performed.

Staphylococcus culture filtrate containing potent alpha hemolysin and dermonecrotic activity was injected into rabbit skin immediately after preparation with 10 μg. *E. coli* endotoxin. The alpha hemolysin was diluted 1:10, 1:100, and 1:1000 with 0.85 per cent NaCl and each dilution was inoculated separately into endotoxin-prepared skin and into normal skin. Two groups of 3 rabbits were treated in this way.

The sterile culture filtrate containing alpha hemolysin activity produced dermonecrosis in normal skin of all rabbits when diluted 1:10, but when diluted 1:100 produced dermonecrosis in only 50 per cent of animals, and when diluted 1:1000 produced only erythema in normal skin.

There were no differences in the skin lesions produced by alpha hemolysin when inoculated into normal skin or into rabbits immediately after preparation of skin with endotoxin. Similar experiments were done in which the alpha hemolysin was injected 30, 60, and 90 minutes after preparation of skin with endotoxin and still no effect of the endotoxin upon the reaction to the alpha hemolysin was demonstrable. It was concluded, therefore, that the reaction, produced in rabbit skin prepared with endotoxin, to pathogenic staphylococci was not attributable to a potentiation of the dermonecrotic effects of sterile culture filtrate containing alpha hemolysin.

Other experiments were performed to determine if the endotoxin potentiated some extracellular toxin other than the alpha hemolysin produced by the pathogenic staphylococcus *in vivo*. To evaluate this, sterile, clarified peritoneal exudate from rabbits with staphylococcal peritonitis was injected in varying dilution into endotoxin-prepared rabbit skin but did not result in discernible differences from the lesions induced by such sterile exudate when injected into normal rabbit skin.

The possibility that endotoxin may interact with some constituent of the bacterial cell of the pathogenic staphylococcus to produce dermonecrosis was investigated by injecting heat-killed, washed staphylococci into rabbit skin prepared with endotoxin, but no detectable necrosis of skin was seen.

From these experiments, therefore, it was concluded that pathogenic, viable

staphylococci were required to produce hemorrhagic, necrotic lesions in rabbit skin prepared with bacterial endotoxin. This suggested, furthermore, that endotoxin may have enabled accelerated multiplication of the staphylococcus producing infection and that production of excess alpha hemolysin by the staphylococci could be responsible for the dermonecrosis. Histopathological study of the lesions in rabbit skin prepared with 10 μg. *E. coli* endotoxin and immediately inoculated with 10^7 pathogenic bacteria seemed to confirm these conclusions. Biopsy of such lesions at 6, 12, and 24 hours showed necrosis in skin injected with endotoxin and staphylococci, and in the area of necrosis at 6 hours no leukocytic response was visible but there were masses of unphagocytosed staphylococci. This area was sharply demarcated from the remainder of the skin by a surrounding wall of polymorphonuclear leukocytes. In the skin injected with endotoxin alone or staphylococci alone, a mild inflammatory polymorphonuclear response was visible at 6 hours and no staphylococci were visible in the tissues. At 12 and 24 hours the skin inoculated with 10^7 staphylococci only revealed beginning abscess formation with many leukocytes but few bacteria. A similar picture was seen at 12 and 24 hours in the skin injected with endotoxin and staphylococci although the area of necrosis and abscess formation was much more intense and extensive (Figs. 3 to 6). From these observations, therefore, it seems certain that endotoxin injected into rabbit skin or injected intravenously enables accelerated multiplication of pathogenic staphylococci. It then seems not at all unlikely that production of alpha hemolysin by the staphylococci was responsible for the extensive dermonecrosis.

That the alpha hemolysin produced by staphylococci inoculated into endotoxin-prepared rabbits was responsible for the hemorrhagic, necrotic reaction was confirmed in the following studies.

Two rabbits were immunized against alpha hemolysin by passive transfer of 15 ml. serum from animals immunized with alpha hemolysin toxoid. Another 2 rabbits were given 15 ml. of normal non-immune rabbit serum as control. 24 hours later these 4 animals were challenged with endotoxin and pathogenic staphylococci as described in the preceding experiment.

Animals passively immunized with rabbit serum containing antitoxin to staphylococcal alpha hemolysin failed to develop hemorrhagic, necrotic reactions, but the animals given normal serum containing no antitoxin developed such lesions in the same way as before.

An additional experiment was performed in which a group of 3 rabbits was immunized with alpha hemolysin toxoid and after 4 weeks, during which the animals had received 3 weekly injections of toxoid in adjuvant, they had anti-alpha hemolysin titers of between 6 and 8 units per ml. serum. At the completion of immunization the animals were resistant to the dermonecrotic effects of the culture filtrate containing alpha hemolysin. The rabbits were then injected intracutaneously into 2 sites with 10 μg. *E. coli* endotoxin. Immediately there-

after 10^7 and 10^6 staphylococci were injected into the endotoxin-prepared skin and into normal skin. At the same time the same inoculations were made in a group of non-immune rabbits.

The non-immune animals developed the same hemorrhagic, necrotic reactions as described above in endotoxin-prepared skin injected with pathogenic staphylococci, while the immune animals did not develop such lesions.

From these studies, therefore, it was concluded that endotoxin influenced rabbit skin to enable accelerated multiplication of viable pathogenic staphylococci and that the resulting dermonecrosis was attributable to production of alpha hemolysin by the infecting bacteria.

Mechanism by Which Endotoxin Enables Accelerated Multiplication of Pathogenic Staphylococci in Rabbit Skin.—As described in the preceding experiments the influence of endotoxin inoculated intracutaneously or intravenously upon the infectivity of pathogenic staphylococci was strictly limited to the 2 to 3 hour period after the endotoxin was injected. This critical interval suggested that the effect of endotoxin probably was attributable to some briefly active biological effect of the toxin and was probably unassociated with inflammation. Soon after endotoxin was injected intradermally a mild inflammatory reaction might develop locally, characterized by vascular dilatation or constriction and some leukocytic infiltration, but there was no reason to suspect that this inflammation was responsible for the change in infectivity of staphylococci, since, in other experiments (2), it had been shown that non-specific inflammation produced in a variety of other ways failed to influence staphylococcal infection to the same degree as did endotoxin. Furthermore, the ability of intravenous endotoxin to affect the infectivity of staphylococci in rabbit skin to the same degree as intracutaneous endotoxin excluded inflammation as a factor responsible for its effects.

The short period of time during which endotoxin exerted an effect upon cutaneous staphylococcal infection suggested that an interruption of natural mechanisms of host defense might be involved. It seemed likely that one of two possible mechanisms might be responsible, including the effect of endotoxin upon leukocytes and its effect upon blood vessels. Furthermore, the similarity between the effects of the interaction of staphylococci and endotoxin to the effects of adrenalin upon endotoxin-prepared skin of rabbits (8) suggested that the interference with cutaneous blood flow might be the mechanism involved. To evaluate these possibilities the following experiments were done.

Ten rabbits were given 0.5 mg. *dibenamine* intravenously 30 minutes prior to injection of *E. coli* endotoxin and pathogenic staphylococci into the skin, as described in previous experiments. 2 additional rabbits were treated in the same way with dibenamine but given staphylococci intracutaneously without preparation with endotoxin. Another 2 rabbits were given, in the usual way, 10 μg. endotoxin intracutaneously and immediately thereafter the staphylococci in dosage of 10^7, 10^6, and 10^5 bacteria were injected into the endotoxin-prepared skin, as in the other animals of the experiment, and as done before.

No differences were detected in the hemorrhagic, necrotic reaction of skin treated with endotoxin and staphylococci in the rabbits given dibenamine when compared to the control animals not given dibenamine. There was no effect of the dibenamine upon the staphylococcal lesions in the control animals. Similar experiments were performed in which animals were given 5 mg. *chlorpromazine* intravenously, 30 minutes before injection of endotoxin and staphylococci, and there was no effect upon the dermonecrotic reaction.

The possibility that *cortisone* might inactivate the effect of endotoxin upon cutaneous staphylococcal infection was investigated by treatment of a group of rabbits with 10 mg. cortisone injected intramuscularly for 3 days prior to injection of endotoxin and staphylococci. Rather than inactivating the effect of endotoxin upon staphylococcal infection of rabbit skin the cortisone treatment exaggerated the hemorrhagic, necrotic reaction. In addition, cortisone treatment of control rabbits given only endotoxin intracutaneously resulted in slightly hemorrhagic, erythematous, and indurated lesions at the site of endotoxin in the same way as previously described by Thomas (9).

The influence of intracutaneous endotoxin upon infiltration of intravenous Evan's blue dye into the endotoxin-prepared skin was re-evaluated and, as reported by others (10), the area injected with endotoxin remained pale in contrast to the diffuse blue discoloration of the animal's skin, indicating impaired permeability or interference with vascularity at the site of endotoxin injection. No apparent differences were observed, however, between the diffuse generalized bluish coloration of rabbit skin following intravenous injection of Evan's blue dye and endotoxin, when compared with animals given Evan's blue dye alone.

Rabbits were given 10 μg. *E. coli* endotoxin intracutaneously into 5 different sites and then temperature and blood leukocyte counts were examined at one-half hour intervals. No fever or leukopenia was apparent. Similar experiments were performed following intravenous injection of 10 μg. *E. coli* endotoxin into a group of 3 rabbits, and, as expected, they developed a typical febrile response and transient leukopenia (Text-fig. 1).

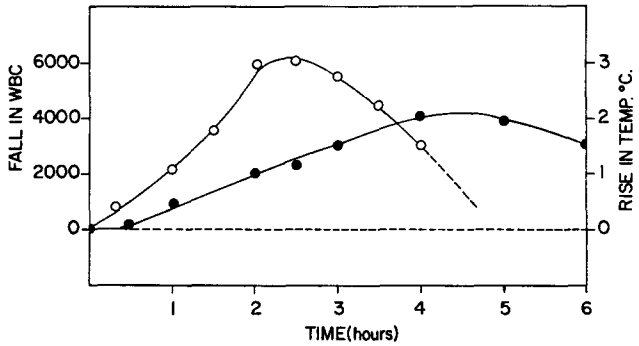
To determine if acquired tolerance or resistance to the pyrogenic action of endotoxin inhibited the enhancement of staphylococcal skin infection in endotoxin-prepared animals, the following experiment was done.

Three rabbits were made tolerant to 10 μg. *E. coli* endotoxin, as described in Methods. The tolerant rabbits and 3 normal rabbits were injected intracutaneously with 10 μg. endotoxin in 2 separate sites and immediately thereafter 0.1 ml. of the pathogenic staphylococcus in a dosage of 10^6 organisms was injected into 1 of the endotoxin-prepared sites and into normal skin on the opposite side of the animals.

No hemorrhagic, necrotic reactions were observed at the site of endotoxin and staphylococcus injection in the endotoxin-tolerant rabbits, whereas such reactions were observed in similar sites in the control animals, indicating that

acquired tolerance or resistance to the pyrogenicity of endotoxin was associated with inactivation of the effect of endotoxin upon staphylococcal infection of rabbit skin.

Effect of Intravenous Injection of Endotoxin upon Experimental Staphylococcal Infection of Rabbit Kidney.—To determine if endotoxin exerted an effect upon staphylococcal infection in other tissues than skin and to obtain quantitative information as to the ability of endotoxin to enable accelerated growth of staphylococci *in vivo* the following experiment was performed.



TEXT-FIG. 1. Comparison of the leukopenia (○) and febrile (●) response of a group of rabbits to a single intravenous injection of 10 μg. *E. coli* endotoxin, showing that this effect on leukocytes was limited to the first 4 hours after injection.

Five groups of 4 rabbits were given injections into their kidneys as described in Methods. In the first group, 2 of the animals were injected in one kidney with 10 μg. *E. coli* endotoxin mixed with 10^8 pathogenic staphylococci and the opposite kidney was injected with endotoxin alone. In the other 2 rabbits of the first group, staphylococci were inoculated into one kidney and saline was injected into the other. In subsequent experiments 2 animals in each group were injected into one kidney with staphylococci in a dosage of between 1500 and 4500 bacteria mixed with 10 μg. endotoxin and the opposite kidney was either not injected at all or injected with sterile saline. The other 2 rabbits in each group were injected into one kidney with 1500 or 4500 bacteria in sterile saline and the opposite kidney was not injected or injected only with sterile saline. After 18 or 24 hours the animals were sacrificed and the kidneys removed aseptically. Each kidney was homogenized in 5 ml. 0.85 per cent NaCl and then diluted for agar pour plate cultures to quantitate the number of bacteria. In addition, prior to homogenization the kidneys were bisected for gross examination and small sections taken for histopathological study.

With the largest inocula (10^8) of staphylococci mixed with endotoxin hemorrhagic and enlarged kidneys were occasionally produced. Histopathologically, the kidneys injected with endotoxin and staphylococci showed tubular degeneration and casts as well as medullary hemorrhage, and focal necrosis. The kidneys inoculated with endotoxin alone showed no abnormalities in the gross or histologically at 24 hours, and kidneys inoculated with staphylococci

alone showed in one instance a single small focal abscess in the kidney. Quantitative cultures of the kidneys showed no growth of staphylococci unless the bacteria had been directly inoculated into the organ, except when 10^8 bacteria were injected, and then the opposite kidney occasionally grew staphylococci as well. With smaller inocula the kidneys not injected with staphylococci were always sterile on culture. Comparison of the bacterial counts from kidneys inoculated with endotoxin and staphylococci, with kidneys inoculated with staphylococci only showed the former to contain between 0 and 4 logs more bacteria, with an average difference in numbers of bacteria of 10^2. This finding supports the suggestion that endotoxin enables accelerated multiplication of staphylococci *in vivo*.

DISCUSSION

There are innumerable examples of the profound effects of changes in non-specific resistance upon experimental and naturally occurring infection. Specifically, it is now clear that staphylococcal infection occurs more frequently and is of greater severity in persons with certain types of associated diseases in which decreases in non-specific resistance are known to be present (4). The increased frequency of such infection in surgical wounds, in patients with exfoliative dermatitis and at the site of intravenous catheters documents these situations as predisposing causes of staphylococcal infection (4). Furthermore, Elek (3) has shown precisely the importance of retained foreign bodies in human skin in locally increasing the infectivity of staphylococci.

The implication of experimental inflammation as a local factor influencing the infectivity of staphylococci has been investigated (2), and although non-necrotic inflammatory reactions of rabbit skin have been shown to increase susceptibility to staphylococcal infection, the magnitude of the change is not great. The way in which inflammation increases the infectivity of staphylococci and other microorganisms, however, is not known. Necrotic tissue also is more susceptible to infection by staphylococci (1), and this is not surprising, as one might anticipate that such tissue would not demonstrate the usual mechanism of non-specific resistance. In addition to the local effects of inflammation and necrosis upon infectivity of staphylococci it also is known that nutritional, hormonal, and other influences may adversely affect the resistance of experimental animals and human beings to staphylococcal infection.

The mechanisms of "natural" resistance to staphylococcal infection are not particularly well understood. Furthermore, the role of acquired resistance in staphylococcal infections has been thought to be of low order or non-existent, but may be considerable in certain instances (1). It is likely, however, that phagocytosis is the principal means by which invasion by the staphylococcus is controlled (11). If this is true, an impairment of phagocytic function might be expected to decrease resistance to infection, and improvement in phagocytic

function might be expected to improve resistance to infection by the staphylococcus.

In a previous study of infection in necrotic burns by staphylococci it was shown that antibody neutralization of the alpha hemolysin produced by the microorganism was associated with control of infection (1). Of particular interest in those experiments was the observation that when the staphylococcal toxin was not neutralized leukocytic infiltration into the infected necrotic burn was absent. On the other hand, neutralization of the toxin with serum antibody was associated with intense leukocytic infiltration into the necrotic burn inoculated with staphylococci. This suggested that the production of alpha hemolysin by the multiplying microorganisms lysed the leukocytes, thereby further interfering with the capacity of the animal to control the infection. This was suggested again by the limitation of the infection to the necrotic tissue and contiguous areas by extensive leukocytic infiltration at the margin of the lesion. From these studies, therefore, it was possible to indicate that, provided the opportunity for initiation of bacterial multiplication by induction of necrosis, the elaboration of alpha hemolysin potentiated the infection by destroying the polymorphonuclear leukocytes migrating into the area of bacterial growth. It seems likely, therefore, that one of the important mechanisms of host injury in staphylococcal infection is the extracellular toxin of the bacteria and that the toxin potentiates the infection by destroying one of the essential mechanisms of resistance; the phagocyte. That alpha hemolysin does, indeed, destroy polymorphonuclear leukocytes has recently been confirmed in our laboratory in some unpublished observations.

Recognition of the mechanisms involved in the studies described above is essential to an understanding of the events described in this publication. For example, it has been shown here that intradermal inoculation of staphylococci into the endotoxin-prepared rabbit results in hemorrhagic and necrotic lesions not dissimilar to the lesions produced by staphylococci inoculated into necrotic burns. Furthermore, inoculation of the microorganism into endotoxin-prepared animals is associated with accelerated bacterial multiplication, the lesions produced are necrotic, there are no leukocytes found initially in the area of bacterial multiplication, only pathogenic staphylococci will induce the lesions, and the infection can be controlled by antitoxic immunity. In the endotoxin-prepared rabbit, therefore, the mechanism by which the staphylococci produce host injury appears to be the same as in infections induced in necrotic burns. This further suggests the importance of the extracellular toxin of the microorganism in the pathogenesis of at least certain types of staphylococcal infection.

A crude culture filtrate containing potent alpha hemolytic and dermonecrotic activity was used in the experiments described here. It is possible that other antigens elaborated by the staphylococcus during growth could be responsible for the injurious effects of staphylococcal infection in endotoxin-prepared rab-

bits, but in more recent studies using an immunochemically purified toxin the importance of the alpha hemolysin in the pathogenesis of this infection seems established.

The way by which endotoxin prepares the rabbit so that it is more susceptible to staphylococcal infection is not as clear as the probable mechanism by which the staphylococcus produces host injury. Endotoxin has a variety of biological effects in the rabbit and other species of animals (5). Among the effects of endotoxin that might be incriminated as possible causes of decreased resistance to staphylococcal infection are: (*a*) its ability to cause inflammation, (*b*) its effect upon vascular reactivity, (*c*) its effect upon leukocytes, and (*d*) its effect upon humoral substances involved in host resistance.

A peculiar feature of the effect of endotoxin upon staphylococcal infection in these experiments is its short duration. It was impossible to demonstrate an enhancement of staphylococcal infection when more than 4 hours lapsed between injection of endotoxin and injection of staphylococci. Dubos and Schaedler (12) made similar observations in studying the effect of endotoxin upon lethal staphylococcal infection and upon accelerated bacterial multiplication in the viscera of mice. This critical period after injection of endotoxin leads to the probability that the effect of endotoxin upon blood vessels or leukocytes is most likely involved in changing the susceptibilty of animals to staphylococcal infection.

Depending upon the dose of endotoxin given to an animal either vasoconstriction or vasodilatation may be observed (5). In addition, endotoxin is known to sensitize blood vessels to the action of adrenalin (8). Many of these actions of the toxin can be inhibited by chlorpromazine or dibenamine (8), but in the experiments reported here it was not possible to influence the effect of endotoxin upon staphylococcal infection with these drugs. Although this finding suggests that the vascular effects of endotoxin characterized by vasodilatation and vasoconstriction may not be responsible for changing the infectivity of staphylococci, it does not exclude them as possibilities. Further studies will be required to better understand this relationship. The wide effects described by Dubos and Schaedler (12) upon the multiplication and lethal effects of staphylococci in mice, however, are not readily explained by the effects of endotoxin upon blood vessel constriction, but are better explained by an effect of endotoxin upon some other function involved in host resistance.

The close time relationship between the effects of endotoxin upon leukocytes and upon staphylococcal infection suggests that impaired diapedesis or phagocytic activity of white blood cells may be responsible for the effect of the toxin upon staphylococcal infection. There are conflicting reports on the effect of endotoxin upon diapedesis and phagocytosis by leukocytes, but Cohn and Morse (13) have recently shown that under certain circumstances the toxin may

enhance phagocytic function *in vitro*. Delaunay *et al.* (14) reported, however, that endotoxin may interfere with leukocyte diapedesis, and Fekety (15) has recently been able to confirm this effect of endotoxin upon leukocytes using the glass window technique described by Rebuck (16). Furthermore, Fekety has been able to show an impairment of leukocyte diapedesis with the same doses of endotoxin required in this report to enhance staphylococcal infection of rabbit skin. It seems likely, therefore, from the indirect evidence available, that endotoxin enhances the infectivity of staphylococci by interfering with normal leukocytic function.

Endotoxin has been shown to change the surface activity of leukocytes, as illustrated by the adherence of white blood cells to each other and to vascular endothelium (17), but whether or not this action of endotoxin could be responsible for impaired diapedesis is not known. The failure of Evan's blue dye to infiltrate an area of endotoxin-prepared skin suggests a general impermeability of blood vessel walls to protein and it is possible that the impaired migration of white blood cells into extravascular tissues is attributable to alterations in vascular function as well as to an alteration in leukocytes. Whatever the mechanism is by which endotoxin transiently influences the infectivity of staphylococci, however, it is clear that induction of tolerance to the pyrogenic action of endotoxin in rabbits is associated with a nullification of the toxin's effect upon the microorganism's infectivity.

It is unlikely that the mild inflammatory reaction elicited by endotoxin inoculated into rabbit skin is responsible for the toxin's profound effect upon staphylococcal infection, as inflammation of a comparable degree produced with several other agents fails to increase similarly the infectivity of the bacteria (2).

Condie, Zak, and Good (18) have reported an increase in the severity of pneumococcal infection in mice by prior injection of endotoxin, and Dubos and Schaedler (12) have shown a similar effect upon other microorganisms. The full extent of the influence of endotoxin in producing a transient increase in the infectivity of a variety of types of bacteria, however, has not been elucidated. It would be of interest if this effect of the toxin could serve to better define the mechanisms of host resistance in infection and the mechanisms of host injury induced by other microorganisms.

SUMMARY

Intracutaneous and intravenous injection of pyrogenic, non-lethal doses of bacterial endotoxin were found to increase the infectivity of pathogenic but not non-pathogenic staphylococci in rabbit skin. The increased infectivity of the microorganism was characterized by accelerated multiplication at the site of inoculation and by the production of necrosis and hemorrhage locally. Histo-

logically, the infection of skin in endotoxin-prepared animals was characterized by necrosis, masses of bacteria, but absence of leukocytic infiltration into the area of bacterial growth.

The infectivity of staphylococci in skin of endotoxin-prepared rabbits could be controlled by antibody to the alpha hemolysin of the microorganism.

The effect of endotoxin upon staphylococcal infection was demonstrable only within 4 hours after injection of the endotoxin. It could not be prevented with chlorpromazine or dibenamine and was closely related to the effect of endotoxin upon leukocytes.

It was suggested that the effect of endotoxin upon leukocytes was probably responsible for its influence upon staphylococcal infection.

The implications of these findings in the pathogenesis of staphylococcal infection are discussed.

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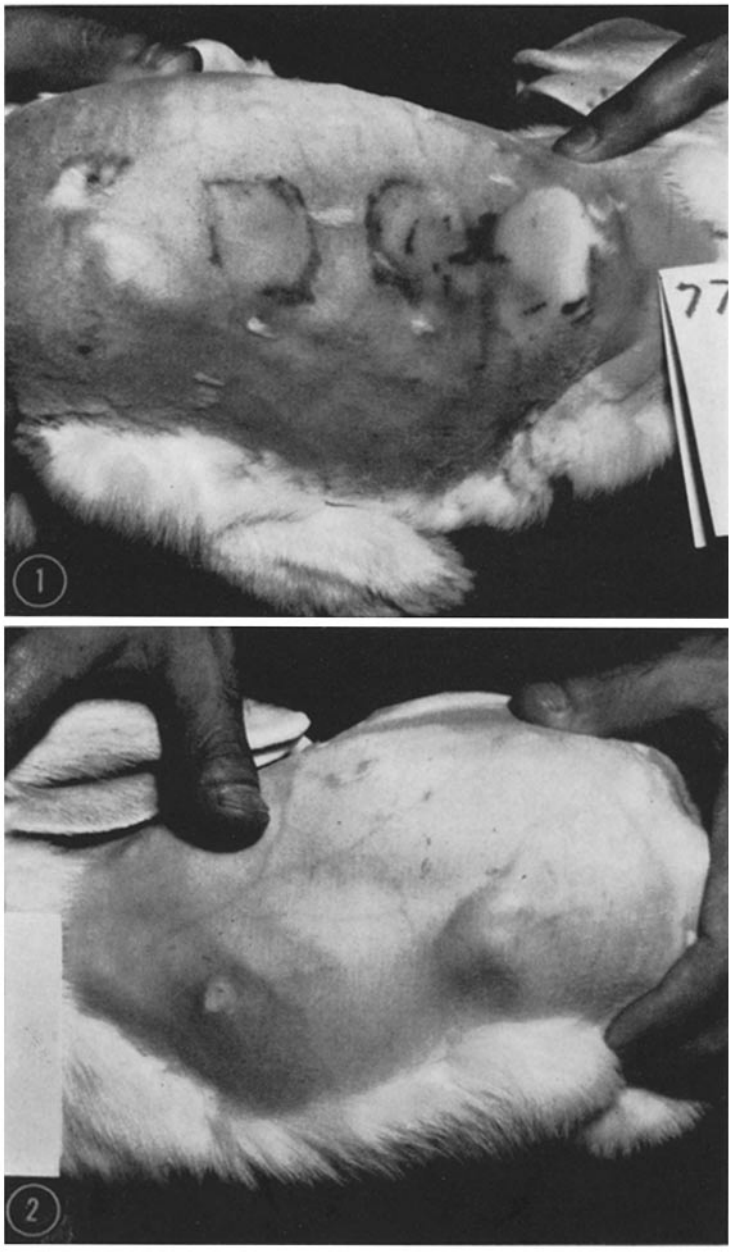
EXPLANATION OF PLATES

PLATE 85

FIG. 1. Hemorrhagic necrosis induced by intradermal injection into rabbits of 10 μg. *E. coli* endotoxin with 10^6, 10^7, 10^8, and 10^9 pathogenic staphylococci, and the slight pallor induced with endotoxin alone (lower left).

FIG. 2. Lesions induced in rabbit skin injected with 10^9, 10^8, 10^7, and 10^6 pathogenic staphylococci showing small abscesses only at the site of inoculation of 10^9 and 10^8 bacteria.



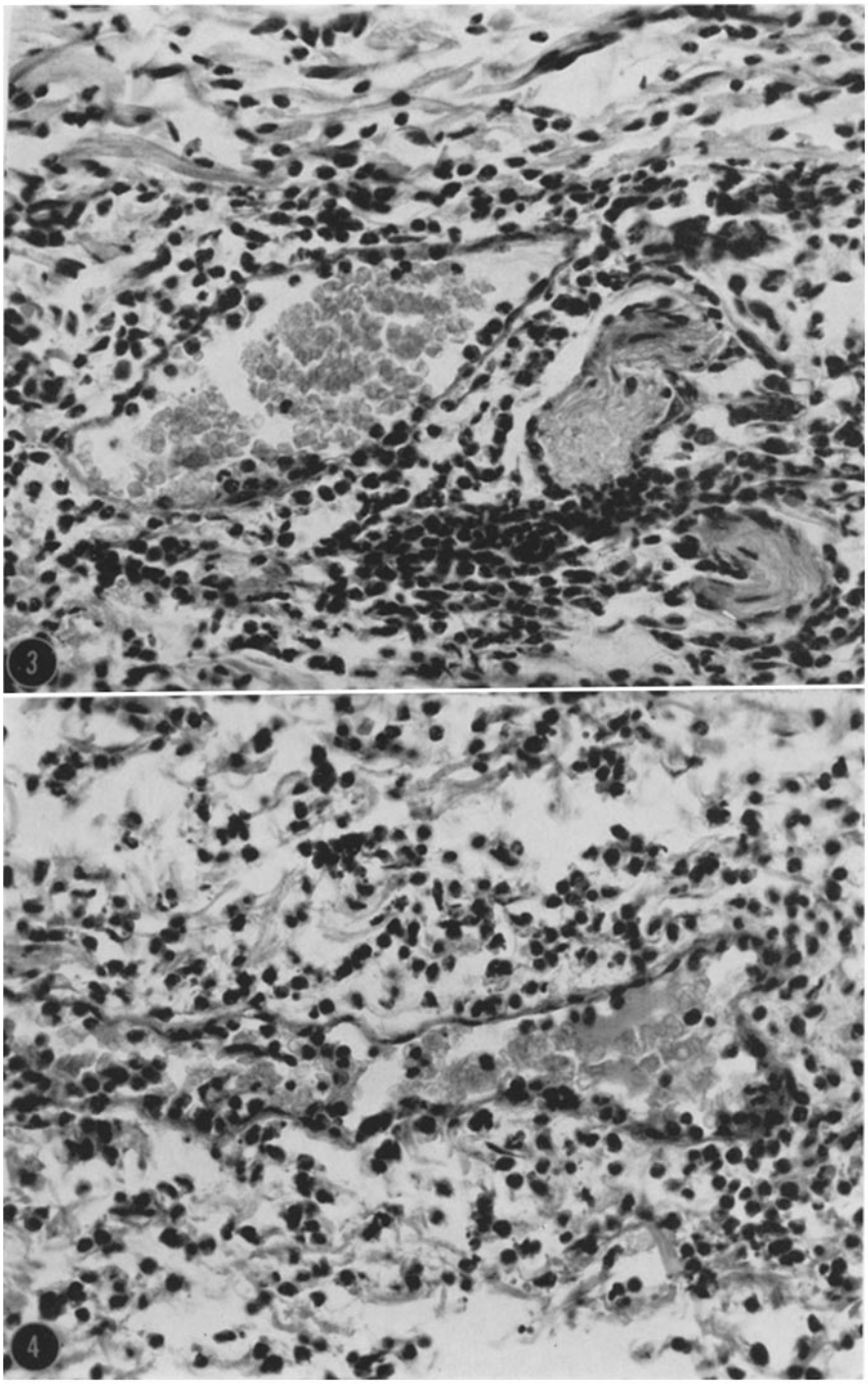
(Conti *et al.*: Pathogenesis of staphylococcal infection)

PLATE 86

Microscopic sections of skin removed 6 hours after injection and stained with hematoxylin-eosin.

FIG. 3. Skin injected with 10^7 pathogenic staphylococci. × 350.

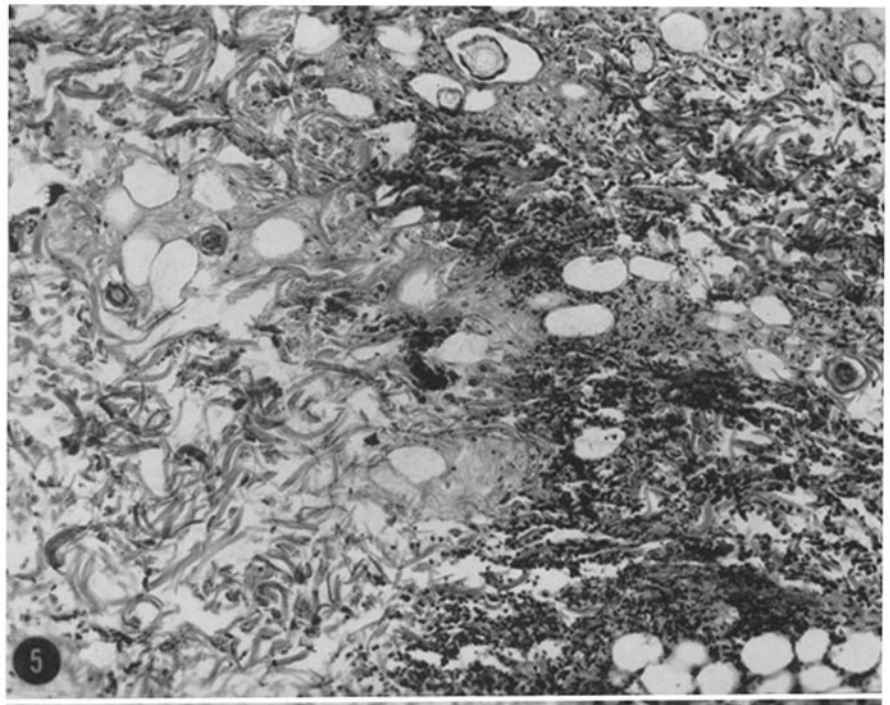
FIG. 4. Skin injected with 10 μg *E. coli* endotoxin. × 350.

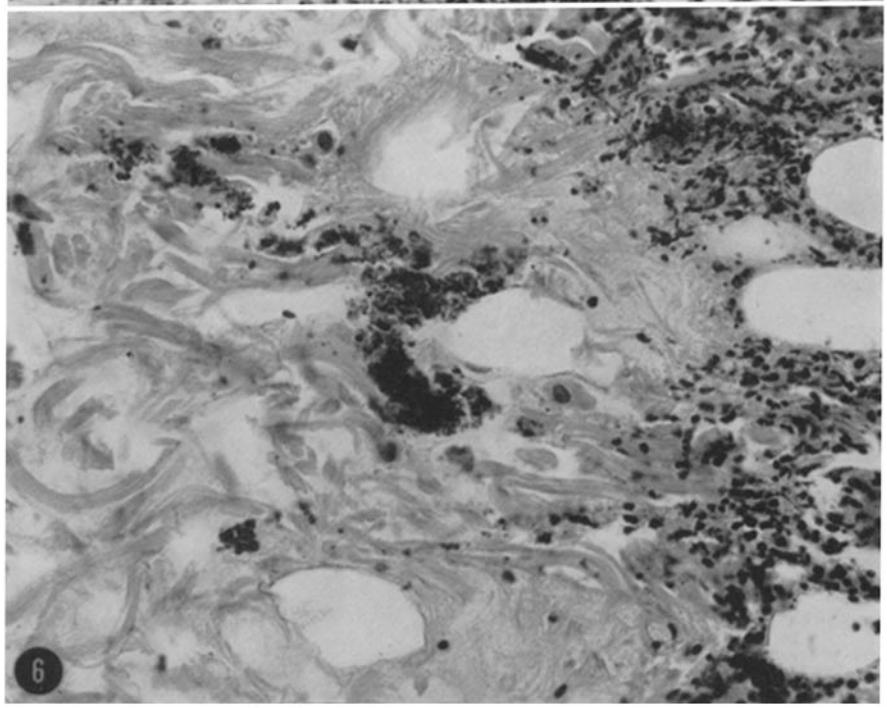


(Conti *et al.*: Pathogenesis of staphylococcal infection)

PLATE 87

FIGS. 5 and 6. Skin injected with 10 μg. *E. coli* endotoxin and 10^7 pathogenic staphylococci, showing necrosis, masses of bacteria, absence of leukocyte infiltration in the area of bacterial growth, and marked leukocyte infiltration at margin of lesions. Fig. 5, $\times$ 150 and Fig. 6, $\times$ 350.





(Conti *et al.*: Pathogenesis of staphylococcal infection)