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Formation of Intraperitoneal Abscesses by *Staphylococcus aureus*

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Certain *Staphylococcus aureus* strains, when inoculated into the peritoneal cavity of mice, were clumped and surrounded by a thick layer of leukocytes. After being enclosed with a connective tissue capsule, the structures histologically resembled staphylococcal abscesses. Of four strains examined, all were destroyed within abscesses, although at different rates. Abscess homogenates possessed bactericidal activity toward staphylococci, and this activity was associated with the sedimentable fraction of the homogenates. Leukocytes did not appear to be responsible for the bactericidal activity. Appreciable quantities of alpha toxin accumulated in these abscesses even without multiplication of the organisms. This model infection offers opportunities for studying some aspects of staphylococcal host-parasite interactions occurring in localized lesions.

Although the abscess is the most common type of *Staphylococcus aureus* infection in humans, little is known about factors which influence the organism's behavior within such lesions. In part, this is due to a lack of models which can be manipulated in the laboratory.

While studying the fate of staphylococci in vivo, we came to realize that the intraperitoneal inoculation of staphylococci can lead to the formation of abscesses which are recoverable with ease. With this type of model, it was possible to monitor both the organism's survival and the accumulation of alpha toxin within the lesion. Later studies also revealed the appearance of a staphylocidal system which may contribute to the destruction of the organism.

When nonencapsulated *S. aureus* strains possessing the clumping factor are inoculated into the peritoneal cavity of mice, they are promptly clumped through the interaction of clumping factor with the fibrinogen in the peritoneal fluid (3). Neutrophils entering the region during the ensuing inflammatory response surround the aggregated organisms, but phagocytosis appears to be minimal, presumably because only those organisms at the periphery of the clumps are readily accessible to the leukocytes. After about 6 h, the clumps, together with associated leukocytes, coalesce into a few large clusters containing almost the entire inoculum (3).

Unless the strain is encapsulated, the organism's ability to clump is important for its immediate survival. Strains lacking clumping factor remain dispersed and are subject to phagocytosis. However, even strains possessing clumping factor may not be clumped effectively if fewer than 2×10^8 cocci are inoculated, and therefore the organisms remain accessible to leukocytes (3). Although the administration of large numbers promotes efficient clumping, such doses usually release so much alpha toxin that the host is killed within 6 to 24 h (3, 4). Host survival can be assured by using nonhemolytic strains, but these strains would not be representative of human isolates, which usually elaborate the alpha and delta toxins (2).

During other studies, we found that toxigenic strains cultured in the absence of carbon dioxide were subsequently retarded in their elaboration of alpha toxin when introduced into the peritoneal cavity (unpublished data). Capitalizing on these findings, it was possible to administer doses that were sufficient for maximal clumping but unable to release a lethal dose of alpha toxin. Under these circumstances, the long-term behavior of the organism within the host could be evaluated.

MATERIALS AND METHODS

Strains. *S. aureus* 18Z (3) produced soluble coagulase, clumping factor, and both alpha and delta toxins. Strains 674 and 689, isolated from human sources, produced soluble coagulase, clumping factor, and only alpha toxin. Strain 689, however, produced more alpha toxin than did strain 674 (61 50% hemolytic doses [HD_{50}] per 10^9 cocci versus 3.3 HD_{50} per 10^9 cocci when grown in Trypticase soy broth; BBL Microbiology Systems).

The alpha toxin-negative variant, 18Z-G, was derived from the parent 18Z strain after UV irradiation.

The variants 18Z Sm^r and 18Z-G Sp^r, resistant to either streptomycin (Sm) or spectinomycin (Sp), were derived from their respective parents by plating on Trypticase soy agar containing either 60 μg of streptomycin per ml or 600 μg of spectinomycin per ml.

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Inocula. Staphylococci were grown for 24 h in Trypticase soy broth with constant shaking. Cultures were continuously gassed with 100% oxygen to minimize alpha-hemolysin production. The organisms were harvested by centrifugation, washed in saline containing 1% Trypticase soy broth (vol/vol), and resuspended in the same diluent to give a concentration of approximately 1×10^{10} to 5×10^{10} cocci per ml. Suspensions were sealed in ampoules and stored at −70°C until needed. The bacterial concentration was confirmed by plate counts each time the suspensions were used.

Animal inoculations. Groups of female white Swiss mice (20 to 30 g) were inoculated intraperitoneally with 0.5 ml of suspension containing 1×10^9 to 2×10^9 staphylococci. Beginning at 3 h, and at various intervals thereafter, subgroups of infected animals, selected at random, were sacrificed, and the clumped organisms or abscesses were aseptically removed from the peritoneal cavity.

To enumerate staphylococci present in the lesions, all clumps or abscesses from a single animal were placed in a glass tissue grinder fitted with a motor-driven Teflon pestle and homogenized in 5 ml of saline containing 1% Trypticase soy broth. The suspension was serially diluted, and the bacterial population was determined by plate counts.

To measure alpha toxin associated with lesions, homogenates were similarly prepared except that 1 ml of saline was used as the diluent. After centrifugation, the clear supernatant fluid was assayed for alpha toxin as previously described (5).

Histological sections. For microscopic studies, the clumps of staphylococci with associated material or abscesses were removed from the peritoneal cavity and placed in neutral-buffered Formalin. After fixation, specimens were embedded, sectioned, and stained with hematoxylin-eosin.

RESULTS

Abscess formation. Staphylococci inoculated into the peritoneal cavity were promptly clumped and within 4 h became surrounded by neutrophils as previously described (3). After 24 h, the leukocyte-covered clumps were surrounded with a layer of eosinophilic material which contained some cells (Fig. 1A). The nature of this eosinophilic material was not determined, but it was presumed to be fibrin. Evident by 48 h, and becoming progressively more patent over the next 2 days, was the deposition of connective tissue around the eosinophilic layer (Fig. 1B). At this time, the lesions consisted of (from center to periphery) a core of closely packed cocci, a region of degenerate leukocytes, a broad zone of intact leukocytes, the eosinophilic layer, and a vascularized connective tissue capsule.

This overall organization was still apparent in abscesses removed 2 to 3 months after infection (Fig. 1C). However, by then the abscesses were somewhat larger, and the central region contained only scattered islands of basophilic-staining staphylococci (Fig. 1E and F). The zone of intact polymorphonuclear leukocytes remained prominent (Fig. 1D), but, in view of the short life-span of these cells, probably was maintained by a continuous influx of new cells.

Survival of staphylococci in abscesses. Beginning 3 h after inoculation, groups of infected mice were sacrificed, their lesions were removed, and the cocci present were enumerated by plate counts. The survival of the organisms within abscesses was studied for approximately 40 days.

Of the strains examined, all were eliminated, but at different rates. Both strains 674 and 689 were progressively destroyed, but the former much more quickly than the latter (Fig. 2 and 3). *S. aureus* 18Z presented a more complicated pattern of survival (Fig. 4). After a period of rapid decline during the initial 4 days, the population increased, but did not return to its original level. After about 10 days, the population again decreased, although more slowly than before. This peculiar pattern has been consistently observed with this strain in repeated studies.

Strain 18Z-G was destroyed within abscesses as readily as was strain 674.

Regardless of the strain involved, bacterial counts obtained from different animals sacrificed at the same time were in fairly close agreement among specimens removed a few days after infection; however, in later samples there was greater animal-to-animal variation.

Throughout the course of study the only organisms recovered from these abscesses were the staphylococci originally inoculated.

Bactericidal activity associated with abscess homogenates. To determine whether abscesses might contain a substance responsible for destruction of staphylococci, abscess homogenates were examined for bactericidal activity. Mice were inoculated with 10^9 *S. aureus* 18Z, which is sensitive to both spectinomycin and streptomycin, and the resultant abscesses were aseptically removed and homogenized in saline. To this homogenate was added a test mixture consisting of an equal number of the antibiotic-resistant 18Z Sm^r and 18Z-G Sp^r strains. The mixture was incubated at 37°C with shaking, and samples were periodically removed. By plating sample dilutions on Trypticase soy agar containing either streptomycin or spectinomycin, each test organism could be enumerated without interference from the 18Z strain used to generate the abscess and which was still present in the homogenates. Under these conditions, it was found that the abscess homogenates were bactericidal for the nonhemolytic 18Z-G Sp^r strain, whereas the hemolytic 18Z Sm^r strain was unaffected (Fig. 5). Subsequent studies revealed

that this preferential bactericidal activity could be demonstrated in homogenates prepared from abscesses collected 1 to 16 days after inoculation. However, homogenates prepared from leukocyte-coated staphylococcal clumps removed 4 h after intraperitoneal inoculation lacked bactericidal activity, suggesting that neutrophils alone did not account for the observed bactericidal effect.

By centrifuging the abscess homogenates at 40,000 × *g* for 30 min it was found that the bactericidal activity resided in the sedimented fraction (Fig. 6). Similar experiments using abscess homogenates prepared from abscesses collected at various times after infection indicated that the bactericidal activity always resided in the pelleted fractions, while supernatants remained devoid of this activity.

Accumulation of alpha toxin within abscesses. Mice were infected with 10^9 cocci of strains 674 or 689, the resultant abscesses were harvested and homogenized, and the supernatant fluid was assayed for alpha toxin. Little alpha toxin was associated with leukocyte-covered clumps removed 3 h after infection with strain 674 (Fig. 7), but the amount increased rapidly as abscesses matured, reaching a peak level of approximately 100 HD_{50} per animal by day 3. With strain 689, larger amounts of toxin were associated with early samples and even larger amounts accumulated within abscesses, but peak levels (1,000 HD_{50} per animal) were not attained for 7 days. In both instances, once maximum concentrations were achieved, the amounts of toxin within abscesses steadily declined with a half-life of about 8 to 10 days.

DISCUSSION

The prior growth of staphylococci in the absence of CO_2 sufficiently retards alpha toxin release into the peritoneal cavity so that 10^9 cocci can be inoculated without killing the mouse. With this dose, clumping of the organisms is rapid and efficient (3). The subsequent deposition of leukocytes and connective tissue about the organisms leads to the formation of lesions that histologically resemble staphylococcal abscesses. This model has the advantage that lesions are localized and easily recoverable with a minimum of extraneous tissue. The most serious deficit of the model rests with its lack of an early phase involving multiplication of the inoculum. Although in this study only the organisms' survival and the accumulation of alpha toxin were monitored, the model offers opportunities for studying still other processes involved in the later stages of the host-parasite interaction.

Most staphylococci become incorporated into clumps within a few minutes after inoculation into the peritoneal cavity. Organisms not included are subject to phagocytosis by leukocytes initially present or arriving during the inflammatory response (3). Whereas phagocytosis should eliminate the unaggregated cocci, it is possible that some organisms can escape to enter the circulation. Since the kidneys are particularly prone to infection with small numbers of staphylococci (10^3 to 10^6) cleared from the blood (4), it was conceivable that organisms not confined to the peritoneal cavity could give rise to renal lesions. That this kind of spread can occur was apparent from the observance of renal abscesses in animals sacrificed more than 5 days after infection. However, this occurred in only about 5% of mice infected with strains 18Z and 689, and was not seen in animals given the other strains.

Even though they had been grown in the absence of CO_2 before inoculation into mice, strain 674 and 689 staphylococci were able to elaborate alpha toxin in abscesses. Furthermore, this occurred without obvious multiplication of the organisms. Since the median lethal dose of preformed alpha toxin for mice is known to be 20 to 25 HD_{50}, the amounts accumulated within abscesses (100 to 1,000 HD_{50}) are impressive. However, once peak concentrations were attained, the toxin concentration within abscesses declined steadily. It is not known whether this represents diffusion of toxin from the lesion after production has ceased or whether toxin is de-

FIG. 1. *Histological sections through staphylococcal abscesses produced in the peritoneal cavity of mice by inoculation of* 2×10^9 *S. aureus 18Z. Hematoxylin-eosin stain. (A) Twenty-four hours after infection. Deposition of eosinophilic material (em), presumably fibrin, around the neutrophil layer. The center of the lesion is toward the lower left. (B) Four days after infection. Vascularized connective tissue (ct) now surrounds the eosinophilic layer (em). Center of abscess is toward lower right. (C) to (F) Sections from abscess removed 82 days after infection. (D), (E), and (F) are at the same magnification. (C) Successive layers are still evident. The central region (lower left), originally composed of clumped staphylococci, now appears eosinophilic. Around this are layers of disintegrated leukocytes (dl), intact leukocytes (il), the eosinophilic material (em), and connective tissue (ct). A single giant cell can be seen in the region between the two outer layers. (D) Enlargement of leukocyte layer showing intact neutrophils. (E–F) Islands of staphylococci located within central portion of abscess.*

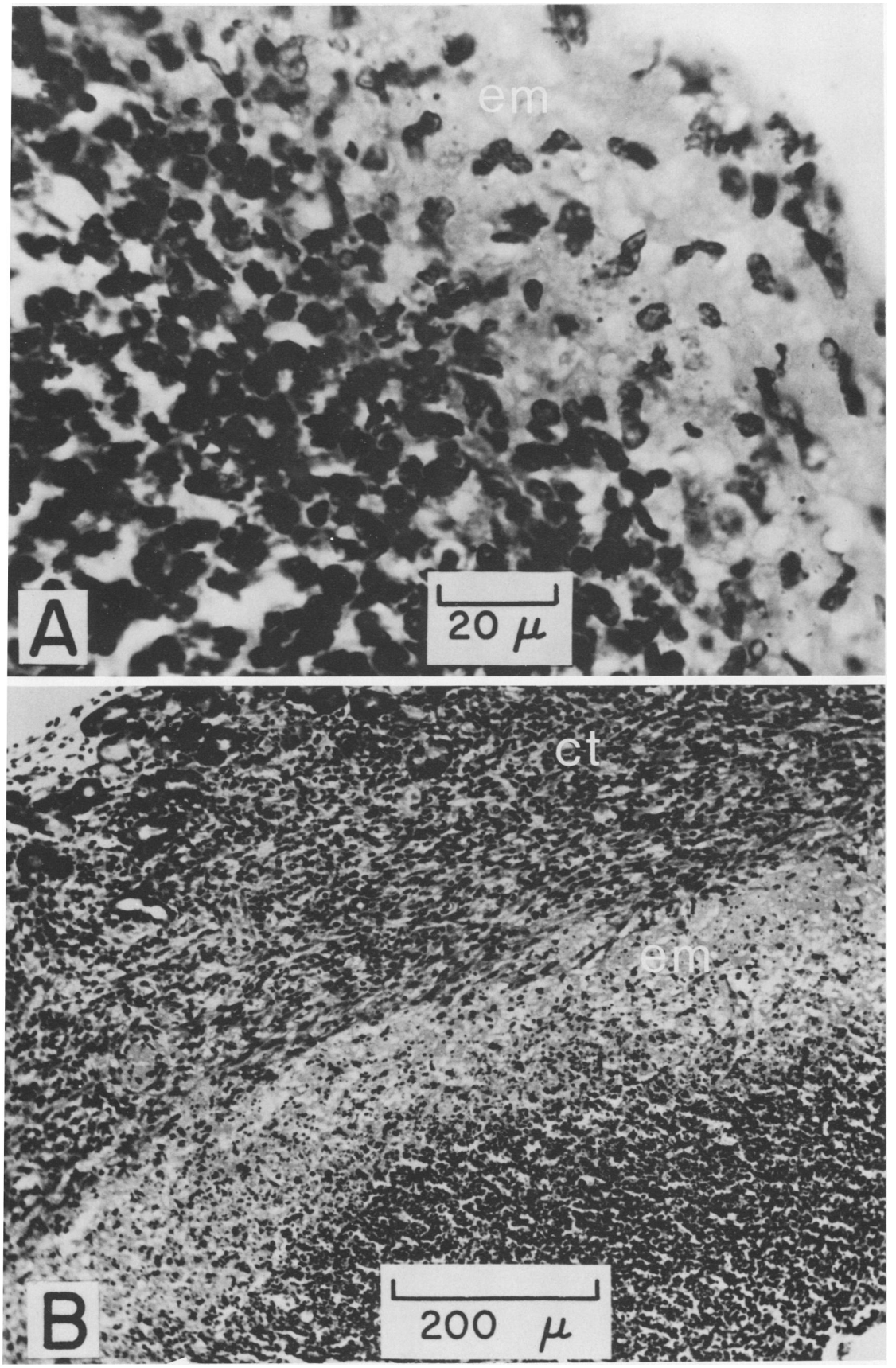

FIG. 1A–B.

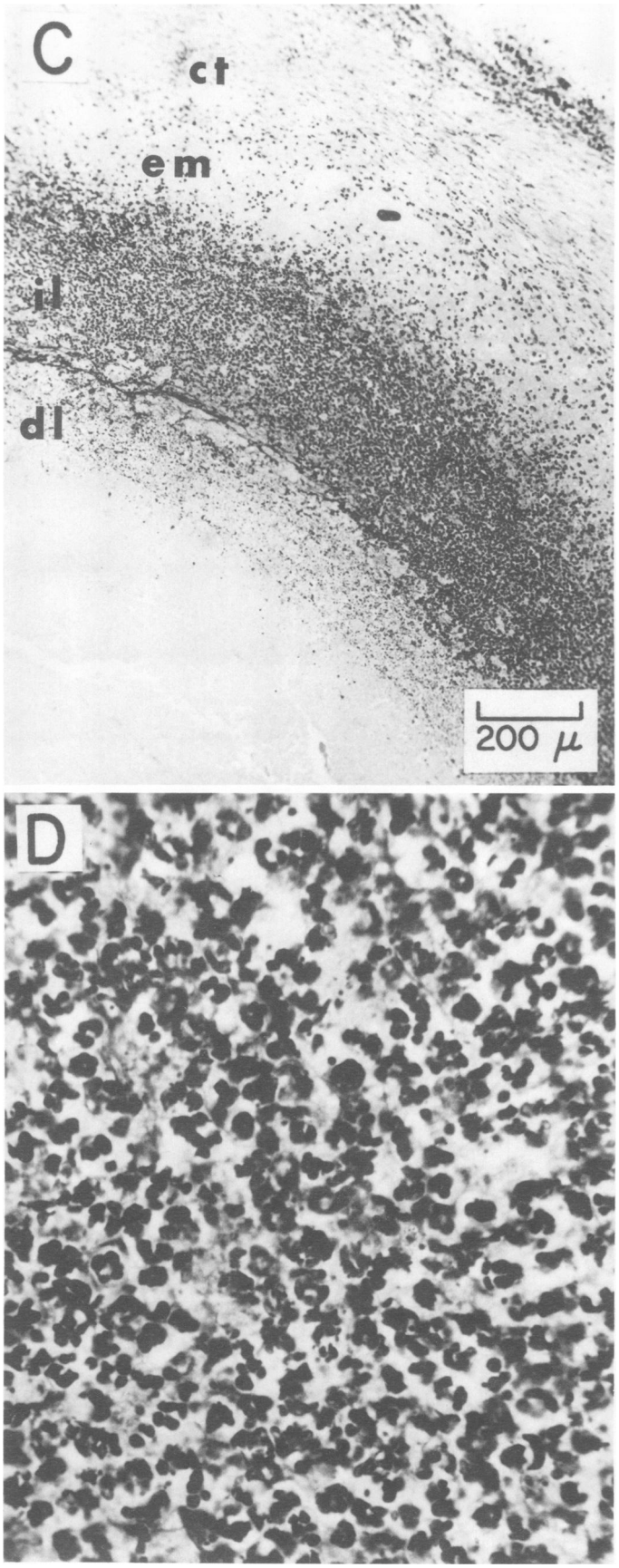

FIG. 1C–D.

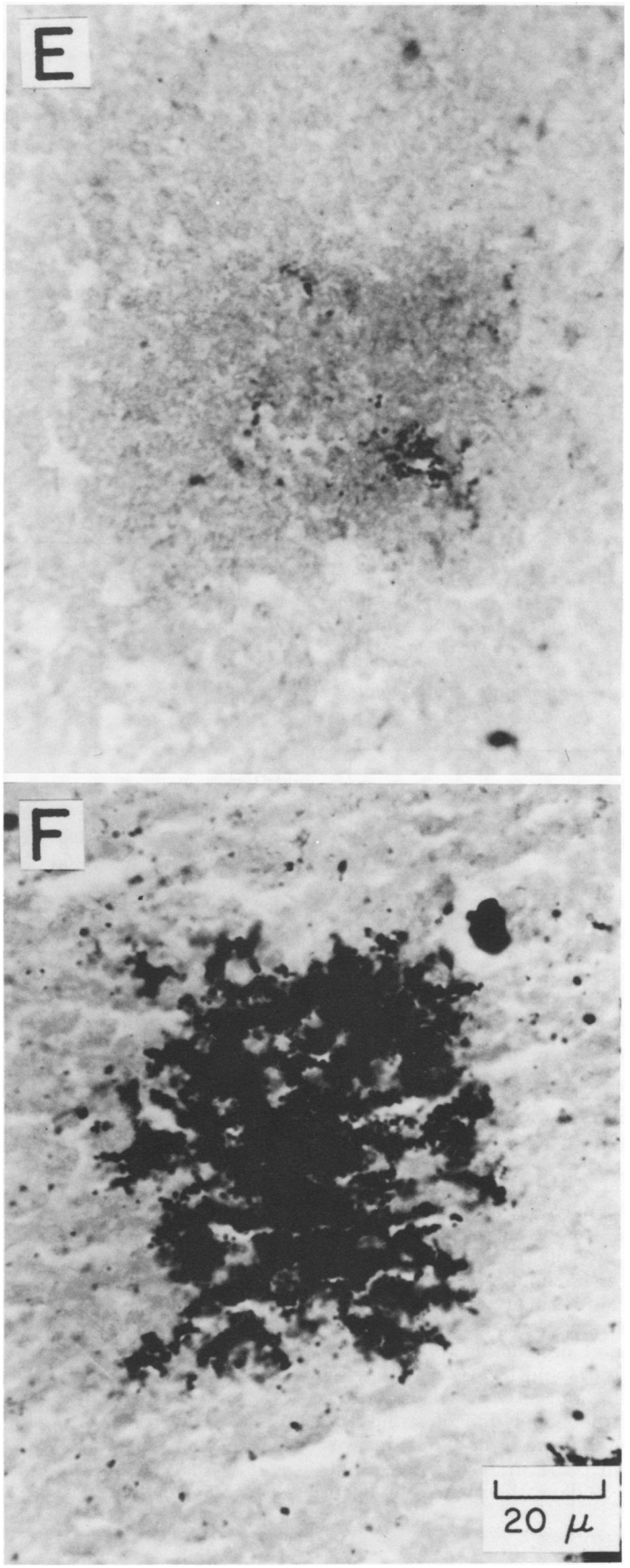

FIG. 1E-F.

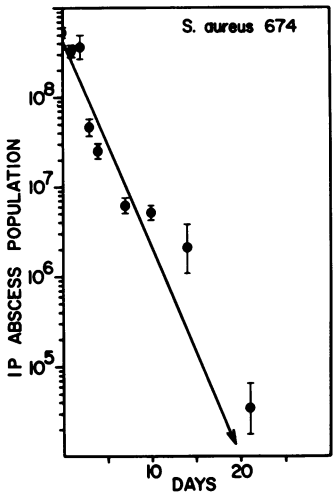

FIG. 2. *Persistence of S. aureus 674 within abscesses produced in peritoneal cavities of mice after inoculation of 10^9 organisms. Each point represents geometric mean (±standard error) of counts derived from 8 to 10 animals. Arrow indicates that the regression line is also based upon data points obtained after 21 days, but which are not shown.*

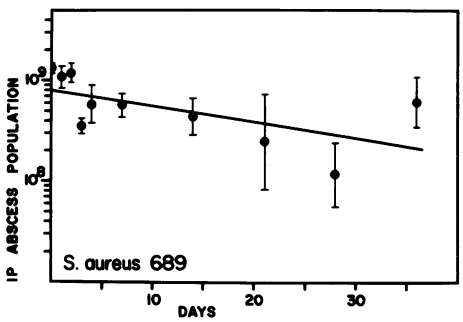

FIG. 3. *Persistence of S. aureus 689 within abscesses produced in peritoneal cavities of mice after inoculation of 10^9 organisms. Each point represents geometric mean (±standard error) of counts derived from 12 to 16 animals.*

graded or inactivated. Although antibody to alpha toxin was not detected in serum removed from mice 14 days after intraperitoneal infection, nevertheless it is possible that disappearance of toxin within abscesses could represent neutralization from influx of antibody.

Although all strains were destroyed in abscesses, the rates of destruction differed. The half-life for strains 674 and 18Z-G was about 1.5 days, and that for strain 689 was 22 days. During the first 4 days, the population of strain 18Z decreased rapidly (half-life, 1 day), but this was followed by an increase in recoverable organisms. After 8 to 10 days, the population again declined, but more slowly than before (half-life, 5 days). We presently have no explanation for the population changes seen with this strain, but the changes have been consistently observed.

The means whereby staphylococci were destroyed within abscesses did not appear to depend upon phagocytosis. As early as 12 h after inoculation, intact leukocytes were no longer found in the immediate vicinity of the staphylococci, and, although a broad band of intact leukocytes was always evident near the periphery of the abscess beneath the connective tissue capsule, staphylococci could not be detected in their proximity.

By using *S. aureus* 18Z and its nonhemolytic variant 18Z-G as indicator strains, abscess homogenates were shown to possess bactericidal

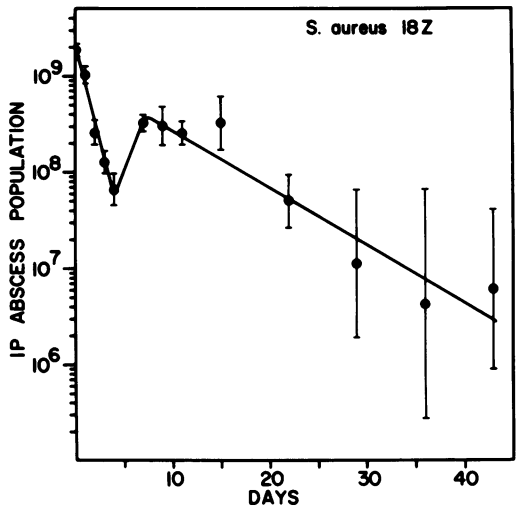

FIG. 4. *Persistence of S. aureus 18Z within abscesses produced in peritoneal cavities of mice after inoculation of 2×10^9 organisms. Each point represents geometric mean (±standard error) of counts derived from 8 to 10 animals.*

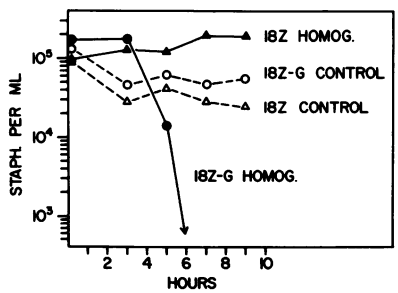

FIG. 5. *Survival of S. aureus strains 18Z (streptomycin resistant) and 18Z-G (spectinomycin resistant) in abscess homogenate. Suspensions of organisms in homogenate diluent served as controls.*

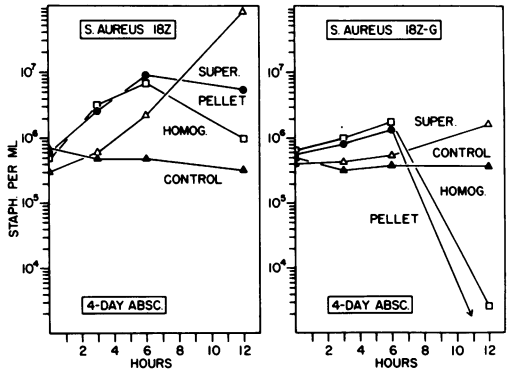

FIG. 6. *Survival of S. aureus strains 18Z (streptomycin resistant) and 18Z-G (spectinomycin resistant) in fractionated homogenates from 4-day-old abscesses. Strains were distinguished by plating samples on Trypticase soy agar containing either streptomycin or spectinomycin. Whole homogenate (□); pellet after centrifugation (●); supernatant fluid after centrifugation (△); organism in diluent only (▲).*

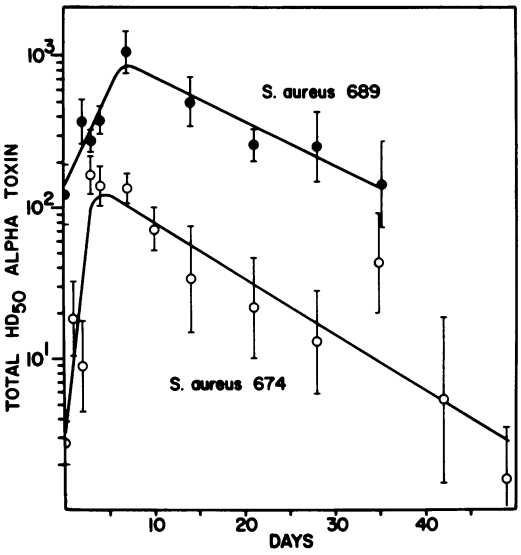

FIG. 7. *Accumulation of alpha toxin within abscesses produced with S. aureus strains 674 and 689 after inoculation of 10^9 cocci. Each point represents geometric mean (±standard error) of titers derived from 6 to 12 animals.*

activity which acted preferentially on the nonhemolytic strain. This bactericidal activity was subsequently shown to reside in the sedimentable fraction of abscess homogenates and could be detected in abscesses collected 1 to 16 days after infection. Although it was possible that destruction of organisms was mediated by lysosomal substances released from disintegrating leukocytes, further studies suggested that this was not the case. These findings are reported elsewhere (1).

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