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THE SURVIVAL OF STAPHYLOCOCCUS AUREUS IN RENAL ABSCESES

The Ohio State University

PH.D.

1981

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THE SURVIVAL OF STAPHYLOCOCCUS AUREUS
IN RENAL ABScesses

A Dissertation

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

by

Karen Elaine Grandel, B.S.

The Ohio State University
1981

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To my parents, whose patience and faith in me encouraged me greatly.

To TRS, JKJ and FSN whose companionship, and friendship made my stay here more pleasant and memorable.
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My sincerest appreciation and thanks go to Dr. Frank A. Kapral, my advisor and mentor, for his guidance and wisdom. My gratitude also extends to my committee members, Dr. B.U. Bowman, Dr. A.C. Ottolenghi and Dr. N.L. Somerson for their time and expert advice. My thanks also goes to John Chinn for his technical assistance and his friendship.
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INTRODUCTION

When a dose of about $10^9$ nonencapsulated *Staphylococcus aureus* are inoculated into the peritoneal cavity of mice, the organisms clump due to the interaction of fibrinogen present in the peritoneal cavity and the bound coagulase present on the surface of the organisms. Macrophages initially present in the peritoneal cavity and neutrophils subsequently arriving during the inflammatory response collect around these clumped organisms, but phagocytosis is minimal since most of the organisms are protected within the interior of the clump. When less than $2 \times 10^8$ cocci are inoculated, clumping is ineffective and the dispersed organisms are readily phagocytized (31-34).

Toxins produced by the staphylococci can be elaborated into the peritoneal cavity only during the interval between time of inoculation and the time when the clumped cocci are surrounded by leukocytes. If a lethal dose of either alpha or delta toxin is elaborated, the mouse will die several hours later. However, staphylococci can be grown in such a way as to reduce subsequent toxin production in the peritoneal cavity (33). Under these circumstances, the animals survive and the staphylococcal lesions have been shown to develop into typical abscesses. Histologically, the
abscesses consist of a core of staphylococci and acellular debris which is surrounded by a layer of viable leukocytes. The entire structure is further enclosed in a vascularized connective tissue capsule.

Dye et al., studying the survival of various strains of *S. aureus* in peritoneal abscesses, found that three patterns of survival occurred. Some strains persisted for several weeks whereas others were promptly and continuously eliminated. With still other strains, the population at first decreased and then increased to original levels, but then were progressively and permanently eliminated. The elimination of staphylococci within abscesses did not depend on the previously characterized lysosomal mechanisms; rather it appeared to be due to a staphylocidal fatty acid which accumulated during the course of the infection. Staphylococci varied considerably in their sensitivity to the bactericidal fatty acid and their resistance to this lipid correlated with their ability to survive in peritoneal abscesses.

Abscess homogenates possess staphylocidal activity due to the presence of the bactericidal fatty acid. The amount of activity demonstrable can be increased four to tenfold by prior incubation of homogenates with either live staphylococci or cell-free staphylo-
coccal culture supernatants. This phenomenon, called activation, is presumed to result from the conversion of an inactive lipid precursor to the bactericidal fatty acid.

Histologically, considerable amounts of stainable lipid accumulate within abscesses during the course of maturation. Although the
largest lipid droplets are found at the periphery of the leukocyte layer, immediately adjacent to the connective tissue capsule, smaller droplets are scattered throughout the lesion, even at the core where the staphylococci are located (Shryock, Kapral and Dye unpublished results).

Many free fatty acids, particularly unsaturated fatty acids, are known to exhibit bactericidal activity. Although the particular fatty acid associated with the destruction of staphylococci in abscesses has not been identified, it is distinguished from other fatty acids with similar activities by (1) its greater specific activity, (2) its resistance to catalytic reduction, (3) its differential bactericidal activities towards certain indicator strains (12).

The intraperitoneal infection model has provided a simple way of generating staphylococcal abscesses free of extraneous tissue and has permitted the detection of a heretofore unrecognized mechanism responsible for the elimination of staphylococci from such lesions. The major deficiency of the model rests with the lack of multiplication by the inoculum during the early stages of lesion development. It was for this reason that a study of the behavior of staphylococci within renal tissue was undertaken.

In contrast to the intraperitoneal infection, the renal infection in the mouse usually results in multiplication of the organisms deposited in the kidney. When staphylococci are injected intravenously, more than 99% of the inoculum is cleared by the liver and spleen and the cocci removed by these organs do not multiply (39,46). However, approximately 1/3000 of the inoculum will lodge in the kidney and it is these organisms which may multiply (34).
Many groups have investigated the host-parasite interaction of staphylococcal pyelonephritis (1, 3, 10, 17, 20-24, 34, 39, 46, 52). Since Staphylococcus aureus produces a variety of biologically-active products, certain products have been studied to determine their role in the kidney infection. The role of soluble coagulase and clumping factor has been studied in the renal infection model using mice (39) and rabbits (46). It was found that coagulase negative variants were able to proliferate in the kidney just as well as were the parental strains. However, some other coagulase positive strains could not multiply under the same conditions.

The role of the alpha and delta toxins was also studied. It was found that alpha toxin negative variants failed to proliferate and were eliminated (34), whereas the same dose of the parental strains multiplied. These data suggested a possible role for alpha toxin in establishing the renal infection. When delta toxin negative variants were tested for their capacity to multiply, the results were less clear cut. One delta toxin negative mutant multiplied to some extent, but never as much as the parental strain, while another nonhemolytic variant was rapidly eliminated (34).

One aim of the present study was to determine which, if any, of these toxins might play in the development of renal abscesses. In addition, an endeavor was made to determine what host factors may be involved in determining multiplication and destruction of staphylococci in renal tissues. Accordingly, normal and infected kidney homogenates were examined for the presence of staphylocidai
activity during the course of infection. When such activity was detected, the nature of the material responsible was compared to that of the bactericidal fatty acid in peritoneal abscesses. The bactericidal material from renal tissue was shown to reside in the free fatty acid fraction and demonstrated some differential bactericidal activity toward selected indicator strains, but was present in both normal and infected kidneys.
MATERIALS AND METHODS

Animals

ICR white Swiss female mice, weighing 25-30 gm, were used for this study.

Staphylococcal Strains

*S. aureus* strains 18Z, P78, PGL14, TG, 689, 674, 18Z-G, P78-22 and 303, which differed in the hemolysins produced, have been described previously (31,35,36,37,38). Strain TG produces the alpha, beta and delta hemolysins while strains 18Z and P78 produce alpha and delta hemolysins. Strain 689 produces alpha hemolysin only, strain PGL14 produces delta hemolysin only, and strain 303 produces only the beta-hemolysin. Strains 18Z-G, PGL14-1, PGL14-2 and P78-22 are non-hemolytic variants derived from their respective parental strains following UV irradiation. Strain 674 produced only alpha hemolysin but it produces 20-fold less than strain 689.

Strain 18Z-D, a variant of strain 18Z, also produced alpha and delta hemolysins. Strain 18Z-D was originally distinguished from the parental strain by its inability to multiply in the kidney (46). The strain occurred as a yellow pigmented (18Z-DY) and a non-pigmented variety (18Z-DW).

The variants 18Z-Sm and 18Z-G-Sp, resistant to streptomycin and spectinomycin respectively, were derived from the parental strains.
after plating on tryptase soy agar (TSA) containing either 60 μg/ml streptomycin or 600 μg/ml of spectinomycin. All strains produced the soluble coagulase and the clumping factor.

Cultures

For purposes of intravenous or intraperitoneal animal inoculation, the various strains were grown in tryptcase soy broth (TSB) under a steady flow of O₂ on a shaking incubator at 37°C. After 24 hours of incubation, the cultures were centrifuged at 4°C and the pelleted cells were washed three times in a diluent consisting of 0.1% tryptcase and 0.1% skim milk. The organisms were resuspended in the same diluent at a concentration of about 10⁹ cocci/ml and stored at -70°C.

For use in the bactericidal and inhibitory assays, S. aureus strains TG, 18Z-G, PGI14 and 303 were grown in TSB for 24 hours. For some experiments, strain 303 was harvested after 8 hours of growth while in the log phase. The organisms were centrifuged, washed three times in saline containing 8% TSB and resuspended in the same diluent to give a concentration of 10⁸ cocci per ml. The suspensions were stored in ampules at -70°C until needed.

Generation of Renal Abscesses and Enumeration of Viable Cocci

Groups of 30-40 mice were inoculated intravenously with 2-6 x 10⁶ staphylococci. At various intervals after inoculation, 3-6 animals were sacrificed and the kidneys aseptically removed, weighed, and homogenized in 5 ml saline containing 1% TSB. Plate counts were done on homogenates prepared from each kidney. In these studies, the zero time samples were obtained 1-4 hours after inoculation.
In some studies, to determine the effect of reinfection, mice were infected as before and subsequently reinfected with $2 \times 10^8$ cocci of the same strain.

In some experiments, mice were infected with a mixture of 18Z-8m and 18Z-G-8p. In these cases, the same procedure was used as described above except that aliquots of the kidney homogenates were plated on TSA containing either 60 μg/ml streptomycin or 600 μg/ml spectinomycin in order to enumerate each strain separately.

**Preparation of Infected Kidney Homogenates**

Groups of mice were inoculated intravenously with 2-4x10$^6$ *S. aureus* strain 18Z or 689. At various intervals after inoculation, 3-10 animals were sacrificed and the kidneys aseptically removed. The kidneys were freed of extraneous tissue and were homogenized in sterile saline. The homogenates were centrifuged at 40,000 x g for 30 min. at 4°C. The supernatant fluid was removed taking care to retain the floating film with the pelleted portion. The pellet together with the floating film were rehomogenized in saline and centrifuged as before. This washing procedure was repeated twice more and the washed pellets were stored in sterile ampules at -70°C.

**Normal Kidney Homogenates**

Uninfected mice were sacrificed and their kidneys removed and processed as were the infected kidney homogenates.

**Generation of Intraperitoneal Abscesses**

Mice were inoculated intraperitoneally with $10^9$ cocci, *S. aureus* strain 18Z, in a volume of 0.25 ml. Seven days after the inoculation, the mice were sacrificed and the intraperitoneal abscesses aseptically
removed, taking care to eliminate any fat tissue and mesentery. The abscesses from one animal were pooled and homogenized in 1 ml of sterile saline. The homogenates from several animals were combined and centrifuged at 40,000 x g for 30 minutes. The insoluble material was washed three times with saline. The washed pelleted abscess homogenates together with floating film (PAH) were resuspended in saline and stored at -70°C.

**Activation of PAH and Kidney Homogenates**

Washed suspensions of PAH were boiled for 30 minutes to destroy the remaining viable staphylococci. In order to generate maximal levels of bactericidal lipid, the boiled PAH was mixed with 3 volumes of a membrane filter-sterilized culture supernatant from *S. aureus* strain 18Z grown in TSB. Activation was accomplished by incubating the mixtures at 37°C for 24 hours (12). This homogenate, now termed "activated" peritoneal abscess homogenate (AcPAH) generally possessed a 5-fold greater bactericidal activity than it did prior to activation.

In those instances where activated kidney homogenates were desired, activation was performed in a similar manner.

**Bactericidal Assays**

The procedure for measuring bactericidal activity has been described previously (12). Briefly, material being evaluated for bactericidal activity was serially diluted in a diluent consisting of 2 M NaCl with 2 mM EDTA, adjusted to pH 6.8 with 1M NaOH. To each 0.5 ml dilution was added 0.5 ml suspension of the appropriate indicator strain containing 500 cocci. Control tubes, containing 0.5 ml diluent plus 0.5 ml of indicator organisms suspension were also included.
All tubes are incubated for one hour at 37°C and 0.5 ml aliquots of the mixtures are plated on TSA to enumerate surviving organisms. One unit of bactericidal activity (ED₅₀) was defined as that amount of material which killed 50% of the indicator organism under the test conditions. Each assay was done in duplicate. The 50% endpoints were determined by regression line analysis using the method of probits. For the assays recorded in Tables 5-9, the diluent contained in addition to NaCl and EDTA, 1 mg/ml gelatin. The diluent was adjusted to pH 6.8 with NaOH and buffered with 0.05 M phosphate buffer.

**Inhibitory Assay**

Material being tested for inhibitory activity (to the bactericidal lipid) was serially diluted in the same diluent used in the bactericidal assay. To 0.25 ml of each dilution was added 0.25 ml of either AcPAH or a suspension of the free fatty acid fraction derived therefrom (containing 1 LD₁₀₀ cidal activity). In some studies, the free fatty acid fraction derived from infected kidneys was used instead. After standing at room temperature for 30 minutes, 0.5 ml of the appropriate indicator organism suspension was added and the mixtures were incubated at 37°C for 1 hour. After incubation, the surviving organisms were enumerated by plating 0.5 ml aliquots from each tube on TSA. One unit of inhibitory activity was defined as that amount of material that allows 50% of the indicator organisms to survive in the presence of 1 LD₁₀₀ of bactericidal material. Each assay was done in duplicate. Included with each assay were control tubes that contained either the indicator organisms with diluent or the indicator organism with AcPAH or the free fatty acid fraction.
For data recorded in Table 4, the diluent in addition to NaCl and
EDTA also contained 1 mg/ml gelatin and was buffered at pH 6.8 with
0.05 M phosphate buffer.

For both the bactericidal and inhibitory assays, a two-fold or
greater difference in titer was determined to be significantly
different.

Histological Sections

Mice were infected with *S. aureus* strain 689 or 18Z by intraven-
ous inoculation. At various intervals after infection, the mice were
sacrificed and the kidneys removed. The kidneys were divided longi-
tudinally (through the hilus), the halves were placed in neutral
buffered formalin, and frozen sections were prepared. Sections were
obtained from different regions in the kidney and were stained such
that one section was stained with Hematoxylin-Eosin and an adjacent
section was stained with Oil Red O stain.

Solvents

All solvents used for extraction and separation of lipids were
reagent grade. All except hexane and diethyl ether were redistilled
in glass just prior to use.

Extraction of Lipids from Tissue Homogenates

The Bligh and Dyer modification of the Folch procedure was used
to extract lipid from kidney homogenates (5). One volume of kidney
homogenate (containing about 1 gm tissue) was mixed with 10 volumes
of chloroform and methanol (1:2). The mixture was homogenized for
about 2 minutes, centrifuged, and the supernatant fluid was placed in
a separatory funnel. The pellet was suspended in water, re-extracted
with chloroform:methanol (1:2), and the supernatant combined with the first extraction. Two volumes of water and two volumes of chloroform were added to the separatory funnel and the contents were shaken vigorously. After phase separation occurred, the lower phase was collected and dried under nitrogen. When necessary, lipids from peritoneal abscess homogenates were extracted in the same way.

Nonlipid contaminants were removed from the extracts by partition chromatography Sephadex G-25 according to the method of Rouser et al. (53). Ten to eighty mg portions of lipid were dissolved in a small volume of chloroform:methanol (19:1) saturated with water (CMS), and added to a 1x10 cm Sephadex G-25 column equilibrated in methanol and water (1:1). The lipids were eluted with 25 ml of CMS and the eluate dried in a Buchi rotary evaporator under vacuum. The lipids were dissolved in chloroform, quantitatively transferred to weighed vials, and dried to a constant weight under nitrogen.

Separation of Lipids into Major Lipid Classes

Unisil (silicic acid, 100-200 mesh) was activated at 120°C for 2 hours, slurried in chloroform and poured into a 1.2 cm diameter column to a height of 8-10 cm. The column was washed with 10 column volumes of chloroform prior to application of 10-80 mg lipid dissolved in chloroform. Neutral lipids were eluted with 10 column volumes of chloroform, glycolipids were eluted with 40 column volumes of acetone, and phospholipids were eluted with 10 column volumes of methanol. Each fraction was collected and dried in the rotary evaporator. The lipids were dissolved in a small volume of chloroform or methanol
and transferred to a preweighed vial, and dried to a constant weight under nitrogen.

**Fractionation of Neutral Lipids**

Portions of the neutral lipid fraction were further fractionated on silicic acid column according to the procedure of Carroll and Serdarevich (6). Unisil was activated at 120°C for 2 hours, slurried in hexane and a 1x20 cm column prepared. The column was washed with at least 100 ml of hexane prior to application of the sample dissolved in hexane. The various neutral lipids were separated using increasing concentrations of diethyl ether in hexane (Table 10) (40). Each fraction was dried under N₂. The free fatty acids were consistently recovered in fraction 4 (hexane-diethyl ether 92:8).
RESULTS

Thirteen *S. aureus* strains were selected and examined for their ability to survive within the kidneys of mice. The combinations of hemolysins produced by these strains are shown in Table 1.

The survival of these 13 strains in mouse kidney is shown in Figure 1. Although the zero time counts obtained 1-4 hours after inoculation varied by only 1 - 1-1/2 logs, the subsequent behavior of the strains in the tissues did differ and three patterns of survival were noted. Strains 689 and PG114-2 both exhibited rapid multiplication in the kidney with population increases of about 4-5 logs over a 3 day period. Thereafter, the population remained relatively stable for about 2 weeks before decreasing. With strains PG114-1, PG114, 18Z-DY, P78, 18Z, TG, and 303, there was a 1-4 log population increase during the first five days after infection, but peak populations were not maintained and the organisms were eliminated by 10-14 days. In the case of strains, P78-22, 674, 18Z-G and 18Z-DW there was no multiplication in the kidney and the initial population declined steadily until no organisms could be recovered from the samples. In some cases where the original inoculum was eliminated (strains 674, 18Z-G, and P78-22), mice were reinfected with the same strain, but with a 100-fold greater dose. It can be seen that the
second inoculum was eliminated at the same rate as was the first inoculum (Figure 1).

It should be noted that the variation (standard error) among early samples was small, but the variation among later samples was quite large suggesting greater animal-to-animal variation with time. There was no obvious correlation between the pattern of survival exhibited by a strain within the kidney and the hemolysins or combination of hemolysins produced. Furthermore, there was no complete correlation between a strain's survival in kidneys and its sensitivity to AcPAH (Table 1).

**Simultaneous Infection**

To determine whether a strain capable of renal multiplication could assist a strain incapable of multiplication, mixtures of such strains were inoculated. In order to enumerate each strain separately, strains were selected that differed in their antibiotic sensitivities. *S. aureus* strain 18Z-SMR, capable of renal multiplication, and 18Z-G SpR, a non-proliferator in the kidney were mixed in equal numbers and inoculated into a group of mice. Kidney homogenates were prepared as usual and the organisms enumerated by plating aliquots on TSA with either 60 μg/ml of streptomycin or 600 μg/ml spectinomycin. The results are shown in Figure 2. It is apparent that the two strains behaved in the same way when present in the kidney together as when present alone. The presence of strain 18Z did not aid the survival of strain 18Z-G, and the presence of the latter did not alter the growth of the former.
Bactericidal Activity in Kidneys

Since previous work had shown that a bactericidal fatty acid accumulated in peritoneal abscesses and that it appeared to be responsible for the killing of the staphylococci in these lesions (12,36), we elected to assay for the presence of bactericidal activity in homogenates prepared from kidneys removed at various intervals after infection with strain 303. However, no staphylocidal activity was detected even in homogenates which were prepared from kidneys removed at times when the organisms were being killed in vivo. The same was true when mice were infected with strains TG or 18Z.

Histology

Since no bactericidal activity was demonstrated, it was decided to determine whether any accumulation of lipid in the vicinity of renal abscesses could be revealed by histological means. Frozen sections from infected kidneys were stained with hematoxylin-eosin to locate abscesses. An adjacent section, stained with Oil Red O, was then examined for lipid accumulation in the same area. It was found that lipid accumulation occurred as early as 2-3 days post inoculation and the regions of accumulation coincided with regions of PMN infiltrates (Plates I-IV). The sections illustrated were prepared from kidneys removed 9 and 13 days after inoculation with strain 689. In those instances where connective tissue deposition occurred around the periphery of the abscesses, larger lipid droplets were seen just within the outer connective tissue capsule while smaller droplets were distributed toward the core of the lesion. In contrast to the infected kidneys, little or no stainable lipid was seen in sections from normal kidneys.
Inhibitory Activity in Kidneys

Since the visual evidence supported the concept of lipid involvement, but examination of kidney homogenates had failed to reveal any bactericidal activity, the possibility was considered that kidney homogenates contained an inhibitor of the bactericidal lipid. Both Ca\(^{++}\) and albumin were known to inhibit the bactericidal fatty acid from peritoneal abscesses (12), therefore it was possible that these or some other substances might be present in the kidney homogenates and mask any bactericidal activity.

Using the inhibitory assay, it was found that crude homogenates of either normal or infected kidneys could neutralize the bactericidal activity of the AcPAH (Table 2). Most of this neutralizing activity resided in the pelleted fraction of the kidney homogenates. Therefore, if any bactericidal fatty acid was indeed produced in renal abscesses during the course of the infection, it was likely to remain undetected because of the inhibitor present in the kidney homogenates.

Characterization of the Inhibitor

From Table 2, it can be seen that increasing the concentration of EDTA, from 1 mM to 5 mM did not decrease the inhibitory activity found in a particular kidney homogenate, thereby suggesting that Ca\(^{++}\) was not responsible. Furthermore, heating the kidney homogenate to 121°C for 30 minutes did not significantly alter its inhibitory activity. Therefore, it appears unlikely that the inhibitor was albumin or a similar heat labile protein. In other studies, it was found that the inhibitor was unable to negate the bactericidal activity of AcPAH if the
indicator organisms were exposed first to the AcPAH. In view of these findings and because most of the inhibitory activity resided in the insoluble fraction of kidney homogenates, consideration was given to the possibility that the inhibitor was also a lipid.

Lipids were extracted from pelleted homogenates of normal and infected kidneys using the Bligh and Dyer procedure (5). The lipids were separated from non-lipid contaminants and fractionated into major lipid classes by silicic acid chromatography. Of the three major lipid classes, the phospholipid fraction contained most of the inhibitory activity against AcPAH (Table 3). The neutral lipid fraction had no measurable inhibitory activity, but in one instance there was a low level of inhibitory activity in the glycolipid fraction from normal kidney. This may have been due to an incomplete separation of glycolipids from phospholipids in this instance. The inhibitory activity was observed in phospholipid fractions derived from both normal and infected kidneys. Furthermore, the inhibitory activity of the phospholipid fraction was observed irrespective whether activated and unactivated kidney homogenates were used as starting material.

**Phospholipid Content of Renal Homogenates**

Mice were intravenously infected with either *S. aureus* strain 18Z or 689. During the course of renal infection animals were sacrificed, their kidneys removed and homogenized, and the lipids extracted. The lipids were fractionated and the amount of each major lipid class determined (Figures 3 and 4). The amount of phospholipid recovered increased during the period when multiplication of staphylococci
occurred and when neutrophilic infiltrates were histologically observable. However, when total inhibitory activity was calculated from assays done on the phospholipid fractions, there appeared to be no consistent trend in the amount of inhibitory activity observed during the course of infection (Table 4).

In some cases, kidney homogenates from infected mice were activated prior to lipid extraction and fractionation. There appeared to be little change in the amount of phospholipid recovered under these conditions and there was no significant increase in the total inhibitory activity during the course of infection (Table 4).

**Analysis of the Neutral Lipid Fraction**

The three lipid classes obtained from kidneys removed from mice infected with *S. aureus* 18Z were examined for the presence of any bactericidal activity (Table 3). It can be seen that bactericidal activity was present and the activity reside primarily in the neutral lipid fraction.

Unlike the amount of phospholipid, the amount of neutral lipids recovered increased only slightly during the course of infection (Figures 3 and 4). However, the total amount of bactericidal activity present in the neutral lipid fraction did not increase (Table 5). This was also true for samples prepared from kidneys as much as 28 days after infection. The amount of bactericidal activity observed was generally greater in neutral lipid fractions obtained from activated homogenates than in comparable samples obtained from homogenates which were not activated. When the neutral lipid fractions were examined for differential bactericidal activities toward certain
indicator strains, some differential activity was evident (Table 6). In some instances, the differences were not marked but the differentiated trend was consistently found.

Also shown in Table 6 are the results obtained with neutral lipid samples recovered from normal kidneys. Although little or no differential bactericidal activity was present in these neutral lipids from unactivated homogenates, after activation there was a marked increase in both specific activity as well as demonstrable differential bactericidal activity.

The neutral lipid fractions from kidneys removed from mice infected with *S. aureus* 689 were also examined (Table 7). Throughout the course of the infection, there was a low level of bactericidal activity which was demonstrable only towards strains 182-G and 303. Because of the low degree of bactericidal activity present, the neutral lipid fractions were further fractionated into the free fatty acid fraction. When examined, not only did the free fatty acid pool exhibit a greater specific activity than did the neutral lipid fraction, but differential bactericidal activity toward the indicator strains TG, PG114 and 303 was also evident (Table 8). However, the free fatty acid fraction obtained from normal kidneys exhibited about the same amount of activity as did samples from infected animals. When the total bactericidal activity in infected kidneys was calculated, there was no significant change throughout the infection (Table 9), nor was there any significant change in the amount of free fatty acid recovered (Figure 5).
Analysis of the Glycolipid Fractions

The amount of glycolipid fraction recovered was usually less than that of the other fractions. During the course of infection with either S. aureus strains 18Z or 689, the amount of glycolipid present changed very little (Figures 3 & 4). In certain samples, the glycolipid fraction exhibited some bactericidal or inhibitory activity, but this was considered most likely to represent an incomplete separation of the lipid classes. These activities were not consistently seen, and no specific role was attributed to this fraction.
DISCUSSION

Many investigators have studied the renal infection with staphylococci in mice (3,9,10,17,20-24,39,46,52). The basic features of this type of infection were confirmed in this study. After IV inoculation of approximately $10^6$ staphylococci, a small number of cocci lodged in the kidneys and, depending upon the strain of S. aureus inoculated, multiplication occurred. Typical abscess formation was found and eventual elimination of the organisms was observed.

A variety of staphylococcal products have been studied in an endeavor to determine their role in the renal infection. Several investigators concluded that coagulase and clumping factor are not virulence factors since mutants lacking these substances multiplied in the kidney as well as did the parental strains (1,23,39,46). Previous studies have suggested a role for the alpha toxin since alpha toxin-negative variants were unable to multiply while the toxigenic parental strains could multiply in the kidney (34). Foster has shown the accumulation of alpha toxin in kidneys after inoculation with either viable organisms or purified alpha toxin (18,19). It is well known that alpha toxin is cytotoxic (59) and has the ability to cause necrosis in many tissues including the kidney (56). Thus, it may play a role in the kidney infection.
The role of the delta toxin in the pathogenesis of renal lesions is more uncertain. In one study, a delta toxin-negative variant was able to multiply in the kidney, although not as well as the hemolytic parental strain, whereas another non-hemolytic variant was incapable of multiplication (34).

Such factors as protein A, have been studied, but by themselves have not been solely implicated as playing a role in this type of infection (17). In mice lethally infected with \textit{S. aureus}, nuclease has been shown to accumulate in the kidneys more so than in other organs, but no definite role has been established for it in the pathogenesis of renal infection.

In the present study, there was no correlation between the hemolysins produced by a strain and the ability of the strain to survive in the kidneys (Table 1). Although most of the non-proliferating strains were non-hemolytic or produced very little toxin, 182-DW produced both the alpha and delta toxins. Strain 689, one of the strains best able to multiply, produced only the alpha-toxin, but strain PG114-2, also a strain able to proliferate well in the kidney, produced only trace amounts of delta hemolysin. Strain PG114-1, a non-hemolytic variant, multiplied as well as the parent strain PG114 which produces large amounts of delta toxin. Strain 303 which produced only the beta hemolysin, multiplied, but only to a limited extent. Therefore, it was not possible to implicate any single hemolysin, or combination of hemolysins, as responsible for multiplication in the kidneys of mice.
Data from this study, however, suggested that there were some inherent differences between proliferating and the non-proliferating strains which governed their ability to survive in the kidney. The simultaneous inoculation of strains 18Z and 18Z-G (Figure 2) indicated that the multiplication of strain 18Z could not prevent the elimination of strain 18Z-G, and strain 18Z-G could not reduce the multiplication of strain 18Z. It must be realized that with the doses inoculated, it is unlikely that representatives of both strains would be deposited in close proximity to each other in the tissues. Therefore, even if a particular product affected multiplication or destruction in the kidney, the product might not effect the kidney as a whole or act at a site distant from the site of production.

The preferential multiplication of staphylococci in the kidneys has been often noted. One reason put forth to explain this phenomenon relates to the observation that phagocytosis is depressed in hypertonic environments such as found in the kidney (8). The presence of a hypertonic environment may thus predispose the kidney to disease caused by various bacteria, including staphylococci (8). Others who have emphasized the role of phagocytic mechanisms in preventing and curing experimental staphylococcal pyelonephritis, have noted that the circulatory anatomy and physiology of the kidney predispose the papillary region to bacterial proliferation because of the sub-optimal functioning of the phagocytic system (3). It has been pointed out that there is a lowered hydrostatic pressure in the capillary bed of this region and it has been suggested that the higher concentration of protein (colloid) present may enhance bacterial growth (24). Others have suggested that
bacteria within the kidney tubules are protected from the normal phagocytic clearance since granulocytes cannot gain entrance until tubular integrity has been disrupted. Antibody deficiencies in the tubules exist and phagocytosis may be less than optimal (52). Phagocytosis may also be depressed due to the acid-base conditions, the osmolarity and electrolyte states which exist in the renal tissue (8). Furthermore, it has been shown that renal tissue contains an anti-complementary factor, apparently high ammonia concentrations, which may depress any serum bactericidal activity present (4).

Some workers have demonstrated the presence of locally synthesized immunoglobulin produced in the normal human bladder (15) and during experimental pyelonephritis induced by E. coli in rabbits (48,49,54). IgM, IgG and secretory IgA have been found to be synthesized locally by lymphoid cells with specificity for the infecting organisms. However, even though the levels of local antibody rise and persist after systemic levels have declined, the role of these immunoglobulins in renal infections is not as clear. Hubert et al., (29) in studying the effect of the renal physico-chemical milieu on the bactericidal activity of serum containing complement concluded that increased osmolarity, and increased concentrations of NaCl, KCl, or urea, as well as any pH changes away from neutrality depressed the bactericidal activity of the serum for E. coli.

Since phagocytosis or antibody have not been shown to be responsible for the elimination of staphylococci from the kidneys, we elected to examine infected renal tissue for the presence of a bactericidal
factor similar to that present in peritoneal abscesses. This bactericidal factor was originally demonstrated in peritoneal abscess homogenates and was later shown to be a free fatty acid (12).

However, when crude infected kidney homogenates were examined, no staphylocidal activity was found. Although the pattern of survival exhibited by a strain within the kidney was not necessarily analogous to that exhibited by the same strain in peritoneal abscesses, six of the nine strains tested in both sites behaved similarly. Similarly, a strain's ability to survive in the kidney correlated only partially with its sensitivity to the staphylocidal fatty acid (Table 1).

It should be remembered that the two infection models differ in certain respects. Although the peritoneal abscess model is useful for studying the late events in abscess formation, no proliferation of the organism occurs and most strains are rapidly eliminated after inoculation. In the renal infection model, most strains undergo a period of multiplication before being eliminated. Also, whereas peritoneal abscesses cannot be generated with doses less than $10^8$ cocci because of inadequate clumping, renal abscesses can be initiated by less than $10^3$ organisms deposited in the kidney. Therefore, it is not unreasonable to expect different mechanisms of defense to occur within the different locations in the same animal.

In the kidney, the foci of staphylococci and surrounding cellular infiltrates are widely scattered and much intervening normal tissue is evident. Therefore, whereas peritoneal abscess homogenates are essentially free of normal tissue, homogenates of infected kidney
consist mainly of normal tissue elements. The presence of normal tissue is important since homogenates of uninfected kidney were capable of inhibiting the cidal action of AcPAH (Table 2). Whether the phospholipids responsible for the inhibition can assist the survival of staphylococci in the kidney is not known, but the presence of normal tissue in homogenates of infected kidney could easily explain our inability to demonstrate staphylocidal activity in unfractionated kidney homogenates.

Although it was not possible to detect bactericidal activity in homogenates of infected kidneys, histologically it was possible to demonstrate the accumulation of lipid around renal abscesses (Plates I-IV). The lipid accumulation was not marked until the second day of infection, but once evident, was associated only with the foci of infection, and did not occur in normal regions of the kidney or in uninfected kidneys. Lipid droplets were larger at the periphery of the lesion, but finer droplets were dispersed throughout the abscess and extended to the core where the staphylococci were located. The presence of stainable lipid in the vicinity of renal abscesses resembled the situation seen in histological sections of peritoneal abscesses in mice (Shryock, Kapral and Dye, unpublished data).

A number of investigators have demonstrated the bactericidal or bacteriostatic action of lipids (7,12,25,27,30). Some unsaturated fatty acids of chain length C₁₆ to C₂₀ and some saturated fatty acids having a chain length up to C₁₆ were shown to be inhibitory to the in vitro growth of Neisseria gonorrhoeae (47). Walstad et al. (58) have shown that certain strains of N. gonorrhoeae produce free fatty
fatty acids and phospholipids, and that these lipids have an inhibitory effect on other strains of *N. gonorrhoeae* or other *Neisseria* species. Some gram negative organisms such as *E. coli* (14) and *B. pertussis* (16) were also shown to be inhibited by fatty acids.

Kodicek and Worden (44) demonstrated that linolenic, linoleic and oleic acids could inhibit *Lactobacillus helveticus* and other gram positive organisms, and found that certain other lipids such as lecithin and cholesterol could reverse this inhibition. Wynne and Foster (60) and Tripathi et al. (57) found that some of these same fatty acids inhibited *S. aureus*. In another study, Group A streptococci were shown to be killed by oleic acid, presumably by altering the cell membrane integrity and leading to a loss of RNA (55).

Some workers have suggested an involvement of various lipids in certain host-parasite interactions. It has been postulated that skin lipids influence the survival of *S. aureus* and the streptococci on the skin surface. Even though these organisms are often encountered in skin lesions, they appear unable to survive on the skin for long periods, possibly because of the lethal effects of the lipids present (2, 26, 27, 50). However, in one study, the effects of four commonly encountered skin lipids upon strains of staphylococci and streptococci were tested (26). They found that all strains were susceptible to these lipids, and that there were no differences in susceptibility between strains isolated from the skin and those isolated from other sources. This would suggest that strains recovered from the skin are not better able to survive there because of an increased resistance to the skin lipids (26).
Kochan et al. (41-43) has described the occurrence of anti-
mycobacterial lipids (free fatty acids) which arise during infection
or immunization with mycobacteria or its products. The anti-mycobac-
terial activity was also noted in extracts of immune activated
macrophages or membrane components of liver cells from infected
animals. It has been postulated that this lipid may play a role
in the host's defense against the organism.

Hedstrom (20) found that S. aureus strains isolated from chronic
cases of osteomyelitis that were not being successfully treated with
antibiotics often did not possess significant amounts of lipolytic
activity. Strains with demonstrable lipolytic activity in vitro were
more often associated with acute osteomyelitis cases which generally
were more treatable with antibiotics. His explanation for this
finding was that strains with lipolytic activity were able to break
down lipids in the bone tissue thereby releasing bactericidal fatty
acids that would accumulate in the area and assist in the killing
of the staphylococci. In contrast, strains lacking lipolytic enzymes
enzymes would not cause the release of bactericidal fatty acids
and thus the organisms would have a better chance for survival.

Recently, Dye et al. (12,36) suggested that free fatty acid(s)
found in peritoneal abscesses were responsible for the elimination of
S. aureus in these lesions. S. aureus strains differed widely in their
ability to survive within abscesses and in their sensitivity to the
bactericidal fatty acids recovered from these abscesses. Those strains most
sensitive to the lipid were also more readily destroyed within abscesses,
whereas those strains more resistant to the lipid survived best.
Although attempts to demonstrate bactericidal activity in crude homogenates of infected kidneys failed, histological examination revealed the accumulation of lipid around renal abscesses similar to the situation observed in peritoneal abscesses. Therefore, it was possible that the same processes found in peritoneal abscesses might lead to the production of bactericidal lipids in renal abscesses.

However, since the bactericidal fatty acid in peritoneal abscesses has not been identified, only its biological properties could be used to determine its presence in renal abscesses. Therefore, the characteristics of the bactericidal activity in kidney homogenates were compared with those associated with the bactericidal fatty acid in AcPAH.

It was found that the bactericidal activity in kidney homogenates was present in the neutral lipid fraction, but the specific activity of this fraction from infected kidneys was not as active as the comparable fraction from AcPAH. After separation of the neutral lipid fraction, the bactericidal activity was found in the free fatty acid pool (Table 8). It should be noted that isolation of the free fatty acid fraction from kidney homogenates resulted in a marked increase in specific activity over that found in the neutral lipid fraction. This is probably due to the fact that in kidney extracts, free fatty acids accounted for only about 20% of the neutral lipid fraction, whereas in the case of AcPAH free fatty acids comprised about 42% of the neutral lipid fraction.

Another property shared by kidney homogenates and peritoneal abscess homogenates is their ability to be activated. In both cases, activation resulted in an increase in specific activity (Table 5) (36).
This suggests that both preparations contain a precursor which can be converted to the bactericidal material.

Activation can be accomplished by incubating homogenates with either live staphylococci or their culture supernatants. Of the 13 *S. aureus* strains originally studied for their ability to survive in renal tissue, all could activate PAH. Although activating activity was not specifically quantitated, there were differences in the amount of activation seen with culture supernatants from different strains. It is not known whether staphylococci in abscesses are involved in the production of the cidal fatty acid from the precursor, but if this is the case, then differences in activating ability could influence survival of the organisms within these lesions. Therefore, the survival of a strain might be dependent not only upon its sensitivity to the cidal fatty acid, but also upon its activating ability and the presence of inhibitors in its vicinity.

The activation process has not been adequately characterized, but may involve lipolytic enzymes produced by the staphylococci which might release free fatty acids from more complex lipids. It is also possible that activation results from the breakdown of an inhibitory substance which normally masks part of the bactericidal lipid present. Since phospholipids were shown to inhibit the bactericidal fatty acid and since staphylococci have been reported to elaborate phospholipases (11,51,61), this possibility is not remote. Some data in Table 4 suggest that there is a decrease in phospholipid inhibitory activity after activation.
The other property exhibited by the free fatty acid pools from AcPAH and kidney homogenates is the differential bactericidal activities towards the different indicator strains (Table 8). Although lipids from adipose tissue or mesentery, as well as certain free fatty acids, were shown to have staphylocidal activity. These lipids did not manifest any differential bactericidal activities toward the different indicator strains (Dye and Kapral, unpublished data). Therefore, expression of the differential bactericidal activities has been an important characteristic distinguishing the bactericidal fatty acid in peritoneal abscesses from other lipids in normal tissues. However, free fatty acids with differential bactericidal activity were recovered not only from infected kidneys, but also from uninfected kidneys, and the amounts present did not change significantly during the period of study (Figure 5). This is in contrast to findings in peritoneal abscesses where the bactericidal fatty acid is not present initially, but appears and increases during abscess development before again declining as the organisms are eliminated (Shryock and Kapral, unpublished data). If the bactericidal fatty acid were responsible for the elimination of staphylococci in the kidney, one might expect to see the amount present to increase significantly during the course of the infection, especially at the times when the organisms are eliminated.

It is possible that an increase occurred, but was not detectable. This could occur if lipid extracts of renal tissue contained a large excess of the more common unsaturated fatty acids. Further fractionation of the fatty acid fraction might be helpful, but definitive studies would require identification of the bactericidal fatty acid.
It is also possible that the differential activity is due to the combined effects of several uncommon fatty acids. If this were the case, separation of the fatty acids mixture would result in the loss of the differential activity.

It is important to recognize that whereas peritoneal abscesses are generally harvested free of extraneous tissue, homogenates of infected kidneys consist primarily of normal tissue. Histologically, it can be seen that renal abscesses comprise only a small portion of the kidney and that the lesions are widely scattered in the organ. Therefore, the extraction of infected kidney includes not only extraction of lipids from renal abscesses, but also extraction of lipids from undiseased portions as well. Thus, it is possible that normal tissues possess lipids which resemble the cidal fatty acid from peritoneal abscesses. If present in large amounts, such fatty acids might mimic the activity of the bactericidal fatty acid from abscesses while not being directly involved in the host-parasite interaction.

The amount of phospholipid recovered from kidneys increased during the course of infection, but whether this increase is of any special significance is not known. The phospholipid increase coincided with the period of multiplication by the staphylococci, but this would not necessarily imply that the phospholipids were responsible for the observed multiplication. Since there is an influx of neutrophils around sites of multiplication, the increase in phospholipid content could merely result from extraction of neutrophils entering the kidneys. Elsbach has noted that during phagocytosis, rabbit granulocytes increase their synthesis of phos-
pholipids (13). Therefore, the increase in phospholipids might result from more cells (membranes) being present in the kidney or from an actual increase in phospholipid synthesis. On the other hand, the phospholipids might negate the bactericidal activity of fatty acids initially present or subsequently produced, and thereby permit the organisms to proliferate.
SUMMARY AND CONCLUSIONS

Thirteen *S. aureus* strains, differing in the hemolysins produced, were examined for their ability to survive in the kidneys of mice. Although three distinct patterns of survival were noted, no correlation could be found between the hemolysins produced and the survival pattern exhibited by a particular strain. Some strains multiplied in the kidney and maintained a large population for about 3 weeks before being eliminated. Other strains also multiplied in the kidney, but the population was abruptly eliminated. Still other strains were unable to multiply and were eliminated promptly. The latter strains were destroyed just as rapidly when animals were reinfected with a much larger dose. The infection of mice with mixtures containing a strain capable of multiplication and a strain unable to multiply, revealed that the behavior of the strains in the kidney was the same when both strains were present together as when each strain was present alone.

Attempts were made to ascertain whether the destruction of staphylococci in renal abscesses was mediated by the same bactericidal fatty acid previously detected in peritoneal abscesses. Although no bactericidal activity could be demonstrated in homogenates of kidneys removed during the course of infection, histological studies did reveal the accumulation of lipid droplets around renal abscesses in
the kidney. Subsequent studies indicated that kidney homogenates
contained an inhibitor of the bactericidal fatty acid and that the
inhibitor consisted of phospholipids.

When lipids extracted from infected kidneys were fractionated
on silicic acid columns to eliminate phospholipids, bactericidal
activity was found in the neutral lipid fraction. Further work showed
that the bactericidal activity was associated with the free fatty
acid fraction and that this fraction exhibited differential bacteri-
cidal activities toward certain indicator strains, as did the bacteri-
cidal fatty acids from peritoneal abscesses. However, a fatty acid
pool with these characteristics was also found in uninfected kidneys
and the fatty acids present did not change significantly during the
course of infection. Although the bactericidal fatty acid in kidneys
may be identical to that found in peritoneal abscesses, this can not
be determined until the latter is chemically identified. It also
remains to be determined whether the bactericidal fatty acid in
kidneys influences the survival of staphylococci in this site.
Table 1

Comparison of a Strain's Survival in the Kidney and its Sensitivity to AcPAH

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hemolysins Produced</th>
<th>Pattern of Survival in the Kidney&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Relative Sensitivity to Cidal Fatty Acid (PAH) LD&lt;sub&gt;50&lt;/sub&gt;/ml&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>689</td>
<td>α</td>
<td>Persistent survival</td>
<td>3200</td>
</tr>
<tr>
<td>PG114-2</td>
<td>None</td>
<td>Persistent survival</td>
<td>ND</td>
</tr>
<tr>
<td>PG114-1</td>
<td>None</td>
<td>Intermediate survival</td>
<td>2300</td>
</tr>
<tr>
<td>PG114</td>
<td>δ</td>
<td>Intermediate survival</td>
<td>350</td>
</tr>
<tr>
<td>18Z-D yellow</td>
<td>α,δ</td>
<td>Intermediate survival</td>
<td>ND</td>
</tr>
<tr>
<td>P78</td>
<td>α,δ</td>
<td>Intermediate survival</td>
<td>1600</td>
</tr>
<tr>
<td>18Z</td>
<td>α,δ</td>
<td>Intermediate survival</td>
<td>120</td>
</tr>
<tr>
<td>TG</td>
<td>α,β,δ</td>
<td>Intermediate survival</td>
<td>40</td>
</tr>
<tr>
<td>303</td>
<td>β</td>
<td>After one log proliferation, immediate elimination</td>
<td>8000</td>
</tr>
<tr>
<td>P78-22</td>
<td>α(trace)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Immediate elimination</td>
<td>2700</td>
</tr>
<tr>
<td>674</td>
<td>α&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Immediate elimination</td>
<td>ND</td>
</tr>
<tr>
<td>18Z-G</td>
<td>None</td>
<td>Immediate elimination</td>
<td>2200</td>
</tr>
<tr>
<td>18Z-D white</td>
<td>α,δ</td>
<td>Immediate elimination</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Immediate elimination refers to strains that are unable to multiply in the kidney. Intermediate survival describes those strains that multiply and are then eliminated in about 10 days. Persistent survival refers to those strains which multiply and maintain the population for 2-1/2 - 3 weeks before being eliminated.

<sup>b</sup> The greater the LD<sub>50</sub>/ml the more sensitive the strain. (Dye and Kapral, unpublished data).

<sup>c</sup> The amount of α toxin produced by strain 674 was significantly less than that produced by the other strains listed, with the exception of P78-22.
Table 2

Characteristics of the Inhibitor in Kidney Homogenates

<table>
<thead>
<tr>
<th>Inhibitor Source</th>
<th>Inhibitor Activity (ED$_{50}$/ml)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IN 1 mM EDTA</td>
</tr>
<tr>
<td>Normal Kidney</td>
<td></td>
</tr>
<tr>
<td>Pelleted Homog.</td>
<td>980</td>
</tr>
<tr>
<td>Filtered Supernatant</td>
<td>200</td>
</tr>
<tr>
<td>Infected Kidney</td>
<td></td>
</tr>
<tr>
<td>Pelleted Homog.</td>
<td>1200</td>
</tr>
<tr>
<td>Filtered Supernatant</td>
<td>140</td>
</tr>
</tbody>
</table>

$^a$ Measured against 1 LD$_{100}$ activated peritoneal abscess homogenate. The indicator organism used in this assay was 18Z-G.
Table 3

Inhibitory and Cidal Activity of Kidney Lipids

<table>
<thead>
<tr>
<th>Lipid Source</th>
<th>Cidal Activity ED₅₀/ kidney</th>
<th>Inhibitory Activity ED₅₀/ kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal Kidney</td>
</tr>
<tr>
<td>Neutral lipid</td>
<td>17*</td>
<td>---</td>
</tr>
<tr>
<td>Glycolipid</td>
<td>---</td>
<td>49</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>---</td>
<td>1290</td>
</tr>
<tr>
<td>Activated Normal Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral lipid</td>
<td>83</td>
<td>---</td>
</tr>
<tr>
<td>Glycolipid</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>---</td>
<td>740</td>
</tr>
<tr>
<td>Infected Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral lipid</td>
<td>12</td>
<td>---</td>
</tr>
<tr>
<td>Glycolipid</td>
<td>17</td>
<td>---</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>---</td>
<td>167</td>
</tr>
<tr>
<td>Activated Infected Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral lipid</td>
<td>169</td>
<td>---</td>
</tr>
<tr>
<td>Glycolipid</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>---</td>
<td>374</td>
</tr>
</tbody>
</table>

* Strain 18Z-G was used as the indicator strain.*
Table 4

Total Inhibitory Activity of Phospholipids from Kidney Homogenates

<table>
<thead>
<tr>
<th>Strain</th>
<th>Days after Infection with 18Z</th>
<th>Uninfected Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
<td>1</td>
</tr>
<tr>
<td>228*</td>
<td>66</td>
<td>151</td>
</tr>
<tr>
<td>18Z-G</td>
<td>196</td>
<td>113</td>
</tr>
<tr>
<td>303</td>
<td>126</td>
<td>47</td>
</tr>
</tbody>
</table>

* Values are total activity expressed as ED_{50}/kidney.
Table 5

Total Bactericidal Activity in Neutral Lipid Fraction From Kidney Homogenates

<table>
<thead>
<tr>
<th>Indicator Strain</th>
<th>Neutral Lipids From Kidney Homogenates After Activation</th>
<th>Uninfected Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days After Infection with 18Z</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0  1  6  9</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>21* 35 106 7</td>
<td>84</td>
</tr>
<tr>
<td>18Z-G</td>
<td>42 64 172 13</td>
<td>206</td>
</tr>
<tr>
<td>303</td>
<td>110 116 222 18</td>
<td>550</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Indicator Strain</th>
<th>Neutral Lipids From Kidney Homogenates Without Activation</th>
<th>Uninfected Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days After Infection with 18Z</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0  1  9</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>7 17 6</td>
<td>46</td>
</tr>
<tr>
<td>18Z-G</td>
<td>22 28 7</td>
<td>17</td>
</tr>
<tr>
<td>303</td>
<td>119 81 30</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

* Values are total activity expressed as ED$_{50}$/kidney.
### Table 6

**Specific Bactericidal Activity of the Neutral Lipid Fractions from Kidney Homogenates**

<table>
<thead>
<tr>
<th>Indicator Strain</th>
<th>Neutral Lipid from Unactivated Homogenates</th>
<th>Uninfected Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days After Infection with Strain 18Z</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TG</td>
<td>65*</td>
<td>33</td>
</tr>
<tr>
<td>18Z-G</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>303</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

* Values are μg/ED₅₀.*
Table 7

Specific Bactericidal Activity of the Neutral Lipid Fractions from Kidney Homogenates

<table>
<thead>
<tr>
<th>Indicator Strain</th>
<th>Neutral Lipid From Unactivated Homogenates</th>
<th>Uninfected Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days After Infection with Strain 689</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>TG</td>
<td>&gt;50*</td>
<td>&gt;50</td>
</tr>
<tr>
<td>PG114</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>18Z-G</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>303</td>
<td>28</td>
<td>34</td>
</tr>
</tbody>
</table>

* Values are μg/ED_{50}.

ND = Not done.
Table 8

Specific Bactericidal Activity of the Free Fatty Acid Fraction In Kidneys Removed from Mice Infected with S. aureus 689

<table>
<thead>
<tr>
<th>Indicator Strains</th>
<th>Free Fatty Acid Fraction from Unactivated Homogenates Days After Infection with 689</th>
<th>Uninfected Kidney</th>
<th>AcPAH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>TG</td>
<td>22*</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>PGL14</td>
<td>10</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>303</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* Values are μg/ED$_{50}$. 


Table 9

Total Bactericidal Activity of Free Fatty Acid Fraction
In Kidneys Removed from Mice Infected with \textit{S. aureus} 689

<table>
<thead>
<tr>
<th>Indicator Strains</th>
<th>Free Fatty Acid Fraction From Unactivated Homogenates</th>
<th>Uninfected Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days After Infection with 689</td>
<td>1</td>
</tr>
<tr>
<td>TG</td>
<td>134*</td>
<td>98</td>
</tr>
<tr>
<td>PGL114</td>
<td>308</td>
<td>217</td>
</tr>
<tr>
<td>303</td>
<td>1756</td>
<td>791</td>
</tr>
</tbody>
</table>

* Values are total activity expressed as ED$_{50}$/kidney.
Table 10
Fractionation of Neutral Lipids on Unisil Column

<table>
<thead>
<tr>
<th>Eluting Solvent</th>
<th>MLS Eluante</th>
<th>Neutral Lipids Eluted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hexane</td>
<td>45</td>
<td>HC, squalene</td>
</tr>
<tr>
<td>2. Hexane-Diethyl Ether (99:1)</td>
<td>100</td>
<td>Steryl ester, methyl esters</td>
</tr>
<tr>
<td>3. Hexane-Diethyl Ether (95:5)</td>
<td>70</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>4. Hexane-Diethyl Ether (92:8)</td>
<td>90</td>
<td>FA &amp; alcohols</td>
</tr>
<tr>
<td>5. Hexane-Diethyl Ether (85:15)</td>
<td>120</td>
<td>Cholesterol, diglyceride</td>
</tr>
<tr>
<td>6. Diethyl Ether</td>
<td>50</td>
<td>Monoglycerides</td>
</tr>
</tbody>
</table>

*a* 1 x 20 cm, unisil (Silicic Acid) column (40).
Figure 1. The survival of S. aureus strains in the kidneys of mice. Each point represents the geometric mean of counts (± 2 S.E.) in kidneys obtained from 3-6 animals. Each kidney was processed separately.
Figure 1, Con't. The survival of *S. aureus* strains in the kidneys of mice. Each point represents the geometric mean of counts (± 2 S.E.) in kidneys obtained from 3-6 animals. Each kidney was processed separately.
Figure 2. Simultaneous infection of a mouse kidney with *S. aureus* strains 18Z-G Sp<sup>r</sup> and 18Z Sm<sup>r</sup>. The organisms were mixed in equal amounts and inoculated intravenously. Samples were plated in TSA containing either 600 μg/ml spectinomycin or 60 μg/ml streptomycin to enumerate each organism independently. Each point represents the geometric mean of counts (+ 2 S.E.) in kidneys obtained from 3 animals.
Figure 3. Lipid fractions recovered from kidneys removed from mice infected with S. aureus strain 18Z. Each point represents the weight of lipid obtained from 6 kidneys. The geometric mean counts of staphylococci present in the kidneys are shown for comparison.
Figure 4. Lipid fractions recovered from kidneys removed from mice infected with S. aureus strain 689. Each point represents the weight of lipid recovered from 20 kidneys (not activated). The geometric mean counts of staphylococci present in the kidneys are shown for comparison.
Figure 5. Neutral lipid subfractions recovered from kidneys removed from mice infected with *S. aureus* strain 689. Each point represents the weight of lipid recovered from 20 kidneys (not activated). Fraction 3 was eluted from Unisil with diethyl ether-hexane (95:5), fraction 4 with diethyl ether-hexane (92:8), and fraction 5 with diethyl ether-hexane (85:15). The geometric mean counts of staphylococci present in the kidneys are shown for comparison.
Plate I: Histological section of a kidney from a mouse after 9 days infection with *S. aureus* strain 689. H & E stain. Same area as shown in Plate II.
Plate II: Histological section of a kidney from a mouse after 9 days infection with *S. aureus* strain 689. Oil Red O stain. Same area as shown on Plate I. Lipid droplets appear intense black.
Plate III: Histological section of kidney from a mouse 13 days after infection with S. aureus strain 689. H & E stain. Same area as shown in Plate IV.
Plate IV: Histological section of kidney from mouse 13 days after infection with *S. aureus* strain 689. Oil Red O stain. Same area as shown in Plate III. Lipid droplets appear intense black.
BIBLIOGRAPHY


