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DETERMINANTS OF INFECTION IN THE PERITONEAL CAVITY

I. Response to and Fate of *Staphylococcus aureus* and *Staphylococcus albus* in the Mouse**

The introduction of bacteria into an animal host initiates a series of events which culminates in either the multiplication or destruction of the microbes. In general, the outcome of infections is influenced by cellular and humoral bactericidal factors as well as by the properties of the bacteria which are related to virulence, i.e., toxins, capsules, etc. The interplay between these factors when studied *in vitro* can often be misleading in terms of the pathogenesis of a given infection in the living animal.

The analysis of infections in the intact host is fraught with technical difficulties. Some of the more important of these are: (1) the quantitation of both bacterial and cellular populations, (2) the determination of the rates of bacterial inactivation by humoral and cellular mechanisms, and (3) the transport of bacteria away from the initial injection site with subsequent influences in other parts of the body. Certain of these problems were discussed by Miles[1] in relation to skin infections, and were considered in the present studies, in terms of a choice of models. Both the intradermal and intravenous routes of infection were considered too complex to permit adequate quantitation and the peritoneal cavity was selected as the site best suited for this purpose.

This article will delineate certain of the defense mechanisms operative in the mouse peritoneal cavity and will compare the effectiveness with which they cope with infections produced with both *S. aureus* and *S. albus*.

MATERIALS AND METHODS

Mice. All animals employed in the present investigation were from the NCS colony maintained at the Rockefeller Institute. This strain and its attributes have been thoroughly described by Dubos and Schaedler in a prior communication.[2] The animals, weighing 20 ± 2 g. were fed commercial pellets and received water *ad lib.* during

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the experimental period. Mice of both sexes were employed without any notable difference in results.

Bacteria. A variety of strains of both *Staphylococcus aureus* and *Staphylococcus albus* were employed as 16-hour stationary cultures in penassay broth. The prototype strain of *S. aureus* was the Smith strain and of *S. albus,* the Greaves strain. Both organisms have been described previously.[3] Other strains of *S. aureus* included: (a) NYH-3 and NYH-6—both coagulase positive, hemolytic, phagetype 80/81 human pathogens obtained from Dr. Edward Hook of New York Hospital; (b) diffuse Smith—the mouse virulent variant described by Hunt and Moses[4] and obtained from Dr. Donald Luria of Bellevue Hospital. Strains of *S. albus* included: (a) Air, (b) Mendita, (c) Prengle and (d) McGaffrey, all of which have been described.[5]

A strain of *Mycobacterium smegmatis* was obtained from Dr. Russell Schaedler of the Rockefeller Institute and propagated in the manner outlined previously.[3]

Preparation of bacteria. Bacteria to be injected into the peritoneal cavity were either diluted with phosphate buffered saline (pH 7.5) or collected by centrifugation and resuspended in phosphate buffered saline. In the case of *M. smegmatis* two washing steps were included to remove traces of Tween 80 present in the medium. It should be noted that when small volumes of fluid containing bacteria, i.e., 0.2 ml., were injected, there was no demonstrable difference between organisms which were washed and those still suspended in culture medium.

Technique of studying the peritoneal cavity. In general, a group of mice of similar weight were injected intraperitoneally with a given inoculum of prepared bacteria and subsequently sampled at hourly intervals for the determination of bacterial and cellular populations. In most experiments two or three animals were sacrificed at each time interval, and a sufficient number of extra animals were employed as mortality controls. In this way, the dynamics of the intraperitoneal interaction as well as the ultimate outcome of the infection could be studied simultaneously.

The experiment was initiated by the injection of 0.2 ml. of bacteria into the peritoneal cavity by means of a 1.0 ml. syringe fitted with a No. 26 gauge, ½ inch needle. Two or three animals were killed immediately after injection and the contents of their peritoneum harvested to give the zero time sample. Following the rapid death of the animal with chloroform, the mouse was pinned on a wooden block and the abdominal surface flooded with 70 per cent ethanol. The skin of the abdomen was then reflected, care being taken not to open the peritoneal cavity during the procedure. The peritoneum was grasped with a fine forceps, tented, and 4.0 ml. of phosphate buffered saline injected forcefully by means of a No. 20 gauge needle. This volume of fluid as well as the force of injection insured adequate mixing. The distended abdomen, still grasped by forceps, was then punctured with a Pasteur pipette and the peritoneal fluid withdrawn and transferred aseptically to appropriate tubes. At least 3.0 ml. of fluid could be recovered with this procedure. The injection of smaller volumes of fluid resulted in less consistent recovery of microorganisms and leucocytes. The incorporation of anti-coagulants such as heparin and versene did not materially influence the agglutination of leucocytes and was omitted from the lavage fluid in the majority of experiments.

Processing of peritoneal fluid

(a) *Determination of total and extracellular bacteria:* The method of partitioning leucocyte-bacteria mixtures by low speed centrifugation has been described in a previous

publication.[3] In brief, it consists of centrifuging the fluid at 400 r.p.m. for three to four minutes in siliconed, Kolmer centrifuge tubes. This sediments the leucocytes, leaving extracellular bacteria in the supernate. For the determination of total and extracellular organisms, samples were taken from the uncentrifuged fluid and the supernatant fluid respectively. The processed samples were then plated on the surface of quadrant plates and incubated at 37° C. for 18 hours. At least four tenfold dilutions were plated and the number of bacteria was calculated from quadrants containing 10-100 organisms. To evaluate the adequacy of bacterial recovery, the inoculum for each experiment was routinely plated and compared with the number of bacteria present in the zero time sample. These values were usually within the experimental error of the plating method. The results of the bacterial enumeration technique are expressed as *Total* or *Extracellular Recoverable Bacteria.* This value was calculated by multiplying the number of bacteria per milliliter of fluid by a factor of four (the total volume of lavage fluid). The amount of fluid present in the peritoneal cavity prior to lavage did not introduce a significant error in the calculation.

(b) *Enumeration of peritoneal fluid leucocytes:* Total white cell counts were done promptly on harvested peritoneal fluids using standard techniques. The number of *Total Recoverable Leucocytes* was the product of leucocytes/ml. times 4. It was of importance to sample exudates immediately after harvesting and prior to the aggregation of cells which occurred on prolonged standing at room temperature.

A variety of techniques were initially employed to determine the distribution of mononuclear and polymorphonuclear leucocytes in the lavage fluids. The most satisfactory was the differentiation of cells in the hemocytometer chamber with the 43 x objective under conditions in which the nuclei were stained with methylene blue. From the differential and total leucocyte counts, calculations were made of the number of both mononuclear and polymorphonuclear cells in the sample. No attempt was made to differentiate lymphocytes from macrophages, since in many cases this was extremely difficult because of transitional cell types. The presence of intracellular staphylococci could easily be distinguished under these conditions.

Stained smears. Stained smears were employed as an additional index of cell morphology and of the distribution of bacteria within leucocytes. The peritoneal cavity was lavaged with 1.0 ml. of buffer in order to maintain a high cell density. Drops of fluid were then placed on clean glass slides, housed in a moist chamber. After 10-15 minutes at 37° C., to allow for adherence of the cells to the glass, the cells were flash dried.[5] The slides were then fixed in methanol and stained for 30 minutes with a dilute buffered Giemsa stain.

Determinations on peripheral blood. Total and differential leucocyte counts were performed on tail vein blood and evaluated by the methods described for peritoneal fluids.

The quantitation of bacteremia was made by a method similar to that described by Berry.[6]

Estimation of hemolysin in peritoneal fluids. Hemolysin was determined on fluids collected after introducing 1.0 ml. of phosphate buffer into the peritoneal cavity. This small volume was necessary in order to minimize the initial dilution of the toxin. After harvesting, the exudates were centrifuged at 2,500 r.p.m. for 20 minutes to remove leucocytes and other debris and the clear supernatant fluid removed for assay.

Sheep erythrocytes were obtained from Cappel Laboratories (West Chester, Pa.) and washed three times in Gey's solution prior to use in the test. Rabbit and mouse erythrocytes were obtained from heparinized cardiac blood and washed in a similar fashion. All erythrocytes were adjusted to a concentration of 1 per cent in Gey's solution. For assay, twofold dilutions of the peritoneal fluid were made in normal saline. To these dilutions was added an equal volume of 1 per cent erythrocytes (0.5 ml.). The tubes were then shaken and incubated at 37° C. for 1.5 hours. The results are expressed as the highest dilution exhibiting complete or partial hemolysis.

Immune serum. Rabbit immune serum prepared against phenol-killed Smith strain was employed.[8] In the first type of experiment mice were injected i.p. with 0.2 ml. of a 1/100 dilution of immune serum one hour prior to the injection of viable *S. aureus*. The second procedure was to pre-opsonize the viable bacteria with a 1/100 dilution of immune serum prior to their injection. Washed organisms were suspended in a 1/100 dilution of serum and allowed to remain at 25° C. for 10 minutes. They were then sedimented at 2,500 r.p.m. resuspended in phosphate buffered saline and injected i.p. Control experiments were performed with the same lot of immune serum which had been absorbed three times with a one-half volume of packed heat-killed bacteria at 4° C. Residual bacteria were removed from the serum by centrifugation at 10,000 x g.

RESULTS

GENERAL EFFECTS OF *S. AUREUS* AND *S. ALBUS* INJECTED INTO THE PERITONEAL CAVITY

Staphylococcal infection of the mouse by the intraperitoneal route displayed certain characteristics not apparent with other routes of administration. The most striking of these was the rapid death following large doses of organisms. Animals surviving the first 24 hours of infection invariably did not succumb at later time periods. Prior to the more detailed analysis of intraperitoneal events, a dose response study was conducted with the two prototype strains. These experiments are outlined in Table 1. It is apparent that with the mouse virulent Smith strain a relatively large number of bacteria were required to produce a fatal infection, and that small differences in inoculum size produced a pronounced effect on the mortality of animals. The mean survival time was as short as six hours, whereas smaller inocula, i.e., 1×10^8, produced infections which were fatal at approximately 10 hours and never longer than 24 hours. With each of the lethal doses a relatively constant number of bacteria (5×10^9—1.7×10^{10}) could be recovered from the peritoneal cavity at the time of death. In contrast, *S. albus* even at doses of 5×10^9 failed to kill any of the mice. It was of interest that the diffuse variant of the Smith strain had an LD_{50} identical to that of the stock strain of Smith employed in this laboratory.

Mice which received sublethal doses of bacteria and which were maintained for as long as 21 days exhibited no gross pathology in the viscera, including the kidneys. Animals dying the acute death from lethal inocula also showed no gross pathology other than in the peritoneal cavity. Characteristically, this showed a purulent exudate, occasionally hemorrhagic, in which numerous extracellular staphylococci were observed in stained smears.

TABLE 1. THE COURSE OF THE INTRAPERITONEAL INFECTION OF MICE WITH *S. aureus* AND *S. albus*

Organism	*Dose**	*Survival at 24 hrs. Alive/total*	*Mean survival time in hrs.*	*Bacteria in peritoneal cavity at death†*
S. aureus	$8x10^8$	0/20	7.1	$5x10^9$-$1.7x10^{10}$
"Smith"	$4x10^8$	0/20	8.1	" "
	$2x10^8$	4/20	8.3	" "
	$1x10^8$	12/20	10+	" "
	$8x10^7$	20/20	..	.. ..
S. albus	$5x10^9$	20/20	..	.. ..
"Greaves"	$1x10^9$	10/10	..	.. ..
	$5x10^8$	10/10	..	.. ..

* Total number of viable bacteria injected in a volume of 0.2 ml.
† Values of six animals in each group, sampled just prior to death or within ½ hour after death.

DISTRIBUTION OF BACTERIA FOLLOWING INTRAPERITONEAL INJECTION

For accurate evaluation of results it was first necessary to obtain quantitative information on the extraperitoneal dissemination of the injected bacteria, and on possible extracellular killing in exudate fluid or serum.

Figure 1 shows the results of a typical experiment in which various numbers of the Smith strain and *S. aureus* were introduced into the peritoneal cavity and the numbers entering the blood stream assayed. Each of the points on the graph represents the mean value of four mice, whereas the bars indicate maximum and minimum values.

The intraperitoneal injection of 3 x 10^8 staphylococci resulted in an early bacteremia which diminished briefly after one hour and then continued to increase until time of death. In this particular experiment two-thirds of the animals died prior to the eight-hour sample. With smaller inocula the bacteremia was transient in nature, with a progressive decline

in organisms. The injection of 3 x 10^6 and 3 x 10^7 organisms was never lethal to the normal NCS mouse. Less complete studies on the organ census of bacteria in the liver, spleen, lungs and kidneys revealed populations which were always 70 per cent or less of that present in the peripheral circulation.

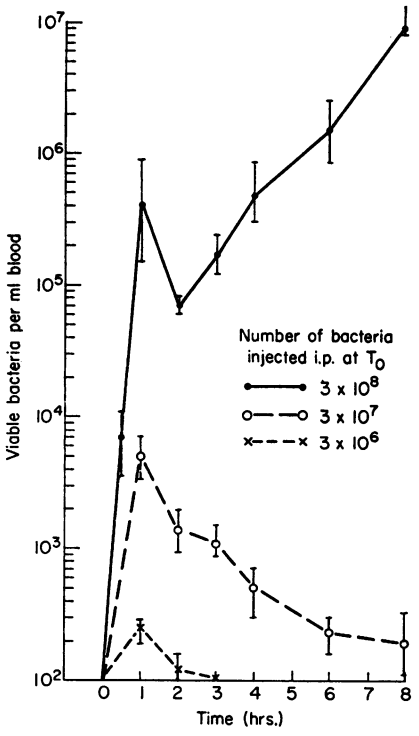

FIG. 1. Bacteremia in mice inoculated intraperitoneally with various numbers of *S. aureus* "Smith."

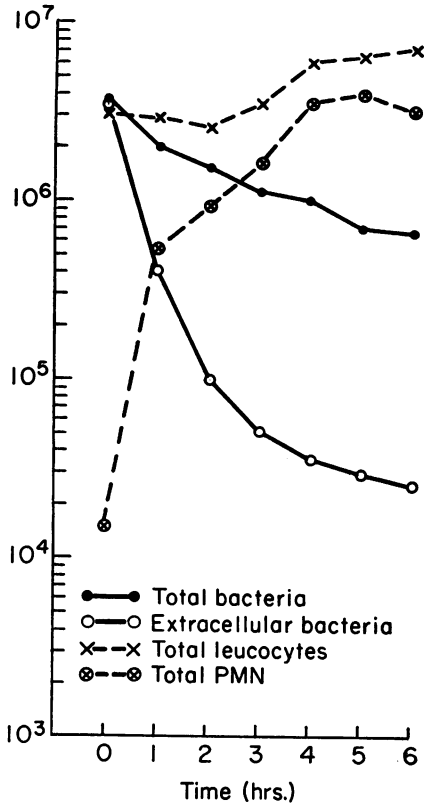

FIG. 2. The fate of *Mycobacterium smegmatis* in the peritoneal cavity of the mouse.

These results indicate that a certain number of bacteria escape from the peritoneum, enter the peripheral circulation and are present in the various organs. The number of these organisms, however, is only a small proportion (1% or less) of those present in the peritoneal cavity at any given time period (see "D" in Fig. 4).

In order to evaluate, in another manner, the retention of injected bacteria in the peritoneal cavity, an organism was employed which (1)

was not inactivated within mouse peritoneal leucocytes during a six hour period and, (2) did not multiply to any significant extent during the same interval. This was in essence a "viable label" and any reduction in the number of recoverable bacteria would be an index of the removal of bacteria from the peritoneal cavity. Figure 2 illustrates one such experiment utilizing *Mycobacterium smegmatis,* an organism previously employed for analagous experiments *in vitro.*[8] During the experimental period there

TABLE 2. INFLUENCE OF MOUSE SERUM, PLASMA AND EXUDATE FLUID ON SURVIVAL OF *S. aureus* AND *S. albus*

Material	*Concentration** *(per cent)*	*S. aureus "Smith"* *T zero*	*T 90'*	*S. albus "Greaves"* *T zero*	*T 90'*
Mouse serum	10	$3x10^6$	$7.2x10^6$	$2.1x10^6$	$9.6x10^6$
"	50	"	$9.8x10^6$	"	$1.3x10^7$
"	80	"	$1.4x10^7$	"	$2.5x10^7$
Mouse plasma	10	"	$9.6x10^6$	"	$8.4x10^6$
"	50	"	$1.0x10^7$	"	$1.0x10^7$
"	80	"	$1.2x10^7$	"	$1.3x10^7$
Peritoneal exudate fluid†					
1 hr.	100	"	$8.7x10^6$	"	$9.0x10^7$
4 hr.	"	"	$9.4x10^6$	"	$1.4x10^7$
6 hr.	"	"	$9.6x10^6$	"	$1.9x10^7$

* Material diluted in phosphate-buffered saline pH 7.5 to which a washed inoculum of bacteria was then added. Tubes incubated at 37° C. in reciprocating water bath, and sampled.

† Hours after the injection of 10^8 heat killed *S. aureus.* Exudate fluids were collected with 2.0 ml. of saline and centrifuged at 2,000 rpm/15' to remove intact leucocytes. The supernatant fluid was then assayed for its effect on staphylococci.

was a gradual reduction in the total number of viable Mycobacteria with approximately 20 per cent of the original inoculum recoverable at the end of six hours.[7] At the same time there was a rapid reduction in extracellular organisms indicative of phagocytosis by peritoneal leucocytes. Assuming that the results with a Mycobacterium are analagous to the transport of staphylococci out of the cavity, marked reductions in viable staphylococci, and not dissemination, would be the result of intraperitoneal killing.

It was also necessary to evaluate the influence of noncellular constituents of the peritoneal and blood fluids on the viability of both *S. albus* and *S. aureus.* Table 2 presents one such experiment, in which staphylococci were suspended in various concentrations of NCS mouse serum, plasma and peritoneal fluid. In all instances both *S. albus* and *S. aureus* survived,

and even multiplied in these media. It was concluded, therefore, that any rapid reduction in the number of staphylococci in the mouse peritoneum would probably be the result of cellular factors.

S. AUREUS AND *S. ALBUS* IN THE MOUSE PERITONEUM

The next series of Figures will illustrate the fate of both *S. albus* and *S. aureus* in the mouse peritoneum, as well as the leucocyte response which occurs subsequent to the injection of different inocula. The data

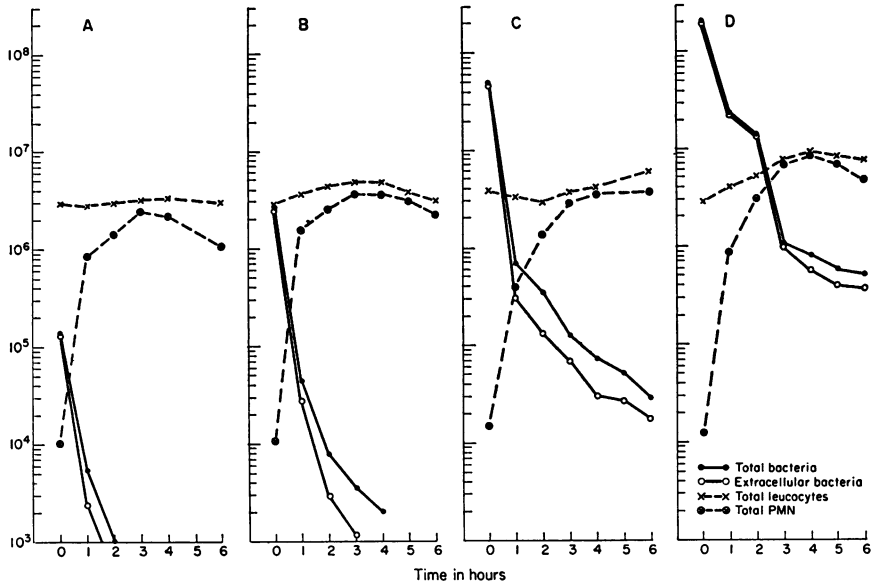

FIG. 3. The fate of and response to the intraperitoneal injection of *S. albus* "Greaves." (A) 10^5, (B) 10^6, (C) 10^7, (D) 10^8.

are presented in a similar fashion throughout, and allow a comparison of the dynamics of the interaction and the relationship between the total number of leucocytes and bacteria at time periods up to six hours.

The injection of 10^5 *S. albus* ("A" in Fig. 3) was followed by a rapid fall in the number of total and extracellular bacteria with the disappearance of over 95 per cent of the inoculum in one hour. Within two hours there had been a 99 per cent reduction in bacterial numbers. During the entire period there was essentially no change in the total number of leucocytes. However, within 60 minutes there had been a polymorphonuclear response (30% of total leucocytes), which reached a maximum at three

hours (78%) and then gradually fell. Initially, the mouse peritoneum contained, at most, 2 per cent polymorphonuclear leucocytes and in most instances none were seen at zero time. The number of normally occurring peritoneal mononuclear cells was relatively constant and varied between 2-4 x 10^6/20 g. NCS mouse. Mice of larger size, however, often had as many as 8-10 x 10^6 cells in their peritoneal cavities.

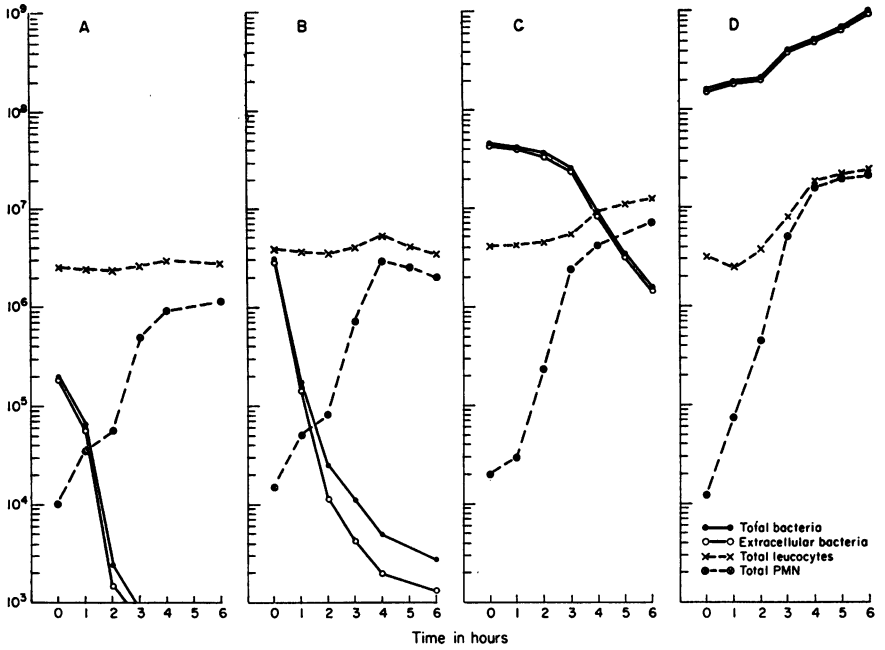

FIG. 4. The fate of and response to the intraperitoneal injection of *S. aureus* "Smith." (A) 10^5, (B) 10^6, (C) 10^7, (D) 10^8.

"A" in Figure 4 illustrates the events following the injection of 10^5 bacteria of the Smith strain. Although rapid destruction of this inoculum occurred, there were differences in both the rate of inactivation and leucocyte response when compared with *S. albus*. During the first hour there was a delay in bacterial inactivation, but thereafter a marked reduction in both total and extracellular bacteria occurred. In addition, a delay in the influx of granulocytes was noted, reaching 20 per cent in two hours and never exceeding 50 per cent in the succeeding periods. The relatively similar reduction in the total and extracellular bacterial population with both *S. albus* and *S. aureus* suggested that phagocytosis was the rate-

limiting step in bacterial inactivation. The difference in the apparent rates of ingestion between the Smith and Greaves strains, at this inoculum size, was of no consequence to the fate of the animal.

When larger inocula were employed ("B" in Figs. 3 and 4), i.e., 4 x 10^6, to give an initial ratio of approximately one bacterium/leucocyte, both *S. albus* and *S. aureus* were effectively phagocyted and killed by the cells in the mouse peritoneum. The rates of these reactions were greater with *S. albus*. The leucocyte response to this tenfold larger inoculum was more vigorous. The delay in granulocyte influx following the injection of *S. aureus* was again apparent. It was of interest that during the first two hours, when there was a relatively rapid reduction of the virulent strain, only a small number of granulocytes were present in the peritoneum. This suggested the bactericidal efficiency of the normally occurring mononuclear cells and their ability to cope with an equal number of bacteria.

The injection of a still larger inoculum, (10 organisms per mononuclear cell) accentuated the differences between the two strains of staphylococci. "C" in Figure 3 represents an experiment in which 5 x 10^7 *S. albus* were injected at time zero. Rapid phagocytosis and intracellular killing occurred and less than 0.1 per cent of the bacteria were recoverable at six hours. Following an initial slight decline, the number of total leucocytes rose slowly and was accompanied by the entry of granulocytes. Examination of stained smears at one hour revealed that the majority of cells contained intracellular staphylococci.

"C" in Figure 4 illustrates the events when a similar number (5 x 10^7) of *S. aureus* were injected. During the first three hours only a slight reduction occurred in the number of total and extracellular bacteria. Between the fourth and sixth hours there was a more striking reduction in bacteria; this reduction being temporally associated with a rapid increase in the number of polymorphonuclear leucocytes. Although phagocytosis had occurred during the early phase of the interaction, the existing mononuclear cell population was incapable of containing these microorganisms, in contrast to the situation with a similar challenge of *S. albus*. Although a delay in the entry of polymorphonuclear cells again occurred, the total cellular population of the peritoneal cavity eventually rose to greater and more sustained heights than with the avirulent strain. This inoculum of either strain did not produce any mortality in the animals.

The final study of this series was conducted with a multiplicity of approximately 60 bacteria per initial mononuclear cell. "D" in Figure 3 reveals that an inoculum of 2 x 10^8 *S. albus* was ingested and killed efficiently, but at a slower rate than with the smaller inoculum. Ninety

per cent of the organisms were destroyed in the first hour. There was then a short lag, followed by another episode of rapid killing, concomitant with the attainment of a high granulocyte count. In four hours the total leucocyte count rose to about three times the initial value and the vast majority of cells at this time were polymorphonuclear leucocytes.

TABLE 3. THE INFLUENCE OF VARIOUS MICROORGANISMS ON THE RAPIDITY OF THE POLYMORPHONUCLEAR RESPONSE IN THE PERITONEAL CAVITY

Strain	*Inoculum size*	*Percentage PMN leucocytes in the peritoneal cavity at**		
		60′	*120′*	*180′*
S. aureus				
Smith	4×10^6	3	7	26
"	4×10^7	2	11	32
"	2×10^8	8	18	69
NYH-6	4×10^7	..	4	..
NYH-5	4×10^7	..	2	..
Stovall	4×10^7	..	8	..
S. albus				
Greaves	4×10^6	44	51	65
"	4×10^7	27	58	78
"	4×10^8	20	58	84
Air	4×10^7	..	47	..
McGaffrey	4×10^7	..	39	..
Prengle	4×10^7	..	45	..
E. coli				
K-12	4×10^6	28	43	51
M. smegmatis	4×10^6	23	32	39

* Each time point represents the mean of four mice.

Figure 4 ("D") illustrates a similar experiment performed with the Smith strain. At this multiplicity, the bacterial population increased slowly for the first two hours and then rose more rapidly. This was in essence the multiplication of extracellular bacteria, which in this particular experiment killed six out of eight similarly inoculated mice. Here again the total leucocyte population rose markedly. Experiments in which 4×10^8 organisms were injected resulted in a more rapid increase in bacteria and an even shorter survival time.

The difference in the rapidity of the polymorphonuclear response to the virulent and avirulent staphylococci was further evaluated and extended

to other strains and bacterial species. Table 3 presents these data and demonstrates the relative delay in polymorphonuclear entrance when strains of *S. aureus* were injected into the peritoneal cavity. A strain of *E. coli* and of *M. smegmatis* resembled the avirulent strain in this regard.

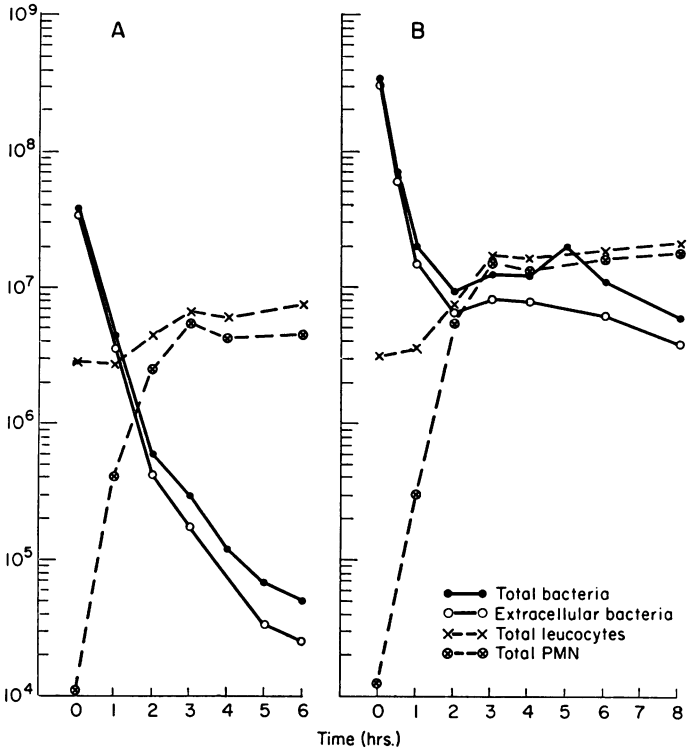

FIG. 5. The influence of immune serum on the fate of *S. aureus* "Smith" in the peritoneal cavity of the mouse. (A) 10^7, (B) 10^8.

THE INFLUENCE OF IMMUNE SERUM ON THE INTRAPERITONEAL FATE OF *S. AUREUS* "SMITH"

The comparative delay in the phagocytosis of the Smith strain in the peritoneal cavity was thought to be one of the factors allowing extracellular multiplication of this organism and the subsequent death of the animal. This resistance to ingestion was a considerably greater threat to the animal under conditions in which a large inoculum of bacteria was employed. It seemed of interest, therefore, to evaluate the effect of immune serum on the dynamics of the intraperitoneal reaction.

Figure 5 ("A" and "B") represents the result of two experiments in which 10^7 and 10^8 *S. aureus* were preopsonized with immune serum and injected intraperitoneally. Comparing Fig. 5(A) to the previously described Fig. 4(C), it can be seen that the rates of phagocytosis and bacterial inactivation were considerably more rapid. In the case of the larger inoculum, a prompt reduction of viable organisms occurred, followed by a rather abrupt cessation in clearance. The preopsonization of this size inoculum was found to protect nine out of twelve mice. In contrast, absorbed serum did not influence the fate of the staphylococci when used at similar concentrations, nor did it protect animals injected with 4×10^8 bacteria.

THE RELATION BETWEEN HEMOLYSIN PRODUCTION AND THE DEATH OF MICE INFECTED INTRAPERITONEALLY WITH *S. AUREUS* "SMITH"

In view of the symptomatology and acute demise of mice infected with a large dose of virulent staphlococci, studies were undertaken to demonstrate the presence of staphylococcal toxins in the peritoneal cavity. Previous studies of others[8] had demonstrated that staphylococcal culture filtrates contained a mouse lethal factor which had properties similar to the hemolysin and dermonecrotic factor. The isolation of such material in pure form and the demonstration of these three activities in associaton with the same molecule has not, however, been accomplished. Attention was turned primarily to the hemolysin, since its assay seemed most suitable to this study.

Initial experiments revealed that a hemolysin was produced in the peritoneal cavity of mice inoculated with a lethal dose of the Smith strain. This hemolysin was found to lyse both sheep and rabbit erythrocytes at 37°C. to approximately the same extent, but did not lyse mouse red cells. Time course studies following injection of a lethal dose of the Smith strain are presented in the upper portion of Table 4. Following the injection of 4×10^8 organisms no demonstrable hemolysin was present in the peritoneum until about the fifth hour. This is the time period when the intraperitoneal population has approached 10^9 and is roughly the point when mice begin to look ill and display weakness. In this particular experiment mice died betwen 7.8 and 8.2 hours after infection, a time when the hemolysin concentration was maximal in the peritoneum. Uninfected controls and animals inoculated with heat-killed organisms failed to show any demonstrable hemolysin. It was of interest that 16-hour culture supernates of the Smith strain failed to hemolyze red cells, suggesting that the production of the hemolysin was in some way stimulated in the peritoneum. It is also apparent that to produce an effective concentration of this sub-

stance *in vivo,* uninhibited extracellular growth of the organism must occur. The injection of sublethal inocula of viable Smith organisms, i.e., 7 x 10^7, has never been accompanied by the formation of detectable toxin. The hemolysin produced in the peritoneal cavity: (1) has a spectrum of hemolytic activity similar to that of the α-toxin, (2) was inactivated by heat at 100°C., and (3) was inhibited by a human serum containing antitoxin.

TABLE 4. THE PRODUCTION OF α-HEMOLYSIN IN THE PERITONEAL CAVITIES OF MICE INFECTED WITH *S. aureus* "SMITH"

Treatment	*Time at which exudates harvested*	*Reciprocal of dilution of peritoneal exudate supernatant fluid producing:**	
	hrs.	*Complete hemolysis*	*Partial hemolysis*
$4x10^8$ bacteria injected i.p.	zero	..†	..
	1	..	..
	2	..	..
	3	..	..
	4	..	undilute
	5	undilute	2
	6	2	8
	7	4	16
	8	4	16
$7x10^7$ bacteria injected i.p.	0–8	..	..
Uninjected control	..	..	..
$1x10^9$ heat killed bacteria injected i.p.	2, 4, 6	..	..
Undiluted 16 hr. culture fluid $5x10^9$ bacteria/ml.	..	..	..

* Rabbit erythrocytes employed.
† Denotes no hemolysis in undiluted exudate fluid.

DISCUSSION

The present study, conducted in the environment of the mouse peritoneal cavity serves to compare the interactions between two strains of staphylococci and those factors in the host which lead to their ultimate destruction or multiplication. Previous studies *in vitro*[8] demonstrated that in a system composed of rabbit granulocytes and staphylococci, the determining factor

in the reduction of viable bacteria was the rate at which organisms were ingested by leucocytes. In the *in vivo* situation, in which phagocytosis and intracellular killing are prominent factors in the disappearance of bacteria from the peritoneal cavity, similar determinants appear to be active. The mouse's peritoneum, however, has certain variables not present *in vitro*. One of these is the probable participation of surface and intercellular phagocytosis, which increases the efficiency of ingestion and which seems to be particularly apparent when small numbers of bacteria are employed. A second is humoral factors, e.g., opsonins, which are normally present in mouse serum and whose entry into and concentration in the peritoneal cavity is unknown. When a large inoculum is introduced, the difference in rates of phagocytosis between the virulent and avirulent strains is accentuated and compares with the *in vitro* findings. Although a small proportion of the virulent strain may survive intracellularly, as emphasized by Rogers and Melly,[9] this fraction cannot be responsible for the virulence of the Smith strain under these circumstances. It appears that the extracellular population is the more threatening and when present in large enough numbers can rapidly multiply and kill the animal.

The presence of a constant number of intraperitoneal bacteria at the time of death is analogous to studies of *Salmonella typhimurium* in the whole mouse carcass.[10] This number of extracellular bacteria, liberating large amounts of toxin, may account in part for the rapid death of the animals. Although the major source of bacteria is the peritoneal cavity, the influence of bacterial products, e.g., toxins, is no doubt the result of extraperitoneal action. In this regard, the toxin assayed in the peritoneal cavity may represent only a small proportion of total toxin production, since this material might be expected to diffuse constantly into the circulation.

The resistance of the Smith strain to ingestion by rabbit leucocytes *in vitro* has been clearly shown to be the result of an acidic polymer on the surface of the bacterium.[11] This substance may also play a role in the peritoneal cavity, and the protection afforded by the passive administration of immune serum is suggestive evidence that this is the case. The direct leucotoxicity of coagulase-positive staphylococci[12] may represent another factor which could decrease the effectiveness of a given phagocyte population to ingest and destroy large numbers of organisms. The presence of leucotoxin has, however, not been determined under these conditions.

The delay in the granulocyte response following the injection of *S. aureus* may be related to certain early observations of Delaunay[13] in which there was little leucocyte infiltration early in the course of rabbit skin infections.

This was thought to be related to the liberation of "toxins" by the bacteria. The more recent observations of Neter,[14] describing "endotoxic" substances in the Smith strain, are pertinent in that gram-negative endotoxins delay the emigration of granulocytes into areas of inflammation.[15] Although this phenomenon has not been shown to be of critical importance in the experiments described, it could conceivably be an adjunct in the initiation of localized lesions, as in the skin.

From a general perusal of the rates of bacterial killing and the changes in cell distribution it appears that both mononuclear and polymorphonuclear phagocytes are effective in the destruction of staphylococci. The initial exposure of the staphylococci is to the mononuclear cell which seems capable of handling inocula of 10^6 *S. aureus* and 10^7 *S. albus*. Larger numbers of organisms require the added influx of polymorphonuclear leucocytes from the peripheral circulation. The combined efforts of both cell types in the unaltered peritoneal cavity are, however, still not adequate to cope with more than 10^8 virulent staphylococci. The over-all efficiency of these cells is increased by the addition of immune serum to the system.

SUMMARY

A procedure is described for the analysis of certain host and bacterial factors which influence the course of infection in the peritoneal cavity. Employing this method, a study was conducted on the ability of the mouse to eliminate a strain of both *S. albus* and *S. aureus* from the peritoneum. Initial studies indicated that a large proportion of the injected organisms remained within the peritoneal cavity and were not influenced by humoral or local extracellular factors. The major determinant for the destruction of bacteria was found to be the phagocytic cells: either the mononuclear cells normally present in the cavity or granulocytes which entered in response to the infection.

The injection of various doses of S. albus (10^5—10^8) resulted in a prompt granulocyte response and was accompanied by the phagocytosis and destruction of the majority of bacteria within a six-hour period. None of the animals injected with as many as 5×10^9 *S. albus* succumbed to the infection.

In contrast, the inoculation of large numbers of *S. aureus* resulted in the acute death of mice, usually within a 10-hour period. A detailed study of this interaction revealed that *S. aureus* at lethal inocula was not effectively phagocyted and was able to multiply extracellularly. A relative delay in polymorphonuclear leucocyte influx was noted following

the injection of *S. aureus*. The extracellular multiplication of *S. aureus* was accompanied by the production of a factor, demonstrable in the peritoneal cavity, which had the properties of the α-hemolysin. This material was considered to account in part for the early deaths produced by large numbers of intraperitoneally injected *S. aureus*.

The passive administration of specific immune serum resulted in increased phagocytosis of *S. aureus in vivo* and protected mice from an LD_{100} inoculum.

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