ZANVIL A. COHN* | *The Rockefeller Institute, New York*

DETERMINANTS OF INFECTION IN THE PERITONEAL CAVITY

II. Factors Influencing the Fate of *Staphylococcus aureus* in the Mouse**

The ability of many types of stimuli to alter the resistance of man and animals to bacterial infection has been the subject of renewed interest in recent years.[1, 2] Whereas in most instances the over-all influence of host modification is clear-cut in terms of mortality and survival time, the mechanisms underlying these phenomena are often obscure. Unfortunately, many examples of either increased or diminished resistance occur under complex conditions which render them not readily amenable to analysis.

Employing the relatively simple infection of the mouse peritoneum by *Staphylococcus aureus,* attempts were made to modify the environment of the peritoneal cavity and compare the findings with those obtained in the normal animal. This communication will describe results with a lipopolysaccharide endotoxin, cortisone and X-irradiation, along with a brief description of the effects of starvation, ammonium chloride ingestion, and alloxan diabetes.

MATERIALS AND METHODS

The basic methods of phagocyte and bacterial enumeration, harvesting peritoneal fluids, etc. were identical with those described previously.[3]

Lipopolysaccharide. The endotoxin employed for all the following experiments was obtained from Dr. Otto Westphal, Freiburg, Germany, and was a highly purified lipopolysaccharide extracted from *Escherichia coli 0111.* A few comparative experiments performed with Dr. Westphal's "pyrexal," obtained from *Salmonella abortus equi,* gave similar results. The lipopolysaccharide (LPS) was suspended in pyrogen-free saline or phosphate-buffered saline (pH 7.5) at a stock concentration of 1.0 mg./ml. and stored at —20° C. for periods never exceeding two weeks. Suitable dilutions were then made in the same media and injected intraperitoneally in volumes of 0.2 ml.

In each experiment, 16-hour cultures of staphylococci were centrifuged, diluted and adjusted to concentration with phosphate buffered saline containing lipopolysaccharide. The intraperitoneal injection of 0.2 ml. of this mixture delivered the illustrated number

---

* Associate Professor.

** This investigation was supported by a research grant, E-3454, from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

Received for publication 26 March 1962.

of bacteria and 20 μg. of *E. coli 0111* endotoxin. A total of 25 mice were used in each study, two being sacrificed at each time interval and the remaining six kept for mortality data. Each point represents the average of two animals. A comparison between the results of this study and the fate of a similar number of staphylococci in the normal mouse can be made with Figure 4 (C and D) in the previous paper.[8] Mice pretreated with endotoxin received 20 μg. of LPS either intraperitoneally or intravenously, 48 hours prior to the intraperitoneal challenge with *S. aureus* Smith.

Hydrocortisone. A variety of dosages and routes of administration were employed. Only results with the subcutaneous and intraperitoneal routes at a constant dose level will be presented. A saline suspension of hydrocortisone acetate (Merck, Sharp and Dohme) was employed throughout.

Depressed resistance to the intraperitoneal inoculation of staphylococci was obtained by the subcutaneous administration of 2.5 mg. of hydrocortisone for four consecutive days prior to challenge on the fifth day. When given intraperitoneally in a volume of 0.2 ml., a somewhat smaller total dose was found to be effective. At 96 and 48 hours prior to an experiment 3.0 mg. were injected i.p., so that the peritoneum had not been disturbed for two days prior to the experiment.

Animals receiving this dosage schedule did not die of spontaneous infection during the pretreatment, nor for at least five days following it. Cultures of the peritoneal cavity during this nine-day period were negative for extraneous microorganisms.

X-irradiation. NCS mice weighing 20 g. were irradiated through the kindness of the Department of Radiation Therapy, The New York Hospital. The animals were placed in the individual compartments of commercial egg containers and exposed to a total dose of 650 r. from a 250 KV X-ray machine. This dose represented at least an LD_{50} for certain mouse strains, but under these conditions did not result in the spontaneous death of a single NCS mouse during the 21 days post-irradiation. The efficacy of irradiation was followed by peripheral leucocyte counts which showed a marked depression on the second post-irradiation day. Animals were employed for experiments on the 5th, 7th, and 11th day after exposure without any significant differences being noted in the results. The experiments to be described were performed on mice on the seventh day after irradiation.

Ammonium chloride ingestion, alloxan diabetes and starvation. Ammonium chloride at concentrations of 1.5 and 2.0 per cent was added to the drinking water of mice and the animals maintained for 10 days prior to challenge. Commercial pellets were available *ad lib.* On the eleventh day, groups of animals were challenged i.p. with 10^6 and 10^7 staphylococci.

Alloxan (Nutritional Biochemical Corp., Cleveland, Ohio) was administered intravenously to mice at dosages of 75, 100 and 150 mg./kg. Two hours later the animals received 1.5 ml. of 20 per cent glucose subcutaneously. Glycosuria was present on the day following injection and blood sugar levels ranged from 250-550 mg./100 ml. Acetonuria and acetonemia were never present. All those dosage groups were challenged i.p. five days after alloxanization with 10^6 and 10^7 staphylococci.

Mice were housed in metabolism cages, without wood shavings, and maintained without food for 24, 36 and 48 hours. Water was supplied *ad lib.* and the animals challenged with 10^6 and 10^7 staphylococci.

RESULTS

THE INFLUENCE OF A BACTERIAL LIPOPOLYSACCHARIDE ENDOTOXIN

Effects on peritoneal and peripheral leucocytes. Previous studies[4,5] indicated that the simultaneous intraperitoneal injection of the "Giorgio" strain of *S. aureus* and bacterial endotoxin resulted in enhanced susceptibility of the mouse and a rapidly progressive infection. Other investigators

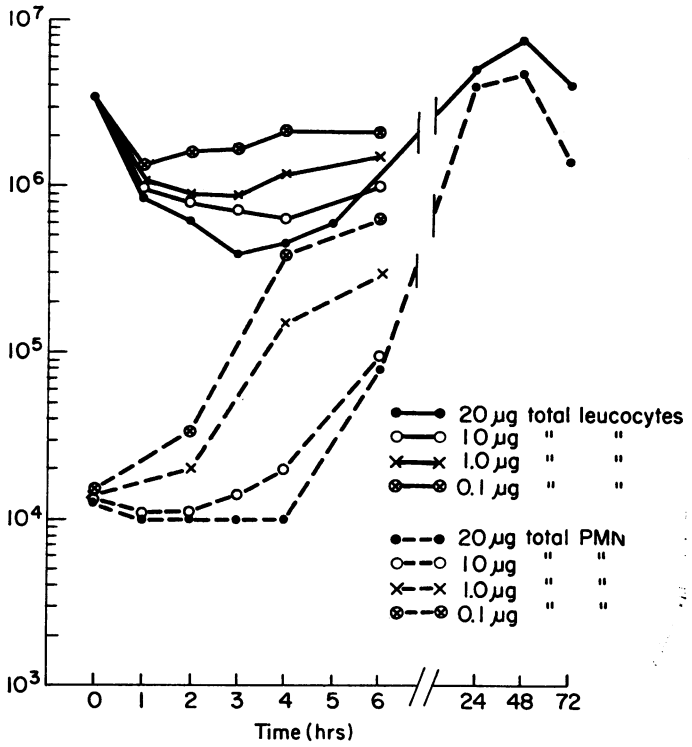

FIG. 1. The effects of lipopolysaccharide on the leucocyte populations in the peritoneal cavity.

have also noted a phase of decreased resistance following the administration of lipopolysaccharide, which was dose dependent and was followed by increased resistance to bacterial infection. These results have been explained in various ways, including fluctuations in properdin titers.[6]

Figure 1 illustrates the sequential influence of various concentrations of lipopolysaccharide on the intraperitoneal cellular populations of 20 g. NCS mice. Each point represents the mean of three animals. Following the

injection of endotoxin at time zero, there was a progressive decrease in the number of recoverable leucocytes. This response was dose dependent in terms of both the absolute reduction in leucocytes and the duration of the effect. At the lower concentrations of 0.1 and 1.0 μg. there was a relatively early granulocyte response, while at higher doses this was markedly delayed. Animals injected with the 20 μg. dose, and examined at 24, 48 and 72 hours respectively, contained larger numbers of both mononuclear and granulocytic leucocytes in their peritoneal cavities. The mechanism underlying the reduction in the peritoneal cell population is

TABLE 1. THE EFFECT OF THE INTRAPERITONEAL INJECTION OF 20 μG. OF LPS ON THE PERIPHERAL BLOOD COUNT OF MICE*

Time (hrs.)	*Total leucocytes per mm.³*	*Total PMN per mm.³*
0	12,450	2,540
1	6,215	658
2	5,150	710
3	5,280	1,339
4	8,400	4,676
5	8,700	4,921
6	9,275	5,759
24	10,425	3,026
48	16,380	2,820

* Mean value of four mice per time interval.

not clear, but might be: (1) lysis of cells, (2) transport out of the peritoneum or perhaps, (3) aggregation and firm binding to the peritoneal surface.

Table 1 shows the effect of intraperitoneally administered LPS on the peripheral white count. A maximum decrease in total leucocytes occurred at one to two hours and was accompanied by an absolute granulocytopenia. Thereafter, the granulocyte count rose rapidly, exceeding pretreatment levels at four hours, whereas the total number of leucocytes required more than six hours to reach normal levels. After the third hour, therefore, sufficient numbers of granulocytes were present in the peripheral circulation.

Lipopolysaccharide and the fate of S. aureus in the peritoneal cavity. Prior to the more detailed evaluation of the effects of endotoxin on the course of staphylococcal infection, a series of titrations was performed in which various challenging doses of staphylococci were employed. The

composite results of this study are presented in Table 2. The simultaneous administration of LPS and staphylococci resulted in increased susceptibility of the mouse. Little effect was noted at inocula of 10^6, but at 10^7 all animals receiving endotoxin died. Control animals injected with the identical number of bacteria, but without LPS, all survived the infection. At 10^8 both treated and untreated mice died in a similar fashion, although the time of death was approximately one to two hours shorter in the endotoxin-treated animals.

TABLE 2. THE INFLUENCE OF LIPOPOLYSACCHARIDE ON THE MORTALITY OF MICE INJECTED INTRAPERITONEALLY WITH VARIOUS DOSES OF *S. aureus* SMITH

Mice injected with 20 μg. E. coli 0111 *LPS*	*Challenge inoculum injected i.p.*	*Treated mice, survivors/total 48 hrs.*	*Untreated controls, survivors/total 48 hrs.*
I.p., simultaneously	4×10^8	18/20	20/20
I.p., simultaneously	4×10^7	0/18	20/20
I.p., simultaneously	4×10^8	0/15	0/16
I.p., 48 hrs. previously	4×10^8	40/42	0/20
I.v., 48 hrs. previously	4×10^8	5/25	1/20

The administration of 20 μg. of LPS 48 hours before challenge with a 10^8 inoculum resulted in a marked increase in the resistance of mice to staphylococcal infection. This inoculum was almost invariably lethal to normal NCS mice as indicated in the previous paper.[3] In this case, 95 per cent of the animals pretreated with LPS survived the infection and did not demonstrate any gross lesions in their organs, including the kidneys, at periods up to 21 days after infection. The prior administration of endotoxin by the intravenous route was much less effective in protecting animals from an intraperitoneal challenge and resulted in the survival of only 20 per cent of the mice. Employing these data as a guide, experiments were then performed on the intraperitoneal factors responsible for the above alterations in host resistance.

Figure 2 illustrates the influence of simultaneously administered lipopolysaccharide and inocula of 2.5×10^6 and 2.5×10^7 *S. aureus* Smith.

The results with a 10^6 staphylococci-LPS mixture, a combination which did not kill mice, is shown in Figure 2(A). For the first four hours the

total and extracellular bacterial populations remained at the same level with only slight fluctuations. Between the fourth and sixth hours multiplication commenced and the titer rose threefold. Thereafter, a precipitous reduction in bacteria occurred, with extracellular organisms falling at a rate similar to that of the total population. An examination of the phagocyte population revealed a decrease in total leucocytes to one third of original values and then a rapid increase between the sixth and eighth hours. This late

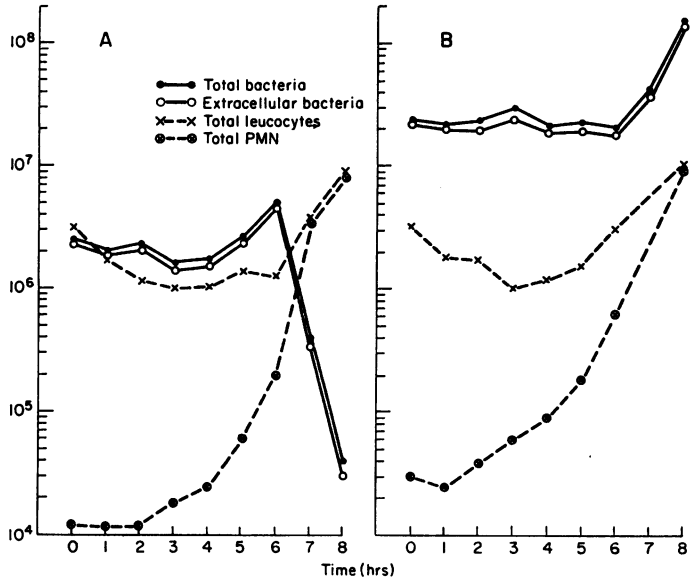

FIG. 2. The effect of the simultaneous administration of lipopolysaccharide and staphylococci. (A) 10^6 staphylococci, (B) 10^7 staphylococci.

increase was almost entirely the result of the influx of granulocytes. An additional finding was a marked delay in polymorphonuclear leucocyte entry during the first six hours. These results suggest that the initial population of mononuclear cells was inhibited in phagocytic activity by virtue of decreased numbers, a direct toxic action on the cells, or perhaps by decreasing the level of intraperitoneal opsonic factors. Thereafter, an inhibited inflammatory response and consequent reduced influx of granulocytes allowed bacteria to multiply. Coincident with the late, sudden granulocyte response, effective numbers of functional cells became available, and prompt phagocytosis and killing took place. The "decisive period" under these conditions was as long as six hours.

A larger challenge dose, 10^7 ("B" of Figure 2), brought about a different end result. In this case, all the animals died and endotoxin had reduced the lethal dose to 1/10 of that required in the untreated mouse. After a long lag phase bacterial multiplication became exponential and at eight hours their number had increased tenfold. The leucocyte response to this inoculum was similar to that in Figure 2(A) with a late increase in

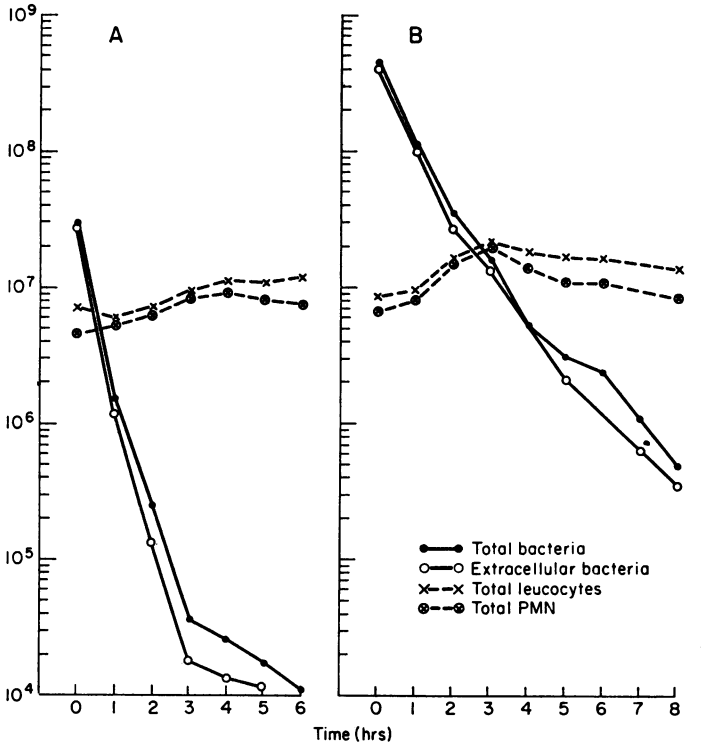

FIG. 3. The influence of the previous administration of lipopolysaccharide on the interactions in the peritoneal cavity. (A) 10^7 staphylococci, (B) 10^8 staphylococci.

polymorphonuclear cells. The total cellular response at eight hours was essentially maximal for the mouse, and at this time could not cope with a log phase culture which exceeded it in numbers by a factor of ten.

Results of experiments in which endotoxin had been given intraperitoneally 48 hours previously are presented in Figure 3. In Figure 3(A) the inoculum of 10^7 staphylococci was rapidly ingested and killed by the peritoneal cells. The lag period in bacterial inactivation observed in the normal mouse was not present, and the final microbial census at six hours

was considerably lower. The initial total leucocyte count was 2.5-fold greater than in the untreated animal, with more than half of the cells being granulocytes. Both cell types increased in number, and at four hours there were more than 10^7 cells recoverable from the peritoneal cavity.

The injection of endotoxin pretreated animals with 4 x 10^8 *S. aureus,* a dose lethal for the normal mouse, resulted in a markedly different series of intraperitoneal events. Whereas this size inoculum was able to multiply extracellularly and kill the untreated animal, striking ingestion and

TABLE 3. THE INFLUENCE OF CORTISONE TREATMENT ON THE MORTALITY OF MICE INFECTED WITH VARIOUS DOSES OF *S. aureus* "SMITH"

	*Peripheral blood counts**		*Challenge inoculum i.p.*	*Treated mice, survivors/total 48 hrs.*	*Untreated controls, survivors/total 48 hrs.*
Cortisone treatment	*WBC/mm.³*	*Per cent PMN*			
3.0 mg. injected i.p.			4 x 10^5	10/10	10/10
96 & 48 hours	14,260	63	4 x 10^6	1/10	10/10
prior to challenge			4 x 10^7	0/10	10/10
2.5 mg. injected s.c.					
for 4 days prior	12,350	83	4 x 10^6	0/10	10/10
to challenge			4 x 10^7	0/10	10/10

* Mean value of 10 determinations, immediately prior to the injection of staphylococci.

inactivation took place. This continued in a linear fashion for eight hours, after which time only 10^5 viable bacteria were present in the peritoneum. The initial leucocyte population was again elevated, and, in response to the larger number of bacteria, rose rapidly to 2 x 10^7 total cells. During the first 2 to 3 hours after the infection many more cells were available in the peritoneum as compared to normal controls. No demonstrable α-hemolysin was present in the peritoneal cavity throughout the course of similar experiments. The exponential and continued reduction of viable bacteria is in contrast to the results reported in the previous paper (see Fig. 5(B) of that paper) with specific immune serum.[3]

Animals pretreated by the intravenous route had a normal number of peritoneal cells, i.e., 3 x 10^6, all of which were mononuclears. These animals survived a lethal inoculum only slightly better than untreated controls. This suggested that the local inflammatory response produced by the intra-

peritoneal administration of LPS was a major factor in the accelerated destruction of staphylococci.

THE INFLUENCE OF HYDROCORTISONE ON THE INTRAPERITONEAL FATE OF STAPHYLOCOCCI

The ability of large doses of cortisone to depress host resistance has been amply documented in the literature.[7,8] The mechanisms underlying this phenomenon have recently been discussed by Hirsch and Church,[9]

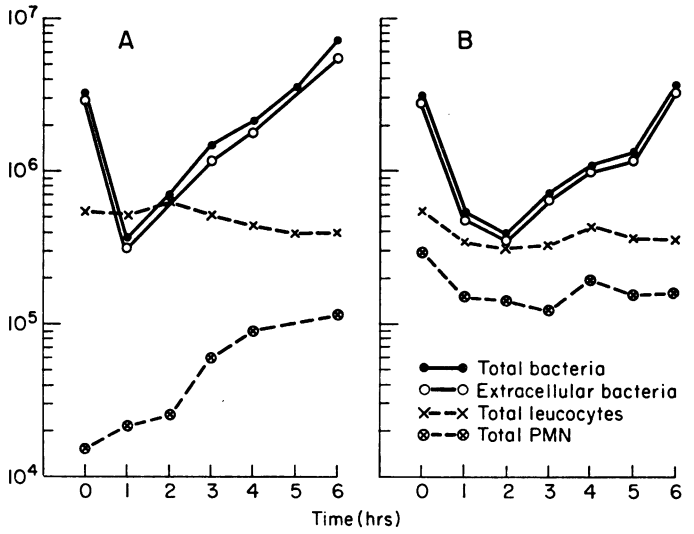

FIG. 4. The effect of cortisone on the fate of and response to 10^6 *S. aureus*. (A) subcutaneous administration, (B) intraperitoneal administration.

who cast doubt on any deficiency in the phagocytic or bactericidal functions of the polymorphonuclear leucocyte, or on the absence or diminution of serum opsonic factors. The present system presented an interesting example for the study of certain of these hypotheses.

Preliminary titrations revealed that considerably fewer staphylococci were able to produce fatal infections in cortisone-treated mice than in normal controls. The results of two such studies are presented in Table 3. Animals treated first by either the subcutaneous or intraperitoneal routes and challenged with varying numbers of staphylococci i.p. succumbed to a fulminating infection with inocula as small as 10^6. With both routes of administration an inoculum of 10^5 failed to kill any of the animals. An examination of the peripheral blood counts of mice at the time of challenge showed an absolute granulocytosis with up to 80 per cent poly-

morphonuclear cells. It appeared, therefore, that the absolute number of circulating phagocytes was not a limiting factor in the outcome of the infection.

Intraperitoneal events in the cortisone-treated mouse. An evaluation of the fate of staphylococci and the cellular responses in the cortisone-treated animal was then performed, employing inocula similar to those discussed in Table 2. Figure 4 presents the results of experiments in which 10^6 staphylococci were inoculated intraperitoneally. Animals receiving cortisone by the subcutaneous route (Fig. 4A) according to the previously described schedule were unable to clear the bacteria. After an initial one hour phase in which phagocytosis and killing took place, the staphlococcal population rose exponentially and subsequently killed mice between 10-14 hours. At the time of death, four of the animals were sampled, and had intraperitoneal populations which were identical to those reported for normal mice injected with 10^8 bacteria.[8] The presence of α-hemolysin could also be demonstrated at this time, in titers similar to those found in normal animals infected with a lethal inoculum, i.e., 1:16. The rapid multiplication observed in this instance was the result of a large sustained extracellular population of bacteria.

In the cortisone-treated animal the initial cell counts at time zero were greatly reduced when compared to the normal mouse. The population of mononuclear cells normally present in the peritoneum (3×10^6) was depressed to only 10-20 per cent of this value. In Fig. 4(A), the original number of bacteria/phagocyte, which in the normal animal would have been 1:1, was now increased to 7:1. During the six hours following inoculation, there was no increase in the total number of leucocytes and even a slight fall. This lack of leucocyte response was apparently due to the marked inhibition of granulocyte diapedesis, since large numbers of polymorphonuclear leucocytes were present in the circulation. It appeared therefore, that there were two factors related to enhanced bacterial multiplication in the animals receiving cortisone: (1) the small number of initial mononuclear cells, and (2) the subsequent lack of any cellular inflammatory response.

Figure 4(B) shows a similar experiment performed with mice which had received hydrocortisone intraperitoneally. The fate of the bacteria was essentially the same as in Fig. 4(A) and resulted in the death of the animals. The cellular pattern was, however, somewhat different. At time zero there was again a reduced number of phagocytes, but the population was a mixture of both mononuclear and polymorphonuclear cells. This presumably resulted from the irritating effect of the previous

injections of steroid. Both the total and polymorphonuclear cell counts remained stationary throughout the experiment, again emphasizing the almost complete inhibition of granulocyte diapedesis.

Additional studies of the phagocytic activity of peritoneal cells present in the cortisonized animals were performed by means of stained smears. Table 4 illustrates one experiment in animals previously treated by

TABLE 4. THE DISTRIBUTION OF INTRACELLULAR STAPHYLOCOCCI IN CELLS FROM CORTISONE-TREATED MICE

No. staphylococci/cell	*No. mononuclear cells*	*No. polymorphonuclear cells*
0	24	4
1	8	2
2	16	24
3	6	14
4	12	26
5	10	8
6	14	12
7	4	4
8	4	0
9	8	2
10	2	2
11–20	6	4
	114	102

Total number of leucocytes = 216.
Total number of intracellular bacteria = 910.
Number intracellular bacteria/leucocyte = 4.21.
Number intracellular bacteria/mononuclear = 4.28.
Number intracellular bacteria/polymorph = 4.14.

the i.p. administration of steroid, which had approximately 50 per cent granulocytes in the peritoneum at time zero. A total of 216 cells were evaluated and contained 910 staphylococci. The average number of bacteria per mononuclear or polymorphonuclear leucocyte was essentially the same, suggesting that there was no difference between the phagocytic activity of either cell type in the cortisonized animal. When these data were compared to a similar experiment performed in the normal animal, it appeared that a larger proportion of the phagocytes contained a greater number of staphylococci per cell.* This was the result of an increased multiplicity of

* Normal mice inoculated with 4.1 x 10^6 *S. aureus*, and harvested 45 minutes later, had 3.8 x 10^6 mononuclear cells (100%) recoverable from the peritoneal cavity. Examination of stained smears revealed a total of 532 intracellular staphylococci in 400 leucocytes, or an average of 1.33 bacteria/cell.

total bacteria/ total leucocytes, rather than an enhanced phagocytic activity of cells from cortisonized animals. This suggested that the over-all process of intraperitoneal phagocytosis was not substantially impaired in the steroid-treated animal, and that the ingestion of this number of bacteria was probably related to the early phase of bacterial inactivation.

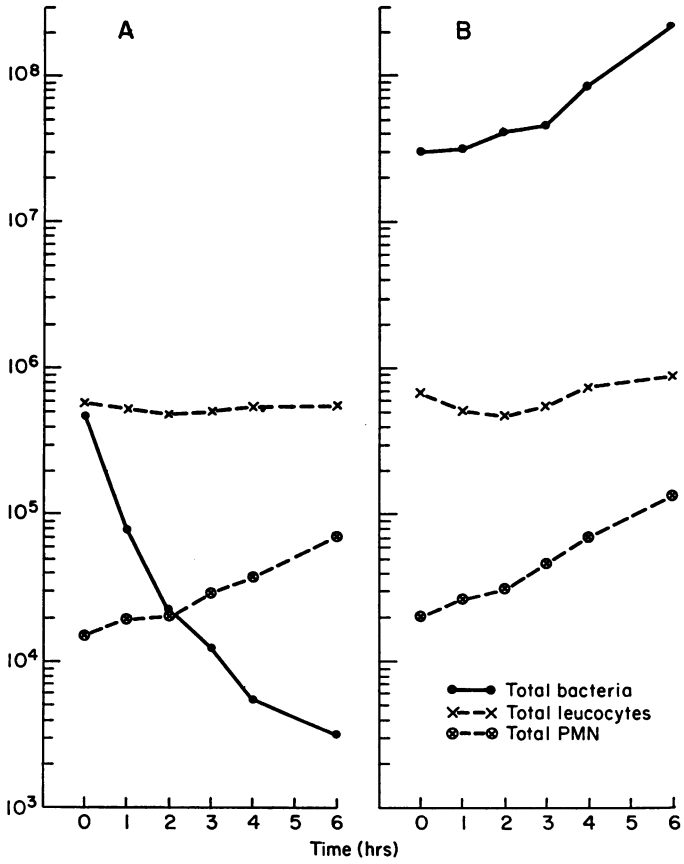

FIG. 5. The effect of the subcutaneous administration of cortisone on the course of staphylococcal infection. (A) 10^5 staphylococci, (B) 10^7 staphylococci.

The inoculation of smaller numbers of bacteria, i.e., 10^5, gave the data presented in Figure 5(A). In this instance the number of bacteria and leucocytes was roughly proportional at time zero, and rapid ingestion and killing took place with a reduction of more than 99 per cent of the original inoculum. Under these conditions, with a 1:1 multiplicity of

bacteria/cell, the reduced initial phagocyte population and an inhibited inflammatory response were nonetheless adequate to handle the challenge. This experiment is comparable to the injection of 10^6 staphylococci in the normal 20 g. NCS mouse.

When 10^7 bacteria were employed as the challenge (Fig. 5B) no reduction in bacterial numbers occurred and rapid extracellular multiplication ensued. In terms of numbers of bacteria per phagocyte, this experiment corresponds to the injection of 10^8 bacteria in the normal mouse.

THE INFLUENCE OF TOTAL BODY X-IRRADIATION ON THE FATE OF *S. AUREUS* IN THE MOUSE PERITONEUM

The previous studies with endotoxin and steroid indicated the importance of changes in the number and rate of entry of peritoneal phagocytes. It was next of interest to investigate the influence of peripheral leukopenia on the dynamics of the intraperitoneal reaction. Various mechanisms have been postulated for the increased susceptibility of animals following total body irradiation.[10] These include, in addition to the obvious depression in total numbers of circulating leucocytes, decreased leucocyte mobility,[11] the inability of the fixed and wandering macrophages to kill ingested bacteria,[12] and the depression of the immune response.[13]

The use of various inocula of *S. aureus* Smith resulted in the mortality data presented in Table 5. Irradiated mice injected i.p. with 10^7 staphylococci succumbed to the infection, whereas 10^6 bacteria resulted in the death of only one out of 15 animals. This represented a reduction of the lethal dose to approximately 1/10 of that required to kill the normal mouse. The total circulating leucocytes in this group of animals was markedly depressed with a mean value which was only 5 per cent of normal. It was of interest, however, that the percentage of granulocytes was essentially within normal limits.

A portion of the same group of animals was then used for studies of events within the peritoneal cavity. Two sets of experiments performed with 10^6 and 10^7 staphylococci, respectively, will serve to illustrate the findings (Fig. 6). Following a two-hour period (Fig. 6A) in which 85 per cent of the original inoculum was inactivated, there was a sharp increase in the rate of phagocytosis and killing. This coincided with the entry of a small number of granulocytes from the periphery, the total number being only 10 per cent of that observed with a similar number of staphylococci in the normal animal. These cells were apparently functional and when confronted with the residual population of extracellular organisms at ± 3 hours, were able to reduce rapidly the number of viable staphylococci.

TABLE 5. THE INFLUENCE OF X-IRRADIATION ON THE MORTALITY OF MICE INFECTED WITH *S. aureus* SMITH

Treatment	*Peripheral blood count**			*Challenge inocula i.p.*	*Irradiated mice survivors/total 48 hrs.*	*Control untreated survivors/total 48 hrs.*
	WBC/mm.³	*Per cent PMN*	*PMN/mm.³*			
650 r. total body irradiation administered 7 days previously	633	12	76	4×10^5	15/15	10/10
				4×10^6	14/15	10/10
				4×10^7	2/15	10/10
				4×10^8	0/15	0/10

* Mean value of 15 determinations. Individual counts varied between 300 and 900 WBC/mm.³.

The shape of the granulocyte response curve was of normal appearance, implying that there was no inhibition of leucocyte diapedesis. Assuming a circulating blood volume of 2.0 ml., the total number of polymorphonuclear leucocytes entering the peritoneum accounted for almost all the intravascular granulocytes. In contrast, little effect was noted on the normally occurring peritoneal mononuclear cells either in terms of numbers or functional activity.

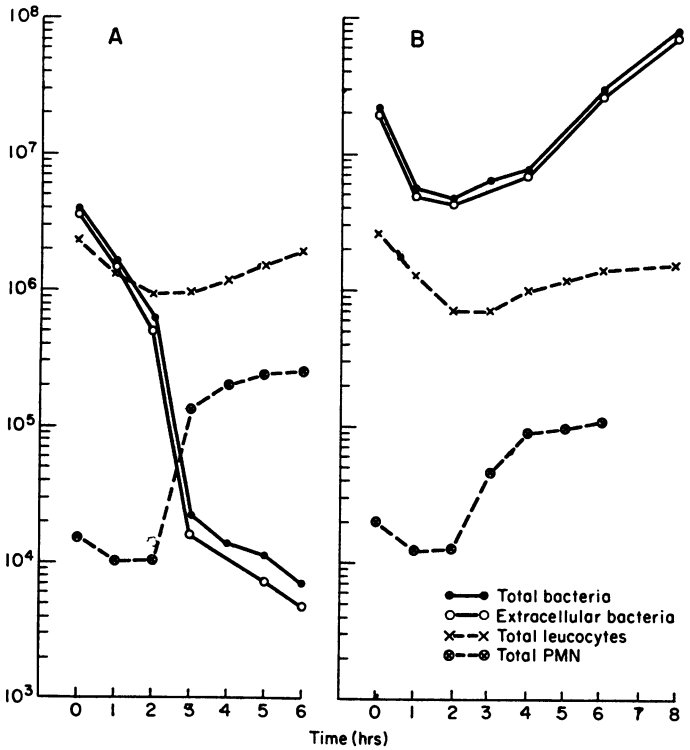

FIG. 6. The influence of 650 r. of total body X-irradiation on the fate of and response to *S. aureus*. (A) 10^6 staphylococci, (B) 10^7 staphylococci.

Figure 6(B) illustrates the fate of 10^7 staphylococci—a lethal dose for the irradiated animal. Under these conditions, after a prompt reduction in bacteria, extracellular multiplication ensued and could not be interrupted by the small numbers of granulocytes entering the peritoneum. The total granulocyte influx was not augmented by the larger number of bacteria, as it had been in the normal mouse, suggesting that the 10^6 inoculum had evoked a maximal response.

STUDIES ON AMMONIUM CHLORIDE INGESTION, ALLOXAN DIABETES AND STARVATION

Ammonium chloride,[14] starvation[15] and alloxan diabetes[16] have all been reported to increase the susceptibility of experimental animals to bacterial infection. Under the conditions employed in the present investigation, mice stressed by each of these means survived an intraperitoneal challenge with as many as 5×10^7 staphylococci. The examination of intraperitoneal events showed that, in all cases, the ingestion and killing of bacteria as well as the leucocyte response was within normal limits. This was the case when either 4×10^6 or 4×10^7 staphylococci were administered.

DISCUSSION

The major emphasis in the preceeding experiments has been on the role of cellular factors in the control of intraperitoneal staphylococcal infection. Two distinct populations of phagocytes combine to form an effective bactericidal system in the unaltered mouse. The first, in terms of the temporal sequence of events, are the naturally occurring mononuclear cells of the peritoneum, while the second are the granulocytes which emigrate into the cavity in response to the challenge inoculum. In the normal mouse the number of mononuclear cells within the peritoneum is able to carry out the primary destruction of staphylococci. There was, however, a limit to the number of bacteria which these cells could effectively destroy—as expressed by a net reduction in viable count during the first two hours after challenge. When the mononuclear cells were exposed to an equal number of bacteria (1 bacteria/1 leucocyte at time zero), a prompt reduction occurred in the total bacterial population. In contrast, a larger inoculum, i.e., 10 bacteria/leucocyte, although phagocyted and killed to some extent, was able to survive extracellularly in large numbers. Under these circumstances, the influx of new phagocytes became of critical importance in controlling extracellular multiplication. It was apparent that both the rate and total numbers of granulocytes entering the peritoneum were of importance in this regard.

In many instances, it appeared that a given number of leucocytes was required to bring about the effective inactivation of a given number of bacteria. The ratio of numbers of leucocytes/numbers of bacteria can be considered an estimate of the over-all efficiency of the phagocytic and intracellular bactericidal processes. This ratio would be influenced by concentration of phagocytes, activity of individual phagocytes, opsonic factors, bacterial capsules and toxins, bacterial growth factors, etc. Factors such as the first three would tend to decrease the ratio, whereas the last two would increase it. Such a relationship, although descriptive in nature,

is useful under conditions in which it is difficult to analyze simultaneously each of the above variables, or in which such factors could fluctuate during the experimental period.

A comparison between the results obtained in the unaltered and modified animals revealed that in most cases the over-all efficiency of the bactericidal systems was the same. The depression of resistance with endotoxin, cortisone and X-irradiation was best correlated with a decreased total number of available phagocytes rather than a decreased effectiveness of the phagocyte. A few examples will serve to illustrate this conclusion for both the mononuclear and polymorphonuclear leucocytes. In the cortisone-treated mouse a tenfold reduction occurred in the number of original mononuclear cells. These cells (5×10^5) were able to inactivate an equal number of bacteria as readily as the mononuclear cells of the normal mouse (3×10^6) inactivated a 4×10^6 inoculum. In both instances a tenfold increase in bacterial numbers resulted in ineffectual killing of bacteria.

The ratio between the number of granulocytes and bacteria was of a similar magnitude. This was illustrated by the effectiveness of the late influx of granulocytes in the endotoxin-treated animal to control a 3:1 multiplicity and their inability to cope with a 10:1 multiplicity (see Fig. 2, 6th to 8th hours).

There were, however, two instances in which the efficiency of the cellular bactericidal system varied from this norm. Both occurred in experiments involving the administration of lipopolysaccharide. The first occurred during the early hours following the simultaneous administration of endotoxin, and was evidenced by the depressed activity of the mononuclear cells below what one would have expected on the basis of the reduction in cell numbers. The second was the enhanced activity of the mixed cellular population following the injection of endotoxin 48 hours previously. In this case a combination of polymorphonuclear and mononuclear leucocytes was able to inactivate a 50:1 multiplicity, suggesting some direct enhancement of cell activity[17] and/or the appearance of new opsonic factors.[18]

The strictly quantitative aspects of these data can be considered applicable only to the interaction of the Smith strain of *Staphylococcus aureus* in the peritoneal cavity of the 20 g. NCS mouse. Differences in the virulence factors of the microorganism as well as changes in the age and strain of the host could conceivably alter these relationships. It is probable, however, that the over-all mechanisms described in this article apply to a wide variety of bacteria, particularly those which are not primarily influenced by humoral substances and which do not possess striking attributes of virulence.

The three agents, i.e., endotoxin, cortisone and X-irradiation, which brought about the most pronounced changes in the susceptibility of the mouse, also altered the cellular dynamics in the peritoneal cavity. Gross changes in host resistance could therefore be correlated with one or more changes in the sequence of cellular defense mechanisms. Agents which interfered at multiple sites, e.g., cortisone, had the most marked influence on the susceptibility of the host. In contrast, ammonium chloride, starvation, and alloxan diabetes, which had no influence on the susceptibility of the peritoneal cavity to infection, were without effect, under the conditions employed, on these same mechanisms.

For the most part, the cellular alterations were correlated best with the number and availability of phagocytic cells. The direct inhibition of cell function *in vivo* and its influence on host resistance represents a second area of interest which will be dealt with more fully in the following article.

SUMMARY

A study has been performed on the course of staphylococcal infection in the peritoneal cavity of the mouse. The effects of bacterial endotoxin, cortisone and X-irradiation were employed as tools in delineating the factors responsible for the intraperitoneal destruction of *Staphylococcus aureus*.

The simultaneous intraperitoneal administration of lipopolysaccharide and bacteria resulted in a tenfold decrease in the number of organisms required to kill the mouse. This phenomenon was correlated with a decrease in the number and functional activity of the mononuclear cells of the peritoneal cavity, and with a delay in the influx of granulocytes from extraperitoneal sources. The intraperitoneal injection of endotoxin 48 hours prior to staphylococcal challenge resulted in the survival of mice subjected to an ordinarily lethal inoculum. Enhanced resistance under these conditions was considered the result of an elevated number of phagocytes in the peritoneal cavity and possibly an enhanced efficiency of phagocytosis.

Large doses of hydrocortisone reduced the lethal dose of staphylococci to 1/100 of that required in the normal mouse. This agent was found to depress the number of naturally occurring mononuclear cells of the peritoneal cavity to 1/10 of the value present in the unaltered mouse. In addition, cortisone inhibited almost completely the diapedesis of granulocytes, even though large numbers of these cells were present in the circulation. There was no apparent alteration in the over-all phagocytic activity of either mononuclear or polymorphonuclear leucocytes.

A total body irradiation of 650 r. resulted in a tenfold decrease in the

number of staphylococci required to kill the mouse. No significant alteration of phagocytic function or numbers of naturally occurring mononuclear cells of the peritoneal cavity was noted. The major factor involved in increased host susceptibility was a reduction in the number of circulating leucocytes and the subsequent influx of only small numbers of cells. There was no apparent alteration in either the function or rate of emigration of the granulocytes.

Short periods of starvation, the ingestion of ammonium chloride and alloxan diabetes without ketosis did not influence the susceptibility of the peritoneal cavity to staphylococcal infection, nor did they alter the cellular dynamics following infection.

REFERENCES

1. Kiser, J. S. (Ed.): Natural resistance to infections. *Ann. N. Y. Acad. Sci.*, 1956, *66*, 233.
2. MacLeod, C. M. and Cluff, L. E. (Eds.): Nonspecific resistance to infection. *Bact. Rev.*, 1960, *24*, 1.
3. Cohn, Z. A.: Determinants of infection in the peritoneal cavity. I. The response to and fate of *S. aureus* and *S. albus* in the mouse. *Yale J. Biol. Med.*, 1962, *35*, 12.
4. Schaedler, R. W. and Dubos, R. J.: The susceptibility of mice to bacterial endotoxins. *J. exp. Med.*, 1961, *113*, 559.
5. Dubos, R. and Schaedler, R. W.: The effect of bacterial endotoxins on the water intake and body weight of mice. *J. exp. Med.*, 1961, *113*, 921.
6. Landy, M. and Pillemer, L.: Increased resistance to infection accompanying alteration in properdin levels following administration of bacterial lipopolysaccharides. *J. exp. Med.*, 1956, *104*, 383.
7. Thomas, L.: The effects of cortisone and adrenocorticotropic hormone on infection. *Ann. Rev. Med.*, 1952, *3*, 1.
8. Germuth, F. G., Jr.: The role of adrenocortical steroids in infection, immunity and hypersensitivity. *Pharmacol. Rev.*, 1956, *8*, 1.
9. Hirsch, J. G. and Church, A. B.: Adrenal steroids and infection: The effect of cortisone administration on polymorphonuclear leukocytic functions and on serum opsonins and bactericidins. *J. clin. Invest.*, 1961, *40*, 794.
10. Benacerraf, B.: Influence of irradiation on resistance to infection. *Bact. Rev.*, 1960, *24*, 35.
11. Shechmeister, I. L. and Fishman, M.: The effect of ionizing radiation on phagocytosis and the bactericidal power of the blood. I. Effect of radiation on migration of leucocytes. *J. exp. Med.*, 1955, *101*, 254.
12. Nelson, E. L. and Becker, J. R.: The effect of whole body X-irradiation on the bactericidal activity of phagocytic cells. I. Survival of *Pseudomonas aeraginosa* within phagocytes from peritoneal exudates of mice. *J. infect. Dis.*, 1959, *104*, 13.
13. Talmage, D. W.: Effect of ionizing radiation on resistance to infection. *Ann. Rev. Microbiol.*, 1955, *9*, 335.
14. Freedman, L. R. and Beeson, P. B.: Experimental pyelonephritis. VIII. The effect of acidifying agents on susceptibility to infection. *Yale J. Biol. Med.*, 1961, *33*, 318.
15. Dubos, R. J., Smith, J. M., and Schaedler, R. W.: Metabolic disturbances and infection. *Proc. roy. Soc. Med.*, 1955, *48*, 911.
16. Kass, E. H.: Hormones and host resistance to infection. *Bact. Rev.*, 1960, *24*, 177.
17. Cohn, Z. A. and Morse, S. I.: Functional and metabolic properties of polymorphonuclear leucocytes. II. The influence of a lipopolysaccharide endotoxim. *J. exp. Med.*, 1960, *111*, 689.
18. Rowley, D.: Antibacterial systems of serum in relation to nonspecific immunity to infection. *Bact. Rev.*, 1960, *24*, 106.