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

DETERMINANTS OF INFECTION IN THE PERITONEAL CAVITY

III. The Action of Selected Inhibitors on the Fate of *Staphylococcus aureus* in the Mouse**

The preceding paper presented data illustrating the importance of the number of phagocytes on the fate of *S. aureus* in the peritoneal cavity of the mouse. These findings, in many instances, took place under conditions in which there was no major change in the functional activity of the individual mononuclear or polymorphonuclear leucocyte. Previous studies from this laboratory have demonstrated *in vitro* that inhibitors of glycolysis interfere with the phagocytic activity of the rabbit peritoneal exudate granulocyte. Certain of these agents, i.e., arsenite, have been employed *in vivo* by Berry and Mitchell[1] and diminish the survival time of mice infected intraperitoneally with *Salmonella typhimurium.* Other inhibitors and intermediates, e.g., malonate, citrate and succinate, were also studied by the same group,[1,2] and it was suggested that they interfered with the normal functioning of the Krebs, tricarboxylic acid cycle. The type of cell, or more likely cells, influenced by these compounds was not elucidated, nor was the site of action in the animal reported. Infected animals at the time of death exhibited the same microbial titers as those found in untreated animals,[3] but did demonstrate a more rapid and pronounced bacteremia following intraperitoneal injection.[4] Leucocytic function as assayed by the smear technique on blood granulocytes was within a normal range, and this resistance mechanism was not considered to be an influential factor in accelerating the infectious process.[3] More recently, other investigators have reported that malonate interferes with the intracellular killing of gram-negative bacteria by mouse peritoneal macrophages.[5]

In view of the important alterations in host resistance brought about by these agents, a study was conducted aimed at elucidating the mechanisms involved in the enhanced susceptibility to staphylococcal infection of mice treated with metabolic inhibitors.

---

* 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.

MATERIALS AND METHODS

The basic methods for the analysis of events in the peritoneal cavity have been described previously.[6] The Smith strain of *Staphylococcus aureus* was employed for all *in vivo* experiments.

Each of the inhibitors and intermediates was dissolved in pyrogen-free saline or 0.01 M phosphate buffered saline and adjusted to a final pH of 7.2. A variety of concentrations were employed and each was delivered intraperitoneally in a volume of 0.2 ml.

In vitro experiments with rabbit polymorphonuclear leucocytes. Rabbit peritoneal exudate PMN leucocytes were collected and processed under previously described conditions.[7] The particle presented for ingestion was a washed, viable suspension of *S. albus*. The final reaction mixture contained 30 x 10^6 PMN/ml., 33 x 10^6 staphylococci/ml. and 10 per cent fresh normal rabbit serum in a total volume of 2.0 ml. Malonate was incorporated into the suspension of leucocytes at concentrations from 10^{-4} M to 5 x 10^{-2} M and the mixture shaken at 37° C. in a constant temperature bath. After 10 minutes the staphylococcal inoculum was added and samples taken at zero, 60 and 120 minutes for the evaluation of total and extracellular bacteria.

RESULTS

THE EFFECT OF MALONATE ON THE FATE OF *S. AUREUS* IN THE PERITONEAL CAVITY

The initial studies were performed with malonate, a compound which had been previously reported[8] to increase the susceptibility of the mouse to a variety of test pathogens including *S. aureus*. In the current investigation a more detailed examination was performed on the events occurring within the peritoneal cavity, since this was the major locus for the control of the intraperitoneal inoculum of staphylococci in the unaltered mouse.

Preliminary studies employing a variety of malonate concentrations and injection schedules revealed that 20 g. animals receiving 0.1 mmole per hour for six doses died following challenge with 10^6 staphylococci. Larger doses of malonate, i.e., 0.14 mmole, the concentration employed by Berry,[8] resulted in the more rapid death of the animals. Continuation of malonate injections up to the eighth hour and/or a larger challenge inoculum of staphylococci, i.e., 4 x 10^7, also resulted in the more rapid death of the mice. Mice which received 0.1 or 0.14 mmole for six doses did not succumb to the inhibitor alone and no deaths were recorded in a series of 72 animals. A reduction of the malonate dosage to 0.05 mmole x 6 did not result in the death of any of the mice challenged with 4 x 10^6 staphylococci. On the basis of these titrations more detailed studies were performed employing a six dose schedule and a malonate concentration of 0.1 mmole (0.005 mmole/g.).

Figure 1(A) illustrates one such experiment in which mice died between the tenth and twelfth hours after challenge. It is apparent that the repeated

injection of malonate resulted in the quantitative survival of the initial inoculum in the peritoneum for the first few hours. Thereafter, bacterial multiplication occurred and at the sixth hour was exponential in character. Under the stimulus of the repeated injections of malonate there was a vigorous influx of polymorphonuclear leucocytes and a rapid increase in the total leucocyte population. The parallel increase in both the extra-

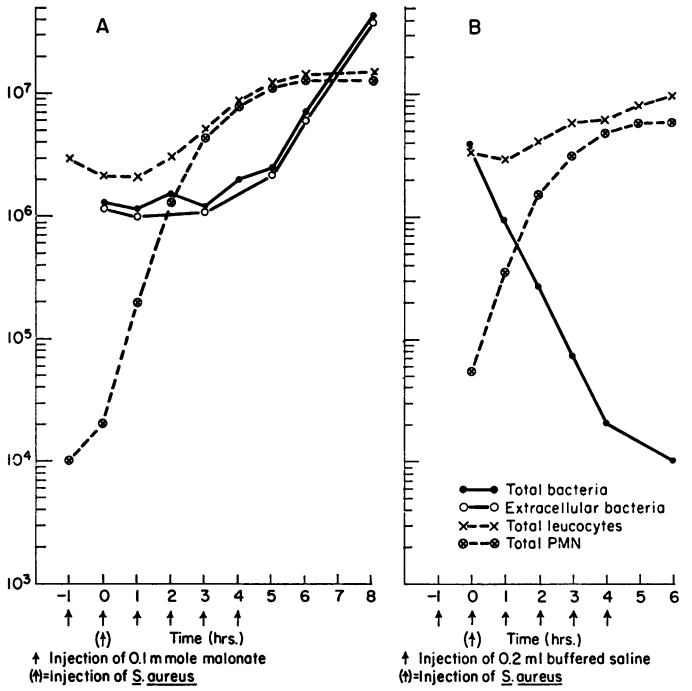

FIG. 1. The influence of the intraperitoneal administration of sodium malonate and physiological saline on the course of staphylococcal infection. (A) Sodium malonate 0.1 mmole x 6, (B) Saline 0.9% x 6.

cellular and total bacterial populations, in the presence of large numbers of phagocytes, indicated that malonate had inhibited the phagocytic process. Repeated examinations of leucocytes in the hemocytometer and on stained smears revealed few intracellular bacteria and supported this conclusion. The primary effect of this concentration of malonate was, therefore, not on the intraleucocytic bactericidal mechanism but on the ingestion phase.

Figure 1(B) represents a control experiment performed with the same number of bacteria but in animals receiving injections of isotonic saline. The phagocytosis and killing of bacteria took place rapidly and was

similar to that reported previously in the unaltered mouse.[6] There was, however, a more rapid and greater influx of granulocytes, which was probably related to the larger total volume of fluid and repeated trauma to the peritoneum. This experiment also demonstrated, that within these limits, the volume *per se* of injectant was without effect on the rate of destruction of *S. aureus.*

In Vitro studies with malonate. Additional experiments were performed *in vitro* employing the rabbit peritoneal exudate granulocyte.

Figure 2 illustrates the influence of various concentrations of malonate on the phagocytosis and killing of *S. albus* by rabbit granulocytes. Line F represents the system in the absence of malonate and indicates that in a two hour period 90 per cent of the original inoculum had been killed. The extracellular population at this time was identical with the total number of remaining bacteria. Phagocytosis and intracellular killing therefore took place promptly. In the presence of 10^{-4} and 10^{-3} M malonate (D, E) there was a slight but insignificant reduction in the ingestion and killing of staphylococci. At higher concentrations, such as 10^{-2} M, inhibition of killing occurred. This inhibition resulted from decreased phagocytosis rather than intracellular survival, as evidenced by the number of extracellular bacteria. Only at 5×10^{-2} M did malonate completely inhibit the phagocytic process and allow extracellular bacteria to multiply. Line B shows that malonate in the absence of leucocytes had no bactericidal effect on this organism. The high concentration of malonate required to inhibit phagocytosis was in marked contrast to other inhibitors such as iodoacetate and arsenite which were effective *in vitro* at 1/1000 of his dose.[9] Other experiments, not shown, in which leucocytes were exposed to 5×10^{-2} M malonate for 60 minutes, then washed and resuspended in a serum-containing medium, revealed that such cells were incapable of phagocytosis. This suggested that malonate effect *in vitro* was directly on the leucocyte and was irreversible after this period of exposure.

The influence of the injection schedule. From the previous experiments it was clear that malonate inhibited the destruction of staphylococci within the peritoneal cavity and that this effect was the result of an altered ingestion phase. Additional studies were then carried out to elucidate the effect on both the mononuclear and granulocytic leucocytes of the peritoneal cavity and to determine the influence of the injection schedule on the fate of bacteria.

The results of one such experiment are presented in Table 1. The administration of malonate for four hours after challenge with staphylococci (Group A) resulted in the subsequent death of 8/10 animals within

TABLE 1. THE INFLUENCE OF THE INJECTION SCHEDULE OF MALONATE ON THE FATE OF *S. aureus* IN THE MOUSE PERITONEAL CAVITY

	A		*B*		*C*		*D*	
Time hrs.	*Material injected i.p.*	*Total bacteria*	*Material injected i.p.*	*Total bacteria*	*Material injected i.p.*	*Total bacteria*	*Material injected i.p.*	*Total bacteria*
minus .25	malonate*		malonate		malonate		malonate	
0	*S. aureus*	4.1×10^6	*S. aureus*	4.1×10^6	*S. aurcus*	4.1×10^6	*S. aureus*	4.1×10^6
1	malonate	....	malonate	....	malonate	....	malonate	3.9×10^6
2	malonate	5.3×10^6	malonate	4.9×10^6	malonate	5.4×10^6	....	5.8×10^5
3	malonate	....	malonate	6.9×10^6	....	8.2×10^5	....	9.3×10^4
4	malonate	8.6×10^6	....	7.6×10^5	....	4.1×10^5	....	4.3×10^4
6	....	7.2×10^7	....	8×10^4	....	5.3×10^4	....	....
Survivors total at 24 hrs.	2/10		8/8		10/10		8/8	

* 0.1 mmole malonate injected at each time interval in a volume of 0.2 ml.

24 hours. The number of bacteria present in the peritoneal cavity rose steadily and at six hours was over a log unit higher. Although malonate injections were discontinued at the fourth hour, the bacteria continued to multiply thereafter and were able to kill the majority of such animals.

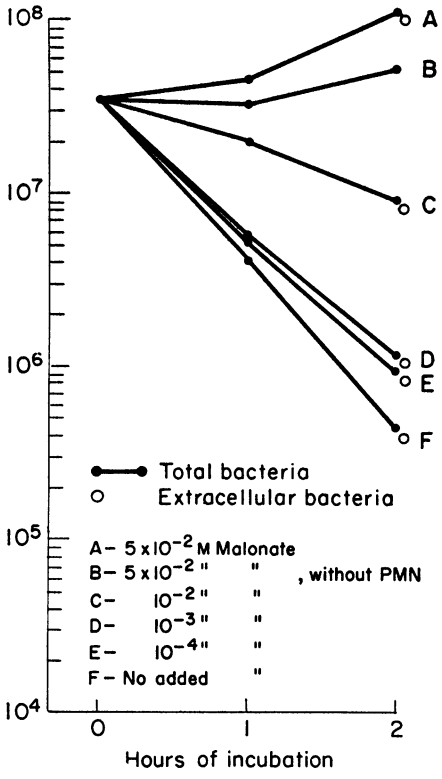

FIG. 2. The *in vitro* influence of malonate on the functional activities of rabbit polymorphonuclear leucocytes.

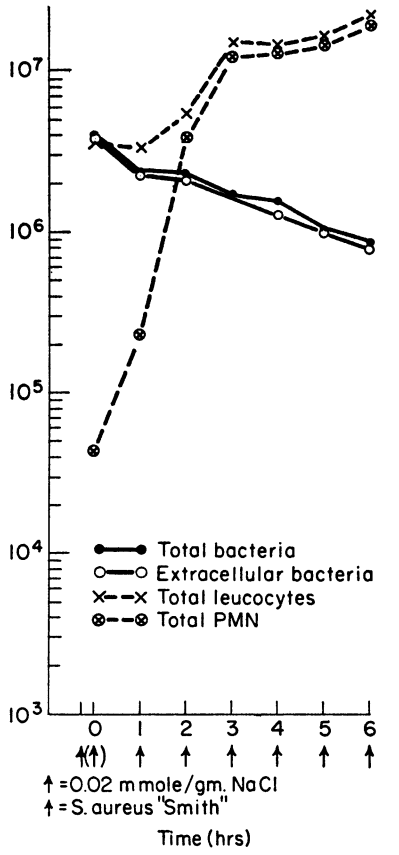

FIG. 3. The influence of hypertonic saline on the fate of *S. aureus* in the peritoneal cavity.

These data are to be contrasted with Groups B-D in which malonate was injected for shorter periods of time. In these cases none of the animals succumbed to the infection and this was mirrored by the fate of the intraperitoneal inoculum. In all cases the cessation of malonate injections from the first (D) to the third (B) hour was followed by a prompt reduction in the total viable bacterial count. During the period of malonate

administration, however, there was no net bactericidal effect in the peritoneal cavity and limited bacterial multiplication took place. The critical time, therefore, under these particular conditions, for the continuing multiplication of bacteria, appeared to be the fourth hour post-infection.

Total and differential leucocyte counts performed in this experiment were similar to those described in Figure 1(A). It was of interest that the maximum total and polymorphonuclear cell counts occurred between the fourth and fifth hours, at the time when the malonate effect was essentially irreversible. During the first three hours after infection, the cessation of malonate injections was followed by the continuing influx of viable leucocytes which were capable of destroying staphylococci. If malonate was continued up to the fourth hour, more than 90 per cent of the phagocytes which accumulated in the peritoneum had been exposed to high concentrations of the inhibitor and were apparently nonfunctional. The small number of cells which entered after the fifth hour and which had not been in contact with malonate, were then unable to control the multiplying bacteria. It also seems clear that malonate exerted an effect on the phagocytic activities of both mononuclear and polymorphonuclear cell populations, since the inhibited bactericidal process during the first hour occurred in the presence of mononuclear cells alone.

The mechanism of action of malonate in the peritoneal cavity. The previous data suggested that malonate at high dosage levels inhibited the phagocytic activity of peritoneal phagocytes. From the *in vitro* studies it appeared that this effect was irreversible and that the injection sequence *in vivo* was dependent upon the inactivation of the majority of leucocytes which were able to enter the peritoneal cavity. The mode of action by which malonate brought about these changes was not clear. Because malonate was effective only at high concentrations, a number of mechanisms were considered. The first tested was the possible specific effect as a competitive inhibitor of succinic dehydrogenase.[10] Experiments were therefore conducted on the ability of succinate to reverse the antiphagocytic activity of malonate.

The results of this experiment revealed that succinate even at the highest ratio did not reverse the inhibition of malonate but instead each of the mixtures was more potent than malonate alone.* In the group receiving malonate alone (No. 1) 8/12 animals died within 24 hours

* Four groups of 20 mice received mixtures of malonate and succinate according to the schedules and volumes presented in Fig. 1: (1) 0.1 mmole malonate alone, (2) 0.1 mmole malonate and 0.05 mmole succinate, (3) 0.1 mmole malonate and 0.1 mmole succinate and (4) 0.1 mmole malonate and 0.2 mmole succinate.

whereas none of the animals of groups No. 2-4 survived the infection. Animals injected with the malonate-succinate mixtures and not challenged with staphylococci did not succumb. These data, although of little use in determining the mechanism of action of malonate, did, however, suggest that the concentration of material injected was of importance. This raised the possibility that malonate was producing a nonspecific effect on peritoneal phagocytosis by virtue of the ionic strength of the injectant or perhaps a chelating effect. The latter possibility was rendered unlikely by the following experiments. Neither calcium, magnesium nor a mixture of the two had any reversing effect on the depressed phagocytosis brought about by this concentration of malonate.* In fact, the presence of the divalent cations lead to a more rapid intraperitoneal multiplication and death of the animals. From this experiment it seemed unlikely that the mechanism of action of malonate was to chelate divalent cations locally and therefore interfere with the phagocytic process.

The speculation that malonate was interfering by means of ionic strength was next evaluated. It was known from previous experiments (Fig. 1B) that isotonic saline had no influence on the fate of staphylococci or on the function of peritoneal leucocytes. More concentrated solutions of saline were then employed under the same experimental conditions.

At a concentration of 24 mg./ml. (Fig. 3) sodium chloride markedly inhibited the phagocytosis of staphylococci and produced a prompt influx of polymorphonuclear leucocytes.** None of the animals, however, died as the result of the infection. At the highest dosage level, 32 mg./ml., the bacteria multiplied within the cavity and were able to kill 7/10 animals within 24 hours. At 18 mg./ml. there was only a slight inhibition of phagocytosis and the peritoneal leucocytes controlled the infection. From these results it appeared that increasing the ionic strength or "tonicity" of sodium chloride solutions produced a progressive inhibition of the phagocytic process in the peritoneal cavity, and was analogous to the data obtained with malonate. The exact ionic strength of the malonate solution is difficult to evaluate in the peritoneal cavity but it is likely that it is at least as high as the 24 mg./ml. solution of saline. These findings would to a large extent explain the influence of malonate on the phagocytic process in the peritoneal cavity.

---

* Six groups of 20 mice were injected according to the schedule described for Fig. 1. Each group received 0.1 mmole malonate along with (1) 0.05 mmole $CaCl_2$, (2) 0.1 mmole $CaCl_2$, (3) 0.05 mmole $MgCl_2$, (4) 0.1 mmole $MgCl_2$, (5) 0.05 mmole $CaCl_2$ and 0.05 mmole $MgCl_2$, (6) malonate alone.

** Mice were injected intraperitoneally with 0.2 ml. of sodium chloride solutions of 9, 18, 24 and 32 mg./ml., according to the schedule outlined for Fig. 1.

THE INFLUENCE OF CITRATE

The use of sodium citrate to depress the resistance of the mouse to staphylococcal infection has been previously reported by Berry and Mitchell.[1] This metabolic intermediate at repeated intraperitoneal doses of 10 mg. reduced the survival time of animals in a similar fashion to that

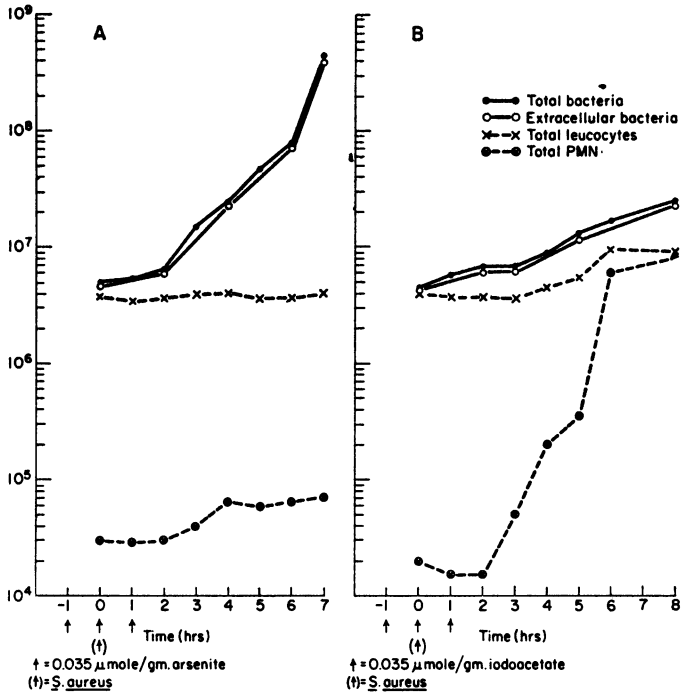

FIG. 4. The effect of arsenite and iodoacetate on the fate of and response to *S. aureus* in the peritoneal cavity. (A) Arsenite 0.035 μmole/g. x 3, (B) Iodoacetate 0.035 μmole/g. x 3.

of malonate. This compound was next evaluated under the following experimental conditions.

Citrate at concentrations of 5 and 10 mg./ml. in isotonic saline was administered according to the injection schedule outlined for Fig. 1. The challenge inoculum of *S. aureus* was between 4-6 x 10^6 organisms. Following the administration of 1.7 μmole/g. for five doses, an intraperitoneal response was obtained which was essentially the same as that with 0.005 mmole/g. of malonate. The rate of influx and numbers of phagocytes was within normal limits yet there was progressive extracellular multiplication

of the bacteria which resulted in the death of 80 per cent of the animals within 24 hours. It was clear that citrate at this concentration severely inhibited the phagocytic process. The influence of citrate could not be reversed by calcium, magnesium or a mixture of the two. A reduction of the citrate dose of 0.85 μmole/g. resulted in a progressive decline of viable bacteria but at a slightly slower rate than in the unaltered mouse. No deaths were produced by either concentration of citrate in 40 uninfected animals. The lower levels of citrate required to produce this effect may in part be a reflection of a higher ionic strength than that of malonate.

TABLE 2. THE INFLUENCE OF ARSENITE, P-CHLOROMERCURIBENZOATE AND IODOACETATE ON THE MORTALITY OF MICE INFECTED WITH *S. aureus* SMITH

Inhibitor	*Quantity inhibitor injected i.p.**	*Challenge inoculum*	*Treated mice: survivors/total 24 hrs.*	*Untreated controls: survivors/total 24 hrs.*
Arsenite	.035 μmole/g. x 3	$2x10^8$ *S. aureus*	0/15	10/10
PCMB	.035 μmole/g. x 3	"	1/18	8/8
Iodoacetate	.035 μmole/g. x 3	"	0/20	10/10

* Same schedule as shown in Figure 4.

THE EFFECT OF ARSENITE, IODOACETATE, AND P-CHLOROMERCURIBENZOATE

The next series of studies were conducted with a group of enzymatic inhibitors which produce metabolic alterations at much lower concentrations and would not be expected to function on the basis of ionic strength.

Figure 4(A) shows the influence of arsenite on the course of a staphylococcal infection in the mouse peritoneum. After the injection of 5 x 10^6 organisms, there was a short lag period followed by a rapid increase in the numbers of both total and extracellular bacteria. The onset of growth was quite abrupt and the rate approached that seen under ideal conditions *in vitro*. Phagocytosis was again inhibited but in contrast to the previous results, drastic changes occurred in the influx of granulocytes. During the course of seven hours there was essentially no change in the number of recoverable phagocytes in the peritoneum and these remained almost entirely mononuclear in type. Animals injected with this amount of arsenite alone appeared ill and exhibited watery diarrhea. It is possible that the animals were hypotensive and that this altered hemodynamic state accounted for the lack of diapedesis of granulocytes.

The results with iodoacetate are described in Figure 4(B). This compound at the same concentration produced similar effects on both phagocytosis and the granulocyte response. Bacterial multiplication ensued but was less rapid than with arsenite. The influx of granulocytes, although inhibited for the first five hours, eventually occurred and was accompanied by a rise in the total cell count. Animals injected with this dose of iodoacetate did not appear ill, in contrast to those receiving arsenite.

The data with p-chloromercuribenzoate are not presented but were intermediate between those obtained with arsenite and iodoacetate. Phagocytosis and diapedesis were again inhibited.

The effect of each of the three compounds on the susceptibility of mice to 2 x 10^6 staphylococci is shown in Table 2. Each of these compounds converted a nonlethal challenge into one which produced deaths within 24 hours. The smallest number of bacteria required to kill the treated mouse was not determined accurately, although with arsenite the challenge inoculum could be reduced tenfold (10^5) to yield similar mortality data.

DISCUSSION

The previous report in this series[11] presented evidence correlating the number of available phagocytes with the rate and efficiency of bacterial inactivation. Under these conditions, in which the phagocytic and bactericidal properties of intraperitoneal phagocytes was essentially normal, there appeared to be a rather constant ratio between the number of bacteria a given leucocyte population could destroy. The limiting factor in most instances was the presence in the peritoneal cavity of insufficient numbers of both mononuclear and polymorphonuclear phagocytes. It is clear that this is not the case in animals receiving the agents examined in the present study. Each of these substances interfered primarily with a single sequence of the peritoneal defense mechanism, namely, the process of phagocytosis. In all instances there was a dissociation between numbers of phagocytes and the bacterial population. The limiting factor in the present situation was the rate of phagocytosis.

Although the over-all site of action was similar, the mechanisms by which inhibitors such as malonate and arsenite influenced particle ingestion were apparently quite different. In this regard, malonate, citrate and hypertonic saline were effective only at high concentrations, much above what one would expect with a selective influence on metabolism. It seems probable that all three compounds influenced the phagocytic process in large measure, by virtue of ionic strength. Earlier reports in the literature

employing *in vitro* phagocytic systems have emphasized the deleterious effects of hypertonicity.[12, 13] Whether ionic strength exerts a major effect on the phagocyte *per se* and/or on the process of opsonization is not clear. A block at either site would, however, lead to similar results and allow extracellular multiplication of bacteria to occur. Factors other than ionic strength may be operative in the case of malonate and citrate although these are not apparent at this time. It is unlikely, however, that malonate brings about a specific metabolic derangement in the leucocyte since the intact cell is (1) relatively impermeable to this substance[14] and (2) malonate would not significantly alter glycolysis—the major energy source for the phagocytic process.[15, 16, 9]

The reversibility of the malonate and citrate block up to the fourth hour following challenge has been interpreted in terms of the influx of new phagocytes from the periphery until the time at which the total leucocyte population reaches maximum values. This association may not entirely explain the phenomenon. Certainly, many other changes have occurred after four to five hours of inflammation and it is conceivable that malonate and citrate may accumulate within the peritoneum and thereby exert a more prolonged effect during the later hours. Although the rate of escape and breakdown of malonate and citrate in the peritoneal cavity is not known, it seems that with the currently employed doses and schedules, that their influence lasts at least one hour. The effects of these compounds on extraperitoneal sites as well as the response of the treated animal to bacterial toxins remains unknown.

The more potent inhibitors such as arsenite, iodoacetate and p-chloromercuribenzoate differed both qualitatively and quantitatively. All three agents exerted a more prolonged influence on bacterial survival in the peritoneum and at considerably lower concentrations than did malonate or citrate. In addition to blocking phagocytosis, each of the compounds inhibited the diapedesis of leucocytes to a greater or lesser extent. This was most clearly demonstrated with arsenite which blocked almost completely and led to a rapidly overwhelming infection. Although the main determinant for leucocyte diapedesis is considered to be the rate of blood flow through capillaries,[17] it is possible that such potent sulfhydryl enzyme inhibitors might also affect the motility of intravascular white cells and the function of the endothelial cell. From *in vitro* studies it seems clear that agents such as arsenite and iodoacetate which effectively block leucocyte glycolysis also interfere with the process of particle ingestion. Such a mechanism if operative *in vivo* could account for the depressed function of both mononuclear and polymorphonuclear leucocytes.

SUMMARY

A study has been conducted on the mechanisms by which selected inhibitors interfere with the normal host defense reactions against staphylococcal infection of the mouse peritoneal cavity.

Malonate at six hourly doses of 0.005 mmole/g. inhibited the rate of bacterial inactivation within the peritoneum and allowed a sublethal inoculum to kill the mouse within 24 hours. This effect was primarily the result of the depressed phagocytic function of both mononuclear and polymorphonuclear leucocytes in the peritoneal cavity. *In vitro* studies with rabbit granulocytes showed that malonate at 5×10^{-2} M concentration completely blocked phagocytosis, whereas at 10^{-3} M it was without effect. Hypertonic saline exerted a similar effect *in vivo,* whereas isotonic saline allowed phagocytosis to proceed normally. The addition of succinate, calcium or magnesium ions to solutions of malonate failed to reverse the *in vivo* blockade and, in fact, accentuated it. It appeared that the malonate inhibition of phagocytosis was largely on the basis of ionic strength rather than of a specific metabolic effect.

Repeated intraperitoneal injections of 1.7 μmole/g. citrate yielded similar data, probably as the result of the same mechanism.

Arsenite, iodoacetate and p-chloromercuribenzoate were all potent inhibitors of intraperitoneal phagocytosis at doses of 0.035 μmole/g. In addition, each agent, particularly arsenite, reduced the influx of granulocytes from the periphery.

REFERENCES

1. Berry, L. J. and Mitchell, R. B.: The relation of the tricarboxylic acid cycle to bacterial infection. II. The effect of fluoracetate, arsenite, citrate and succinate on *Salmonella typhimurium* infections in mice. *J. infect. Dis.,* 1953, *93,* 83.
2. Berry, L. J. and Mitchell, R. B.: The relation of the tricarboxylic acid cycle to bacterial infection. I. The effect of malonate on *Salmonella typhimurium* infections in mice. *J. infect. Dis.,* 1953, *93,* 75.
3. Berry, L. J.: Effect of components of the tricarboxylic acid cycle on bacterial infections. *Ann. N. Y. Acad. Sci.,* 1956, *66,* 370.
4. Berry, L. J. and Mitchell, R. B.: Effect of malonate on bacteremia developed in mice artificially infected with *Salmonella typhimurium. Proc. Soc. exp. Biol. (N. Y.),* 1954, *85,* 209.
5. Nelson, E. L. and Becker, J. R.: The effect of malonate on the bactericidal action of phagocytes. *Bact. Proc.,* 1957, 95.
6. Cohn, Z. A.: Determinants of infection in the peritoneal cavity. I. The response to and fate of *Staphylococcus aureus* and *Staphylococcus albus* in the mouse. *Yale J. Biol. Med.,* 1962, *35,* 12.
7. Cohn, Z. A. and Morse, S. I.: Interactions between rabbit polymorphonuclear leucocytes and staphylococci. *J. exp. Med.,* 1959, *110,* 419.
8. Berry, L. J., Merritt, P., and Mitchell, R. B.: The relation of the tricarboxylic acid cycle to bacterial infection. *J. infect. Dis.,* 1954, *94,* 144.

9. Cohn, Z. A. and Morse, S. I.: Functional and metabolic properties of polymorphonuclear leucocytes. I. Observations on the requirements and consequences of particle ingestion. *J. exp. Med.*, 1960, *111*, 667.
10. Krebs, H. A., Gurin, S., and Eggleston, L. V.: The pathway of oxidation of acetate in baker's yeast. *Biochem. J.*, 1952, *51*, 614.
11. Cohn, Z. A.: Determinants of infection in the peritoneal cavity. II. Factors influencing the fate of *Staphylococcus aureus* in the mouse. *Yale J. Biol. Med.*, 1962, *35*, 00-00.
12. Hamburger, H. J.: *Physikalisch-Chemische untersuchungen über phagozyten.* Wiesbaden, 1912.
13. Wright, A. E. and Reid, S. T.: On spontaneous phagocytosis, and on the phagocytosis which is obtained with heated serum of patients who have responded to tubercular infection, or, as the case may be, to the inoculation of a tubercular vaccine. *Proc. roy. Soc. B*, 1906, *77*, 211.
14. Martin, S. P., McKinney, G. R., and Green, R.: The metabolism of human polymorphonuclear leucocytes. *Ann. N. Y. Acad. Sci.*, 1955, *59*, 996.
15. Becker, H., Munder, G., and Fischer, H.: Über deu leukocytenstoffwechsel bei der phagocytose. *Z. physiol. Chem.*, 1958, *313*, 266.
16. Sbarra, A. J. and Karnovsky, M. L.: The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles of polymorphonuclear leukocytes. *J. biol. Chem.*, 1959, *234*, 1355.
17. Miles, A. A. and Niven, J. S. F.: The enhancement of infection during shock produced by bacterial toxins and other agents. *Brit. J. exp. Path.*, 1950, *31*, 73.

Articles are accepted for publication in *The Yale Journal of Biology and Medicine* on the basis of merit and suitability. The privilege of submitting articles is not limited to those associated with Yale University and contributions from others are cordially invited.

The Board of Editors