# STUDIES ON BACTERIEMIA

## V. The Effect of Simultaneous Leukopenia and Reticuloendothelial Blockade on the Early Blood Stream Clearance of Staphylococci and Escherichia coli*

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The injection of living bacteria into the circulation of experimental animals is followed by their swift disappearance from the blood. Both the reticuloendothelial system and circulating granulocytes have been shown to engulf microorganisms during these initial moments of bacteriemia. This phase of blood stream clearance has appeared relatively inviolate. The production of profound granulocytopenia, reticuloendothelial "blockade," the administration of adrenal steroids, or the simultaneous presence of overwhelming infection or shock have all failed to significantly modify the initial rapid removal process (1).

Study of earlier unsuccessful attempts to modify clearance suggested that the two major cellular systems involved in clearance, the reticuloendothelial tissue and circulating leukocytes might, on occasion, operate reciprocally in the altered host. Impairment of only one removal mechanism might not be detected by usual cultural techniques because of compensatory changes in the clearance capacity of the other. Obviously the importance of circulating polymorphonuclear leukocytes in clearance would depend on the intracellular fate of the bacteria ingested by these circulating cells.

The present studies were designed to determine whether alterations in clearance might be uncovered by simultaneous reticuloendothelial "blockade" and granulocytopenia, using two microorganisms which differ in their fate within polymorphonuclear leukocytes.

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## *Materials and Methods*

Male albino rabbits weighing 2.0 to 3.4 kg. were used in the present experiments. Indwelling polyethylene catheters were placed in the superior vena cava and hepatic vein *via* the right jugular vein under sodium pentobarbital anesthesia (2). Animals were allowed to recover fully from anesthesia before clearance studies were performed.

*Cultures.*—The Smith strain of staphylococcus harvested from 18 hour infusion broth cultures was used as a representative staphylococcus. The characteristics of this strain have been previously reported (3, 4). Inocula ranging from $4.3 \times 10^8$ to $3.1 \times 10^9$ culturable bacteria were injected. This strain has been shown to survive within granulocytes (4). A strain of *Escherichia coli* originally obtained from Dr. Paul Beeson (5) was used. This microorganism does not survive within rabbit granulocytes (6). Inocula ranged from $8.0 \times 10^8$ to $3.0 \times 10^9$ culturable *E. coli*. All intravenous injections were made rapidly into the left marginal ear vein at zero time.

*Production of Leukopenia.*—Leukopenia was induced by a single intravenous injection of mechlorethamine hydrochloride (nitrogen mustard), 1.75 mg/kg. Maximum granulocytopenia was obtained 65 to 96 hours following injection. Total leukocyte counts ranged from 175 to 1475 per $mm.^3$ in all animals immediately before clearance experiments. Differential leukocyte counts revealed no detectable polymorphonuclear leukocytes in all instances. Total and differential white blood cell counts were repeated 30 minutes after the injection of bacteria to assure that a continuing leukopenia was present during clearance. Again differential leukocyte studies failed to reveal detectable circulating granulocytes.

*Production of Reticuloendothelial Blockade.*—Reticuloendothelial "blockade" was accomplished by intravenous administration of 8.0 ml. of thorotrast 19 to 24 hours prior to clearance studies using the method described by Martin, Kerby, and Holland (7). The efficiency of hepatic blockade was checked in 3 animals by repeated simultaneous cultures from superior vena cava and hepatic vein blood. All of these animals showed a significant reduction or complete cessation of hepatic trapping of bacteria.

*Enumeration of Circulating Bacteria.*—Blood cultures were obtained immediately prior to clearance in all animals receiving both thorotrast and mechlorethamine hydrochloride to exclude preclearance bacteriemia. Blood samples were obtained for quantitative culture at appropriate intervals during bacteriemia from the catheter residing in the superior vena cava, using precautions previously described (2). Blood specimens were delivered to sterile $100 \times 13$ mm. tubes containing dried heparin and plating procedures were carried out immediately. Samples were appropriately diluted in serial tenfold dilutions and delivered to infusion agar pour plates.

## RESULTS

### *Studies on the Early Clearance of Staphylococci*

Five normal animals received injections of $1 \times 10^9$ to $3.1 \times 10^9$ staphylococci of the Smith strain. As noted in Fig. 1, such injections resulted in a bacteriemia after 1 minute of approximately $10^6$ culturable staphylococci per ml. followed by rapid disappearance of bacteria during the first 10 to 15 minutes. Bacteriemia then persisted at levels of $10^3$ to $10^4$ culturable microorganisms per ml. As in previous studies, the early removal process appeared uniform and predictable. As shown in Fig. 1, the initial rate of removal and subsequent persistence of the Smith strain in the blood stream mirrored the bacteriemias obtained in 14 rabbits in 1956 using similar inocula of the MAM strain of coagulase-positive staphylococcus (2).

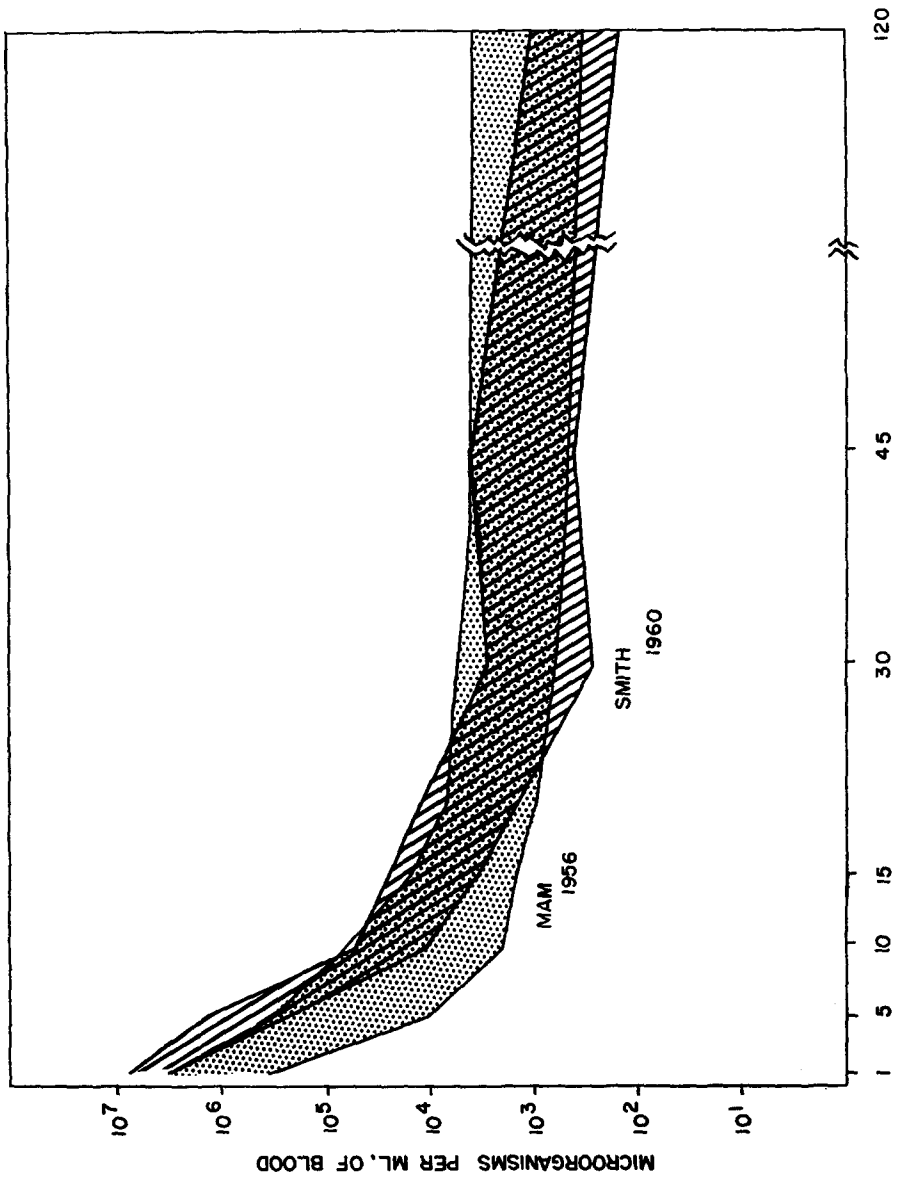


FIG. 1. The blood stream clearance of staphylococci in normal animals. Clearance curves obtained in 5 animals injected with the Smith strain are compared with those obtained in 1956 in 14 animals receiving the MAM strain (2).

*The Effect of Leukopenia.*—Three animals rendered leukopenic with nitrogen mustard failed to show significant impairment of the early blood stream clearance of staphylococci (Fig. 2). Indeed, circulating bacteria appeared to be removed somewhat more swiftly in leukopenic animals during the first 10 minutes following injection. These findings were similar to those obtained with the MAM strain of staphylococcus in previous studies (2).

*The Effect of Thorotrast.*—In contrast, animals receiving thorotrast showed a persistent impairment in early clearance (see Fig. 3). In the 3 animals so studied, bacteriemia ranged 10- to 100-fold higher than that obtained in normal animals during the first 45 minutes, and persisted at a higher level throughout the 2 hours. Despite this impairment, a steady decline in culturable circulating bacteria was noted over the 2 hour period of study.

*The Effects of Simultaneous Leukopenia and Reticuloendothelial Blockade.*—The simultaneous production of leukopenia and the administration of thorotrast failed to increase the impairment in clearance noted with thorotrast alone. Studies on 3 rabbits so prepared are noted in Fig. 4. Thus, in this instance, simultaneous impairment of splanchnic trapping and a profound reduction in circulating granulocytes did not materially increase the impairment in clearance noted with thorotrast blockade alone.

### *Studies on E. coli Bacteriemia*

The initial rate of disappearance of *E. coli* was slower than that of staphylococci in 5 animals receiving injections of $8.0 \times 10^8$ to $3.0 \times 10^9$ culturable bacteria. Clearance curves obtained in these animals showed some differences from the curves obtained with the same microorganism in 6 rabbits studied in 1957 when an inoculum of approximately $5 \times 10^8$ bacteria was used (6). The broad variation in the clearance of *E. coli* noted in past studies was again apparent. In the present experiments, a low grade *E. coli* bacteriemia persisted, in contrast to the total disappearance of *E. coli* often noted in 1957 in animals receiving the smaller inoculum. Comparative results are graphed in Fig. 5.

*The Effects of Leukopenia on Clearance of E. coli.*—Four animals rendered leukopenic showed a suggestive impairment of the early clearance of *E. coli* but the variation from animal to animal was great, and there was sufficient overlap in the bacteriemias noted in leukopenic and normal animals to render the impairment statistically insignificant (see Fig. 6).

*The Effect of Thorotrast on the Clearance of E. coli.*—Similar findings were obtained in 6 animals pretreated with thorotrast. Again, there was suggestive impairment in the early removal process, but the alterations in clearance were not of sufficient magnitude to assume statistical significance (see Fig. 7).

*The Effect of Simultaneous Leukopenia and Reticuloendothelial Blockade on the Clearance of E. coli.*—The simultaneous production of reticuloendothelial blockade and leukopenia resulted in a definite and significant impairment of

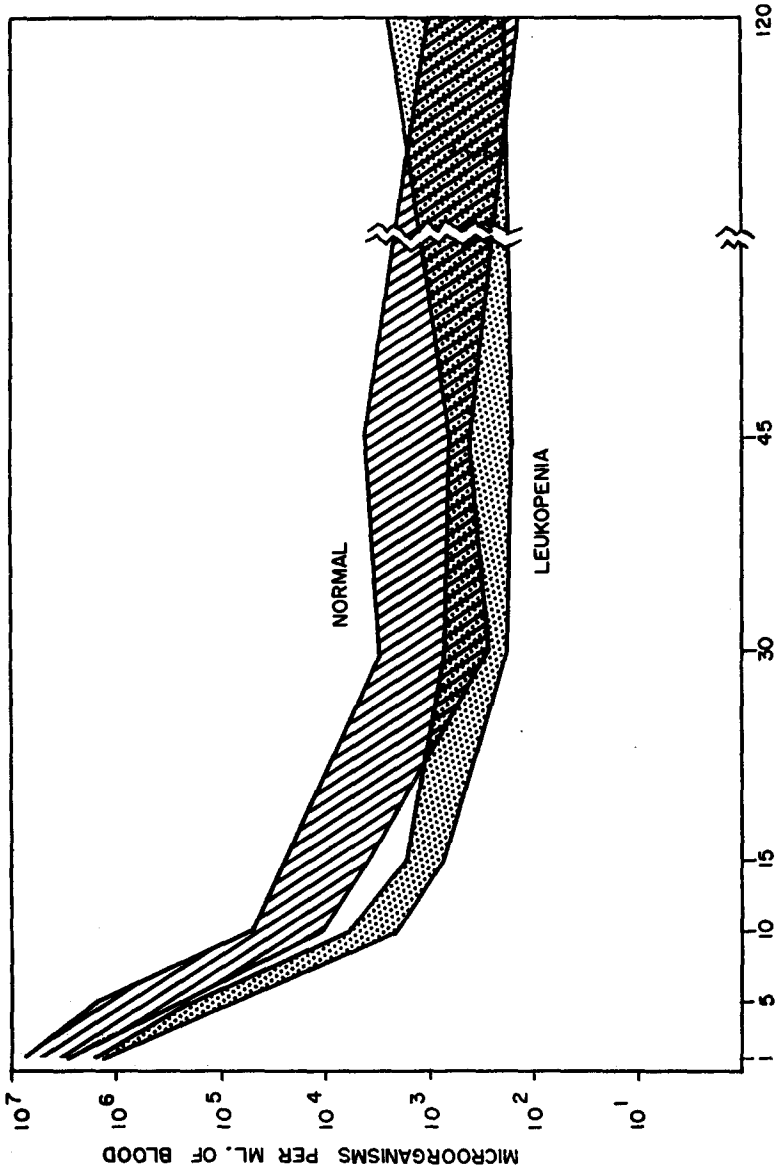


FIG. 2. The influence of leukopenia on blood stream clearance of staphylococci in 3 animals. No impairment in clearance is noted.

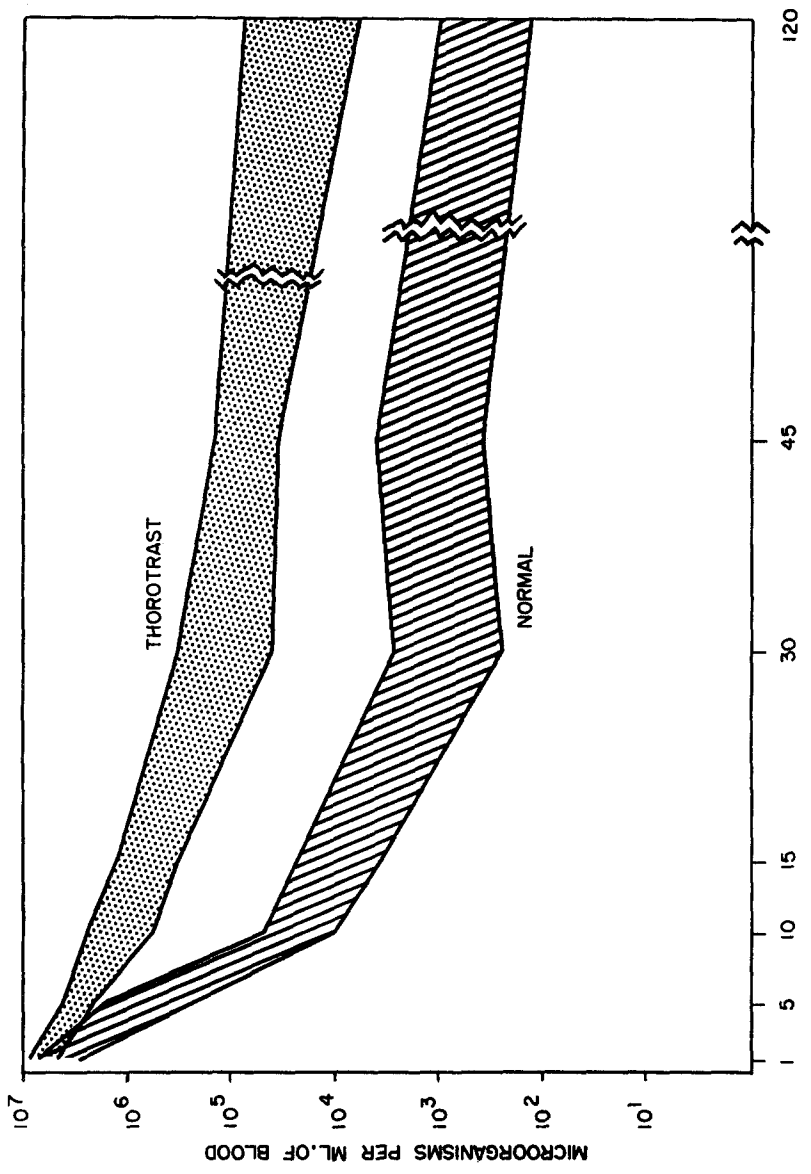


FIG. 3. The influence of thorotrast on the blood stream clearance of staphylococci in 3 animals. Definite impairment in clearance is evident.

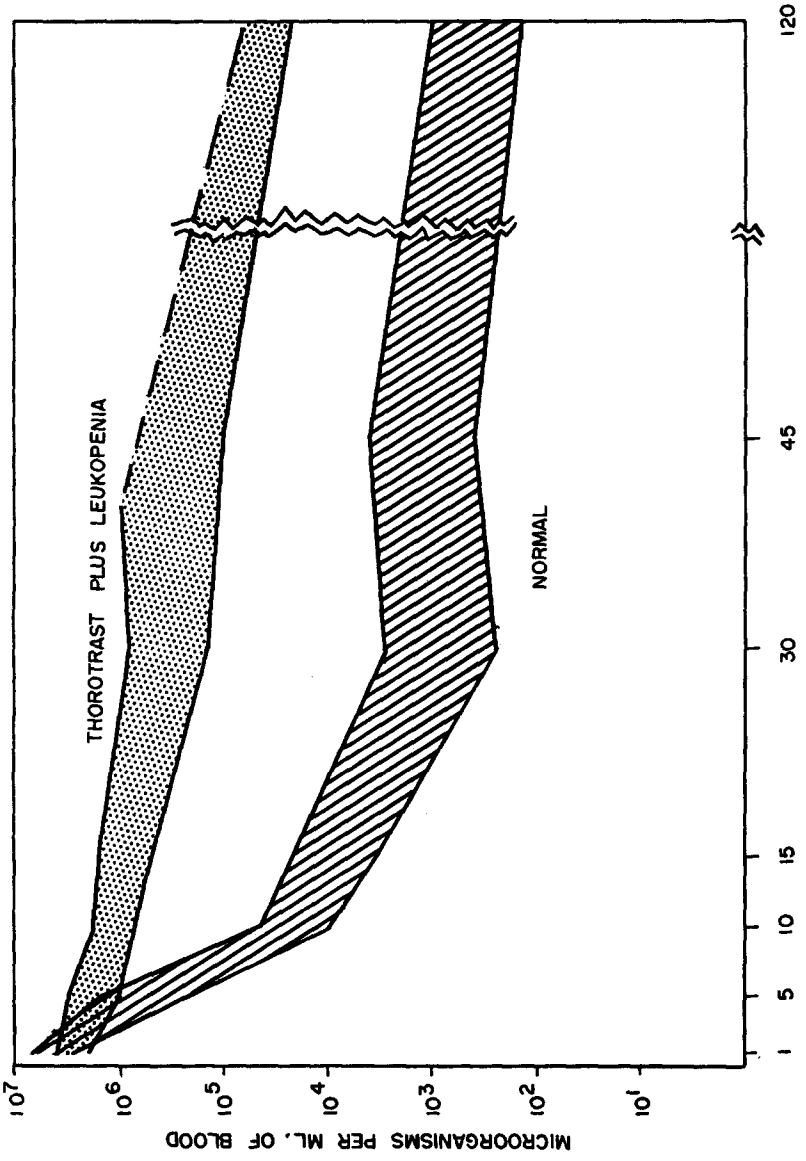


FIG. 4. The effect of simultaneous thorotrast "blockade" of the reticuloendothelial system, and leukopenia on blood stream clearance of staphylococci in 3 animals. The impaired clearance resembles that obtained with thorotrast alone.

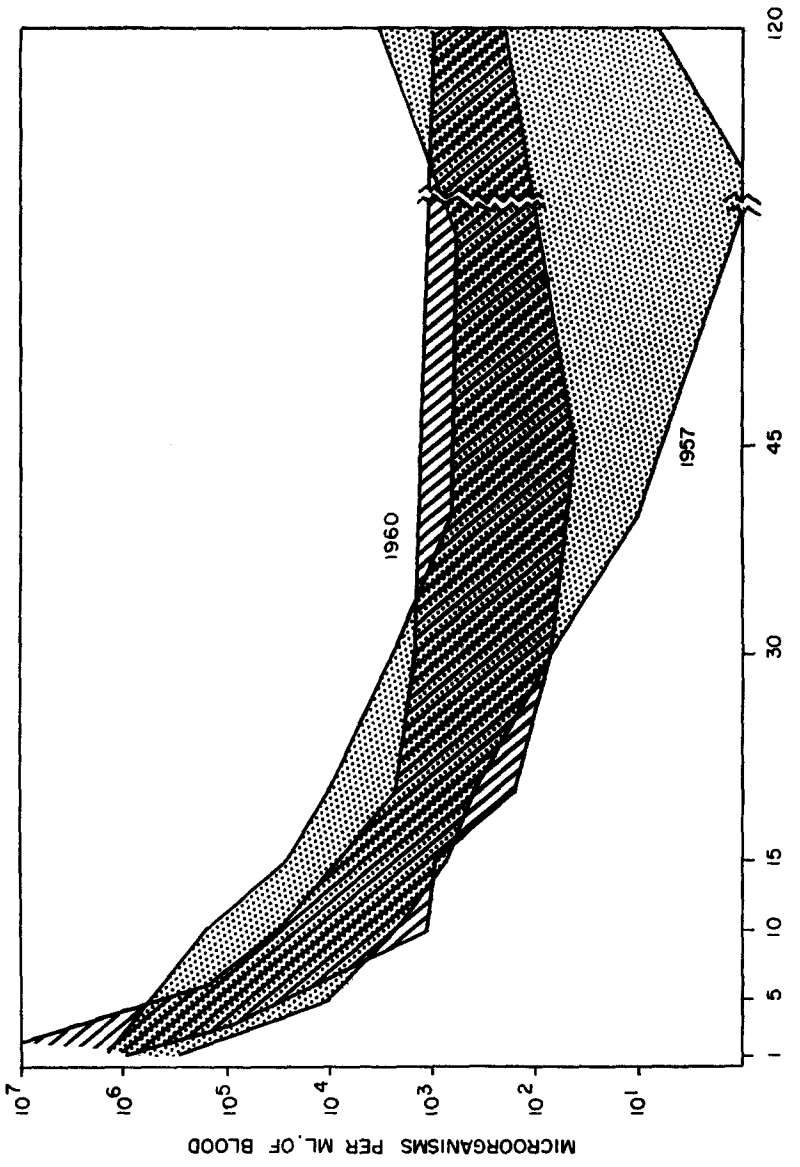


FIG. 5. The blood stream clearance of *E. coli* in normal animals. Clearance curves obtained in 5 animals receiving from $8.0 \times 10^8$ to $3.0 \times 10^9$ microorganisms are compared with those obtained in six animals receiving approximately $5 \times 10^8$ microorganisms in 1957 (6).

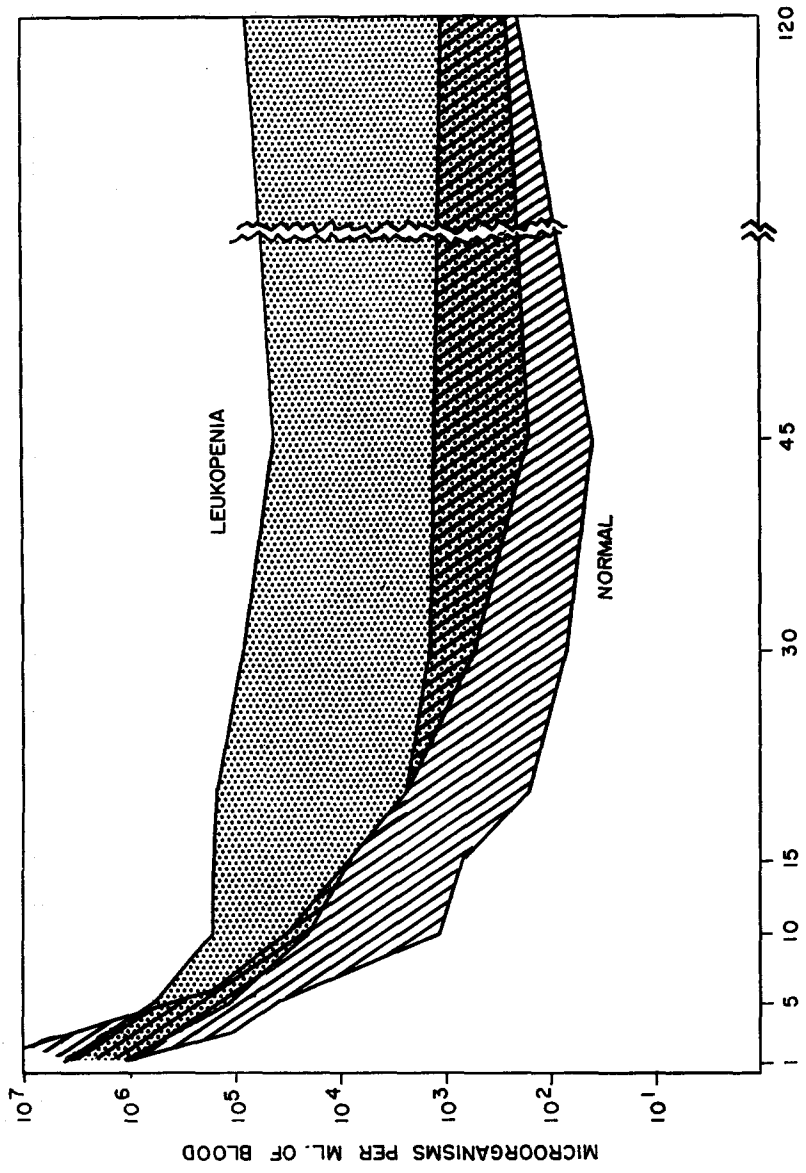


FIG. 6. The influence of leukopenia on blood stream clearance of *E. coli* in 4 animals. The differences in clearance are not statistically significant.

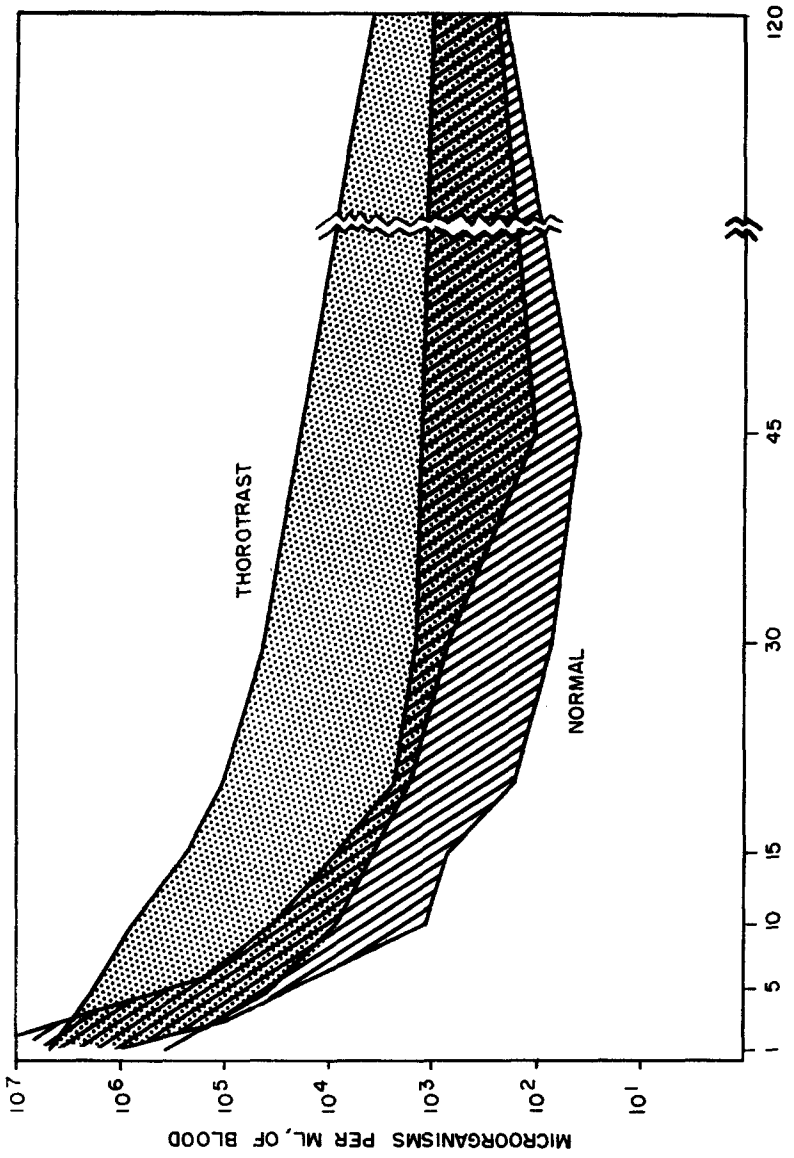


FIG. 7. The effect of thorotrast on the blood stream clearance of *E. coli* in 6 animals. Again the variation is wide and the differences in clearance are not significant.

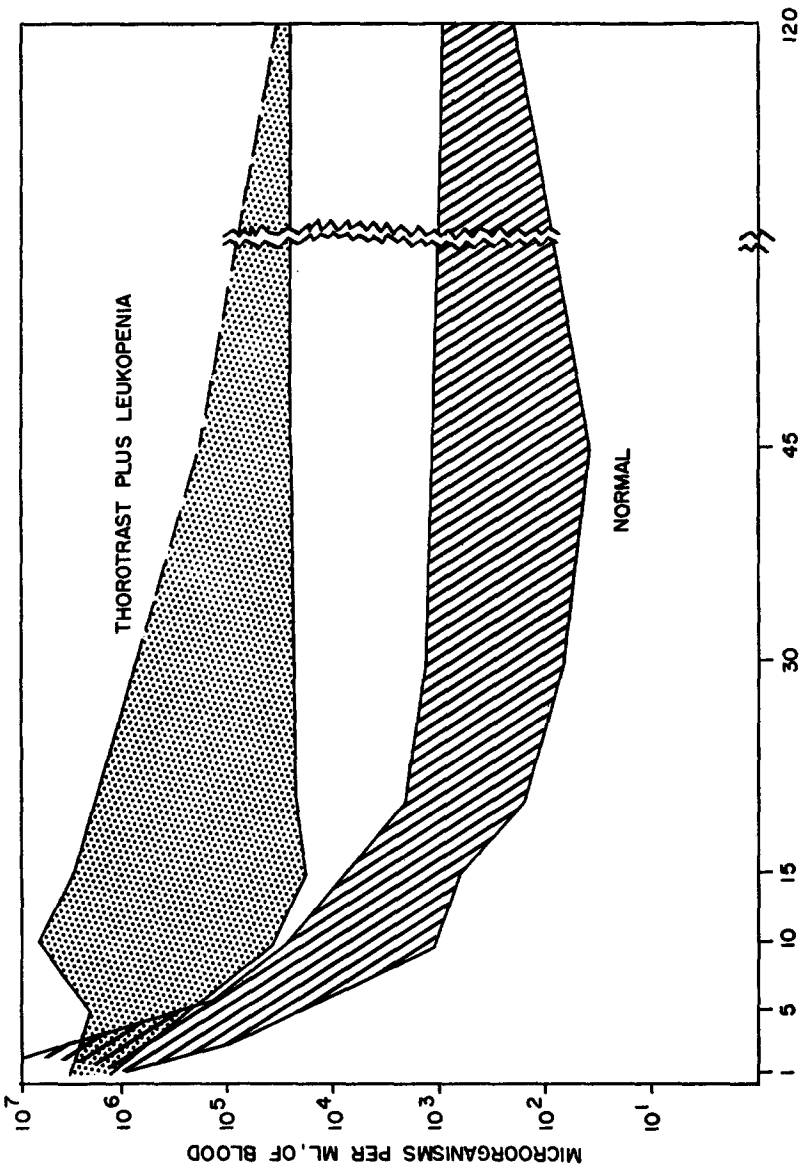


FIG. 8. The combined effect of thorotrast "blockade" of the reticuloendothelial system, plus an induced leukopenia on blood stream clearance of *E. coli* in 4 animals. Definite impairment of early clearance is evident.

early clearance. *E. coli* bacteriemias remained virtually constant in such animals for 30 to 45 minutes with a subsequent slow decline in circulating bacteria during the remainder of the 2 hour period of observation. At 30 to 45 minutes, a 10- to 1000-fold increase in the level of circulating bacteria was noted in the 4 animals so treated when compared with the bacteriemias observed in control animals. Thus, simultaneous reticuloendothelial blockade and severe granulocytopenia virtually abolished the early blood stream clearance of this microorganism.

## DISCUSSION

These studies support the belief that the reticuloendothelial system and circulating leukocytes can act in concert in the removal of certain bacteria entering the blood stream. They further suggest that the relative importance of these cellular clearance mechanisms may differ with different bacteria.

In the current experiments, the reticuloendothelial system appeared of primary importance in the clearance of circulating staphylococci. Severe leukopenia did not impede early staphylococcal clearance and did not enhance the impairment in clearance noted after reticuloendothelial "blockade" with thorotrast. We have previously shown that culturable staphylococci are found in association with the circulating granulocytes in the rabbit blood stream during persistent bacteriemia (2, 8). Furthermore, staphylococci often survive within these phagocytic cells (2). These findings, coupled with the present observations, strengthen the thesis that polymorphonuclear leukocyte ingestion may actually act to protect circulating staphylococci from removal from the circulation.

In contrast, neither profound leukopenia nor thorotrast blockade could alone modify consistently the early removal of *E. coli* from the blood stream. However, when trapping by the reticuloendothelial cells was impaired in animals with profound granulocytopenia, the early clearance of *E. coli* was abolished. The knowledge that this strain of *E coli* is promptly killed after ingestion by rabbit polymorphonuclear leukocytes (6) suggests that in this instance, either cellular system can effectively clear *E. coli* from the blood even in the presence of significant impairment of the other removal mechanism.

Studies by others support the belief that the reticuloendothelial system and circulating leukocytes can play variable roles in blood stream sterilization. Observations in intact animals and perfusion experiments have shown that the liver and spleen avidly sequester certain bacteria in transit, while others pass through the splanchnic circulation without significant removal (1, 2, 6, 9–13). Staphylococci are removed with great efficiency, while pneumococci are only occasionally entrapped in these tissues in the absence of antibody (1, 9, 11, 12). The hepatic trapping of *E. coli* is less effective than that observed with staphylococci (6, 10). Studies on pneumococcal bacteriemia in rabbits and dogs have suggested that clearance of this microorganism depends upon circulating leukocytes rather than the splanchnic tissues (14, 15). The studies of Wood and his

associates have clearly documented the intravascular phagocytosis of this microorganism during pneumococcal bacteriemia (16).

Thus, differences in the intracellular management of bacteria ingested by polymorphonuclear leukocytes, coupled with differences in the avidity of the splanchnic tissues for the microorganism under study, may determine the speed and effectiveness of blood stream clearance.

It should be noted that both agents used to modify host mechanisms in this study are known to produce other changes in the host animal.

Thorotrast administration has been reported to produce fever, increased bleeding times, thrombocytopenia, variable changes in circulating leukocytes and occasional sudden deaths in rabbits (17, 18). Administration of mechlorethamine has been shown to inhibit glycogen synthesis, respiration, and urea synthesis by liver slices *in vitro* (19).

Thus, the administration of either of these agents may alter host mechanisms of importance in clearance which are currently unknown and are unrelated to changes in reticuloendothelial system function or that of the circulating leukocyte mass.

Recognizing that the mechanisms cannot be precisely stated, it is nevertheless clear that striking differences in the clearance of staphylococci and *E. coli* become apparent in animals which receive thorotrast and mechlorethamine.

The circulating polymorphonuclear leukocyte may play a more important role in the control of bacteriemia caused by microorganisms destroyed by intravascular phagocytosis than has been previously recognized.

## SUMMARY

Observations are presented on the course of experimental bacteriemia in rabbits modified by thorotrast reticuloendothelial "blockade" and mechlorethamine induced leukopenia, given singly and together.

The production of severe granulocytopenia did not impair the clearance of staphylococci from the blood stream and did not enhance the impairment of clearance produced by thorotrast reticuloendothelial "blockade."

Neither severe granulocytopenia nor thorotrast blockade alone produced consistent impairment in the removal of circulating *E. coli* from the blood stream, whereas when these conditions were present together, the early phases of clearance in these animals were virtually prevented.

These results indicate that the host mechanisms operative in the control of bacteriemia differ for staphylococci and *E. coli*. It is suggested that the fate of bacteria within polymorphonuclear leukocytes may in part determine the importance of these cells in the removal of bacteria from the blood stream.

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