

## STUDIES ON BACTERIEMIA\*

### I. MECHANISMS RELATING TO THE PERSISTENCE OF BACTERIEMIA IN RABBITS FOLLOWING THE INTRAVENOUS INJECTION OF STAPHYLOCOCCI

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#### PLATE 28

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We have previously reported that pathogenic, coagulase-positive staphylococci are avidly phagocytized by human leukocytes in *in vitro* systems in which pneumococci, streptococci, and *Klebsiella* are rarely ingested (25). These studies further demonstrated that such coagulase-positive staphylococci may survive for long periods of time within the leukocyte.

During the course of experiments on the disappearance of staphylococci from the blood stream of rabbits following a single injection of large numbers of microorganisms, an abrupt reduction in the rate of clearance was consistently noted 10 to 15 minutes following the injection of staphylococci. This abrupt slowing of the clearance rate resulted in a persistent low grade bacteriemia which could not be satisfactorily explained by saturation of known removal mechanisms.

The studies to be reported in this paper indicate that coagulase-positive staphylococci are swiftly ingested by circulating leukocytes in the blood stream of rabbits, and that such intra-leukocytic residence may act to prevent their effective removal from the circulation.

#### *Materials and Methods*

*Rabbits.*—Healthy male rabbits of mixed stock weighing from 2.8 to 3.8 kilograms were used in these experiments.

*Preparation of Rabbits.*—Indwelling venous catheters were used to obtain rapid, repeated blood samples during the period of blood stream clearance. Under light sodium pentobarbital anesthesia supplemented with local 0.5 per cent procaine, a thyroid incision was made, and a polyethylene catheter with multiple terminal holes was inserted in the left jugular and

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passed into the superior vena cava. When simultaneous samples were to be collected from the hepatic veins, a short section of No. 6F to 7F radiopaque Courmand cardiac catheter was also inserted into the right jugular vein. The cardiac catheter was then passed into the right or left lobe of the liver under fluoroscopic control, in a manner similar to that reported by Martin, Kerby, and Holland (20). The distal ends of both catheters were led out subcutaneously through a small skin incision on the back between the shoulder blades, and the thyroid incision closed. Syringe adapters attached to the catheters were firmly sutured to the skin of the back at this site. A roentgenogram showing the usual position of the hepatic catheter is reproduced in Fig. 1. Rigid surgical asepsis was used throughout. The animal was allowed to recover from anesthesia before clearance studies were initiated and was allowed to stand unrestrained on the laboratory table for the removal of blood specimens from the catheters. At the termination of many experiments, the animal was sacrificed and the actual position of the catheters determined directly.

*Collection of Blood Specimens during Clearance Studies.*—In the performance of clearance studies, microorganisms were introduced *via* the left marginal ear vein in a total volume of 2.0 ml. of 0.85 per cent saline. Preliminary studies indicated that the removal of 1.0 to 2.0 ml. of blood completely cleared the catheters, the following sample representing a true specimen of blood in the lumen of the vessel. At each sampling period, 3.0 ml. of blood were removed simultaneously from both catheters and these syringes discarded. Samples were then drawn simultaneously from both catheters. The blood was immediately delivered to sterile  $12 \times 100$  mm. tubes containing sufficient dried heparin to make a final concentration of 1:10,000. These tubes were prechilled and were returned immediately to an ice-salt water bath. All specimens were diluted in distilled water at 4°C. for plating within 5 minutes. When determinations of the degree of intravascular phagocytosis were performed, an aliquot of blood was delivered to 9 volumes of 0.85 per cent saline at 4° immediately following withdrawal from the animal.

*Plating of Specimens.*—Blood samples were serially diluted in 4.5 ml. distilled water blanks. Peptone infusion agar pour plates were made with 1.0 cc. aliquots at the appropriate dilutions. Plates containing from 30 to 300 colonies were used in final calculations. Because of the narrow differential between the numbers of microorganisms obtained from the hepatic vein and the superior vena cava, duplicate serial dilutions and peptone infusion agar pour plates were made in all studies of the splanchnic removal of microorganisms, to increase the accuracy of colony counts. When the clearance of pneumococci was investigated, Todd-Hewitt agar was used for plating. In experiments in which the simultaneous clearance of staphylococci and pneumococci was determined, duplicate specimens were plated in Todd-Hewitt agar, and Todd-Hewitt agar containing 0.015 per cent oleic acid. Control studies to be reported in the body of this paper showed that this concentration of oleic acid completely suppressed the growth of pneumococci, but had no effect on the quantitative recovery of the staphylococcus strain used in these studies.

*Determination of Intravascular Phagocytosis.*—To detect the intravascular phagocytosis of microorganisms, a technique described by Maaløe was used (16). Preliminary experiments were performed to determine a centrifugation procedure which would remove all leukocytes and red cells from the supernatant, but would not significantly alter the distribution of extracellular microorganisms contained in a specimen. After a number of trials, the following procedure was adopted: Following withdrawal of blood from the catheter, exactly 1 volume of blood was immediately delivered to exactly 9 volumes of saline at 4°C. in  $15 \times 120$  mm. conical centrifuge tubes. The contents of the tube were immediately mixed, an aliquot removed, appropriately diluted, and plated. The chilled blood-saline mixture was then centrifuged at 1250 R.P.M. for 3 minutes in a No. 2 International centrifuge, using a No. 233 head. Following centrifugation, an aliquot of the supernatant was removed, appropriately diluted, and plated. The difference in the number of culturable microorganisms

obtained from the total mixture and the supernatant was considered to represent the number of viable microorganisms contained within leukocytes.

Repeated control experiments were performed to determine whether factors other than phagocytosis such as agglutination or adherence to red cells, as described by Nelson (22), could produce a decline in the number of culturable microorganisms remaining in the supernatant. Incubation of the staphylococcus strain used in these experiments in rabbit plasma, plasma-red blood cell mixtures, in blood containing damaged leukocytes, in blood from agranulocytic rabbits, or in blood maintained at 4°C., produced no significant alterations in the numbers of microorganisms obtained before and after centrifugation. Such studies indicated that the presence of viable phagocytic leukocytes, and temperatures and conditions necessary for phagocytosis were required to produce decline in the numbers of microorganisms contained in the supernatant.

*Cultures.—Staphylococcus:* A strain of staphylococcus designated MAM was used in all clearance studies to be reported. This strain was originally isolated from the skin of one of our personnel in 1951. At that time this strain was designated as a hemolytic *Staphylococcus albus*. On repeated study in 1951 it was consistently coagulase-negative, did not ferment mannitol, and did not survive within human leukocytes (25). During the course of subculture in Dr. Dubos' laboratory at The Rockefeller Institute, this strain became weakly coagulase-positive in human plasma, strongly coagulase-positive in rabbit plasma. Concomitantly it acquired the ability to ferment mannitol, became phosphatase-positive (2), and was found to be lysed by phage 52A.<sup>1</sup> The parent New York Hospital strain remained unchanged on simultaneous study, and was not lysed by any combination of staphylococcal bacteriophage. This strain produced a fatal infection in some rabbits, death occurring 5 to 10 days after the intravenous injection of 0.5 ml. of culture with multiple renal abscesses. Studies by Smith and Dubos have indicated that this strain is of intermediate virulence for the mouse (26). Stock cultures were maintained in peptone infusion broth, and overnight cultures were used in these studies.

*Pneumococcus:* A heavily encapsulated Type III pneumococcus originally obtained from Dr. Colin MacLeod was used in some comparative clearance studies. This microorganism was also rabbit-virulent, death occurring in 48 to 72 hours in occasional animals left untreated following the conclusion of clearance studies. The Type III pneumococcus was maintained in 0.5 per cent rabbits' blood broth, and overnight Todd-Hewitt broth transplants were used in clearance experiments.

*Leukocyte Counts.*—White blood cell counts were done using 5 per cent acetic acid colored with methylene blue as a diluent. All cell counts were performed in the usual bright line hemocytometer, and duplicate counts were frequently performed on separate aliquots by different observers in studies of the splanchnic removal of leukocytes. Differential leukocyte counts were performed on coverslips stained with Wright's method, and 100 to 200 white blood cells were counted. Blood smears were also stained by the Gram-Weigert technique for the study of intracellular microorganisms.

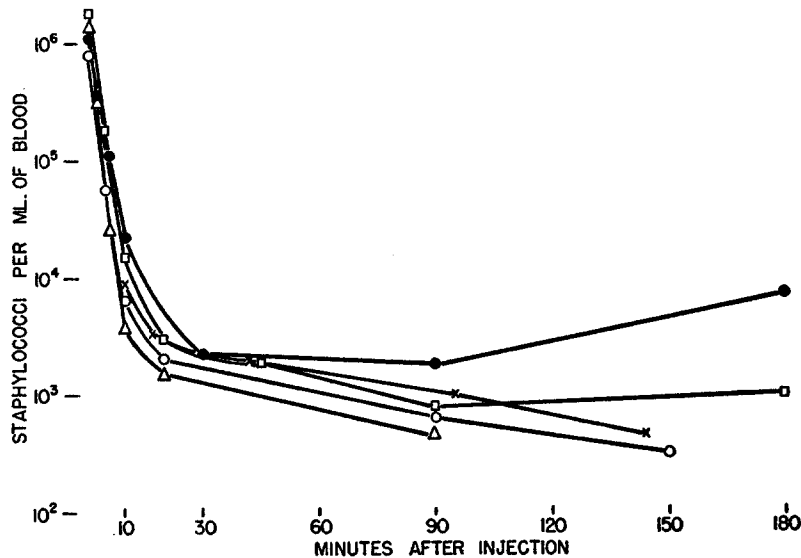
*Production of Leukopenia.*—Leukopenia was induced by the use of mechlorethamine hydrochloride, 1.75 mg. per kilogram give intravenously in a single dose. Daily white blood cell counts and differentials were performed until the total granulocytes were reduced to less than 25 per cmm. before initiating clearance studies. Generally such a granulocytopenia was present 4 days following the injection of nitrogen mustard. Total white blood cell counts ranged from 1200 to 1400 leukocytes per cmm. at this time.

*T-1824 Mixing Studies.*—In studies on the speed of plasma mixing of Evans blue, a predetermined quantity of a 0.1 per cent solution of T-1824 was delivered *via* the marginal ear vein with a specially calibrated syringe and needle, accurate to 0.002 ml. Blood samples

<sup>1</sup> Bacteriophage studies were kindly performed by Dr. Carl Berntsen.

were obtained in the usual manner from the polyethylene catheter lying in the superior vena cava, and delivered to siliconed tubes containing sufficient dried heparin to attain final concentrations of 1:10,000. The plasma was removed by centrifugation, placed in microcuvettes, and the optical density of T-1824 in plasma specimens compared with control plasma specimens in a Beckman spectrophotometer at 625 Angstrom units.<sup>2</sup>

*Thorotrast.*—The thorotrast used in studies of thorotrast blockade of the splanchnic removal system was a 24 per cent to 26 per cent stabilized colloidal suspension of thorium dioxide in 25 per cent aqueous dextrin, containing 0.15 per cent methyl parasept as a preservative. This was obtained from the Testagar Company, Incorporated, Detroit. Using the studies of Martin, Kerby, and Holland as a guide, 7 ml., representing a dose of approxi-



TEXT-FIG. 1. The removal of staphylococci from the superior caval blood of five rabbits following the injection of  $5 \times 10^8$  microorganisms. There is a rapid fall in the number of culturable staphylococci during the first 10 to 15 minutes, followed by an abrupt change to a slower rate of removal which persists for 90 to 180 minutes.

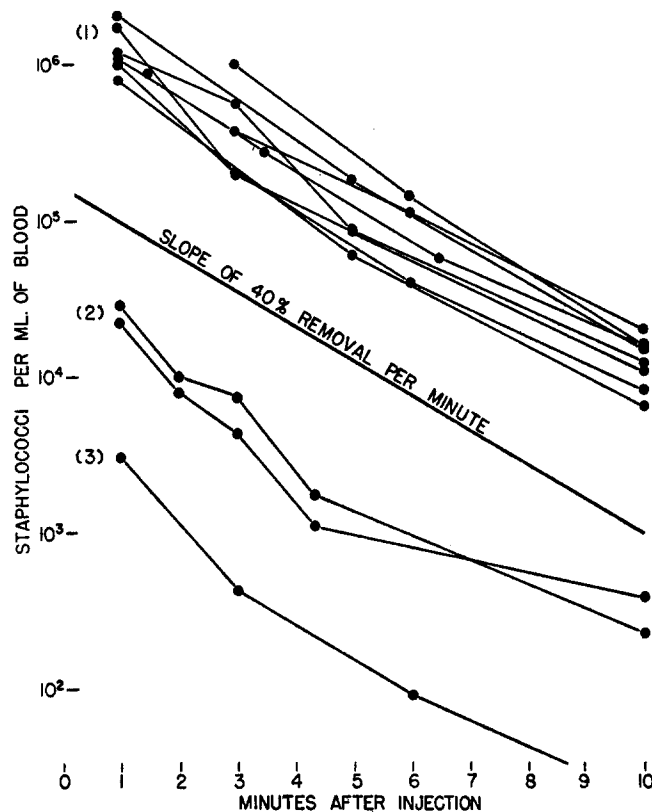
mately 2 ml. per kilogram, was given intravenously 24 hours preceding clearance studies (21). In some animals this dose was given both 48 and 24 hours preceding the clearance experiments.

#### EXPERIMENTAL

*The Disappearance of Staphylococci from Superior Caval Blood Following the Intravenous Injection of a Large Number of Staphylococci.*—Following the intravenous administration of large numbers of staphylococci ( $3$  to  $5 \times 10^8$  microorganisms), blood specimens obtained by rapid repeated sampling from the superior vena cava revealed a striking fall in the number of circulating microorganisms during the first 10 to 15 minutes. In Text-fig. 1, the clearance

<sup>2</sup> These measurements were kindly performed by Dr. Peter Mahrer.

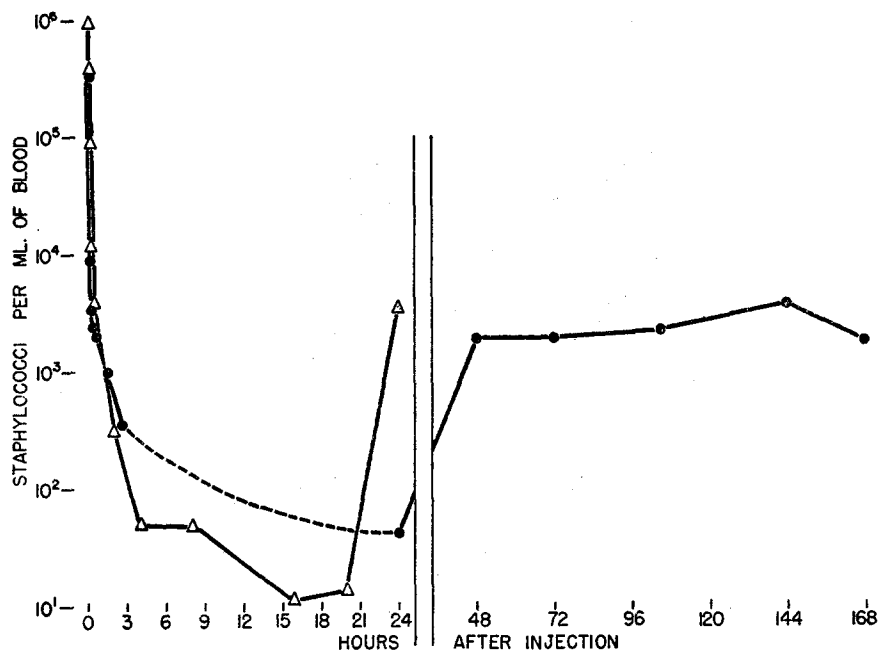
of staphylococci from the blood stream of 5 different animals is charted. Initial 1 minute samples contained from  $9 \times 10^5$  to  $1.8 \times 10^6$  culturable units per ml. During the next 20 minutes there was a profound fall in the number of culturable staphylococci present in superior caval blood to levels of 1 to  $3 \times 10^8$  microorganisms per ml.



TEXT-FIG. 2. Initial rates of removal of staphylococci from the superior caval blood of rabbits. The initial rate of removal of staphylococci is essentially the same following the injection of  $5 \times 10^5$  staphylococci (1),  $5 \times 10^6$  staphylococci (2), or  $5 \times 10^8$  staphylococci (3).

During the first 10 minutes, the decline in culturable staphylococci proceeded as a straight line, semi-logarithmic function, approximating a disappearance of 40 per cent of the microorganisms from the circulation per minute (Text-fig. 2). The rate of removal of microorganisms was surprisingly constant in different animals receiving different numbers of staphylococci intravenously. The number of organisms per milliliter of blood could thus be predicted with reasonable accuracy for any given sampling period during the initial 1 to 3 hours of such experiments.

Ten to 15 minutes after the intravenous administration of staphylococci there was an abrupt decline in the rate of removal of microorganisms, with a subsequent slow progressive fall in the number of circulating staphylococci during the next 2 to 15 hours (Text-figs. 1 and 3). With this large inoculum, the peripheral blood was never completely cleared of staphylococci, bacteriemia of 500 to 1000 culturable units per ml. persisting for many hours in most experiments. Samples obtained beyond 3 hours generally revealed a gradual



TEXT-FIG. 3. The persistence of staphylococci in the superior caval blood of rabbits following the intravenous injection of  $5 \times 10^8$  microorganisms.

decrease in the level of bacteriemia to lowest counts of 30 to 500 staphylococci per ml. 8 to 15 hours after the injection of culture, but complete sterilization of the blood stream did not occur in any animal.

In all animals there was a subsequent rise in the number of circulating staphylococci which was occasionally demonstrable as early as 3 hours after injection. Bacteriemia ranging from  $2 \times 10^3$  to  $2 \times 10^5$  staphylococci per ml. was demonstrable in all animals in samples obtained from the superior cava 20 to 24 hours following the administration of culture. In one instance in which the period of observation was extended to 7 days, staphylococci were consistently demonstrable in the peripheral blood during the entire course of the experiment (Text-fig. 3).

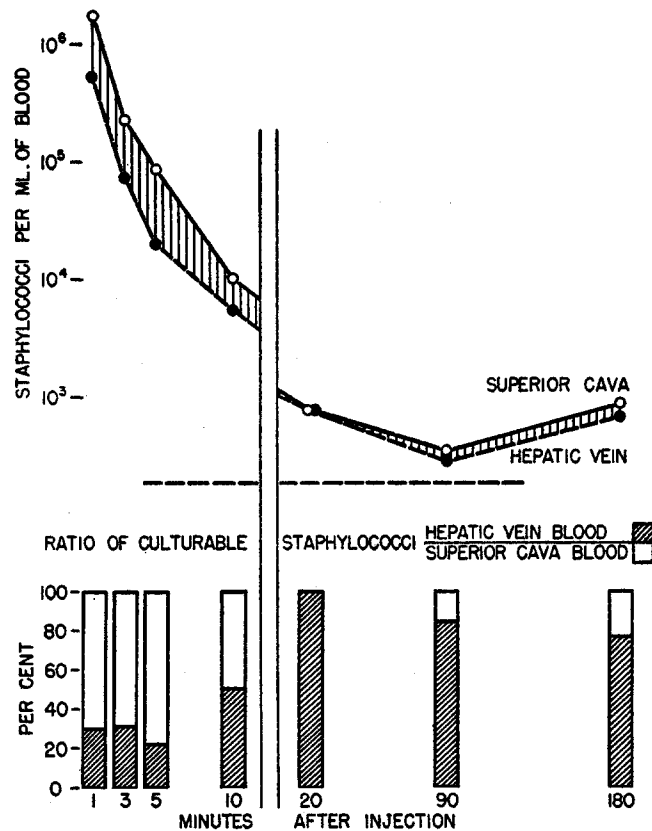
*The Removal of Circulating Staphylococci within the Splanchnic Viscera.*—Hepatic vein samples obtained during the first few minutes of clearance indicated that 70 to 80 per cent of the circulating staphylococci were trapped in splanchnic viscera. This confirmed the observations of Martin, Kerby, and Holland (20). However, as sampling progressed, a reduction in the peripheral hepatic differential was consistently noted. Within 20 minutes, the number of staphylococci cultured from hepatic vein blood approached or equalled the number of staphylococci cultured from peripheral blood. Each of 9 similar experiments showed internal consistency, a steady reduction in the degree of splanchnic trapping occurring over the course of 20 to 90 minutes. Thus average splanchnic removal at 1 to 3 minutes was 77.4 per cent (s.d.  $\pm$  6.9) in these experiments. Twenty to 90 minute specimens revealed an average splanchnic removal of only 26.5 per cent (s.d.  $\pm$  10.6). These findings indicated a gradual cessation of splanchnic trapping. A typical experiment is pictured in Text-fig. 4.

As noted, hepatic vein bacterial counts were 29 and 31 per cent of bacterial counts simultaneously obtained from superior caval blood at 1 and 3 minutes. Ten minutes following the injection of culture, the percentage of culturable staphylococci in the hepatic vein had risen to 50 per cent of the culturable staphylococci present in the superior vena cava. By 20 minutes, equal numbers of microorganisms could be cultured from both hepatic vein blood and superior caval blood. A slight decline in the percentage of staphylococci recovered in hepatic vein blood subsequently occurred. This finding was not in keeping with the studies reported by Martin and his coworkers, in which the splanchnic removal of staphylococci in rabbits was measured during a constant infusion of staphylococci. In such experiments, splanchnic removal remained unchanged over 64 minutes, the total duration of their experiments (2).

*The Removal of Circulating Staphylococci within the Splanchnic Viscera following Two Intravenous Injections of Large Numbers of Staphylococci.*—The decline in the efficiency of the splanchnic removal of large numbers of staphylococci suggested that saturation of splanchnic removal mechanisms might account for the abrupt decline in the rate of removal of microorganisms from the peripheral circulation. This possibility was subjected to study.

Staphylococci were administered intravenously, and simultaneous superior caval and hepatic samples were obtained over a 90 minute period. At this time, when the numbers of staphylococci in superior caval blood were assumed to be relatively constant on the basis of previous experiments, the same dose of staphylococci was again injected. Peripheral and hepatic blood specimens were obtained at identical sampling intervals. It was assumed that if saturation of splanchnic removal mechanisms had occurred, such a secondary load of staphylococci would fail to be cleared from the circulation. The numbers of culturable microorganisms in superior caval blood would thus remain high, while the number of staphylococci cultured from the hepatic blood would approach or equal the number of microorganisms found in superior caval blood.

Experiments in which two injections of  $5 \times 10^8$  staphylococci were administered at 90 minute intervals, failed to reveal any alteration in the speed of disappearance of staphylococci from the superior caval blood following the second injection of staphylococci. Despite the decreasing hepatic superior

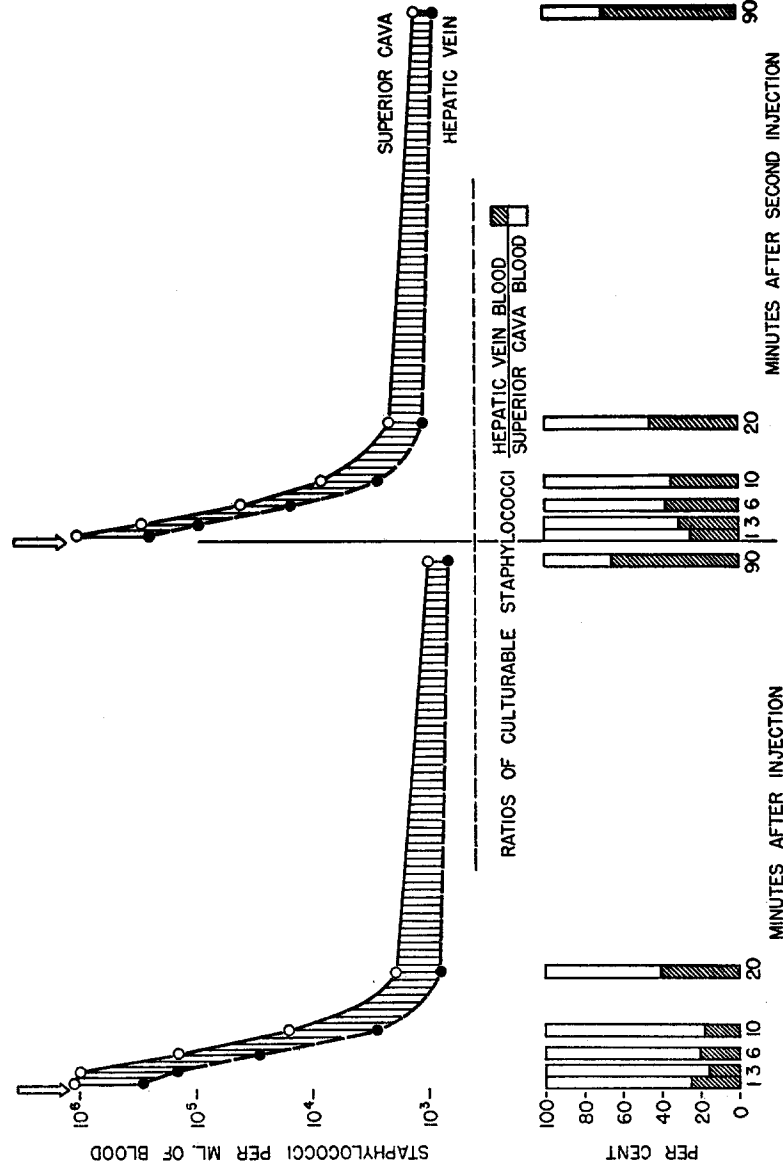


TEXT-FIG. 4. The removal of staphylococci in the splanchnic viscera following the intravenous injection of  $5 \times 10^8$  microorganisms. During the 20 minute period following injection of staphylococci, there is a progressive decrease in the degree of removal of staphylococci in the splanchnic bed.

caval difference in culturable staphylococci present 90 minutes after the initial injection, evidence of efficient splanchnic trapping of microorganisms was again apparent immediately following the second injection of staphylococci. A typical experiment is graphically represented in Text-fig. 5.

As indicated in Text-fig. 5, 75 to 85 per cent of the circulating staphylococci were removed in the splanchnic bed during the first 10 minutes after the injection of  $5 \times 10^8$  staphylococci. Ninety minutes after the initial injection,





TEXT-Fig. 5. The removal of staphylococci in the splanchnic viscera following two spaced intravenous injections of  $5 \times 10^8$  microorganisms. Despite decreasing splanchnic removal during the initial 90 minute period, evidence of splanchnic trapping is again apparent following the second injection of staphylococci.

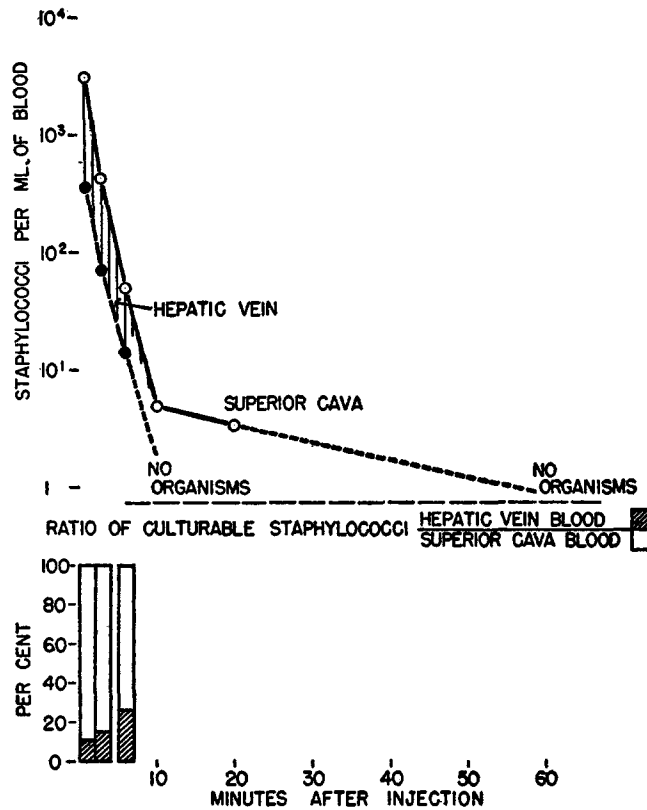
splanchnic removal had significantly declined, only 35 per cent of circulating microorganisms failing to traverse the splanchnic viscera. Following a second injection of  $5 \times 10^8$  staphylococci, superior caval blood was cleared of staphylococci at the same rate as noted after the initial injection, and efficient splanchnic removal was again demonstrable. One minute following the second injection, 75 per cent of the circulating microorganisms were again removed in the splanchnic bed. Ninety minutes after the second injection, splanchnic trapping had again declined to low levels. At this sampling period, 70 per cent of the staphylococci culturable from the superior vena cava appeared in hepatic vein blood.

*The Clearance of Small Numbers of Staphylococci from the Blood Stream of Rabbits.*—In the experiments cited, splanchnic trapping had consistently ceased at low levels of bacteriemia. This suggested that the efficiency of splanchnic removal mechanisms might relate to the opportunity for microorganism-fixed phagocytic cell contact. At initial levels of 1 million circulating microorganisms per milliliter, the opportunity for chance collision between a staphylococcus and the phagocytic cells of the reticuloendothelial system could be assumed to be manyfold greater than at the levels of 1000 circulating microorganisms per ml. persisting in these experiments. It was thus postulated that clearance of staphylococci might be significantly delayed, and splanchnic trapping impaired, if the opportunity for microorganism-fixed phagocytic cell contact were reduced 1000-fold. Rabbits were thus injected with a dose of staphylococci which was adjusted to give 1 minute levels of approximately 1000 circulating microorganisms per ml. Such an experiment is illustrated in Text-fig. 6.

When an inoculum of  $5 \times 10^6$  staphylococci was administered intravenously, the number of culturable staphylococci present in superior caval blood at 1 minute approached 1000 microorganisms per ml. Nevertheless, the numbers of culturable staphylococci present in superior caval blood fell at the same initial rate as observed following injections of  $5 \times 10^8$  microorganisms (see Text-fig. 2). No staphylococci could be recovered from superior caval blood after 20 minutes in such experiments. As noted in Text-fig. 6, simultaneous hepatic vein bacterial counts showed that initial splanchnic removal of microorganisms was of the same magnitude as seen in experiments in which large numbers of staphylococci were injected. This indicated that splanchnic removal mechanisms operated efficiently when presented with smaller numbers of microorganisms per unit of circulating blood. Such experiments showed that initial clearance was not delayed at low initial levels of circulating microorganisms, and that lack of microorganism-phagocytic cell contact could not explain the decline in rate of clearance seen in our high dose experiments.

*Distribution of Microorganisms within the Blood Stream.*—The remarkably constant initial rate of clearance of staphylococci, regardless of the initial dose

of microorganisms (Text-fig. 2), suggested that part of the initial fall observed might relate to the mixing of staphylococci within the circulation rather than actual removal of staphylococci from the blood. To exclude this possibility, the mixing characteristics of T-1824 were studied in two rabbits in whom

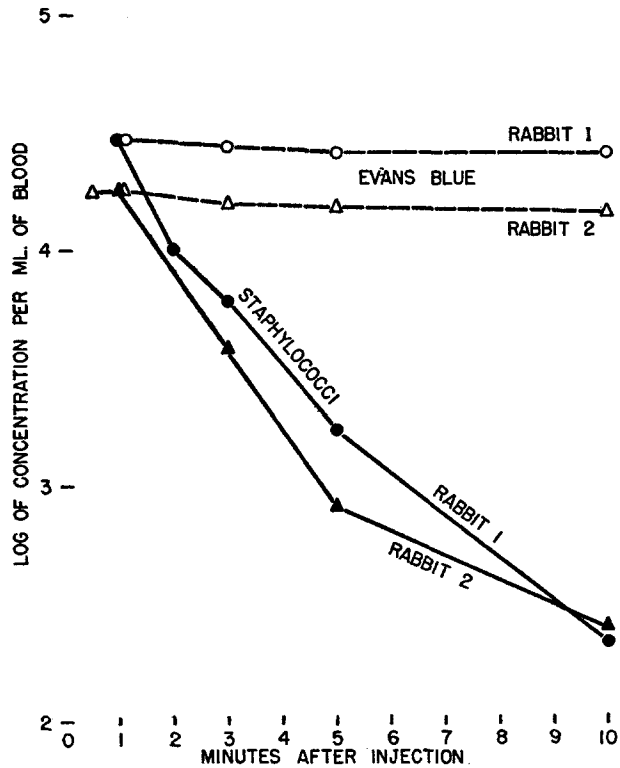


TEXT-FIG. 6. The removal of staphylococci in the splanchnic viscera following the intravenous injection of  $5 \times 10^6$  microorganisms. The rate of removal and degree of splanchnic trapping following injection of small numbers of staphylococci is similar to that obtained following the intravenous injection of large numbers of staphylococci.

clearance studies had been performed. The dye was injected *via* the same marginal ear vein by which microorganisms had been administered. Samples were taken *via* catheter from the same level in the superior vena cava utilized in the clearance experiments.

As illustrated in Text-fig. 7, plasma mixing of T-1824 was essentially complete in 1 minute, and levels remained almost constant during the 10 minute period of study. In contrast, a 100-fold fall in the number of circulating staphylococci occurred during this period.

*The Intravascular Phagocytosis of Staphylococci.*—The studies already documented appeared to eliminate saturation of the clearing mechanisms, lack of microorganism-phagocytic cell contact, or dilution-distribution of staphylococci, as explanations for the persistent bacteriemia observed following the intravenous injection of large numbers of staphylococci. The decreasing



TEXT-FIG. 7. Comparison of the initial rate of removal of staphylococci from the blood stream, with the plasma mixing of Evans blue. Mixing of T-1824 is essentially complete at 1 minute, and plasma levels remain constant during the period of rapid disappearance of staphylococci from the circulation.

splanchnic removal of staphylococci with the passage of time also remained unexplained.

Reviewing these data, it seemed possible that intravascular phagocytosis of staphylococci might account for the phenomena observed. If staphylococci were rapidly phagocyted by granulocytes in the blood, and remained viable within circulating leukocytes, such intracellular microorganisms might pass through the reticuloendothelial system within the cytoplasm of circulating granulocytes. In this way they might escape cellular mechanisms which

normally removed extracellular foreign particulate matter. If such a phenomenon were operative, peripheral-hepatic differential counts would be high immediately following injection of extracellular staphylococci, but would decrease as increasing numbers of staphylococci were situated intracellularly. Such viable intracellular staphylococci might continue to circulate. The decline in the numbers of culturable microorganisms within the blood stream might then be dependent upon slow intracellular destruction of staphylococci, or the removal of leukocytes containing ingested microorganisms from the blood stream at slower rates than obtained with extracellularly situated microorganisms.

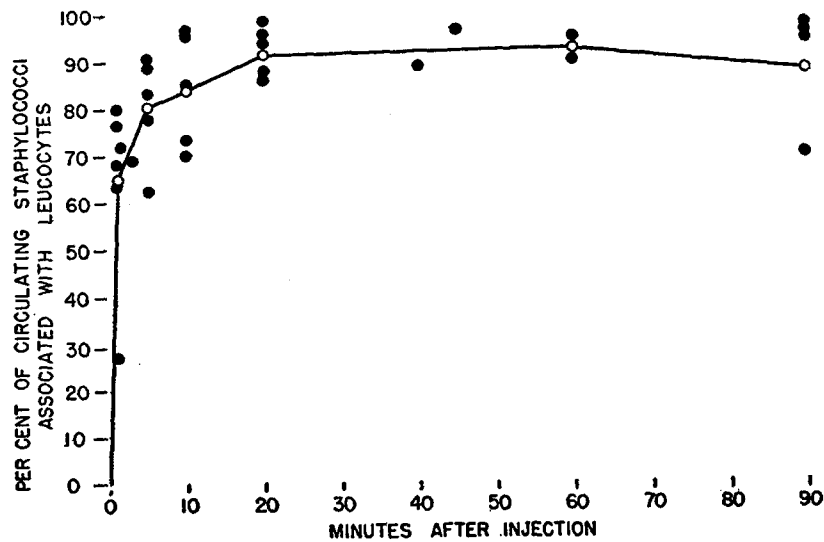
To determine the occurrence of intravascular phagocytosis, a differential centrifugation procedure was used to study the localization of staphylococci within the blood following the intravenous injection of large numbers of staphylococci.

In brief, blood drawn at each sampling period was immediately delivered to saline at 4°C. to halt further phagocytosis. The contents of the tube was then mixed and appropriately plated. The tubes were then centrifuged in a manner previously determined to remove all leukocytes from the supernatant without significantly affecting unphagocytosed microorganisms, and the supernatant carefully removed and plated. The difference in total and supernatant plate counts was assumed to represent viable staphylococci contained in leukocytes. With each experiment, staphylococci from the culture to be used were added to blood obtained from the rabbit immediately prior to clearance studies. These control tubes were maintained at 4°C. and were centrifuged and cultured in identical fashion with the specimens obtained during clearance to check possible alterations in supernatant counts which might result from the centrifugation alone. Control observations indicated that supernatant counts were not significantly altered by such differential centrifugation following incubation of staphylococci in plasma alone, plasma containing leukocytes injured by heating, plasma containing erythrocytes, blood from agranulocytic rabbits, or chilled whole blood.

Studies of the intravascular phagocytosis of microorganisms indicated that staphylococci introduced into the circulation disappeared from the plasma with striking rapidity. Blood samples obtained from the superior vena cava 1 minute following the intravenous injection of staphylococci, consistently revealed that 25 to 70 per cent of the circulating staphylococci were already associated with the cellular layer. Ten to 20 minutes after injection of staphylococci, over 90 per cent of the staphylococci present in the circulating blood appeared to be phagocytosed, less than 10 per cent of the staphylococci remaining culturable in the plasma layer. As indicated in Text-fig. 8, intravascular phagocytosis was extremely swift, with an average of 65 per cent of the staphylococci remaining in the cellular layer at 1 minute. Eighty per cent of the staphylococci appeared to be circulating within leukocytes at 5 minutes. Twenty minutes following their introduction, virtually all of the culturable staphylococci appeared to reside within circulating leukocytes. In over 60 determinations, rapid intravascular phagocytosis was a consistent finding in 12 differ-

ent animals. In studies on granulopenic rabbits to be described, little or no evidence of phagocytosis was apparent in centrifugation studies. Such granulopenic animals served as an excellent control on the centrifugation method.

*The Plasma Leukocyte Partitioning of Staphylococci Obtained from the Hepatic Veins.*—To determine whether circulating leukocytes containing staphylococci could pass through the splanchnic bed without removal, experiments were performed in which the localization of staphylococci in both superior vena cava and hepatic vein blood was studied.



TEXT-FIG. 8. The intravascular phagocytosis of staphylococci. Following intravenous injection, staphylococci are rapidly associated with leukocytes. During the period 20 to 90 minutes after injection of culture, over 90 per cent of the circulating, culturable staphylococci appear to reside within leukocytes.

Simultaneous blood samples from the superior cava and the hepatic veins at each sampling period were centrifuged and cultured in parallel to determine the percentage of staphylococci contained within leukocytes. It was reasoned that if extracellular cocci were preferentially removed within the reticuloendothelial system, blood collected from the hepatic veins should show a higher percentage of staphylococci within leukocytes than in blood obtained from the superior vena cava. Such an experiment is represented in Table I.

As noted in Table I, the number of culturable staphylococci present in the superior vena cava fell rapidly from over 2 million microorganisms per ml. to 11,500 microorganisms per ml. during the first 10 minutes, with a subsequent slow decline to 2,900 microorganisms per ml. at 90 minutes. Plates prepared from hepatic blood revealed splanchnic removal of 75 to 80 per cent of the circulating staphylococci in specimens obtained 1 and 5 minutes after in-

jection, with a subsequent decline in the degree of removal of microorganisms in the splanchnic bed so that the number of culturable staphylococci in superior vena cava and hepatic vein blood was essentially the same at 90 minutes (column 6). Intravascular phagocytosis occurred rapidly, with over 70 per cent of the culturable staphylococci in superior caval blood culturing within the cellular layer at 1 minute. In all samples except the 20 minute specimen, the percentage of staphylococci associated with leukocytes was somewhat higher in hepatic vein blood. Such observations suggested that extracellular microorganisms were preferentially removed in the splanchnic viscera. Nevertheless, calculations of the total number of staphylococci removed indicated that some intracellular staphylococci were also cleared in passage through the splanchnic bed.

TABLE I  
*The Percentage of Staphylococci Contained within Leukocytes in Superior Vena Cava and Hepatic Vein Blood during Blood Stream Clearance of Staphylococci*

Time after injection	Superior vena cava		Right hepatic vein		Ratio
	Staphylococci per ml. blood	Percentage within leukocytes	Staphylococci per ml. blood	Percentage within leukocytes	Staphylococci in hepatic vein superior cava
1 min.	2,100,000	72	440,000	88	0.21
5 "	115,000	89	27,000	92	0.24
10 "	11,500	88	8,100	95	0.70
20 "	4,000	96	2,750	94	0.69
90 "	2,900	Not done	3,200	Not done	1.1
3 hrs.	5,100	76	1,750	>99	0.34
20 "	11,100	Not done	5,300	Not done	0.48

The experiment documented in Table I was of particular interest because sampling was carried over the period at which the number of culturable staphylococci in the superior vena cava began to rise. As shown in Table I, the number of staphylococci present in the superior vena cava rose from 2,900 microorganisms per ml. at 90 minutes to 5,100 microorganisms per ml. at 3 hours. Differential culture of this specimen revealed that the percentage of extracellular staphylococci in superior caval blood had increased from 4 to 24 per cent (column 2). Simultaneously, efficient splanchnic removal of staphylococci was again apparent (columns 3 and 5). At 3 hours, two-thirds of the circulating staphylococci were again removed in the splanchnic bed. In contrast to the decrease in intracellular microorganisms present in superior caval blood, almost all of the staphylococci appearing in hepatic blood were associated with leukocytes at the 3 hour sampling period.

Such findings have suggested that as staphylococci are released from intracellular foci during the period of increasing bacteriemia, splanchnic removal

mechanisms are once again effective as extracellular staphylococci reappear in the circulating blood.

*The Removal of Large Numbers of Staphylococci from the Blood Stream of Granulocytopenic Rabbits.*—

To determine whether the absence of circulating phagocytes would alter the removal of staphylococci from the peripheral blood, 4 rabbits were given 1.75 mg. per kilogram of mechlorethamine hydrochloride intravenously. Clearance studies were performed when circulating granulocyte levels were depressed to less than 25 granulocytes per cmm. Blood cultures taken prior to clearance studies in these animals were sterile. Leukocyte counts and differentials were performed at each sampling period to ascertain whether granulocytopenia was present throughout the course of the experiment.

When granulocytopenic animals were given  $5 \times 10^8$  staphylococci intravenously, the disappearance of staphylococci from superior caval blood during the first 10 minutes proceeded at the same rate of speed as noted in normal animals. However, at 20 minutes, definite differences in the clearance curves were noted. Instead of an abrupt levelling off at  $1$  to  $2 \times 10^8$  culturable microorganisms per ml., the level of circulating microorganisms continued to fall to levels approximately one-fifth that seen in normal animals. Again, the blood was never cleared completely of staphylococci, and an increase in circulating staphylococci was apparent at 3 hours.

Study of the localization of staphylococci within the blood of leukopenic animals showed that over 80 per cent of the microorganisms were free in the plasma layer. Total leukocyte counts and differential smears indicated that granulocytopenia of less than 20 polymorphonuclear leukocytes per cmm. existed during the course of these experiments.

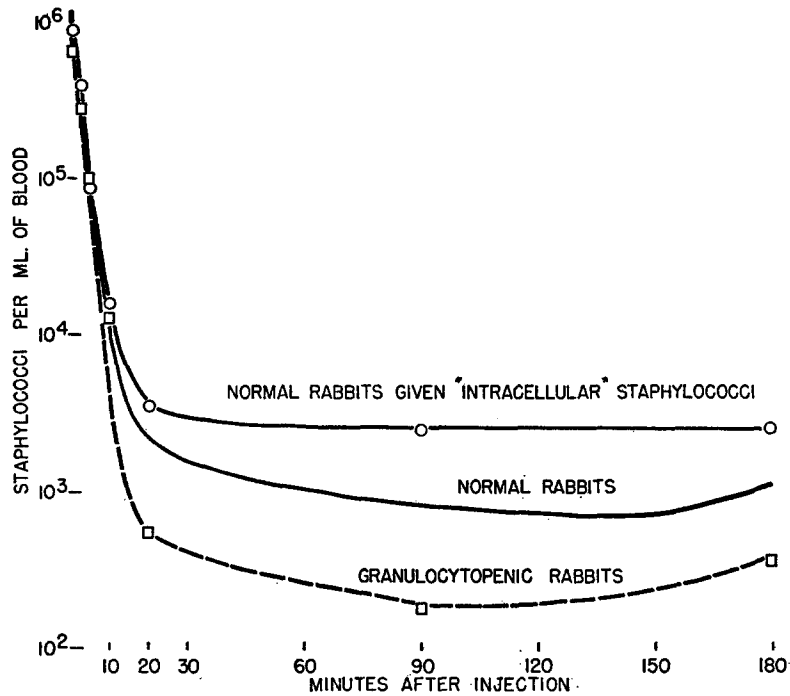
The average clearance curve obtained in 2 granulocytopenic rabbits given  $5 \times 10^8$  staphylococci intravenously is compared with that obtained in 4 normal control rabbits given the same intravenous dose of staphylococci in Text-fig. 9. As can be seen, the number of culturable staphylococci present in the superior caval blood of granulocytopenic animals 20 to 180 minutes after the injection of culture was distinctly lower than that seen in normal animals receiving the same initial inoculum. This increase in the number of staphylococci removed from the blood of leukopenic animals suggested that clearance mechanisms operated with greater effectiveness against staphylococci residing extracellularly in the plasma.

*The Removal of "Intracellular" Staphylococci from the Blood Stream.*—In an attempt to further determine the effect of intracellular residence on the removal of staphylococci from the circulation, experiments were performed in which part of the administered staphylococci were already incorporated within homologous granulocytes.

Rabbits were given an intraperitoneal injection of 200 ml. of warm, normal saline containing 0.1 per cent soluble starch. Four hours later an additional 150 to 200 ml. of normal



saline containing 0.3 per cent sodium citrate was introduced into the peritoneal cavity. The abdomen was gently kneaded, a No. 15 needle with multiple terminal holes inserted, and the fluid allowed to drain into sterile containers. Such exudates were free of blood and yielded a cellular exudate which consisted almost exclusively of polymorphonuclear leukocytes. The cells were removed by gentle centrifugation (1250 R.P.M. for 3 minutes) then resuspended in 2.0 ml. of serum obtained from the donor rabbit. A concentrated, washed suspension of staphylococcus MAM was added to the cell suspension, and phagocytosis allowed to pro-



TEXT-FIG. 9. Comparison of the blood stream clearance of staphylococci in normal and granulocytopenic rabbits, and normal rabbits receiving "intracellular" staphylococci intravenously.

ceed on a rotating drum at 37°C. for 15 minutes. The cells were then removed from the serum by the same centrifugation procedure, washed once in 0.3 per cent citrated saline, and resuspended in 1.0 ml. of donor rabbit serum. Examination of stained smears revealed that almost all of the leukocytes contained large numbers of staphylococci. Despite washing, large numbers of extracellular staphylococci were also present at an estimated ratio of 30 to 50 extracellular staphylococci per each intracellular staphylococcus. Clumping of microorganisms and cells was minimal. Thus such an inoculum represented a mixture containing both intra- and extracellular staphylococci. This suspension of cells and extracellular staphylococci was then injected into the donor rabbit *via* the left marginal ear vein, and blood samples were taken from a polyethylene catheter in the superior vena cava as in previous experiments.

In two rabbits successful experiments were performed in which "intracellular" staphylococci were injected intravenously. Plating of the inoculum revealed that approximately the same number of culturable units had been administered ( $4$  and  $6 \times 10^8$  microorganisms respectively) as used in experiments in which extracellular staphylococci were injected. As noted in Text-fig. 9, the initial removal of this inoculum from the superior vena cava proceeded at the same rate as noted when extracellular staphylococci alone were injected. However, at 20 minutes the number of culturable staphylococci remained higher in animals receiving "intracellular" staphylococci than in animals receiving staphylococcus culture directly. These differences were more striking at 90 minutes. At this sampling period, 3,000 to 5,000 staphylococci per ml. persisted in animals receiving intracellular microorganisms, in contrast to the 600 to 800 microorganisms remaining in superior caval blood of normal animals. Once again, a rise in the number of circulating staphylococci was apparent at 3 hours.

Such experiments have been technically difficult to carry through to completion without error, and these two experiments alone are not sufficient to make the differences noted of statistical significance. Nevertheless, when combined with the results obtained in clearance studies on granulocytopenic rabbits, and the studies of intravascular phagocytosis already documented, these observations support the concept that intracellular residence of staphylococci impairs normal clearance mechanisms.

*The Removal of Type III Pneumococci from Superior Caval Blood.*—To determine whether such intravascular phagocytosis was seen during the blood stream clearance of other microorganisms, a Type III pneumococcus was selected for study. When  $5 \times 10^8$  pneumococci were injected intravenously, striking differences in the clearance of this microorganism from superior caval blood were noted. From similar initial peripheral counts of  $10^6$  culturable microorganisms per ml., the numbers of pneumococci fell steadily in superior caval blood at a slower initial rate than seen during the clearance of staphylococci. However, no significant decline in the rate of clearance subsequently occurred, and the numbers of pneumococci were extremely low or no longer culturable (less than 10 organisms/ml.) 45 to 90 minutes after injection (Table II). As the period of sampling was extended, it was demonstrated that this microorganism was not permanently eliminated. Pneumococci reappeared in the blood stream, and large numbers of pneumococci were again demonstrable in blood specimens taken at 18 to 24 hours.

Studies on the degree of intravascular phagocytosis of viable microorganisms taking place during the sampling periods, again carried out with differential centrifugation, consistently demonstrated that all the culturable pneumococci resided extracellularly and could be quantitatively cultured from the plasma layer following removal of the cells by centrifugation. Culturable pneumococci

reappearing in the blood at 18 to 24 hours were also extracellularly situated (Table II).

*The Simultaneous Removal of Pneumococci and Staphylococci from Superior Caval Blood of Single Animals.*—As a further check on the significance of the differences in superior caval clearance of the strains of pneumococcus and

TABLE II  
*The Removal of Pneumococci from the Rabbit Blood Stream*

Time after injection	Experiment 23		Experiment 32	
	No. of culturable pneumococci per ml.			
	Total blood	Plasma	Total blood	Plasma
1 min.	1,800,000	1,760,000	950,000	1,000,000
5 "	260,000	250,000	480,000	520,000
10 "	64,000	62,000	180,000	130,000
20 "	—	—	13,000	13,000
45 "	100	—	<10	<10
90 "	70	50	<10	<10
3 hrs.	160	—	—	—
20 "	6,200	6,200	7,000	6,800

TABLE III  
*The Effect of Increasing Amounts of Oleic Acid on the Recovery of Staphylococci and Pneumococci in Todd-Hewitt Agar*

Concentrations of oleic Acid	Colonies per plate*	
	Staphylococcus MAM	Pneumococcus III
<i>per cent</i>		
Control	133	98
0.001	150	35
0.005	129	2
0.01	151	0
0.03	146	0
0.06	114	0

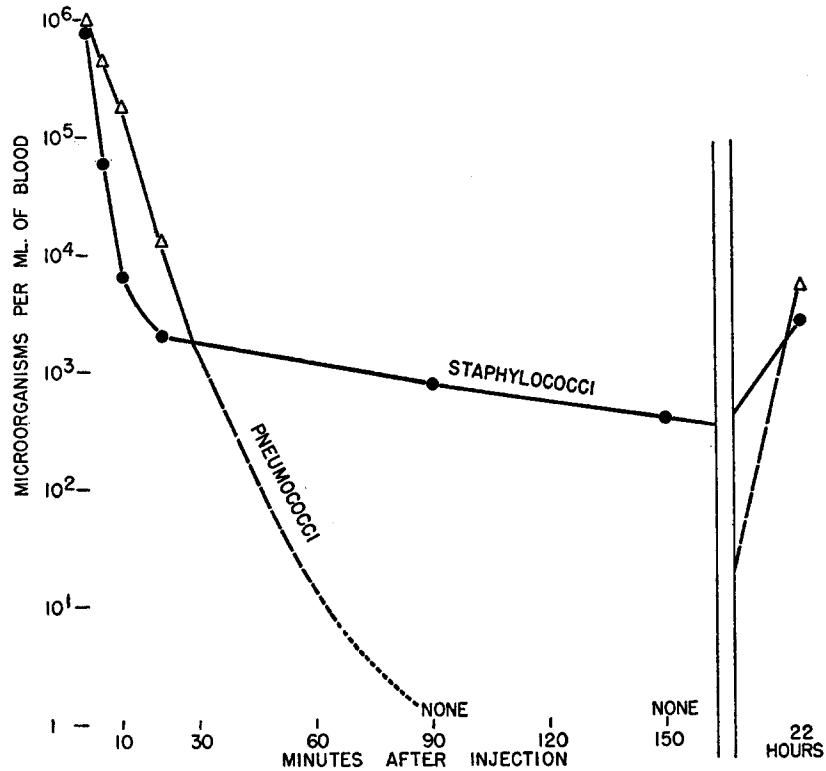
\* A standard inoculum of staphylococci or pneumococci was incorporated in a separate series of pour plates containing the indicated concentrations of oleic acid.

staphylococcus under investigation, the simultaneous clearance of both microorganisms was studied in 2 animals.

Rabbits were injected with a mixture containing approximately  $3 \times 10^8$  staphylococci and  $3 \times 10^8$  pneumococci from 18 hour Todd-Hewitt cultures. At each sampling interval, blood specimens were appropriately diluted, and simultaneously plated in Todd-Hewitt agar, and Todd-Hewitt agar containing 0.015 per cent oleic acid. Numerous preliminary studies demonstrated that oleic acid in these concentrations had no effect on the quantitative recovery of staphylococci and completely eliminated all detectable growth of pneumo-

cocci (Table III). The combination of the two microorganisms in culture did not affect this result.

Such combined clearance studies revealed the same pattern which had been observed with single microorganism injection (Text-fig. 10). Staphylococci swiftly disappeared during the initial 10 minute period. Clearance then abruptly



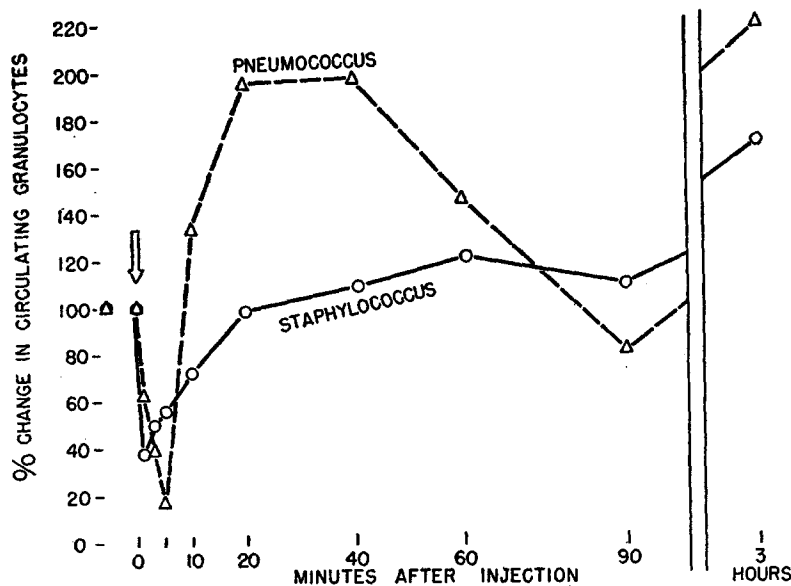
TEXT-FIG. 10. The removal of staphylococci and pneumococci from the blood stream of a single rabbit following simultaneous injection of both microorganisms.

declined in rate, levelling off at about 1000 microorganisms per ml. Differential centrifugation studies showed that most of the staphylococci were promptly situated within leukocytes. In contrast, the pneumococci were removed from the blood stream at somewhat slower rates, but completely disappeared at 45 to 90 minutes. During clearance, all of the culturable pneumococci remained free in the plasma layer.

*Changes in the Number of Circulating Leukocytes Following the Injection of Microorganisms.*—The finding that virtually all the circulating staphylococci were phagocyted within 20 minutes, and the cessation of splanchnic trapping

at this time, made the changes in circulating leukocytes during clearance appear of significance.

Following the injection of either staphylococci or pneumococci, the number of circulating polymorphonuclear leukocytes fell precipitously during the initial 1 to 5 minutes. Leukocytes then swiftly reappeared in the peripheral blood, the number of circulating granulocytes reaching or exceeding pre-injection levels within 10 to 20 minutes. A definite leukocytosis was demonstrable within 3 hours in all animals receiving either microorganism. The rapid



TEXT-FIG. 11. Changes in the number of polymorphonuclear leukocytes present in superior caval blood following the intravenous injection of staphylococci or pneumococci. Average values obtained from 4 rabbits receiving staphylococci, 4 rabbits receiving pneumococci.

onset of leukopenia following the intravenous injection of microorganisms has been previously noted by other investigators (1, 30, 32).

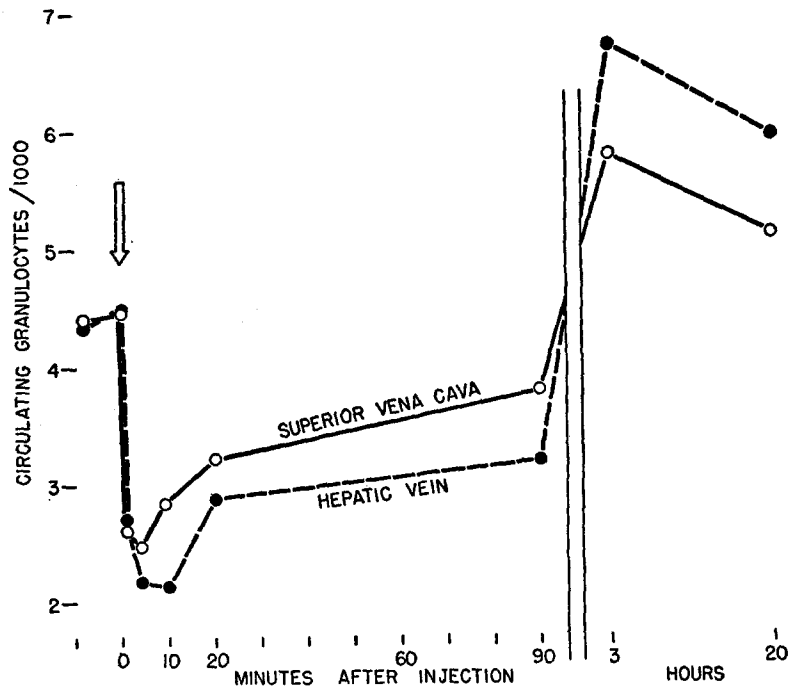
Examination of the granulocytes in stained smears showed little evidence of change in the maturity of the cells during the first 5 to 10 minutes following the administration of culture. Subsequent smears showed an increasing number of immature band forms, suggesting the entrance of new cells into the circulation.

The changes observed in 8 rabbits receiving 0.5 to 1.0 ml. of culture intravenously are summarized in Text-fig. 11. Four animals were given staphylococci, 4 received pneumococci.

Total and differential leukocyte counts were also performed on blood specimens obtained simultaneously from the superior vena cava and hepatic vein,

during clearance studies, to determine whether granulocytes were trapped within the splanchnic viscera following the injection of staphylococci. Such a study is graphically recorded in Text-fig. 12.

As indicated in Text-fig. 12, fewer granulocytes were present in hepatic vein blood than in superior caval blood during the 5 to 90 minute period. This pattern, suggesting the initial splanchnic sequestration of small numbers of granulocytes following injection of staphylococci, was obtained in other similar



TEXT-FIG. 12. Changes in the number of polymorphonuclear leukocytes present in superior caval and hepatic vein blood following the intravenous injection of staphylococci.

experiments. Individual differences were too small, however, (maximum superior caval hepatic difference of 700 granulocytes per cmm.) to make this finding statistically significant. In specimens, obtained beyond 3 hours, the numbers of polymorphonuclear leukocytes present in hepatic venous blood generally exceeded those present in superior caval blood (Text-fig. 12).

*The Appearance of Intracellular Staphylococci in Stained Smears.*—Study of stained blood smears taken at each sampling period revealed that staphylococci could be clearly seen within the cytoplasm of circulating granulocytes within 1 minute, and intracellular cocci could be found on careful search of all subsequent smears. Extracellular staphylococci were not to be found on smears

prepared beyond 10 minutes following injection on repeated examinations. Only the pseudoeosinophilic, polymorphonuclear leukocytes of the rabbit appeared to participate in the immediate phagocytic process, and no staphylococci were noted within monocytes in the peripheral blood. Intracellular microorganisms generally consisted of single pairs of cocci which stained sharply blue-black against the eosinophilic granules of the leukocytes in Wright's stain preparations. Gram-Weigert preparations, which left the cytoplasmic granules of the leukocyte unstained, made intracellular microorganisms stand out prominently. Photomicrographs of circulating polymorphonuclear leukocytes containing staphylococci are shown in Fig. 2.

Microscopic examination of stained smears could not be used for quantitative study of the degree of phagocytosis, however, because of the tedious search required to detect polymorphonuclear leukocytes containing staphylococci. For example, at levels of 1000 circulating staphylococci per ml., one intracellular coccus per 2000 to 4000 granulocytes represented total ingestion of the circulating microbial population.

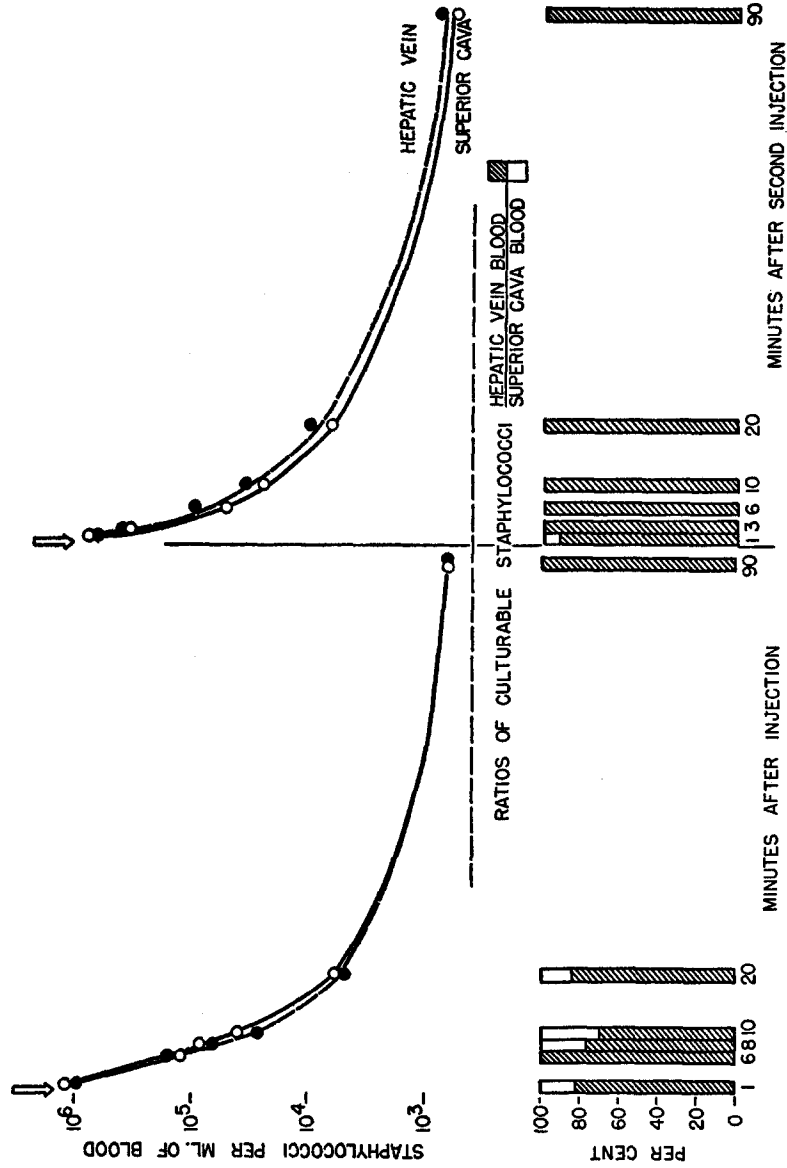
Examination of smears prepared following the intravenous administration of pneumococci also revealed the prompt appearance of intracellular cocci. Pneumococci could occasionally be found within the cytoplasm of granulocytes on smears prepared within 5 minutes after injection and on subsequent smears. This finding was unexpected but appeared in every way to resemble the intravascular phagocytosis of staphylococci. Despite the intracellular location of some pneumococci, differential centrifugation studies, already described, failed to reveal the presence of culturable pneumococci in association with leukocytes.

It thus appeared probable that such intracellular pneumococci were not viable and that the culturable pneumococci recovered from the blood stream represented extracellular microorganisms alone, in contrast to staphylococci which appeared to survive and remain culturable from within leukocytes.

*Platelet Clumping Following the Intravenous Injection of Microorganisms.—*

Smears prepared 1 minute after the injection of either staphylococci or pneumococci showed a marked reduction in number of circulating platelets. Clumping of the remaining platelets was prominent in 1 minute preparations, and an occasional microorganism could be seen trapped in such platelet aggregates. Platelet aggregates rapidly declined in number and size on 3 and 5 minute smears, and were no longer apparent on succeeding smears. Strands resembling fibrin were noted in association with some of the platelet clumps following the injection of staphylococci. In all other respects, platelet clumping and disappearance appeared to be roughly quantitatively and qualitatively similar following the injection of either pneumococci or staphylococci (Fig. 3).

It appeared possible that such platelet clumps were sedimented with the cellular elements of the blood on centrifugation, thus removing extracellular microorganisms from the supernatant. Such a phenomenon would make the apparent degree of intravascular phagocytosis falsely high in 1 and 3 minute specimens. Nevertheless, a similar degree of platelet clumping incorporating an occasional microorganism was noted in smears obtained after the intra-



Text-fig. 13. The effect of thorotrast on the splanchnic removal of staphylococci following two spaced intravenous injections of  $5 \times 10^8$  microorganisms.



venous injection of pneumococci. Centrifugation of such specimens consistently demonstrated that all of the culturable pneumococci remained in the supernatant. Moreover, centrifugation of blood specimens containing platelet clumps obtained from granulocytopenic rabbits following the injection of staphylococci did not reveal a decline in the microorganisms remaining in the supernatant.

Bull has suggested that intravascular agglutination of microorganisms is necessary for their effective removal from the circulation (7-9). No significant agglutination of the microorganisms used in these experiments was noted on examination of stained smears.

*The Effect of Thorotrast "Blockade" on the Peripheral Clearance and Splanchnic Uptake of Staphylococci.*—Studies by Martin, Kerby, and Holland have indicated that the administration of thorotrast 19 to 24 hours prior to injection of microorganisms effectively prevents the uptake of staphylococci within the splanchnic viscera during a constant infusion of staphylococci (21). These studies were repeated, using the same thorotrast dosage as reported by Martin, to determine the effect of thorotrast on the blood stream clearance of large numbers of staphylococci given as a single intravenous injection.

When  $5 \times 10^8$  staphylococci were administered to thorotrast-prepared animals, simultaneous cultures from superior vena cava and hepatic vein blood showed that the splanchnic removal of staphylococci was almost totally prevented. At each sampling period, the number of culturable staphylococci was essentially identical in both superior cava and hepatic samples. When compared with normal clearance curves, there was a slight reduction in the initial rate of clearance, and a more gradual transition to the slower rate of removal in thorotrast-prepared animals. Nevertheless, despite the apparent blocking of this effective removal system, the number of circulating staphylococci fell rapidly from initial levels of  $10^6$  microorganisms per ml. to levels of 600 to 1000 microorganisms per ml., 90 minutes following injection of culture. As in normal animals, a second injection of  $5 \times 10^8$  microorganisms given at 90 minutes, again resulted in the prompt reduction in the number of circulating staphylococci to approach levels of 600 to 1000 microorganisms per ml. 90 minutes after the second injection (Text-fig. 13).

#### DISCUSSION

It has been repeatedly demonstrated that most microbial species are rapidly removed from the peripheral blood following their intravenous administration (6, 7, 10, 12, 14, 29, 32, 33). Many investigators have shown that the majority of circulating microorganisms are removed from the blood in transit through the liver and spleen, while differential arterial-venous sampling across other capillary beds has failed to reveal significant trapping of microorganisms in other organs or the extremities during bacteriemia (4, 17, 23, 28, 33).

Despite considerable study of the mechanisms responsible for the initial dramatic clearance of microorganisms following their intravenous injection, little attention has been directed toward the low level bacteriemia which may

subsequently persist for many hours (13). While quantitatively less impressive, it seems reasonable to assume that host management of such persisters within a microbial population, is of major importance in determining the outcome of infection when bacteria have gained access to the blood stream.

Our studies indicate that the strain of staphylococcus used in these studies is swiftly incorporated within rabbit polymorphonuclear leukocytes. Such intracellular residence appears to protect a significant number of viable staphylococci from removal within the reticuloendothelial system of the liver and spleen. Extracellular staphylococci appear to be preferentially trapped in the reticuloendothelial system during the rapid phase of clearance, and splanchnic sequestration of staphylococci declines as increasing numbers of circulating staphylococci are located within granulocytes.

The observation that splanchnic trapping of staphylococci is again evident when extracellular microorganisms reappear in the circulating blood during the later phase of increasing bacteriemia, supports the belief that failure to trap microorganisms within the splanchnic viscera is related to their intracellular location. Also in keeping with such a concept is the finding that the removal of staphylococci from the peripheral blood is increased in the face of severe granulocytopenia, and is impeded when some of the administered staphylococci are incorporated within leukocytes prior to their intravenous injection. Further evidence of the possible significance of intracellular survival was obtained in studies on a strain of pneumococcus which appears to remain viable only when situated extracellularly. This microorganism was completely cleared from the circulation in simultaneous clearance experiments.

The present studies appear to exclude saturation of splanchnic removal mechanisms as an explanation for this decline in splanchnic uptake of circulating staphylococci. Indeed, there is much evidence to suggest that the capacity of splanchnic removal mechanisms is almost limitless, effective clearance being demonstrable in animals dying of overwhelming bacteriemia (18, 24). Furthermore, decreasing splanchnic trapping does not appear to be due to the small number of staphylococci *per se* which persist in the circulation during the period when relatively constant bacteriemia is demonstrable. A small initial inoculum of staphylococci disappears from the blood stream at a rate equal to that obtained following the administration of large numbers of staphylococci.

The decreasing splanchnic trapping of staphylococci noted 20 to 90 minutes after the injection of culture, is at variance with the findings of Martin and his coworkers who demonstrated a remarkably constant rate of splanchnic trapping during a continuous infusion of microorganisms (18-21). It appears probable that the continuous introduction of extracellular microorganisms in such experiments was responsible for steady rates of splanchnic removal obtained,

and that the effects of intravascular phagocytosis would not be detected in this experimental situation.

While splanchnic clearance appears of major importance in the removal of staphylococci from the blood of normal animals, experiments performed in thorotrast-prepared animals make it clear that effective mechanisms for the removal of circulating microorganisms exist outside the splanchnic bed. Despite the absence of demonstrable splanchnic trapping of staphylococci following thorotrast, staphylococci are cleared from the circulating blood at rates only slightly slower than those obtained in normal animals. Possible extra-splanchnic sites of removal have not been investigated in these studies.

The present studies demonstrate that the intravenous injection of staphylococci produces a swift disappearance of circulating polymorphonuclear leukocytes, followed by a rapid return to preinjection granulocyte levels or above.

It has long been known that the injection of a wide variety of substances, including particulate matter, foreign proteins, bacteria, endotoxin, and pyrogens can produce a rapid reduction in circulating leukocytes (5). The resultant leukopenia may differ in its speed of onset and duration, depending in part upon the substance injected, or previous host experience with the injected agent (1, 5, 29, 32). Studies by Wood (31) suggest that the rapid disappearance of circulating leukocytes following the injection of bacteria may be related to alterations in the surface characteristics of the polymorphonuclear leukocyte, resulting in leukocyte "sticking" to the capillary vascular endothelium. Histologic studies of tissues removed minutes after the injection of leukopenia inducing substances have revealed aggregation of large numbers of polymorphonuclear leukocytes in the capillaries of many organs (27, 30-32). It is thus generally agreed that the leukopenia appearing after the intravenous injection of bacteria or other agents is due to the local sequestering of granulocytes in capillary beds or sites of local vascular injury.

The observations reported here suggest that staphylococci are ingested by polymorphonuclear leukocytes during the phase of leukopenia, perhaps by leukocytes sequestered in various capillary beds. Coincident with the reappearance of leukocytes in the circulation, almost all the circulating staphylococci appear to reside within polymorphonuclear leukocytes. Splanchnic removal mechanisms significantly decline or virtually cease at this time.

It is well known that microorganisms are rapidly and strikingly phagocyted by leukocytes sequestered in various capillary beds (3, 11, 31, 32). It has been generally assumed, however, that such ingested microorganisms are permanently removed from the circulation, and the possibility that leukocytes containing viable bacteria may persist within, or rapidly reenter the circulating blood has received little attention. Nevertheless, it has long been noted by bacteriologists that microbial growth first appears in the buffy coat in blood culture specimens, and occa-

sional reference to the finding of intracellular microorganisms in the peripheral blood of animals and man during bacteriemia has been found in the literature (3, 15). That this phenomenon has not been previously documented is no doubt owing to the extremely small number of granulocytes containing microorganisms apparent on smears during bacteriemias of 1000 microorganisms per ml. or below. At this concentration of microorganisms, one intracellular bacterium per 3000 to 4000 leukocytes represents phagocytosis of the entire circulating microbial population.

It seems probable on the basis of these studies, that intracellular residence of a small fraction of the microbial population may explain the persistence of low grade bacteriemia noted following the injection of large numbers of staphylococci. Phagocytosis and subsequent residence within circulating leukocytes may occur with certain other microorganisms, survival or death within the cytoplasm determining the persistence or disappearance of bacteriemia.

#### SUMMARY

During the course of studies on the characteristics of experimental bacteriemia, staphylococci were swiftly cleared from the blood stream of rabbits during the initial 10 to 15 minutes following intravenous injection of microorganisms. A subsequent abrupt decline in the rate of clearance ensued, resulting in a low grade bacteriemia which was demonstrable for many hours.

The experiments reported have indicated that this strain of staphylococcus is rapidly phagocytosed within the vascular system of rabbits, and that viable staphylococci circulate within the cytoplasm of polymorphonuclear leukocytes.

The removal mechanisms contained within the liver and spleen appear to preferentially trap circulating extracellular staphylococci. When most of the circulating staphylococci are contained within leukocytes, splanchnic removal declines or virtually ceases.

These observations suggest that viable, intracellular microorganisms are responsible for the persistence of staphylococcal bacteriemia in rabbits following the phase of rapid removal from the blood stream.

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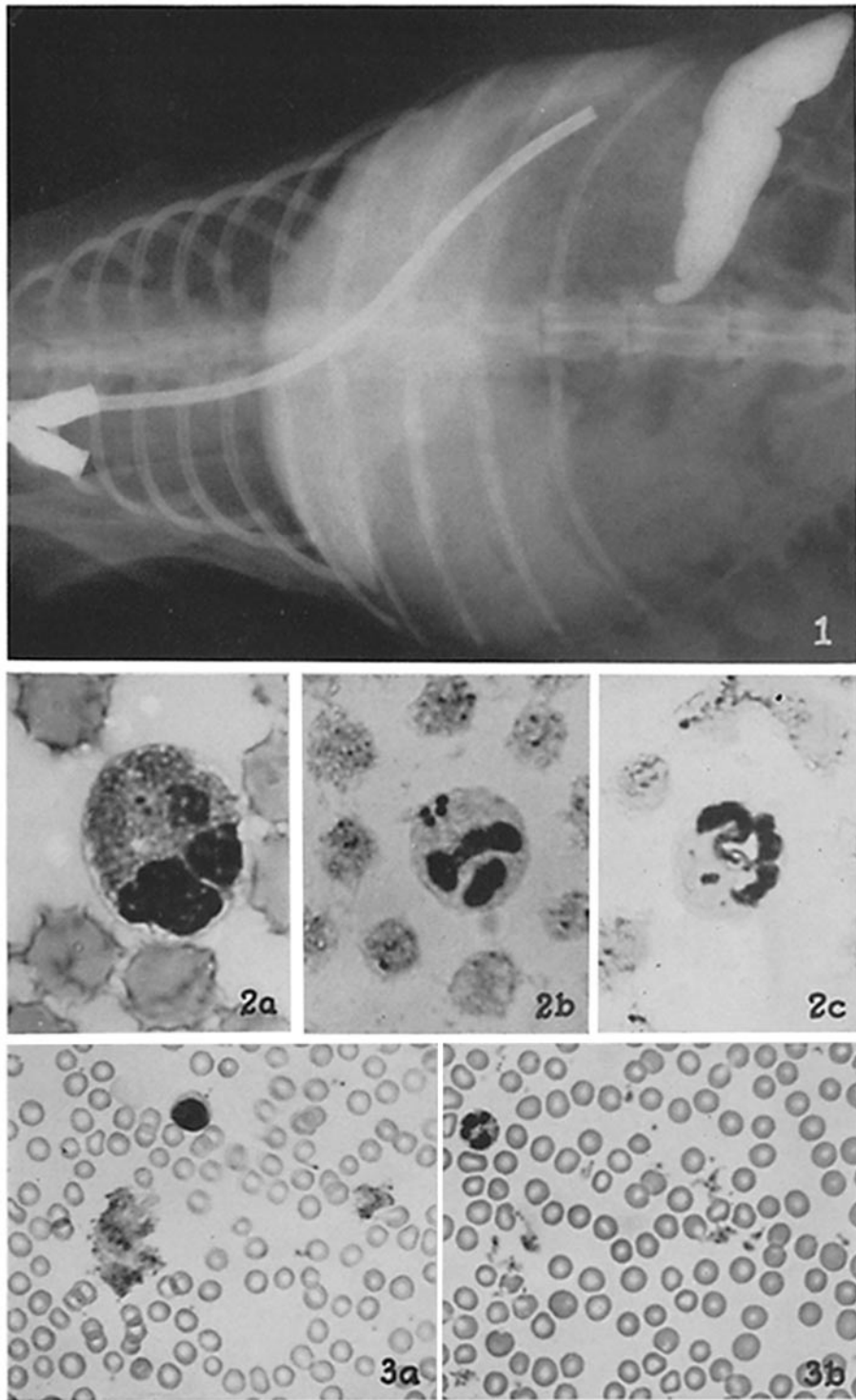
#### EXPLANATION OF PLATE 28

FIG. 1. The radiopaque hepatic vein catheter is shown in position in the left hepatic vein. This animal received thorotrast 24 hours preceding catheterization, which has opacified the spleen, and to a lesser extent, the liver. The second syringe adapter leads to a polyethylene catheter lying in the superior vena cava.

FIGS. 2 *a* to 2 *c*. Photomicrographs of polymorphonuclear leukocytes containing sharply staining staphylococci. These cells were obtained from superior caval and hepatic venous samples, 5 to 20 minutes following the injection of staphylococci. FIG. 2 *a*. Wright's stain.  $\times 2000$ . FIGS. 2 *b* and *c*. Gram-Weigert stain.  $\times 2000$ .

FIG. 3 *a*. Photomicrograph of blood smear taken 1 minute after the intravenous injection of staphylococci. Note the prominent clumping of platelets. No microorganisms can be visualized within these aggregates. Wright's stain  $\times 600$ .

FIG. 3 *b*. Photomicrograph of smear prepared 10 minutes after injection of culture in the same animal. Platelet clumps are no longer evident, and single platelets are again apparent. Wright's stain.  $\times 600$ .



(Rogers: Studies on bacteriemia. I)