

HOST MECHANISMS WHICH ACT TO REMOVE BACTERIA FROM THE BLOOD STREAM

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The body possesses remarkably efficient mechanisms for sterilizing the blood stream. Contrary to popular belief, most microorganisms are less capable of provoking disease when injected intravenously than when administered by any other route (1, 2). With rare exceptions, living bacteria which enter the blood stream of animals or man disappear swiftly from the circulation.

In the 73 years which have elapsed since the initial observations of Wyssokowitsch (3), the events which follow intravascular injections of bacteria have received extensive investigation. Studies on the intravascular behavior of different microorganisms or particulate substances which may mimic bacteria in their initial host management in the circulation have aided in clarifying certain aspects of the blood stream clearance process. Although bacteremia can be profoundly modified by humoral immunity, initial host mechanisms acting to clear the blood stream are probably not dependent on prior experience with the invading microorganism. It is thus proper that the ways in which animals and man handle bacteria which enter the blood stream be included in this symposium on nonspecific host resistance.

It is the purpose of this paper to review what is known of the dynamics of blood stream clearance, the host tissues which participate in the removal of circulating microorganisms, the fate of bacteria lodged in organs which retain them, and the circumstances which may augment, or interfere with, host mechanisms dealing with bacteria which invade the blood stream.

CHARACTERISTICS OF BLOOD STREAM CLEARANCE AS DETERMINED BY PERIPHERAL BLOOD CULTURES

Although the pattern of clearance varies with the particular animal species and the particular microorganism under study, certain general characteristics of the removal process may serve to orient a more detailed commentary on host mechanisms at work during bacteremia.

When large numbers of living bacteria are rapidly injected into the veins of an experimental animal, quantitative blood cultures reveal a pre-

dictable series of events which can be arbitrarily considered in three phases (figure 1).

In the first phase, clearance is rapid. Culturable bacteria disappear swiftly from the blood stream during the first 10 min to 5 hr. When the results of quantitative blood cultures are graphed logarithmically against time, initial clearance rates are a straight line function and quite constant for the microbial parasite under study. A rather constant fraction of circulating bacteria present at any sampling interval is removed in transit through the circulatory system. Ninety to 99.9 per cent of circulating bacteria disappear during this period. This initial removal process appears essentially independent of the nature of the microbial parasite, the animal under study, or the subsequent outcome of infection.

This phase of dramatic disappearance of circulating bacteria is followed by a phase in which microorganisms persist in the circulation at lower concentrations, or by their more gradual removal at slower rates. There may be an abrupt cessation of clearance, resulting in a relatively static low grade bacteremia, or clearance rates may slow progressively, depending on the animal species and particular microorganism under study. Persisting or slowly declining bacteremia may continue for several hours or several days.

Subsequent events appear to relate to the eventual outcome of infection. (The word "determine" will be avoided because it seems probable that the outcome of the bacteremic state is settled prior to this period). During this late phase, there may be a temporary rise in circulating bacteria, but the majority of microorganisms disappear completely from the blood stream. Bacteria producing fatal infections reappear in the peripheral blood in increasing numbers, and bacteremia rises to high levels which persist until the death of the animal. That the virulence of the microbe governs the later blood stream findings was beautifully illustrated in the early studies of Wright (14). The bacteremias produced by three strains of pneumococci of varying pathogenicity for rabbits are redrawn from Wright's studies in figure 2.

With this simplified characterization of clear-

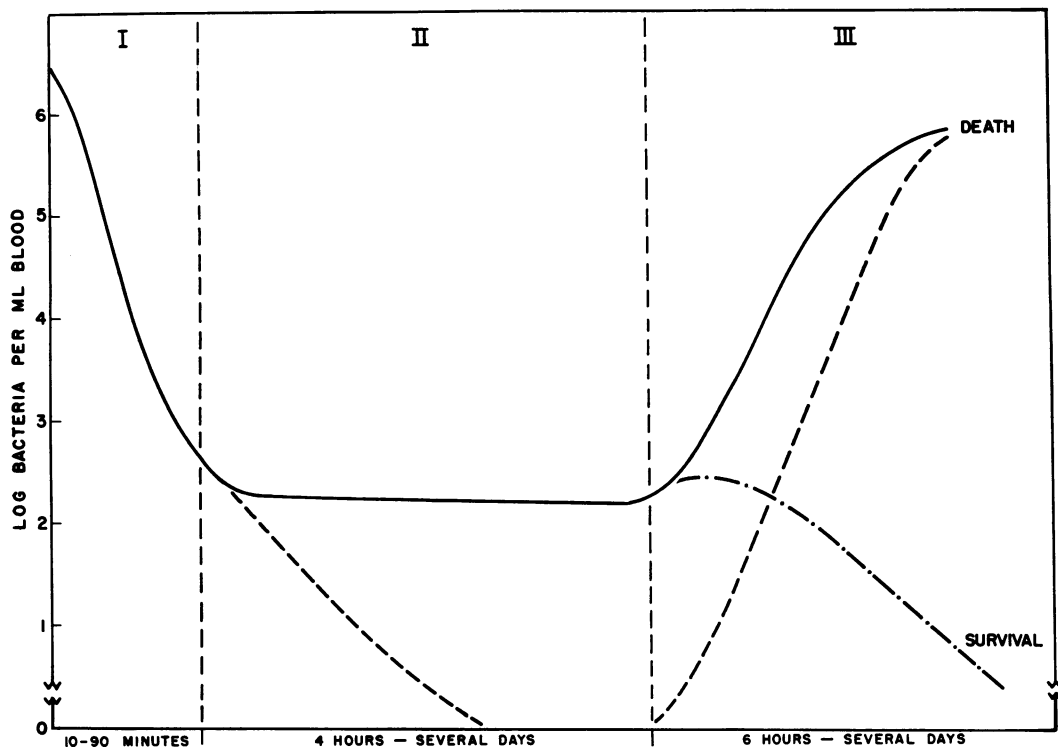


Figure 1. A schema of blood stream clearance as reflected by blood cultures. In phase I, bacteria disappear rapidly from the circulation. During phase II, bacteremia may disappear or persist at low levels. Depending on the virulence of the bacterium under study, resurging bacteremia or sterilization of the blood stream may be observed in phase III.

ance as determined by quantitative peripheral blood cultures, let us turn to an examination of the host mechanisms which appear to produce these changes.

HOST MECHANISMS WHICH REMOVE BACTERIA FROM CIRCULATION DURING INITIAL RAPID PHASE OF CLEARANCE

Splanchnic Tissues

The bulk of microorganisms removed from the circulation are trapped in vascular organs and tissues containing many fixed phagocytic cells. It is generally agreed that the histiocytic cells of the vascular and lymphatic endothelium, characterized by Aschoff (4) by their ability to sequester vital dyes and particulate substances, are also responsible for the trapping of circulating microorganisms (5). The cells of the reticulo-endothelial system which appear most active in dye studies, the Kupffer cells lining liver capillaries, and the phagocytic cells in splenic sinuses appear to be the most important trapping sites in the early

clearance of bacteria, although it is probable that the bone marrow, lymph node sinusoids, and other fixed phagocytic cell depots also participate (6-14). Quantitative studies on the organ distribution of intravenously administered microorganisms indicate that the liver collects the majority of bacteria when clearance is rapid. The spleen is equally avid in sequestering bacteria but stores smaller numbers of microbes during the early hours of clearance because of its relatively smaller size. Between 60 and 95 per cent of bacteria disappearing from the blood stream initially lodge in these two organs (7, 10, 15).

These studies on the principal sites of lodgment of bacteria during clearance are supported by the findings obtained when quantitative cultures are simultaneously obtained from the inflow and outflow blood of different organs during bacteremia. Such studies have consistently shown that only blood emerging from the splanchnic bed (blood draining the liver, spleen, and gut), shows any significant decrease in the number of culturable bacteria (16-20).

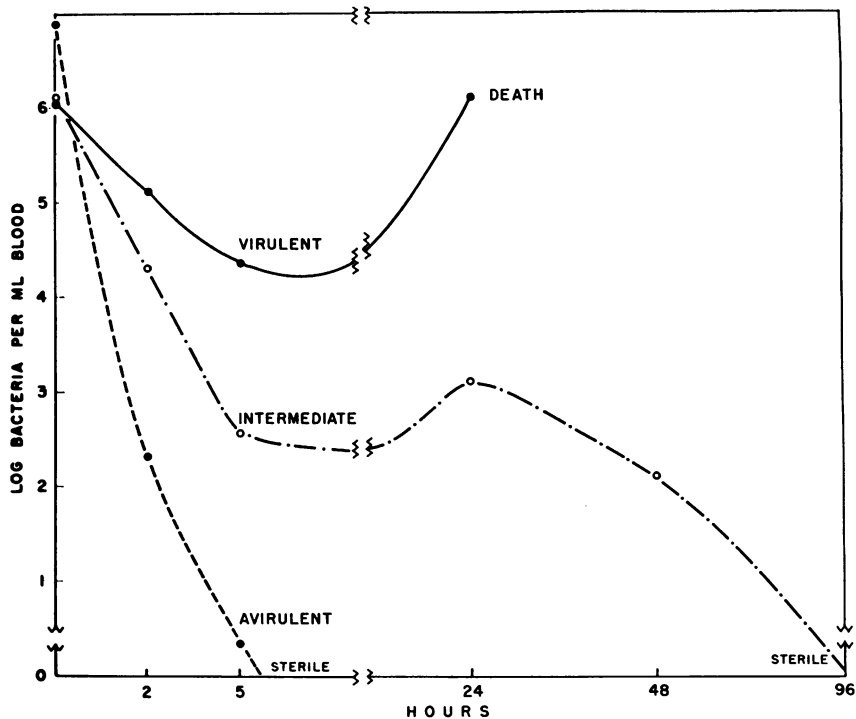


Figure 2. The blood stream clearance of 3 strains of pneumococci of differing virulence for rabbits (redrawn from Wright (14)).

Continuous perfusion experiments in intact animals by Martin *et al.* (17) and Rogers (18) have demonstrated that microorganisms presented to the splanchnic tissues are removed at constant rates which are characteristic for the particular bacteria under study and relatively independent of the concentration of microorganisms presented for removal. Such perfusion studies have further shown that immunization strikingly and specifically increases the trapping of bacteria within the splanchnic bed (20).

Results which correlate well with the findings in intact animals have been obtained when bacterial suspensions are perfused through surgically isolated or excised organs and tissues. A series of experiments of great simplicity and clarity performed by Manwaring and his associates over 40 years ago showed that only the isolated liver and spleen were capable of removing bacteria from a perfusion fluid (22, 23). Components of normal serum were necessary for the hepatic or splenic removal of certain bacteria, whereas others were avidly trapped in transit in the absence of serum. Certain bacteria were better retained than others, and the addition of specific immune serum to the

perfusate greatly increased the hepatic uptake of microorganisms poorly trapped in the absence of antibody.

An experiment illustrating the striking influence of immune serum on the trapping process has been redrawn from the studies of Manwaring and Coe in figure 3. As noted here, encapsulated pneumococci could be passed repeatedly through the isolated dog liver without removal when suspended in normal serum, but were promptly retained when suspended in immune serum. Only the hepatic and splenic trapping mechanisms were rendered more efficient in the presence of antibody, and increased trapping within other organs could not be demonstrated.

These early studies have been confirmed and extended by Wardlaw and Howard (24) in a recent series of similar studies utilizing the isolated rat liver. These experiments indicate that the hepatic trapping of many gram-negative bacteria is augmented by serum, complement, and perhaps properdin. In contrast, certain gram-positive microorganisms, notably staphylococci and streptococci, appear to be avidly sequestered in transit through the liver in the absence of serum con-

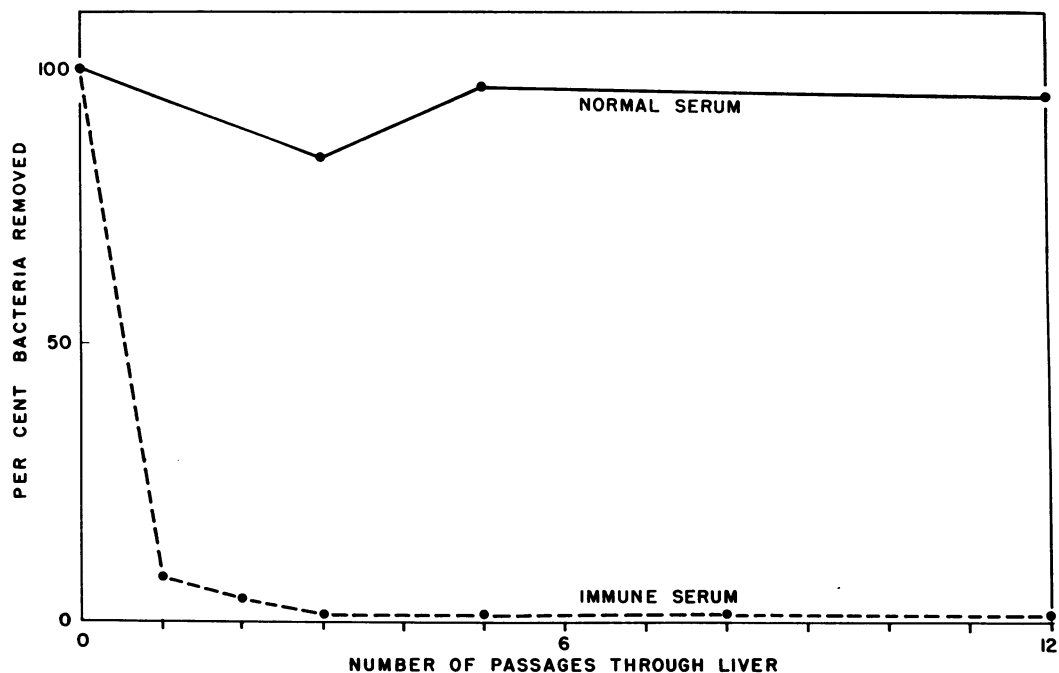


Figure 3. Hepatic trapping of pneumococci. Pneumococci traverse the isolated liver of dogs without removal when suspended in normal serum. The addition of specific immune serum to the perfusate results in prompt hepatic retention of microorganisms (redrawn from Manwaring and Coe (22)).

stituents, and, indeed, the addition of serum to the perfusate appeared to decrease the efficiency of hepatic trapping of these bacteria. The significance of this latter observation is difficult to assess at this time.

Utilizing data accumulated from intact animal and organ perfusion experiments, one can make certain summary statements regarding the early removal of bacteria from the blood stream. First, only the liver and spleen remove large numbers of bacteria from the circulation during the early phases of the clearance process. Secondly, these organs trap different microorganisms with differing degrees of efficiency. Thirdly, the degree of splanchnic retention of bacteria appears to be determined primarily by the nature of the particular microbe under study rather than the animal species in which the study is performed. The splanchnic or hepatic trapping of different bacteria has been found to be quite similar in different animal hosts.

Representative data have been collected in table 1. As noted here, staphylococci are avidly sequestered by splanchnic tissues of the dog, rabbit, and rat. The degree of removal is similar in all three animal species after single injections of

bacteria, continuous perfusion of the intact animal, or perfusion of the isolated liver. *Escherichia coli* are less efficiently trapped in similar experiments performed in the same animals. Pneumococci appear to traverse the splanchnic tissues in such experiments without significant removal when suspended in serum containing no antibody.

Lastly, these differences in the ability of the splanchnic tissues to retain different microbial parasites appear to govern the speed with which microbes disappear from the circulation during the early moments after injection. For example, our own studies show that staphylococci which are avidly trapped in splanchnic tissues disappear from the rabbit circulation more rapidly than do equal numbers of pneumococci which are poorly sequestered (figure 4). Similar findings have been obtained in mice by Thorbecke and Benacerraf (15).

Capacity of the splanchnic reticulo-endothelial system. Studies performed with living bacteria suggest that the trapping capacity of the reticulo-endothelial system is rarely, if ever, exceeded. It has been shown that repeated intravenous injections of bacteria are each cleared with equal speed (9, 14, 18, 25, 26, 32), that overwhelming bac-

TABLE 1
*Splanchnic or hepatic trapping of 3 different microorganisms in 3 animal species**

Microbe	Animal Species	Microorganisms Removed	Experimental Procedure	Author and Reference No.
Staphylococcus	Dog	62-74 ± 15	Continuous infusion, intact animal	Martin and Kerby (27)
	Rabbit	77 ± 7	Single injection, intact animal; decreasing trapping first 10-30 min	Rogers (18)
	Rat	67	Perfusion, isolated liver	Wardlaw and Howard (24)
<i>Escherichia coli</i>	Dog	40	Perfusion of isolated liver	Manwaring and Fritschen (23)
	Rabbit	40-78	Single injection bacteria into intact animal; increasing trapping first 30 min	Rogers and Melly (20)
	Rat	41	Perfusion of isolated liver	Wardlaw and Howard (24)
Pneumococcus	Dog	0-5	Perfusion of isolated liver	Manwaring and Coe (22)
	Rabbit	8 ± 14	Bacteremia from skin focus; intact animal	Martin and Kerby (27)
	Rat	0	Perfusion of isolated liver	Wardlaw and Howard (24)

* Microorganisms suspended in blood or normal heterologous serum.

teremia produced with one microbial species does not change the capacity of the splanchnic tissues to trap other injected bacteria (27), and that animals moribund with metastatic infection can swiftly remove superimposed injections of bacteria from their blood stream (26). Studies on patients with long standing bacterial endocarditis have shown that splanchnic trapping is efficient and unaffected by the protracted bacteremia (16). For these reasons it is generally believed that saturation of or exhaustion of splanchnic trapping mechanisms does not play a role in the course of natural infections.

Studies performed with particulate substances indicate, however, that the capacity of the reticulo-endothelial system to retain injected substances is finite. Benacerraf, Biozzi, Halpern, and their associates have used carbon or other particulate or colloidal substances to show that clearance rates are always maximal when doses of particles similar to those utilized in bacteriologic experiments are injected intravenously (28-31). These observations have helped to explain why the rate of clearance of bacteria appears independent of the numbers of bacteria administered. In essence, all microbial clearance studies are "low dose" experiments. If massive doses of particulate matter are administered, the maximal trapping capacity

of the splanchnic tissues is eventually exceeded. In this range, the rate of disappearance of injected particles then becomes a direct function of dosage. Using such massive doses, the trapping capacity of reticulo-endothelial tissues can be explored in various situations known to affect host resistance.

Other Factors Operating to Clear Bacteria from the Blood Stream

Although the reticulo-endothelial tissues of the liver and spleen are quantitatively most important in removing circulating bacteria, there is evidence to indicate that other host mechanisms participate in blood stream clearance. This is convincingly demonstrated by clearance studies performed in animals whose splanchnic tissues have been rendered incapable of trapping bacteria.

Injections of large doses of certain substances such as colloidal thorium dioxide (Thorotrast), carbon, saccharated iron oxide, lithium carmine, or trypan blue can "blockade" the splanchnic tissues so that bacterial uptake in them is impaired (14, 18, 30, 32). Despite elimination of the splanchnic bed as a trapping site, striking disappearance of circulating microorganisms can often be demonstrated.

Such an experiment is pictured in figure 5. In

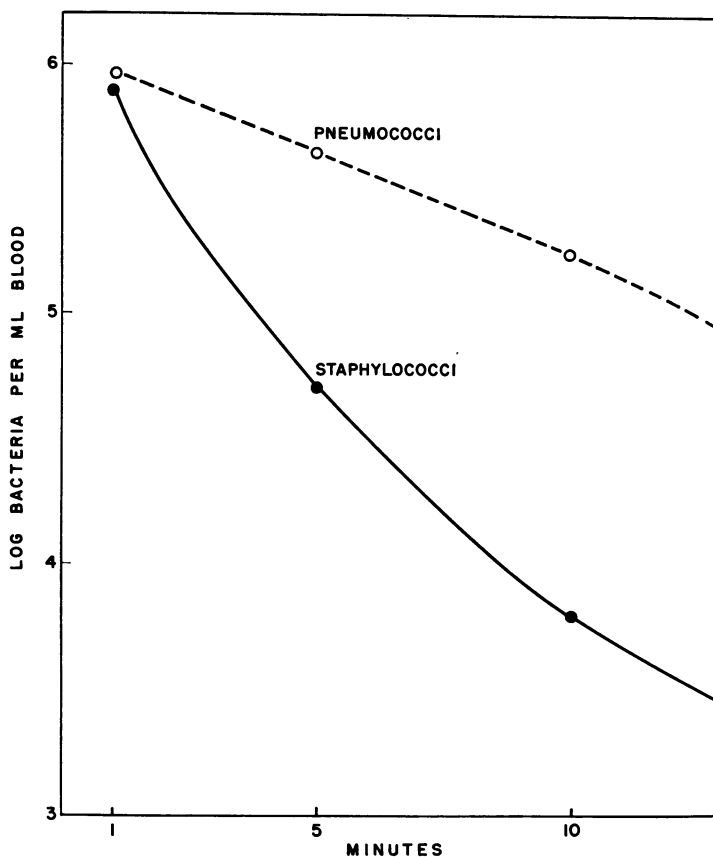


Figure 4. Initial clearance rates of two bacteria. Staphylococci, which are efficiently retained in the splanchnic tissues, disappear from the rabbit blood stream more rapidly than pneumococci, which are poorly trapped in the liver and spleen.

this study a normal rabbit and a rabbit receiving Thorotrast 24 hr previously received similar numbers of staphylococci intravenously. Simultaneous arterial and hepatic vein cultures showed that 70 per cent of staphylococci were removed in transit through the splanchnic tissues of the normal rabbit during the initial minutes of bacteremia. In contrast, no trapping of staphylococci could be detected across the splanchnic bed of the Thorotrast treated animal. Although some slowing of clearance was noted in the Thorotrast treated animal, a thousandfold reduction in circulating bacteria was observed in both animals at the end of 90 min. Clearly other tissues must have participated in such removal.

Intravascular Clumping of Bacteria as a Removal Mechanism

Certain studies have suggested that intravascular agglutination of bacteria may, in part, cause their initial disappearance from the blood stream

(33-36). Many particulate substances are unstable when introduced into the circulation and are rapidly agglutinated into larger masses which are filtered out in finer capillary beds. It has been noted that such intravascular aggregations of bacteria are associated with large numbers of platelets, and the initial moments of bacteremia are often accompanied by profound but transient thrombocytopenia (34-36). Such bacterial-platelet clumps lodge primarily in the capillaries of the lung. Serial observations suggest that aggregates are rapidly invaded by leucocytes which actively phagocytize bacteria at this site.

Although the intravascular aggregation of bacteria has been advanced as an important removal mechanism, current evidence suggests that this is probably not the case. It seems more likely that intravascular clumping is an inconstant phenomena relating to the immense numbers of bacteria administered in experimental studies on bacteremia.

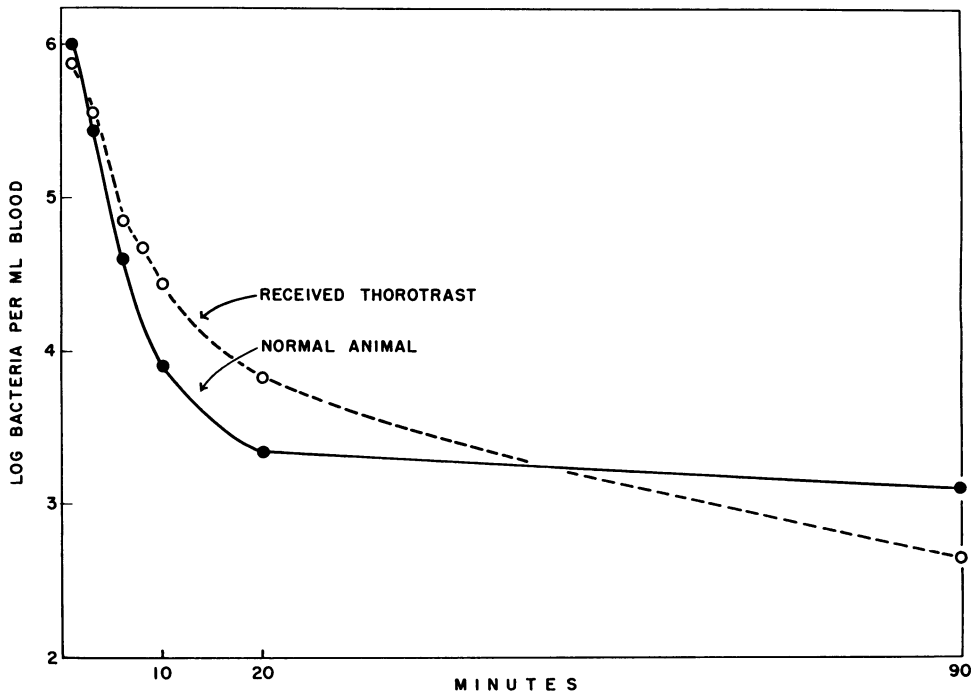


Figure 5. Effect of Thorotrast on blood stream clearance. Despite splanchnic "blockade," staphylococci disappear rapidly from the rabbit blood stream.

Participation of Circulating Leucocytes in Clearance

It is probable that peripheral leucocytes play an important qualitative, if not quantitative, role in blood stream clearance. Wood and his associates have clearly demonstrated that circulating leucocytes adhere to capillary endothelium following intravenous injections of bacteria (37). Motion photomicrographic studies show that such leucocytes are active in the trapping and phagocytosis of circulating bacteria (38).

As noted in figure 6, intravenous injections of staphylococci or pneumococci produce an immediate and profound leucopenia. Virtually all of the circulating granulocytes transiently disappear from the blood, only to re-emerge in the circulation 10 to 20 min later. Examination of stained smears prepared at this time commonly reveals cocci within the cytoplasm of circulating granulocytes. If gram-negative bacteria such as *E. coli* are similarly injected, a prolonged granulocytopenia ensues (39). In this instance, granulocytes disappear from the circulation for long periods, and leucocytosis is not apparent for 4 to 6 hr.

These observations suggest that injections of certain microorganisms like pneumococci and staphylococci cause some alteration in leucocyte or endothelial surfaces which causes transient sticking of the white blood cells in capillary beds. Serial histologic sections and inflow-outflow leucocyte counts show that these leucocytes lodge in lung capillaries and are active in ingesting circulating cocci. The rapid return of normal peripheral leucocyte levels and the appearance of stained smears suggests that some of these granulocytes return to the blood stream containing ingested microorganisms.

In contrast, intravascular injections of gram-negative bacteria appear to produce more intense leucocyte (or endothelial) damage. It seems probable that leucocytes which disappear from the circulation in response to such injections are permanently removed from the blood stream and that newly marshalled leucocytes are responsible for the late leucocytosis.

Current evidence suggests that the mobile polymorphonuclear leucocytes of the blood and the fixed phagocytic cells of the reticulo-endothelial system actively compete for the ingestion of

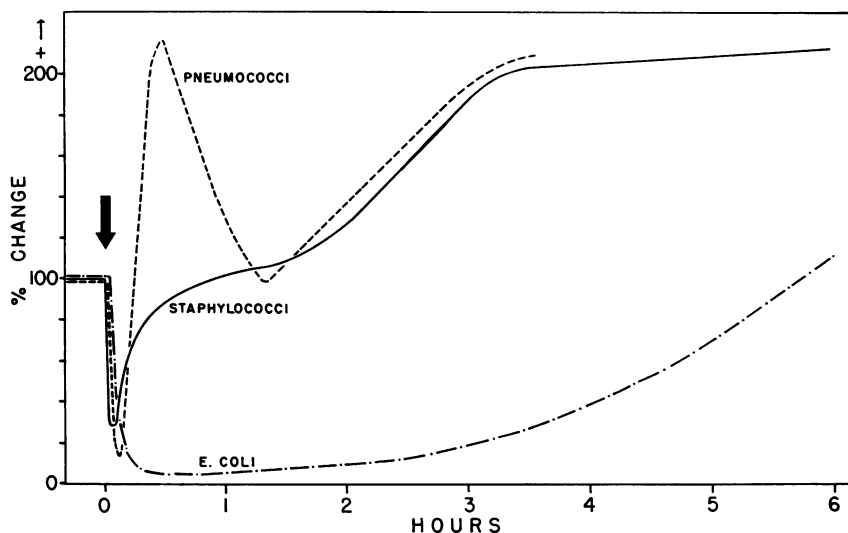


Figure 6. Changes in circulating polymorphonuclear leucocytes following intravenous injection of bacteria. Staphylococci and pneumococci produce a transient disappearance of circulating granulocytes. Injections of *Escherichia coli* produce a prolonged granulocytopenia (from D. E. Rogers, *Cellular Management of Bacterial Parasites, Pasteur Fermentation Centennial, 1857-1957*, pp. 61-73, 1958, reprinted by permission of Chas. Pfizer and Co., Inc.).

circulating bacteria. When splanchnic trapping is avid, polymorphonuclear leucocytes probably play a minor quantitative role in clearance. When bacteria are poorly retained in the splanchnic tissues, granulocytes may be more important in control of bacteremia.

Evidence can also be marshalled to suggest that the intracellular behavior of bacteria ingested by polymorphonuclear leucocytes in the blood stream may, in part, determine whether bacteremia will persist or disappear during the second phase of clearance. This can be illustrated by the intravascular behavior of a strain of coagulase-positive staphylococcus, an organism which survives in significant numbers within leucocytes in our studies (18), and a strain of type III pneumococcus, a microbe promptly destroyed by phagocytosis (40).

When large numbers of staphylococci are administered to rabbits, splanchnic trapping is initially avid, then rapidly declines, and virtually ceases within 15 to 20 min. A low grade bacteremia then persists for many hours. Extensive studies have shown that this cessation of splanchnic trapping is not due to saturation of splanchnic clearance mechanisms, or decreasing bacterial-reticulo-endothelial cell contact (18). Differential cultural studies indicate that virtually all of the

viable staphylococci are associated with the circulating leucocytes at the time that granulocytes reappear in the circulation. It is at this time that splanchnic trapping ceases, suggesting that the incorporation of cocci within leucocytes protects them from trapping within the reticulo-endothelial bed.

This thesis is supported by the observation that rabbits rendered granulocytopenic can remove *more* staphylococci from their circulation than can normal animals. Furthermore, if viable staphylococci are incorporated within rabbit leucocytes *in vitro* and then reinjected into rabbits, the staphylococci remain in the circulation for longer periods than do injected extracellular cocci (18). Thus in this instance the intravascular phagocytosis of a microbe which can survive within the cytoplasm of leucocytes may permit its persistence in the rabbit circulation.

Conversely, while pneumococci are inefficiently removed in the splanchnic circuit, these microorganisms disappear completely from the rabbit blood stream during the first 60 to 90 min after injection. Examination of peripheral blood smears performed on animals receiving intravenous pneumococci also revealed visible cocci within circulating granulocytes, yet in this situation *viable* pneumococci are culturable only from the

plasma layer. It would appear that in this instance leucocytes have rendered the ingested pneumococci nonculturable. Thus in pneumococcal bacteremia it would appear that destruction by polymorphonuclear leucocytes may play an important role in blood stream sterilization.

These observations are supported by earlier workers. Many years ago Wright showed that the blood stream clearance of a particular strain of pneumococcus could be directly related to the bactericidal power of the peripheral blood *in vitro* (14). This finding has been confirmed by others (8).

There is recent evidence to suggest that the leucocytes which disappear from the circulation may still be capable of clearing bacteria from the blood stream. Hollingsworth and Beeson (41) have shown that rabbits rendered leucopenic by irradiation maintain higher bacteremias following intravenous injections of *E. coli* than do normal animals. However, if such leucopenic animals are transfused with healthy rabbit leucocytes, subsequent injections of *E. coli* are cleared normally, despite the fact that the transfused leucocytes have disappeared from the peripheral blood (42). These observations strongly suggest that leucocytes sequestered in capillary beds can, in certain instances, remain active in removing bacteria from the circulation.

Microbial studies on clearance have one serious blind spot. Cultures tell us the location of liv-

ing bacteria. They do not indicate where actual destruction of bacteria occurs, or the initial location of microbes rendered nonculturable during clearance. Here radioisotope labeling may give important leads.

Thorbecke and Benacerraf (15) have recently performed clearance studies in mice using P^{32} -labeled staphylococci and *E. coli*. The distribution of labeled bacteria 30 to 60 min after injection as determined in their studies has been graphed in figure 7.

As shown in figure 7, the bulk of the staphylococcal inoculum is harbored in the splanchnic tissues at this time. Only 6 per cent of the radioactivity remains in the blood stream. In contrast, only 40 per cent of the P^{32} -labeled *E. coli* are contained in the liver and spleen at the same sampling interval, while 25 per cent of the isotope-labeled bacilli remain in the blood.

These studies have been of particular interest to us. As previously noted, our own cultural studies have shown that staphylococci tend to persist in the rabbit circulation while viable *E. coli* generally disappear completely from the blood stream (20) (figure 8).

One might suggest that the surprising radioactivity remaining in the blood stream of animals receiving *E. coli* may represent bacilli rendered nonculturable but remaining within the circulation. Simultaneous radioisotope and cultural studies are needed to determine whether circulating bacteria are actually destroyed by cellular or humoral processes in the peripheral blood or if such "non culturable" bacteria can remain in the blood stream following destruction.

Participation of Other Tissues in Clearance

In certain animal species, or in certain stages of the clearance process, the lung may play a role in the removal of circulating bacteria. The lung of the cat appears to trap large numbers of streptococci or particulate manganese dioxide during the early phases of clearance (9, 43). Rapid phagocytosis of streptococci or manganese dioxide takes place within the pulmonary capillary bed. Thus the lung may be an important initial clearance organ in certain animals.

There is also evidence to suggest that changes may occur within the lung as bacteremia progresses which can lead to the retention of circulating bacteria in pulmonary tissues. This can be illustrated during *E. coli* bacteremia in rabbits. No

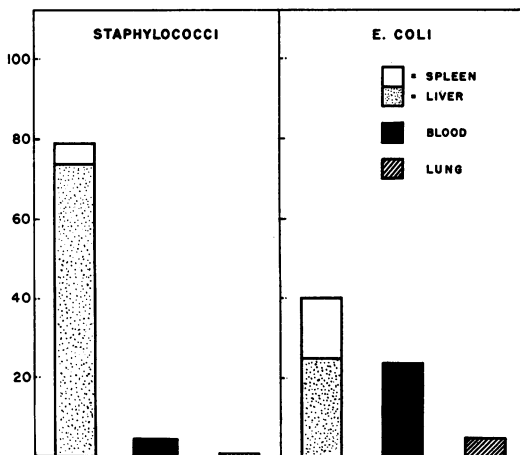


Figure 7. Organ distribution of P^{32} -labelled staphylococci and *Escherichia coli* 30 to 60 min after intravenous injections in mice (drawn from data presented by Thorbecke and Benacerraf (15)).

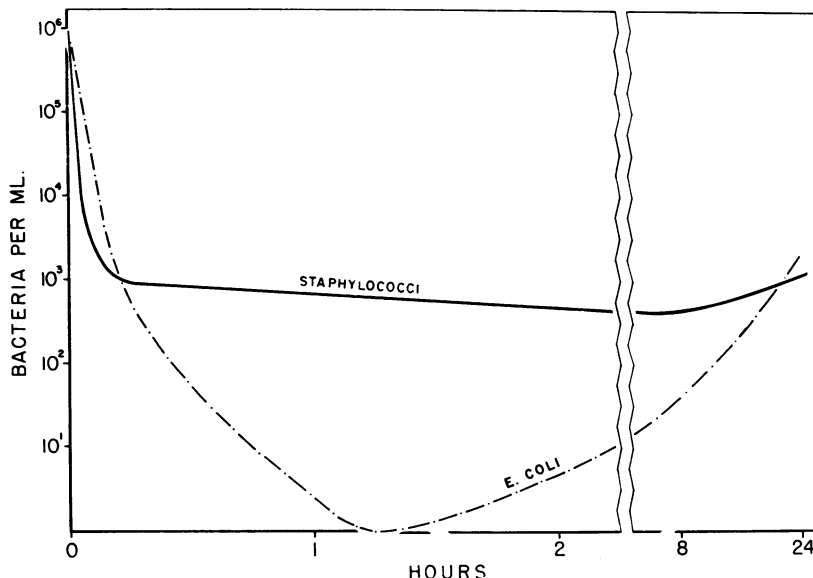


Figure 8. Blood stream clearance of staphylococci and *Escherichia coli* in rabbits. Staphylococci persist while *E. coli* disappear during the first several hours of bacteremia.

trapping of *E. coli* can be demonstrated in passage through the lungs during the initial moments of bacteremia. However, as noted in figure 9, the lung becomes a progressively more efficient filter as bacteremia proceeds and often removes 50 to 75 per cent of the microorganisms present in the pulmonary artery flow 1 to 5 hr after bacteremia has been initiated.

This increasing pulmonary clearance of *E. coli* may relate to leucocyte deposition within the lung. Polymorphonuclear leucocytes are continuously trapped within the pulmonary circulation for the first 4 to 5 hr after injections of *E. coli*. These cells may create a progressively efficient filter within the pulmonary capillary bed as bacteremia proceeds.

The studies of Vejlsen (44) have demonstrated that intravenous injections of particulate substances results in sequestration of leucocytes in terminal capillary and paracapillary beds throughout the body. It seems possible that such areas may develop as effective filtration sites during bacteremic states. The finding of large numbers of unusually active phagocytes in blood from massaged ear lobes in patients with bacterial endocarditis or other long standing bacteremias adds weight to this possibility (45). Perhaps these unusual phagocytes arise in response to the stimulus of a persisting bacteremia to serve as a second-

ary clearing mechanism during prolonged blood stream infections.

PERSISTENCE OR REAPPEARANCE OF BACTEREMIA

Very little is known about the mechanisms which maintain or augment bacteremia following the initial period of clearance. It has already been noted that saturation or exhaustion of the splanchnic clearing mechanisms has not been demonstrated in experimental bacteremic states. Although the evidence is less firm on this point, there is little to suggest that persistence or re-emergence of bacteremia is caused by *in vivo* changes in the microbial parasite. Our own studies on staphylococci or *E. coli* isolated from the blood stream during the phase of persistent or rising bacteremia would suggest that these microorganisms do not differ from those originally injected.

In certain instances the persistence of resurgence of bacteremia may relate to active intravascular multiplication of microorganisms. Studies by Wright showed that virulent pneumococci disappeared from the rabbit blood stream only when injected during the lag phase of growth, and clearance of this microorganism could be prevented by the injection of actively multiplying cocci (15). The presence of enormous numbers of meningococci in the blood of patients with meningococemia in the absence of obvious foci for

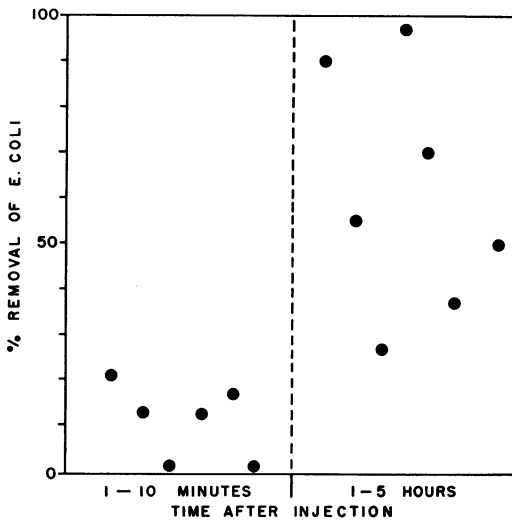


Figure 9. Pulmonary trapping of *Escherichia coli*. During the first 10 min of bacteremia there is little evidence of removal within the lung. As bacteremia progresses, increasing numbers of bacilli are removed in transit through the pulmonary vasculature (reprinted by permission. See figure 6).

seeding the circulation similarly suggests that intravascular multiplication has occurred, but it seems unlikely that this process is a common phenomenon.

Similarly, while staphylococci may persist in the circulation because of their survival within the cytoplasm of circulating leucocytes, this mechanism cannot explain the reappearance of many other bacteria which disappear completely from the blood stream during the early hours of clearance.

The bulk of experimental evidence suggests that bacteremia persists or resurges only when there is active seeding of the blood stream. Studies on streptococcal bacteremia by Hopkins and Parker suggested that small numbers of bacteria trapped in muscles were not subject to immediate destruction and underwent later multiplication in these areas to reenter the circulation (19).

Our own studies indicate that certain bacteria may reseed the circulation from the reticulo-endothelial tissues in which they are initially sequestered. Inflow-outflow cultures obtained across the splanchnic bed during the period of rising staphylococcal or *E. coli* bacteremia often indicate that large numbers of microorganisms reenter the blood from this initial trapping site (table 2).

Whether this represents the usual reason for the reappearance of bacteremia in virulent infections remains to be explored. For the most part the factors which determine whether bacteria ingested by the phagocytic cells of the reticulo-endothelial system will survive or be killed remain totally unknown. It has been demonstrated that profound depletion of certain host metabolites may occur in certain infections (46). One might postulate that changes in host substances essential to phagocytic cell function occur during the course of certain virulent infections which allow microorganisms to emerge from cells that have initially phagocytized them.

FACTORS WHICH ACT TO ALTER HOST CLEARING MECHANISMS

Do changes in the animal host known to affect resistance to infection alter the ability of the host to clear bacteria from the blood stream? Extensive studies have been directed to this question.

Experiments employing bacteria indicate that the initial rapid phase of clearance is virtually inviolate. Starvation (47), massive irradiation (48, 49), the production of agranulocytosis (14, 18, 50), shock (51), experimental diabetes (D. E. Rogers and M. A. Melly, *unpublished data*), splenectomy (19), renal failure (52), or the presence of overwhelming infection (26, 27) do not detectably change the early clearance process. The administration of drugs known to affect susceptibility to infections, including corticotropins (53), adrenal steroids (19), or endotoxin (51), similarly does not alter initial clearance. Changes in blood clotting induced by heparin (52) or removal of platelets with anti-platelet serum (55, 56) are also without effect.

Maximal reticulo-endothelial capacity can, however, be altered by many factors. It has been shown, for example, that depression of maximal reticulo-endothelial uptake of particulate substances occurs in the immediate period following administration of endotoxin (57, 58). Similar changes in splanchnic capacity have been noted in the presence of serious infection (59). Conversely, a definite increase in the ability of reticulo-endothelial tissues to clear circulating carbon particles can be obtained at certain intervals following injections of endotoxin (57, 58), certain estrogenic steroids (60, 61), or choline (62). In the face of findings suggesting that maximal function of the reticulo-endothelial tissues is rarely challenged by bacterial infections, it is currently

TABLE 2

Reappearance of bacteria from the splanchnic tissues of the rabbit following their initial trapping at this site

Staphylococci				<i>Escherichia coli</i>			
Time postinjection	Bacterial count per ml blood		Splanchnic trapping	Time postinjection	Bacterial count per ml blood		Splanchnic trapping
	Superior vena cava	Hepatic vein			Superior vena cava	Hepatic vein	
			%				%
4 min	360,000	40,000	88	1 min	1,805,000	1,100,000	39
10 min	9,000	4,000	56	5 min	400,000	165,000	59
20 min	2,500	2,800	0	10 min	43,800	18,300	58
40 min	2,000	1,800	0	40 min	440	140	69
24 hr	50	1,900	Seeding	60 min	610	430	30
45 hr	40	13,000	Seeding	90 min	620	2,000	Seeding
72 hr	1,900	40,000	Seeding	3 hr	3,700	4,800	Seeding
				5 hr	50,000	65,000	Seeding

difficult to know whether depression or augmentation of maximal phagocytic capacity relates to susceptibility or resistance to infection in any meaningful way.

While initial clearance is little modified by states affecting host resistance, there is much to suggest that the ability of reticulo-endothelial tissues to contain and destroy entrapped bacteria may be considerably influenced by a wide variety of stresses applied to the host animal. Starvation (47), the administration of thyroid, dinitrophenol (63), endotoxin (64), the presence of shock (51), irradiation (48), or the production of agranulocytosis can greatly increase the likelihood of re-surging bacteremia and death during the later phases of the clearance process. A single experiment will demonstrate this point.

If control mice and mice undergoing 24 hr starvation receive equal injections of Friedländer bacilli (*Klebsiella pneumoniae*), the initial speed of clearance and the organs of uptake are similar. No differences in the course of infection in the two groups can be demonstrated at this point. However, as infection proceeds, the behavior of bacteria residing in the liver, the principal site of early removal, is strikingly different (figure 10). Microbial populations remain stationary in the liver of control animals for 10 to 12 hr, then slowly disappear. In contrast, bacilli in the liver of the starved animals begin to undergo vigorous multiplication between 4 and 10 hr after infection. Infection then progresses until the death of the animal.

Many examples of host alteration leading to this type of change in the host management of

bacteria in the later phases of clearance could be cited. All studies emphasize a fact often ignored in literature on bacterial clearance: the disappearance of microbial parasites from the blood stream is not synonymous with their destruction.

RECAPITULATION

It thus emerges that multiple host mechanisms are called into play to clear microorganisms from the blood stream and to destroy them within phagocytic cells. Tissues active in the trapping process probably include the reticulo-endothelial system, the circulating leucocytes, and leucocytes sequestered in various capillary beds.

This removal system has immense reserve capacity which is rarely exceeded. There are indications that the system can adapt variably, depending on the nature of the infection or the duration of the bacteremic stimulus. Past experience with the invading bacteria can greatly enhance the removal, and possibly the destruction, of blood-borne bacteria but is not a prerequisite for effective blood stream clearance.

There is much to suggest that the initial removal process is not significantly altered by situations known to affect host resistance. Rather, the outcome of bacteremia appears dependent upon the ability of fixed and circulating phagocytic cells to retain and destroy ingested microorganisms. There are increasing indications that this intracellular phase can be altered in favor of the parasite by many states known empirically to affect host resistance.

There are many features of the blood stream process which are poorly understood. Bacterio-

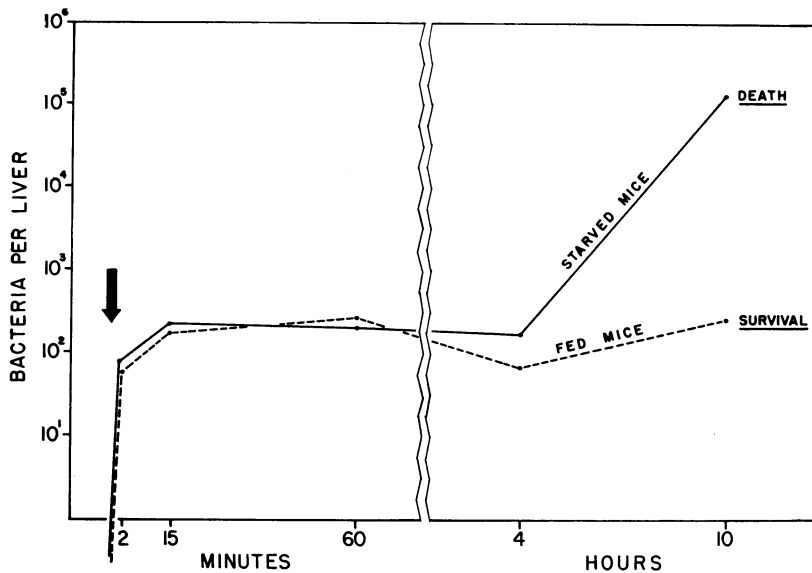


Figure 10. Behavior of Friedländer bacilli within the livers of fed mice and starved mice. A 1000-fold rise in bacterial populations residing in the livers of starved mice occurs 4 to 10 hr after infection (reprinted by permission, see figure 6).

logic studies do not tell us where nonculturable bacteria are localized during the early phases of clearance. We do not know where bacteria go when the splanchnic tissues are experimentally blocked. We do not know whether the various types of fixed and mobile phagocytic cells differ qualitatively in their abilities to ingest or kill different types of bacteria. We do not know the factors which determine whether bacteremia will persist or disappear in a particular animal host.

Perhaps most important, although we can impair intracellular processes associated with microbial killing, to date we know of no way to enhance the intracellular destruction of microbes. Certain of these questions can now be approached using radioisotope techniques, refined tissue culture methods, and biochemical procedures which may pinpoint the intracellular events which determine the outcome of blood stream clearance.

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DISCUSSION

Early reactions of the host to bacterial invasion may or may not be determinants of the eventual outcome of infection. For example, the rapid clearance by the reticulo-endothelial system of bacteria injected intravenously may be the same whether or not later reemergence of bacteremia and death occur. Early reactions of the host, on the other hand, have been shown to be associated with the late events in infection, as illustrated by the relationship of the outcome of infection by virulent and avirulent group A streptococci with the ability of the phagocyte to capture and destroy the bacteria (Rogers, Nashville, and Wood, Baltimore).

Besides the effect of the early antibacterial defenses of the host, the result of induced infection will vary depending upon the route of inoculation. Indicative of this is the observation that a very small number of pneumococci, possibly even one, can produce a lethal infection in mice when inoculated intraperitoneally, whereas very large numbers must be given intravenously to produce the same effect (MacLeod, Philadelphia).

In addition to phagocytosis, blood clearance by the reticulo-endothelial system, and route of inoculation, other factors may play important roles in determining the late events in infection. It was shown, for example, that in the presence of re-emergent bacteremia and lethal infection the reticulo-endothelial system retains its tremendous

reserve capacity to remove heterologous or homologous bacteria injected intravenously. Nevertheless, the reemergent bacteremia is not necessarily or usually controlled by the activity of the reticulo-endothelial system. This fact suggested that the bacteria able to persist in the fixed phagocytic system or able to reinvade the circulation may be resistant to the antibacterial action of the reticulo-endothelial cells. It was pointed out, however, that inoculation of blood containing the reinvading bacteria into normal animals is followed by the usual rapid blood clearing phenomenon, possibly illustrating that the persistent, reinvading, and multiplying bacteria are as susceptible to removal by the reticulo-endothelial system as bacteria grown *in vitro* (Rogers, Nashville). The work of Hedley Wright, however, indicated that pneumococci in the logarithmic phase of growth may evade the rapid initial blood clearing action of the reticulo-endothelial system (Wright, H. D., 1927, Experimental pneumococcal septicaemia and anti-pneumococcal immunity, *J. Pathol. Bacteriol.*, **30**, 185-252). Additional studies will be required to elaborate the influence of the state of the microorganism upon the late and early reactions of the host (Scherp, Bethesda).

The effect of opsonins or antibodies upon the clearance mechanism of the reticulo-endothelial system and upon phagocytosis was discussed and experiments were cited showing the blood clearance of *Escherichia coli* by fixed phagocytes in mice is inefficient, but as little as 0.01 μ g of specific antibody nitrogen per ml blood will greatly increase the clearing action of the reticulo-endothelial system (Benacerraf, New York). The failure of the reticulo-endothelial system to phagocytize and clear a second phase bacteremia could therefore be attributable to depression of serum opsonins. The ability to remove antigenically identical bacteria grown *in vitro*, however, even in the presence of lethal infection, is evidence against this possibility.

Specific antibody facilitates phagocytosis of staphylococci, and human serum after infancy almost invariably contains such antibody. In addition, the serum of rabbits frequently has no demonstrable antibody to the staphylococcus, although these animals may acquire such antibodies in their serum with increasing age, possibly attributable to natural infection. In the study of phagocytosis by leucocytes and fixed

macrophages, therefore, attention must be given to the possible influence of serum opsonins (Rowley, England).

Studies of clearance by the reticulo-endothelial system of bacteria injected intravenously, although necessary to elucidate some of the phenomena of nonspecific host resistance, may represent a relatively inefficient method of inoculating the microorganism into the peripheral tissues. If this is the case, it is probable that the early immediate clearance of bacteria from the blood stream does not take place in natural infection. The late phase of reemergent bacteremia, however, may be comparable to bacteremia occurring under natural conditions. Furthermore, dur-

ing the course of infection, the presence of bacteria in the bloodstream is almost always a reflection of active infection in the tissues, and it may not be the circulating bacteria that render the final outcome poor but the fact that bacteremia indicates a spreading infection in the tissues which is out of control (Bennett, Baltimore).

Finally, the mechanism of the adherence of leucocytes to vascular endothelium was discussed, and the experiments of Allison and Wood were cited as showing that this adherence phenomenon may be as dependent upon changes in the endothelium as in the leucocyte itself (Cluff, Baltimore).