STUDIES ON BACTERIEMIA*

II. Further Observations on the Granulocytopenia Induced by the Intravenous Injection of Staphylococci

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(Received for publication, October 9, 1956)

Pathogenic staphylococci survive for long periods within human and rabbit leukocytes following phagocytosis *in vitro* (6, 11, 13). The significance of this observation was illuminated by recent experiments on staphylococcal bacteriemia in rabbits, which showed that virtually all staphylococci cultured from the peripheral blood during the phase of persisting bacteriemia were contained within circulating granulocytes (9). Further study of this phenomenon suggested that intracellular residence of staphylococci protected them from removal by reticuloendothelial system of the liver and spleen.

Preliminary studies on the behavior of circulating white blood cells showed that the injection of staphylococci resulted in the swift disappearance of polymorphonuclear leukocytes from the circulating blood. Leukopenia was followed by the rapid reappearance of polymorphonuclear leukocytes in the peripheral blood, at a time which coincided with the intracellular location of the total circulating microbial population, and a relatively constant bacteriemia.

The present paper reports more detailed observations on the behavior of circulating leukocytes following the intravenous injection of staphylococci. Studies on the sites of transient leukocyte trapping, and the effects of certain experimental manipulations designed to alter the leukocyte response to the intravenous administration of staphylococci are also reported.

Materials and Methods

The experimental procedures used in these studies have been previously described (9). Healthy male chinchilla rabbits were lightly anesthetized with intravenous sodium pentobarbital. Venous or pulmonary artery catheterization was accomplished by inserting a radiopaque cardiac catheter in one jugular vein and appropriate positioning under fluoroscopic control. All arterial samples were obtained from an indwelling arterial needle placed in the

* This work was supported by research grant No. E 1078 from the National Microbiological Institute of the National Institutes of Health, Public Health Service, Bethesda; and by grants from the Lederle Laboratories Division, American Cyanamid Co., Pearl River; Charles Pfizer and Co., Brooklyn; and The Upjohn Co., Kalamazoo, Michigan.

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left femoral artery. Staphylococcus MAM, a coagulase-positive strain of staphylococcus described in the previous studies, was also used in these experiments. An aliquot of an overnight Todd-Hewitt broth culture, diluted to 2.0 cc. in normal saline, was used as the inoculum and was delivered *via* the left marginal ear vein. Following preliminary clearing of the catheters by the withdrawal of 2.0 cc. of blood, specimens were simultaneously obtained from both catheters during the course of experiments. Blood samples were immediately delivered to chilled siliconed tubes containing sufficient dried heparin to make a final concentration of 1:10,000. White cell pipettes were then filled in the routine manner and smears prepared.

In most experiments, total leukocyte counts were performed in standard fashion in bright line hemocytometers. Counts were frequently checked by two separate observers. In certain experiments, leukocyte counts were performed in a Jarrell-Ash arithmometer to increase counting accuracy. Differential leukocyte counts were performed on coverslips stained by Wright's method. One hundred white blood cells were counted in each differential. Bacteriemia was quantitated by the use of peptone infusion agar pour plates made with 1.0 cc. aliquots at appropriate dilutions.

EXPERIMENTAL

Pattern of Change in Circulating Leukocytes Following the Injection of Staphylococci.—When large numbers of staphylococci (5×10^8 microorganisms) were injected intravenously, the peripheral leukocyte count underwent a predictable series of changes. Within 1 minute there was a profound fall in the total number of circulating leukocytes, leukopenia reaching its maximum in 3 to 5 minutes. Leukopenia was followed by a rapid increase in the number of circulating leukocytes. Within 20 to 40 minutes, leukocyte counts had returned to levels equal to, or exceeding those obtained during the control period.

Differential leukocyte counts indicated that the major changes noted were due to variations in the number of circulating polymorphonuclear leukocytes. Changes in other cell types were of small magnitude. Because of our primary interest in phagocytic cells, attention was focused on changes in circulating polymorphonuclear leukocytes. The remainder of this discussion thus refers to this cell type alone, and the terms polymorphonulcear leukocyte and granulocyte are used interchangeably.

Fig. 1 portrays the change in the number of circulating polymorphonuclear leukocytes which occurred in 14 animals following the intravenous injection of 5×10^8 to 10^9 staphylococci. Granulocyte levels are graphed as a percentage of the initial control values.

As indicated in Fig. 1, there was a marked variation in the magnitude of the initial leukopenia. Nevertheless, the overall pattern of change was consistent. Maximum leukopenia, varying from 50 per cent to less than 1 per cent of the control values, had invariably occurred within 5 to 10 minutes. A rapid rise in circulating granulocytes then ensued, reaching or exceeding the initial control levels within 20 to 40 minutes in most animals. A leukocytosis was present at 3 hours in all animals, granulocytes representing 80 to 90 per cent of the total number of circulating white blood cells.

Leukocytosis generally persisted during the next 2 to 4 hours. 24 hours after

injection of bacteria there was a marked variation in the number of polymorphonuclear leukocytes in the circulation, some animals manifesting a marked leukocytosis, while others showed relatively normal leukocyte counts.

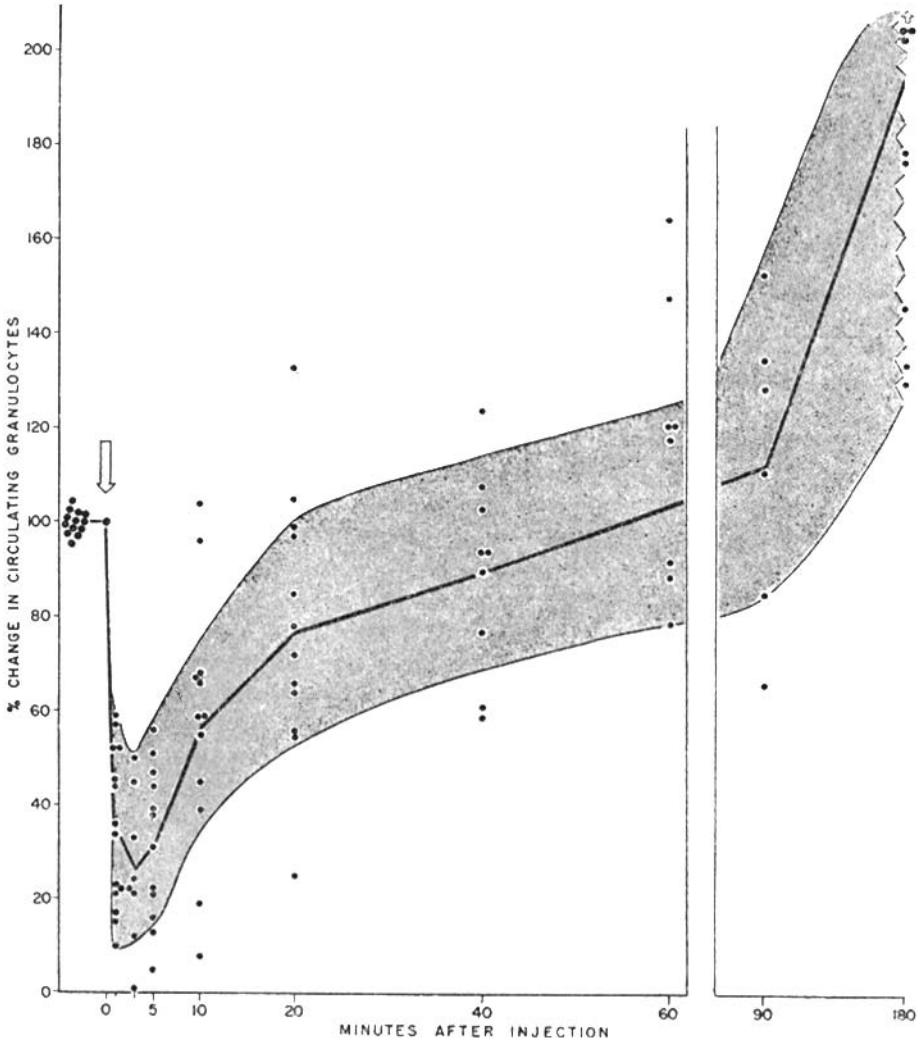

FIG. 1. Changes in the number of circulating granulocytes following the intravenous injection of 5×10^8 to 10^9 staphlyococci. Observations on 14 normal rabbits are recorded. The shaded area includes all animals receiving 5×10^8 microorganisms.

The initial transient leukopenia was associated with the injection of bacterial cells *per se*. When uninoculated sterile peptone infusion broth was injected, no leukopenia ensued. Cell-free culture supernatant also failed to produce a

significant leukopenia when injected intravenously. The administration of washed bacterial cells, both living and heat-killed, consistently produced a marked transient drop in the number of circulating polymorphonuclear leukocytes. Such an experiment is pictured in Fig. 2, in which 3 animals were simul-

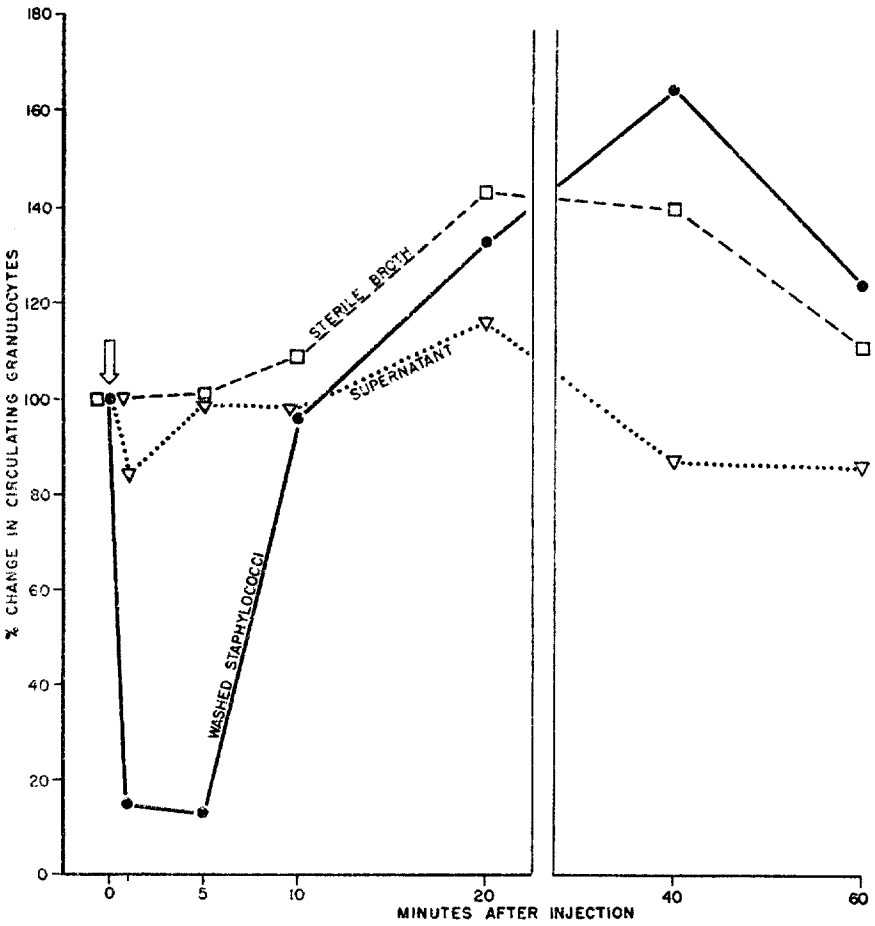

FIG. 2. The effects of sterile infusion broth, culture supernatant, and washed staphylococci on the number of circulating granulocytes. Only the bacterial cells produced a significant granulocytopenia.

taneously injected with sterile broth, culture supernatant, and washed staphylococci, respectively.

Trapping of Leukocytes across Various Capillary Beds.—To determine the sites of leukocyte removal, simultaneous arterial and venous blood specimens were obtained across various capillary beds. These included paired specimens across the pulmonary capillary bed, the liver, the hind extremities, a single front extremity, and the head.

In such differential sampling studies, two sites of leukocyte trapping were detected. Sampling across the pulmonary capillary bed revealed that moderate numbers of granulocytes were consistently removed within the lung during the initial 10 to 15 minutes following the injection of staphylococci. At 20 to 40 minutes and beyond, leukocyte counts in the femoral artery exceeded those in the pulmonary artery, suggesting that leukocytes were reentering the circulation from the pulmonary bed. The differences in leukocyte counts obtained in

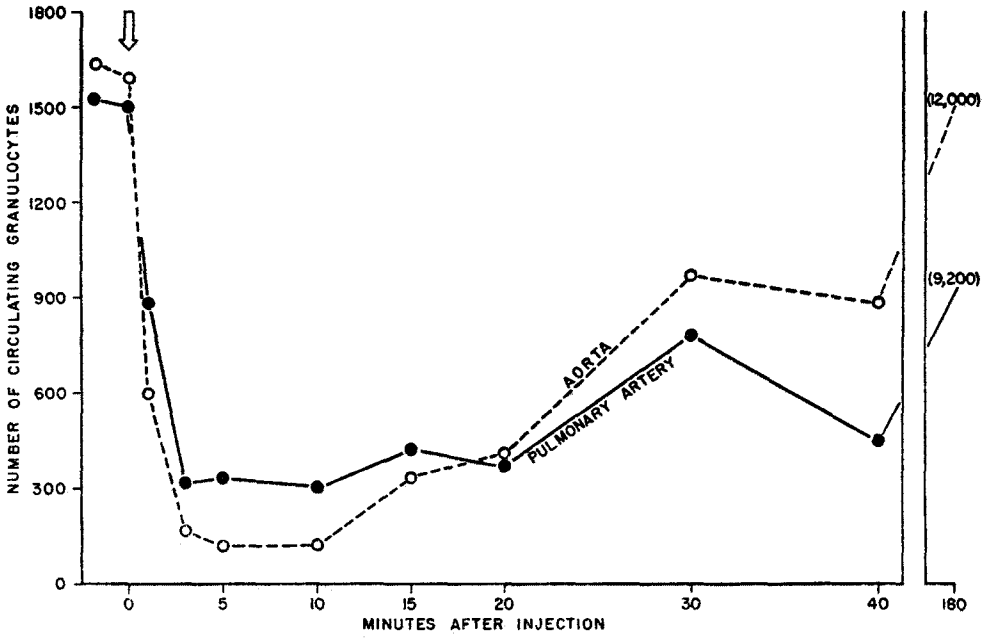

FIG. 3. The trapping of granulocytes within the lung following the intravenous injection of staphylococci. Granulocytes were typically trapped within the pulmonary vascular bed for the first 10 to 20 minutes. Aortic granulocyte counts then exceeded pulmonary artery granulocyte counts suggesting release of sequestered leukocytes.

the pulmonary artery and the femoral artery were never of great magnitude. Nevertheless, evidence of initial trapping was obtained in each of 5 experiments. A typical experiment is pictured in Fig. 3.

A similar sequence of initial trapping of leukocytes and subsequent release of cells was suggested by differential sampling across the splanchnic viscera. Here the differences were of small magnitude, but reasonably consistent evidence of initial sequestration of leukocytes was found in a series of 4 experiments. (See Text-fig. 12, reference 9). No significant degree of leukocyte trapping could be detected across peripheral capillary beds, including the hind extremities, the head, and the front leg. An experiment in which no significant

leukocyte trapping could be detected within the head or right upper extremity is pictured in Fig. 4.

Clearance of Staphylococci across Different Capillary Beds.—Experiments

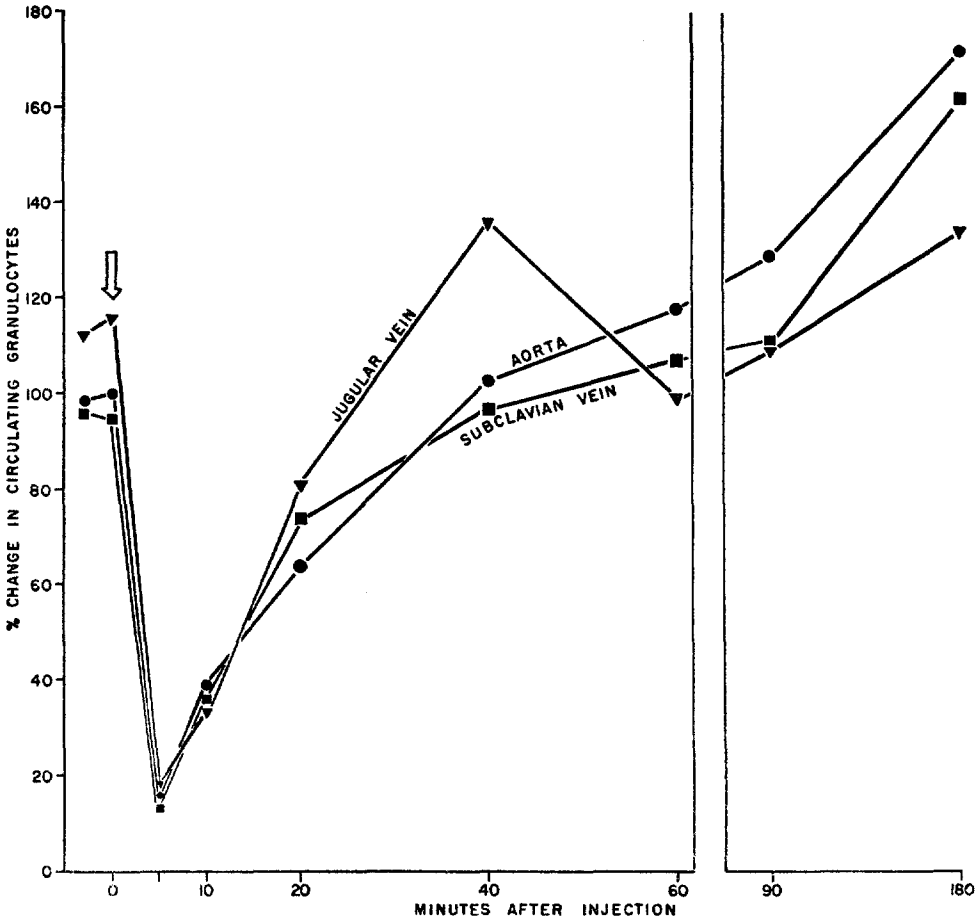

FIG. 4. Simultaneous leukocyte counts on aortic, left subclavian vein, and left jugular vein blood. No trapping of granulocytes could be detected across the head or left upper extremity following the intravenous injection of staphylococci.

previously reported showed that 70 to 80 per cent of the circulating staphylococci were removed in transit across the splanchnic viscera during the initial 15 to 20 minutes following the injection of staphylococci (9). No significant trapping of microorganisms could be demonstrated across any other capillary beds during the course of the present studies. In some experiments, a slight loss of culturable staphylococci appeared to occur across the lung during the

first 10 to 15 minutes following the injection of microorganisms, suggesting that transient trapping of staphylococci might take place in the lung. Nevertheless, the pulmonary sequestration of staphylococci was always of small magnitude (10 to 15 per cent) and was not statistically significant, falling within the range of plating error. No significant loss in culturable staphylococci occurred in simultaneous arterial-venous sampling across the extremities or the head.

Effects of Splenectomy on the Clearance of Staphylococci and the Behavior of Circulating Leukocytes.—It appeared possible that the rapid return of leukocytes to the circulation following the initial leukopenia might be explained by splenic contraction and delivery of granulocytes from a splenic depot to the general circulation. Furthermore, recent studies have suggested that individuals undergoing splenectomy at an early age may be peculiarly susceptible to infections associated with bacteriemia (7, 12). The clearance of staphylococci, and the changes in circulating leukocytes following injection of staphylococci, were thus studied in a small group of animals.

Four rabbits were splenectomized under sodium pentobarbital anesthesia. Following a recovery period of 1 to 8 weeks, the blood stream clearance of a standard number of staphylococci and associated changes in circulating leukocytes were studied as previously outlined.

As noted in Fig. 5, 3 splenectomized rabbits cleared their peripheral blood of staphylococci to the same degree and at rates which paralleled those noted in normal control animals receiving similar numbers of staphylococci. Fig. 5 is constructed to include the bacteriemias observed in normal animals within the shaded area. The clearance curves obtained in splenectomized animals are superimposed.

Simultaneous hepatic vein blood cultures showed that 70 to 80 per cent of staphylococci were removed from the circulation in transit through the livers of splenectomized animals during the 15 to 20 minute rapid phase of clearance. No differences in the intravascular location of staphylococci could be detected in splenectomized animals during the phase of relatively constant bacteriemia which existed 20 to 30 minutes after injection of microorganisms. At this time, the majority of circulating staphylococci appeared to reside within leukocytes as determined by centrifugation procedures previously described (9).

Studies on the behavior of circulating leukocytes following injection of staphylococci in splenectomized animals suggested that the initial leukopenia was less striking. Changes in the number of circulating granulocytes in 4 splenectomized animals following intravenous administration of 5×10^8 staphylococci are pictured in Fig. 6.

As noted in Fig. 6, initial leukopenia was not as marked in splenectomized rabbits as that generally observed in normal animals. Only one of 4 animals showed an initial drop in circulating granulocytes to less than 70 per cent of

control values. All 14 normal animals, shown in the shaded area, demonstrated reduction in circulating polymorphonuclear leukocytes to less than 50 per cent of preinjection granulocyte levels. Nevertheless, the over-all pattern of change was similar, and granulocyte counts again returned to control levels or above within 20 to 40 minutes. Differences in leukocyte response in normal and splenectomized animals did not assume statistical significance.

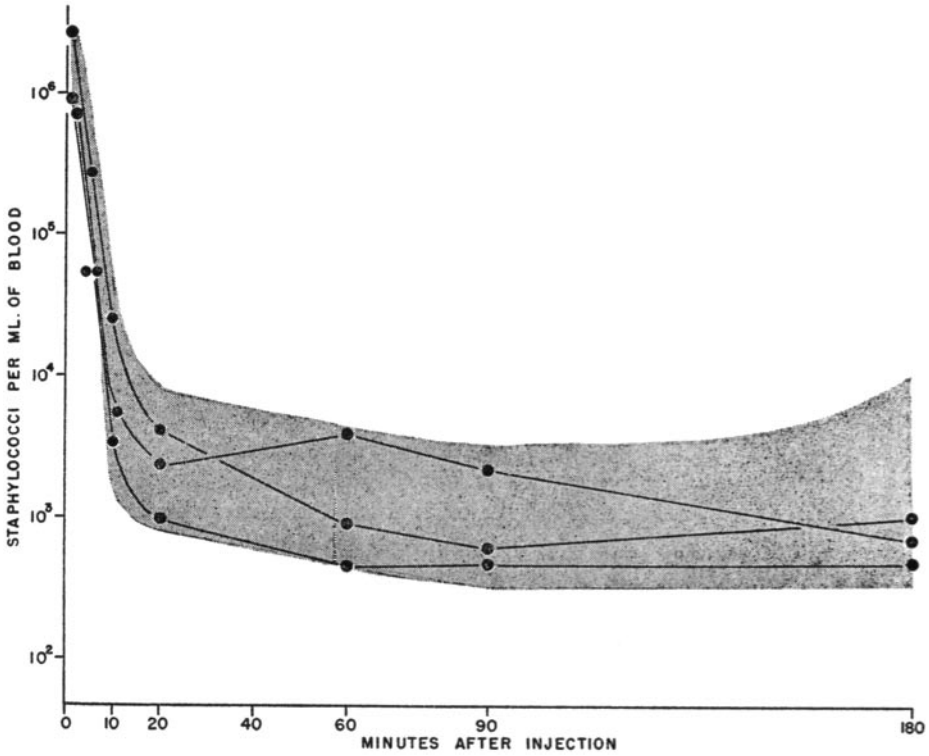

FIG. 5. The blood stream clearance of staphylococci in 3 splenectomized rabbits receiving 5×10^8 staphylococci intravenously. The bacteriemias noted in normal animals are represented in the shaded area.

Effects of Cortisone on the Leukocytic Response to the Injection of Staphylococci.—Allison, Smith, and Wood have recently reported that preliminary treatment of rabbits with large doses of cortisone can abolish the sticking of leukocytes to vascular endothelium in vessels surrounding a local area of thermal injury (1). Using these studies as a guide, a series of rabbits were given 25 mg. of cortisone twice daily for 3 days. On the 3rd day, these animals were injected with staphylococci *via* the left ear vein, and repeated samples of peripheral blood were obtained from indwelling catheters.

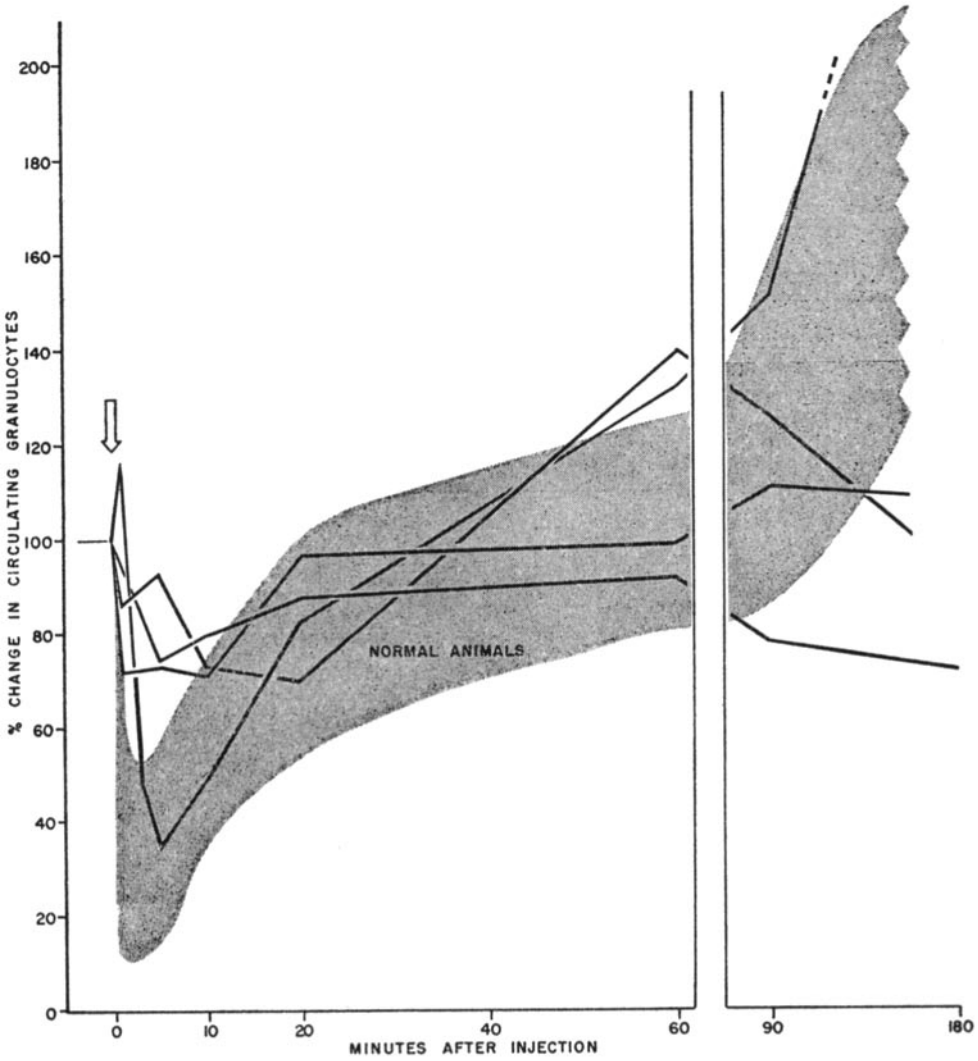

FIG. 6. Changes in the number of circulating granulocytes in 4 splenectomized rabbits receiving 5×10^8 staphylococci intravenously. Values obtained in normal animals receiving similar numbers of staphylococci are denoted by the shaded area.

As noted in Fig. 7, preliminary cortisone treatment failed to abolish the initial phase of leukopenia, 3 of the 4 animals showing a return to control leukocyte levels or above within 10 minutes, in contrast to the 20 to 40 minutes observed in 12 of 14 normal animals. Nevertheless, the small number of animals studied, and the wide variation in response in both normal animals and cortisone-treated animals, rendered these differences statistically insignificant.

Because of the failure to prevent the typical leukopenia, no definitive studies on the clearance of injected bacteria were performed on cortisone-treated animals. A single study on the intravascular location of microorganisms indi-

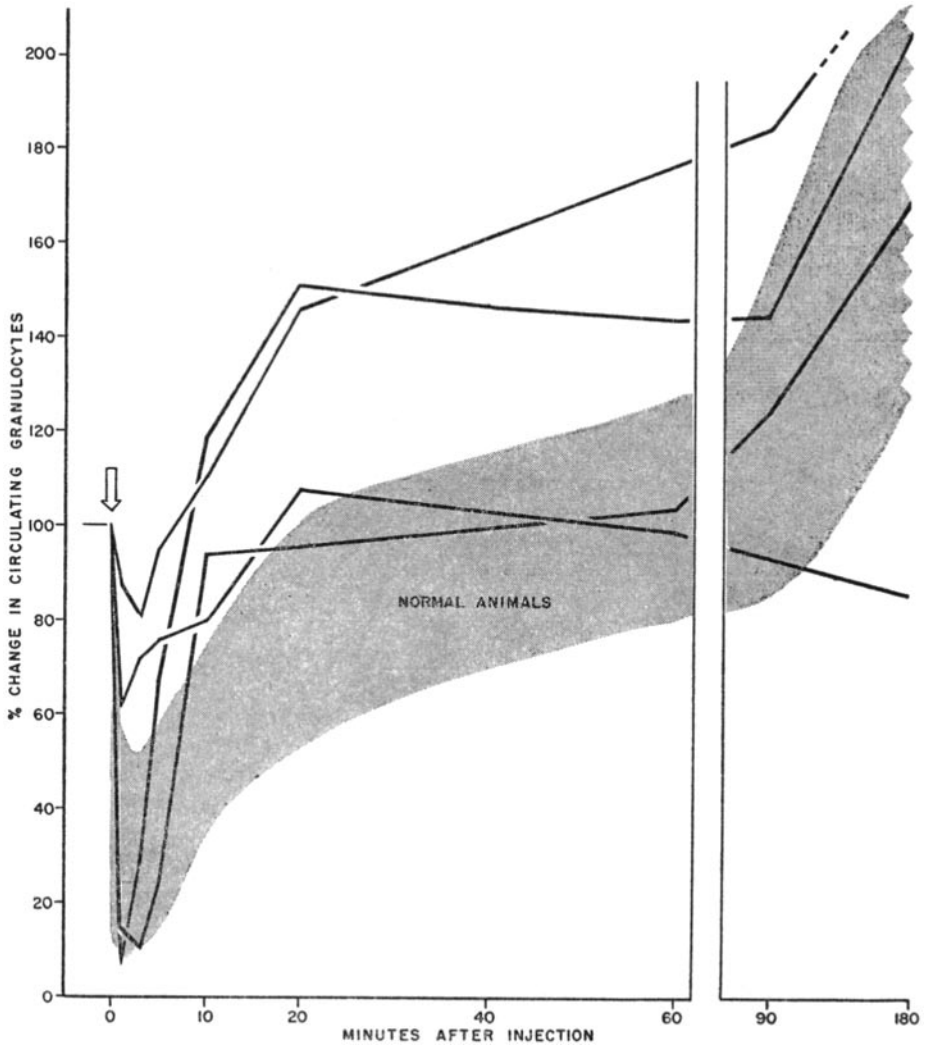

FIG. 7. Changes in the number of circulating granulocytes in 4 cortisone-treated rabbits receiving 5×10^8 staphylococci intravenously. Normal animals graphed as in Fig. 6.

cated that over 90 per cent of the circulating staphylococci resided within the leukocytes of a cortisone-treated animal during the phase of persistent bacteriemia.

Effects of Epinephrine on the Circulating Leukocytes of the Rabbit.—It has

been reported that the injection of epinephrine causes the release of leukocytes from the pulmonary capillary bed and that this area may serve as a site of leukocyte storage in man (4). It thus appeared possible that the trapping of leukocytes in the pulmonary vascular bed in response to the injection of staphylococci might be masked by the simultaneous release of leukocytes already residing in the small vessels of the lung in response to adrenal stimulus.

A series of animals with indwelling catheters in the pulmonary and femoral arteries were thus given 0.1 to 0.25 mg. of epinephrine *via* the left marginal ear vein, and paired leukocyte counts and differentials were performed at appropriate intervals.

TABLE I

Changes in the Number of Circulating Polymorphonuclear Leukocytes in the Pulmonary Artery and Femoral Artery Following the Administration of Intravenous Epinephrine

Time	Rabbit 6-2		Rabbit 6-4	
	Polymorphonuclear leukocytes per c.mm.			
	Pulmonary artery	Femoral artery	Pulmonary artery	Femoral artery
(Controls) 0 min.			4000	4600
0	4370	4970	4000	4060
	Epinephrine 0.25 mg. i.v.		Epinephrine 0.10 mg. i.v.	
1 min.	4150	5000	—	—
3 "	5400	4080	—	—
5 "	7990	6740	3650	3410
10 "	5780	6180	2850	2380
15 "	3940	4040	—	—
20 "	3220	3110	2750	3770
30 "	5800	4690	3850	3570
40 "	—	—	3510	3260
50 "	—	—	3740	3400
60 "	3120	4310	3240	4120

Marked variations in the subsequent leukocyte response were noted. In certain animals, total granulocyte counts rose rapidly and then declined in 15 to 20 minutes to pre-epinephrine control levels. In others a moderate transient leukopenia followed the injection of adrenalin. No consistent release of granulocytes in the pulmonary vascular bed was detected in any of the animals studied. Experiments on 2 animals are recorded in Table I.

DISCUSSION

In the present experiments performed with one strain of staphylococci, the intravenous injection of microorganisms produced a profound, transient granulocytopenia in rabbits. Maximum leukopenia occurred within 3 to 5 minutes. The number of circulating granulocytes then rose rapidly to equal or exceed

control values 20 to 40 minutes following the injection of culture. A decided leukocytosis due to increasing numbers of circulating polymorphonuclear leukocytes subsequently ensued.

Differential arterio-venous sampling across various capillary beds demonstrated that polymorphonuclear leukocytes were trapped within the lung, and to a lesser extent within the splanchnic viscera during the first 10 to 20 minutes following the injection of staphylococci. This phenomenon has been noted by others (2, 3, 5, 14, 15). Granulocyte counts obtained from blood samples taken distal to the lung and splanchnic viscera after 20 minutes were higher than those obtained from the arterial inflow tract, suggesting that leukocytes were being released from these sites to reenter the circulation.

There is much to suggest that secondary return of granulocytes to the circulation represents reentry of the very polymorphonulcear leukocytes initially removed from the blood stream. First, the rebound to normal granulocyte levels occurred within 10 to 40 minutes. There was little evidence of an increasing number of immature cells in blood smears taken at this time, and such rapid mobilization of cells from the bone marrow appears unlikely. Secondly, return of granulocytes to the blood stream could not be explained by splenic delivery of additional leukocytes. The removal of the spleen in 4 animals did not alter the rapid return of circulating granulocytes to control levels following the initial leukopenic phase. Thirdly, we could not obtain satisfactory evidence of peripheral capillary bed depots of leukocytes which could rapidly deliver cells to the blood stream. Differential sampling over various capillary beds failed to reveal a significant rise in leukocytes in the outflow blood over inflow blood during the rebound phase, except from the lung and splanchnic viscera in which, early trapping of leukocytes was apparent. Further, a significant leukocytosis could not be reproducibly obtained with epinephrine administration and no consistent release of leukocytes within the pulmonary bed could be demonstrated following the injection of epinephrine.

It should be noted that our failure to demonstrate leukocyte release from peripheral depots does not exclude this possibility. There is much to suggest that only a small fraction of the total intravascular leukocyte population is actually in circulation (8, 16). A generalized release of small numbers of granulocytes from peripheral capillaries or small venules could occur without detection by the conventional methods of leukocyte counting used in these experiments.

Preliminary treatment of rabbits with cortisone did not prevent the initial leukopenia produced by intravascular injection of staphylococci. We were thus unable to determine whether the initial leukopenia was vital to phagocytosis and subsequent intracellular transport of circulating staphylococci.

It is our present thesis that the following series of events occurs following intravascular injection of staphylococci. The majority of microorganisms are

rapidly removed in the reticulo-endothelial system of the liver and spleen. Granulocytes temporarily sequestered in small vessels of the lung and splanchnic viscera also phagocyte smaller numbers of staphylococci. While it is probable that many of these leucocytes remain attached to vascular endothelium, a certain number of sequestered polymorphonuclear leukocytes return to the circulation. Some of these cells contain viable staphylococci.

Evidence has been presented in a previous report to suggest that such intracellular staphylococci may persist in the circulation (9). Further discussion of the possible reasons for the persistence of microorganisms in the circulation following the initial rapid removal of bacteria are considered in the succeeding paper on the blood stream clearance of *E. coli* (10).

SUMMARY

Intravenous injection of staphylococci produced a marked, transient granulocytopenia in rabbits. Leukopenia was rapidly followed by return of polymorphonuclear leukocytes to the peripheral blood, and normal circulating granulocyte levels were reestablished within 20 to 40 minutes.

Differential arterio-venous leukocyte studies showed that polymorphonuclear leukocytes were trapped within the pulmonary vascular bed and, less constantly, in the splanchnic viscera during the initial 10 to 20 minutes following the injection of staphylococci. Granulocytes were subsequently found in larger numbers in blood leaving the lungs and splanchnic tissues, suggesting that entrapped polymorphonuclear leukocytes rapidly reentered the blood stream. This sequence of changes in circulating granulocytes was not significantly altered by splenectomy or the administration of cortisone.

It has previously been shown that virtually all staphylococci in the blood stream are found within circulating polymorphonuclear leukocytes 10 to 40 minutes after the injection of culture (9). It is during this period that granulocytes return to the blood stream in large numbers.

These observations suggest that staphylococci are phagocyted by polymorphonuclear leukocytes temporarily sequestered in the lungs and splanchnic viscera. It appears probable that some sequestered granulocytes containing living staphylococci subsequently return to the circulation. Such intraleukocytic staphylococci are believed to play a role in the maintenance of bacteriemia.

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