STUDIES ON THE CELLULAR IMMUNOLOGY OF ACUTE BACTERIEMIA

I. Intravascular Leucocytic Reaction and Surface Phagocytosis*

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Bacteriemia is a common complication of acute bacterial infection. Its presence indicates a serious illness from which the patient may fail to recover. Although modern chemotherapy has greatly improved the prognosis of the bacteriemic state (1), the presence of organisms in the blood stream must still be looked upon as a forerunner of possible disaster.

The serious import of bacteriemia has often led physicians to assume that the blood stream is endowed with only the weakest sort of antibacterial defense. That this assumption is incorrect, is indicated by an impressive body of experimental data relating to the ability of the mammalian blood stream to clear itself of injected bacteria (2). There is even evidence that the blood stream is considerably more resistant to bacterial infection than are certain extravascular tissues. For example, Oerskov (3) has shown that injection of 50,000 virulent pneumococci by vein may be survived by 8 out of 10 white mice, whereas the same strain introduced into the peritoneal cavity uniformly causes death even when less than five organisms are injected.

Histologic examinations of tissues from animals infected by intravenous injection have given rise to the belief that blood-borne bacteria are destroyed by reticulo-endothelial cells in the liver, spleen, and bone marrow (4, 5). Under certain conditions capillaries of the lungs are said to filter out bacterial aggregates, and here the polymorphonuclear leucocyte appears to be the active phagocytic cell (4–6). Cultural studies during bacteriemia of various organs (7) and of blood removed from specific sites in the general circulation (8) also suggest that the greatest number of bacteria are removed by the liver and spleen.

These observations, though obviously of great importance, do not indicate

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the mechanism whereby the phagocytic cells of the blood stream succeed in ingesting highly virulent bacteria, particularly those species that are resistant to phagocytosis *in vitro*. It will be recalled that many of the species of microorganisms commonly responsible for acute infections in man possess capsules which tend to protect them from phagocytic cells (9). The virulence of pneumococci, Friedländer's bacilli, and hemolytic streptococci, for example, depends in large part upon their ability to resist phagocytosis and to multiply in an extracellular environment; for once ingested they are usually destroyed.[1] Ever since the discovery of opsonins (11), students of immunology have contended that such fully encapsulated bacteria cannot be phagocyted except when previously opsonized by specific immune bodies (12). Evidence has been presented, however, from this laboratory (13–17) that maximally virulent pneumococci, Friedländer's bacilli, and hemolytic streptococci may be destroyed in the lungs, lymph nodes, and subcutaneous tissues by surface phagocytosis—a mechanism of cellular defense which requires no antibody. Since early clearing of the blood stream in unimmunized animals can be shown to be independent of antibody (4), the question arises as to whether the destruction of encapsulated blood-borne bacteria may not depend upon the same surface mechanisms which operate in extravascular tissues. The present report deals with a direct microscopic study of the intravascular phagocytosis which occurs during the course of bacteriemia produced experimentally with fully encapsulated strains of pneumococci and Friedländer's bacilli.[2]

Methods

The method employed for studying intravascular phagocytosis was a modification of the rabbit ear chamber technique, originally described by Sandison (20). Detailed descriptions of the method are published elsewhere (21).[3] Plexiglass chambers were made according to the specifications of Ahern *et al.* (22), except that the thickness of the base of the chamber was reduced from 1.59 mm. to 1.25 mm., and the space between the central platform and the cover glass was reduced from 50 μ to 20 μ. The mica coverslips recommended by Ahern *et al.* (22) were replaced by "No. 1" glass coverslips. These modifications were designed to improve optical conditions for use of the 40 x oil immersion lens.

Surgical instruments were boiled, and chambers, template, and punch were soaked for 1 hour in zephiran chloride (1:1000 dilution) and were rinsed in sterile salt solution. "Phisoderm" (hexachlorophene, G-11, Winthrop-Stearns, Inc.) was used for cleaning and shaving the ear preoperatively. Each rabbit (Flemish strain, weighing between 2.5 and 4.0 kg.) was

[1] The pathogenicity of the many forms of bacteria that offer no resistance to engulfment obviously depends upon other factors including ability to surivie and multiply within the phagocytes. As emphasized elsewhere (10) such essentially intracellular parasites tend to produce chronic infections (tuberculosis, typhoid fever, brucellosis, etc.), whereas the extracellular parasites give rise more often to diseases of explosive onset and shorter duration (*e.g.*, acute bacterial pneumonia, cellulitis, erysipelas, etc.).

[2] Preliminary accounts of certain phases of this study have been reported elsewhere (18, 19).

[3] The authors are grateful to Dr. Robert Ebert of the Department of Medicine of the University of Chicago for technical advice relating to the use of the chamber technique.

treated for 10 days after operation with daily injections of "syncrobin" (Schenley Laboratories, Inc.), containing 40,000 units of penicillin and 100 mg. of streptomycin.[4] Postoperative infection of the chambers was virtually eliminated by the chemotherapy. Vascularization of the chamber with maturation of capillaries was usually complete within 6 to 8 weeks.

Microscopic studies of capillaries in the living rat were made by the mesoappendix technique of Chambers and Zweifach (23).

The two strains of bacteria used in these studies—pneumococcus type I (strain A-5) and Friedländer's bacillus type A (strain "Chic")—have been described in previous publications (24, 25). Both were stored in rabbit's blood under vaseline (24), and their virulence was maintained by frequent passage through mice. All cultures were made in beef infusion broth containing 0.2 per cent dextrose and 10 per cent sheep serum; they were incubated at 37° C. For purposes of photography stained Friedländer's bacilli were used in some experiments. The bacilli were fixed with formalin and were stained with carbolfuchsin, according to the method of Castaneda (26). Such staining has been shown previously to have no demonstrable effect upon the phagocytability of Friedländer's bacilli *in vitro* (13). All observations recorded in the photomicrographs were nevertheless duplicated with unstained bacilli.[5]

Pneumonia was produced in rats by intrabronchial inoculation of pneumococci suspended in mucin. Details of the methods employed in producing the pneumonia and preparing the lungs for histologic study have already been published (24). Bone marrow was obtained by splitting the femur and removing the intact marrow with a fine probe. The marrow specimens were fixed in Zenker's solution containing 5 per cent acetic acid. Other tissues were fixed in Zenker-formol solution. All were stained by a modification of the Gram-Weigert technique (24).

The Occurrence of Intravascular Phagocytosis during Pneumococcal and Friedländer's Bacillus Bacteriemia—Histologic Evidence

In order to determine whether phagocytic cells in the blood stream are capable of ingesting fully encapsulated bacteria in the absence of antibody, large numbers of virulent organisms were injected into the veins of non-immune animals. When either type I pneumococci or Friedländer's bacilli harvested from 4 hour cultures were injected intravenously into rats, prompt phagocytosis was easily demonstrated in the sinusoids of the liver and spleen and also within capillaries of the lungs. Similar observations have been made in rabbits. The following is a representative experiment.

Friedländer's bacilli (type A, strain Chic) were grown in beef infusion-serum broth for 4 hours at 37° C. The harvested bacilli were washed twice in Locke's solution and resuspended in a final concentration of 2.5 billion organisms per ml. The femoral vein of each of twelve albino rats[6] weighing approximately 250 gm. was exposed by blunt dissection under ether anesthesia, and 4 ml. of the bacterial suspension was injected intravenously. Three animals were sacrificed at each of the following intervals after injection: 30, 60, 90, and 120 minutes. Sections were prepared from the lungs, liver, and spleen of each animal and were stained by

[4] Supplied through the courtesy of Mr. C. B. Taft of the Schenley Laboratories, Inc., New York.

[5] Photomicrographs illustrating phagocytosis of unstained Friedländer's bacilli in the blood stream are presented elsewhere (18).

[6] The strains of rats used have previously been shown to possess no natural opsonins to either type A Friedländer's bacilli or type I pneumococci (27, 28).

the Gram-Weigert method. In all instances bacilli were found in both reticulo-endothelial cells and polymorphonuclear leucocytes within the sinusoids of the liver and spleen (Figs. 1 and 2). Phagocyted bacilli were also demonstrable in polymorphonuclear leucocytes within the pulmonary capillaries (Fig. 3).

It may be argued that the results of such drastic experiments have little bearing upon the cellular immunology of bacteriemia, since the number of microorganisms injected was far in excess of that found in the blood stream of animals suffering from even the most fulminating infections of extravascular origin. Therefore, similar histologic studies were made in animals dying within 48 hours of experimental pneumococcal pneumonia.

Albino rats were inoculated intrabronchially with type I pneumococci suspended in mucin. The resulting pneumonia was uniformly fatal, most of the animals dying within 72 hours (23). All had bacteriemia terminally. From those rats which died within 48 hours of inoculation and were autopsied within less than 10 minutes after death, sections were made of the liver, spleen, lungs, and bone marrow and were stained for bacteria by the Gram-Weigert technique.

As shown in Figs. 4 and 5, phagocyted pneumococci were found in both macrophages and polymorphonuclear leucocytes within the hepatic and splenic sinusoids. Likewise, a surprisingly high proportion of the polymorphonuclear cells within the capillaries of the lungs contained diplococci (Fig. 6). Phagocytosis was not demonstrated in the bone marrow. Since homologous opsonins do not appear in detectable quantities in the serum of rats with pneumococcal pneumonia until after 72 hours (27), and since specific antibody can rarely be detected in the blood in the presence of bacteriemia (29), it is concluded that the intravascular phagocytosis here demonstrated occurred in the absence of antibody.

Many of the sinusoids of the liver and spleen also contained fibrinoid deposits[7] which appeared to be minute thrombi. Within their interstices, bacteria were frequently visible (Figs. 7 and 8). Similar deposits were noted in capillaries of the lungs (Fig. 9).

Intravascular Leucocytic Reaction during Acute Bacteriemia

A prominent feature of the microscopic preparations from the bacteriemic animals was the presence of relatively large numbers of polymorphonuclear leucocytes in the pulmonary capillaries and in the sinusoids of the liver and spleen. The manner in which leucocytes accumulate in small vessels of the body during bacteriemia was demonstrated by the following observations.

When capillaries of the ear chamber in normal rabbits are studied microscopically, the circulating leucocytes are clearly visible and can be seen to roll freely along the walls of the

[7] The reticular deposits were clearly stained by the Gram-Weigert method (an excellent stain for fibrin) and were not stained by hematoxylin and eosin.

patent arterioles, capillaries, and venules (30) (Fig. 10).[8] If microorganisms are then injected in large numbers (100 to 300 billion) into a vein in the opposite ear, the leucocytes begin to stick to the vascular endothelium within a few minutes after entrance of the bacteria into the blood stream. After 15 to 30 minutes many leucocytes have accumulated on the endothelium (Fig. 11); once attached to the vessel wall they become actively motile and migrate about on the endothelial surface both with and against the current (Figs. 12 to 14). The intravascular accumulation of leucocytes is maintained until the animal dies with the bacteriemia. In analogous experiments, the same leucocytic response to bacteriemia has been observed in capillaries of the rat's mesoappendix.[9]

Such sticking of leucocytes has been noted following intravenous injections of a variety of foreign substances (31). It should be emphasized that this reaction, which constitutes an efficient mobilization[10] of potentially phagocytic cells within the blood stream, occurs during the course of natural bacteriemia as well as following the sudden injection of large numbers of bacteria into the blood stream (see Figs. 5 to 9).

Mechanisms of Intravascular Phagocytosis

The mechanisms by which polymorphonuclear cells phagocyte encapsulated bacteria in peripheral capillaries during bacteriemia were revealed by direct microscopic observations using the ear chamber technique.

Rabbits with ear chambers were anesthetized with pentobarbital (nembutal) and inoculated intravenously[11] with either stained or living Friedländer's bacilli. The bacteria used for inoculation were washed twice in Locke's solution and were resuspended in concentrations of between 20 and 50 billion organisms per ml. The volume of suspension injected varied from 5 to 7 ml. Capillaries in the ear chamber were observed continuously for a period of several hours after inoculation. The behavior of the phagocytic cells and the fate of the bacteria in the capillaries were recorded by both still and motion picture photography. Most of the animals injected with such large quantities of bacteria died after 3 to 6 hours.

The intravascular cellular reaction described above, involving the sticking of leucocytes to vessel walls, occurred within 15 minutes after the bacterial injection. In fully patent arterioles, capillaries, and venules, where the flow of blood was relatively rapid, the blood-borne bacteria were rarely phagocyted. Phagocytosis was readily accomplished, however, in capillaries where the flow was sluggish or had ceased altogether (Fig. 15). Ingestion of the blood-borne

[8] The rolling leucocytes can only be shown by motion picture photography (19); because of their progression with the capillary stream, they are not visible as discrete cells in still pictures.

[9] The number of washed pneumococci injected into the femoral vein of each rat varied from 6 to 8 billion.

[10] The term mobilization here refers to the change in the functional capacity of the leucocytes which exhibit ameboid activity only after becoming adherent to the vascular endothelium. As circulating cells they are intrinsically non-motile.

[11] The bacteria were injected into a femoral vein, or into a vein in the opposite ear.

bacteria could be seen taking place within 15 to 30 minutes after inoculation.[12] Repeated observations made in numerous individual experiments demonstrated that the intravascular phagocytosis invariably resulted from one of the three following mechanisms.

(*a*) *Surface Phagocytosis Involving Vascular Endothelium.*—Most of the Friedländer's bacilli were ingested only after being trapped against the wall of the vessel, as shown in Figs. 17–22. Whereas bacteria floating freely in the plasma were frequently pushed along by the pseudopods of the moving leucocytes without being phagocyted, those which were pinned against the capillary endothelium were often ingested. The trapping of an organism against the stationary endothelial surface could be seen to enable the pseudopods of the leucocyte to surround the capsule and ultimately draw the bacterium into its cytoplasm. This general mechanism has been referred to previously as surface phagocytosis (13, 14).

(*b*) *Intercellular Surface Phagocytosis.*—Occasionally bacilli could be seen to be phagocyted in a stagnant capillary by being caught between the surfaces of several adjacent leucocytes. Immobilized in this manner, the trapped organisms were usually ingested by one or more of the leucocytes in the group. The intercellular mechanism is illustrated in the photomicrographs of Figs. 23 to 26. Intercellular surface phagocytosis has already been shown to play an important role in the destruction of encapsulated bacteria in extravascular lesions (10, 32).

(*c*) *Phagocytosis of Bacteria Caught in Intravascular Fibrin.*—The presence of small reticular deposits of what appears to be fibrin within the lumen of capillaries during bacteriemia has been described above. On numerous occasions leucocytes were seen to phagocyte bacteria caught in the interstices of this intravascular reticulum (Fig. 16). The ability of polymorphonuclear cells to phagocyte encapsulated pneumococci and Friedländer's bacilli in the presence of clotted plasma has been previously demonstrated (33).

Thus the same three mechanisms of surface phagocytosis which have been shown to be responsible for the ingestion and destruction of unopsonized pneumococci and Friedländer's bacilli in the lungs, lymph nodes, and subcutaneous tissues (13–17) also operate within the blood stream.

DISCUSSION

Study of the cellular immunology of bacteriemia began with the work of Wyssokowitch in 1886 (34). During the intervening period of over 60 years numerous experiments have been performed under a wide variety of conditions (1). The principal facts which have emerged from the accumulated observations on *non-immune* hosts may be summarized as follows:—

1. Bacteria injected into the blood streams of non-immune animals are rapidly

[12] No natural opsonins to the Chic strain of Friedländer's bacillus could be detected in the serum of rabbits used in these experiments.

removed from the circulation; the maximum fall in bacterial count usually occurs within the first 6 hours (4).

2. The completeness of clearing and therefore the final outcome of the infection depends in part upon the virulence of the injected organisms. In the case of encapsulated bacteria the state of their capsules which serve to protect them from phagocytosis has been shown to be of utmost importance (4, 35). When highly virulent encapsulated bacteria are injected in sufficient numbers, a secondary rise in bacterial count follows the initial clearing. This secondary bacteriemia causes a fatal outcome, unless the animal can survive long enough to develop a specific immunity, in which case recovery may result.

3. The speed and degree of the initial clearing have been shown to vary with each of the following factors: (*a*) the species of host (36, 37), (*b*) the species and type of microorganism (4, 35), (*c*) the state of virulence of the bacteria (4, 35), (*d*) the growth phase of the culture from which the bacterial cells are harvested (4), and (*e*) the number of organisms injected (4).

4. The response of the host to the intravenous injection of bacteria is characterized by a prompt fall in circulating polymorphonuclear leucocytes (4, 38). The degree of leucopenia varies with the size of the inoculum and the type of organism injected (38, 39). An analogous leucopenic phase has been demonstrated during the course of severe bacteriemia in both human patients (40) and laboratory animals (41) with acute infections of extravascular origin.

5. Histologic examinations and cultural studies made on the organs of animals sacrificed during the initial phase of clearing reveal that the disappearance of bacteria from the blood stream is due primarily to phagocytosis by polymorphonuclear leucocytes in both pulmonary and systemic capillaries, and by reticulo-endothelial cells of the splenic and hepatic sinusoids (5, 7, 35, 36). The degree of participation by polymorphonuclear cells and by sessile histiocytes is highly inconstant and varies with different animals (5–7, 37, 42). Polymorphonuclear leucocyctes, particularly in capillaries of the lungs, are active in the early stages of certain bacteriemias (5, 6) whereas cells of the reticulo-endothelial system are primarily responsible for the removal of inert foreign particles, such as dyes, India ink, and manganese dioxide (6, 42).

6. During bacteriemia, blood-borne organisms may be removed from the circulation by the filtering action of minute thrombi which appear to form in systemic and pulmonary capillaries as a result of invasion of the blood stream (43). Platelets are often caught in the thrombi along with the bacteria. The quantitative effect of such mechanical filtration is not known. Attempts to alter the clearing capacity of the blood stream by the use of anticoagulants (43) and by the production of thrombocytopenia have been unsuccessful (44, 45).

In spite of the impressive body of data which is available concerning the cellular defenses of the blood stream, at least one basic question remains unanswered regarding the bacteriemia of acute infections. How are fully encapsulated blood-borne bacteria phagocyted in the absence of circulating antibody?

As previously stated, many of the species of bacteria which commonly cause acute infections in man are known, if maximally virulent, to possess capsules which protect them from phagocytosis. Their virulence indeed may depend

primarily upon the antiphagocytic properties of their capsules. It has been widely taught by immunologists that bacteria possessing such capsules can be phagocyted only if opsonized by specific antibody. This view is based upon the well established observation that phagocytosis fails to take place when suspensions of washed leucocytes and encapsulated bacteria, such as pneumococci or Friedländer's bacilli, are incubated in ordinary test tubes or upon glass slides. Evidence already referred to indicates, however, that negative opsonocytophagic tests performed *in vitro* do not prove that these microorganisms are resistant to phagocytosis *in vivo*. On the contrary, they have been shown to be readily phagocyted in the lungs, lymph nodes, and subcutaneous tissues of living animals (13–17). In addition, they are promptly ingested in leucocyte-bacterium mixtures which are incubated *in vitro* upon almost any tissue surface or upon the surfaces of such inert materials as filter paper, cloth, fiber glass, or agar (13).

This form of phagocytosis, which takes place in the absence of antibody, has been called surface phagocytosis. It occurs *in vitro* under three conditions: (*a*) whenever leucocytes have access to stationary surfaces of suitable texture, against which the bacteria may be trapped and thus ingested (13, 14), (*b*) in highly concentrated suspensions of leucocytes in which the bacteria are phagocyted by being caught between the surfaces of adjacent leucocytes (intercellular surface phagocytosis) (32), and (*c*) in the presence of fibrin clots in the interstices of which leucocytes may likewise trap the bacteria and phagocyte them (33). It should be noted, however, that surface phagocytosis is readily inhibited by dilution of the leucocytic mixture. When the ratio of fluid to cells is sufficiently great, the leucocytes have relatively few contacts with each other or with the stationary surfaces of their environment. Thus diluted they function ineffectively as phagocytes unless aided by opsonizing antibodies. This fact appears to be responsible for the inefficiency of surface phagocytosis in acute infections of the serous cavities and of the subarachnoid space (10, 18).

Each of the three forms of surface phagocytosis listed above has been shown in the present study to take place within the blood stream during experimental bacteriemia. By means of the rabbit ear chamber technique it has been possible to observe directly the exact manner in which leucocytes phagocyte blood-borne Friedländer's bacilli. The bacteria have been seen to be ingested by first being pinned against the walls of the capillaries, by being trapped against adjacent leucocytes, or by being caught in the interstices of intravascular clots. That these same mechanisms operate during bacteriemia resulting from extravascular infection is suggested by the histologic findings recorded in the rats dying within 48 hours of experimental pneumococcal pneumonia (Figs. 4 to 6).

The intravascular phagocytosis observed in the rabbit ear chamber was shown to depend upon a prompt and efficient mobilization of leucocytes within the blood stream. In uninfected animals the polymorphonuclear leucocytes which roll freely along the endothelial surfaces of the vessels are intrinsically non-motile and thus relatively inactive as phagocytic cells. Immediately after the entrance of bacteria into the blood stream, however, their behavior is drastically altered (Figs. 10 and 11). They begin to stick to the endothelium of capillaries, and once adherent to the vessel walls, they become actively motile and potentially phagocytic (Figs. 12 to 14 and 17 to 26). Since the sticking of leucocytes during bacteriemia has been directly observed in capillaries of the rat's mesoappendix, as well as in the ears of rabbits, and since excessive numbers of polymorphonuclear cells are demonstrable in the pulmonary capillaries and the splenic and hepatic sinusoids of animals and patients dying of bacteriemia, it is reasonable to assume that the intravascular leucocytic reaction occurs simultaneously in many parts of the body. Indeed, it appears that the entire vasculature may respond to invasion of the blood stream by a prompt mobilization of intravascular phagocytes. That a transient leucopenia should result seems only natural in view of the extent of the reaction.

It should not be assumed, however, that intravascular phagocytosis takes place at a uniform rate in all capillaries of the body. On the contrary, the efficiency of surface phagocytosis has been shown in the present study to vary widely in different sites. Wherever the flow of blood is rapid, bacteria are rarely ingested; whereas, in vessels in which the flow of blood is slow or has stopped altogether (Fig. 15), phagocytosis is relatively efficient. The dependence of intravascular phagocytosis upon diminished blood flow would appear to account for the efficient phagocytosis noted in the liver and spleen, since in both of these organs relatively large quantities of blood are known to be trapped in closed vessels for considerable intervals (46). The large number of intracellular bacteria found in leucocytes lodged within the capillaries of the lungs is likewise in keeping with the observation that all pulmonary vessels are not patent at any one time (47). The efficiency of surface phagocytosis at any given site within the blood stream appears to vary inversely with the rate of blood flow.

No information is afforded by the present study regarding the relative importance of polymorphonuclear leucocytes and cells of the reticulo-endothelial system in destroying blood-borne bacteria. Certainly under the conditions of the experiments here reported, both groups of cells take part in the ingestion of organisms. Recent observations by Kerby *et al.* (48) confirm the finding of Wright (4) that artificially induced leucopenia does not significantly alter the rate at which bacteria are removed from the blood stream of rabbits. Attempts to block the reticulo-endothelial system have also failed to alter the clearing

capacity of the blood stream (4). Experimental data presently available would appear to warrant only the following general conclusions: (*a*) that both polymorphonuclear leucocytes and reticulo-endothelial cells (particularly of the liver and spleen) participate in the destruction of bacteria within the blood stream, (*b*) that the relative importance of the two phagocytic systems varies in different hosts and in different forms of bacteriemia, and (*c*) that loss of function by one system may be nullified by compensatory activity of the other. Histologic examination of tissues from patients dying with overwhelming bacteriemia reveals that both these systems of intravascular phagocytes operate in man (49).

The results of experiments performed with the rabbit ear chamber must be interpreted with caution in reference to human bacteriemia. The species of host, the number of bacteria in the blood stream, and the speed with which they are introduced sharply differentiate the experimental conditions from those which obtain in human patients. Nevertheless, a limited analogy may be drawn with the aid of certain corollary evidence.

Since essentially the same degree of intravascular phagocytosis by polymorphonuclear cells was observed in rabbits with ear chambers and in rats dying within 48 hours of experimental pneumococcal pneumonia, it appears reasonable to assume that in both sets of experiments the ingestion of the encapsulated bacteria by granulocytes was due to surface phagocytosis. Similarly, there is no reason to doubt that surface phagocytosis operates in man and is of particular importance in clearing the blood stream of organisms possessing protective capsules. The significance of intravascular clotting as a defense reaction, on the other hand, remains obscure, particularly since it has not been demonstrated in humans (49). Although observed at relatively early stages in the rabbit bacteriemia (Fig. 16), the deposition of fibrin within systemic and pulmonary capillaries may represent a terminal phenomenon rather than an early defense of the host.

Particular attention should be drawn to the fact that the present studies afford no definite information regarding the mechanisms by which the Kupffer cells of the liver and the sinusoidal macrophages of the spleen phagocyte encapsulated bacteria during the early stages of bacteriemia. That these cells are extremely active as phagocytes and are able to ingest virulent Friedländer's bacilli and pneumococci without the aid of antibody is clearly shown by the histologic findings. However, the exact mechanisms by which they operate is not known. It is tempting to assume that they too function through surface phagocytosis, but proof of this concept must await direct observation of their activities in the living host.

SUMMARY

Evidence has been presented that the introduction of large numbers of bacteria into the blood stream causes a widespread intravascular reaction, characterized by the sticking of leucocytes to the endothelium of capillaries, arterioles, and venules. The adherent granulocytes promptly become motile and thus potentially phagocytic. This intravascular leucocytic response affords a rapid and efficient mobilization of a vast number of active phagocytes within the blood stream. In some of the smaller vessels of both the systemic and pulmonary circulation the reaction is accompanied by the deposition of what appears to be intravascular fibrin.

Direct observation by the rabbit ear chamber technique has revealed that leucocytes thus mobilized in small peripheral vessels are capable of phagocyting fully encapsulated Friedländer's bacilli in the absence of antibody. Ingestion of the encapsulated blood-borne bacteria results from surface phagocytosis and occurs primarily in those vessels in which the flow of blood is either slowed or has temporarily stopped altogether. Leucocytes can be seen to phagocyte the organisms by first trapping them against the walls of the vessels or against adjacent leucocytes. Bacteria caught in the interstices of the intravascular "fibrin" may likewise be immobilized and readily phagocyted. Thus granulocytes, without the aid of opsonins, are able to ingest and destroy encapsulated blood-borne bacteria by the same mechanisms that operate in extravascular tissues.

It is concluded from these studies that intravascular surface phagocytosis by polymorphonuclear leucocytes supplements the well known phagocytic activities of the reticulo-endothelial cells and therefore serves as an important defense of the host in acute infections caused by encapsulated bacteria and complicated by bacteriemia.

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EXPLANATION OF PLATES

Photomicrographs for Figs. 1 to 9 were made by Mr. Cramer Lewis, Department of Illustration, Washington University School of Medicine. Sections were prepared by Mrs. Alice Hamlin and were stained by the Gram-Weigert technique.

PLATE 33

FIG. 1. Phagocytosis of Friedländer's bacilli in hepatic sinusoid of rat sacrificed 60 minutes after intravenous injection of bacilli. Note outline of polymorphonuclear leucocyte overlying Kupffer cell. Both cells contain phagocyted bacilli. × 1800.

FIG. 2. Phagocytosis of Friedländer's bacilli by polymorphonuclear leucocytes in sinusoid of spleen. Tissue fixed 60 minutes after bacteria were introduced into the blood stream. × 1800.

FIG. 3. Intracellular Friedländer's bacillus is clearly visible in cytoplasm of polymorphonuclear leucocyte contained within capillary loop of alveolar wall. Rat killed 120 minutes after injection. × 1800.

FIGS. 4 to 9. Sections made from rats dying within 48 hours of experimental pneumococcal pneumonia. All had terminal bacteriemia, and each animal was autopsied immediately after death.

FIG. 4. Pneumococci phagocyted by Kupffer cell of hepatic sinusoid. × 1400.

FIG. 5. Polymorphonuclear leucocyte with ingested pneumococci in splenic sinusoid. × 1400.

FIG. 6. Alveolar capillary of lung containing granulocytic leucocyte with intracytoplasmic pneumococcus. × 1400.

FIG. 7. Pneumococci caught in fibrin within hepatic sinusoid. × 1400.

FIG. 8. Similar clot in sinusoid of spleen. × 1400.

FIG. 9. Thrombus in small vessel of lung. Note polymorphonuclear leucocyte containing phagocyted pneumococcus. × 1400.

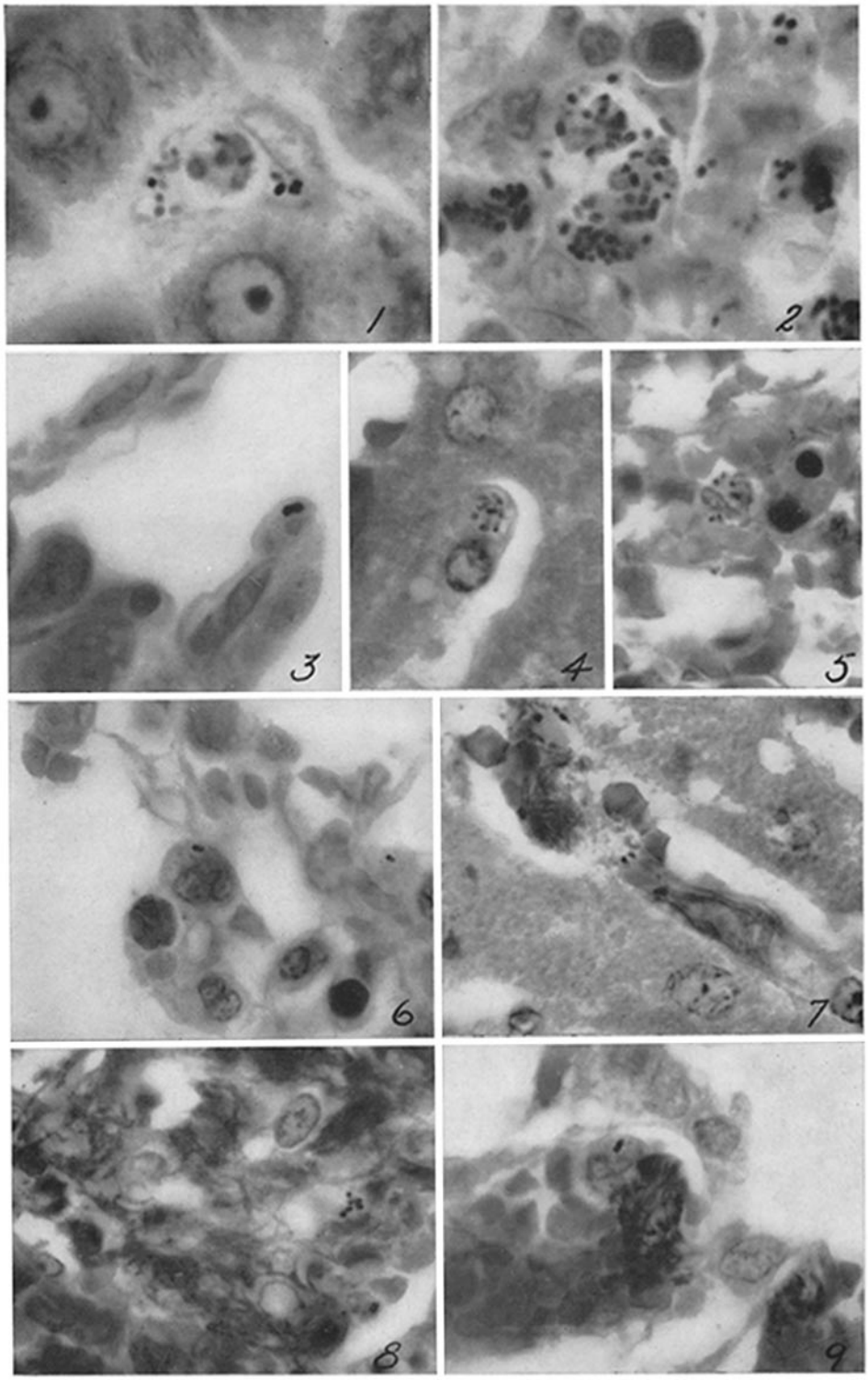

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PLATE 34

FIG. 10. Normal venule in rabbit ear chamber. Because of rapid blood flow and relatively long exposure of film, neither circulating red cells nor leucocytes are visible as individual cells. × 360.

FIG. 11. Same vessel 30 minutes after intravenous injection of Friedländer's bacilli. Granulocytes adhering to endothelium are now clearly visible. Tributary at upper right has become partially plugged by sticking leucocytes. × 360.

FIGS. 12 to 14. Sticking leucocyte moving upward along capillary endothelium. Such motile cells were repeatedly shown to be potentially phagocytic, whereas leucocytes being carried in the blood stream were never observed to phagocyte bacteria. Arrow marks point of reference in vessel wall. Photographs taken at intervals of 60 to 90 seconds. × 1380.

FIG. 15. Stagnant capillary where blood flow has ceased altogether. Note bacilli (arrows) being pushed along by advancing pseudopod of leucocyte. In capillaries such as this surface phagocytosis was most frequently observed. Extravascular granulocyte may be seen just outside lower wall of capillary. × 1380.

FIG. 16. Fibrin clot attached to wall of small venule. Main stream of blood has been side-tracked by thrombus. Note bacilli caught in meshes of clot. The trapped bacilli were frequently observed to be phagocyted by leucocytes entering the interstices of the thrombus. × 1380.

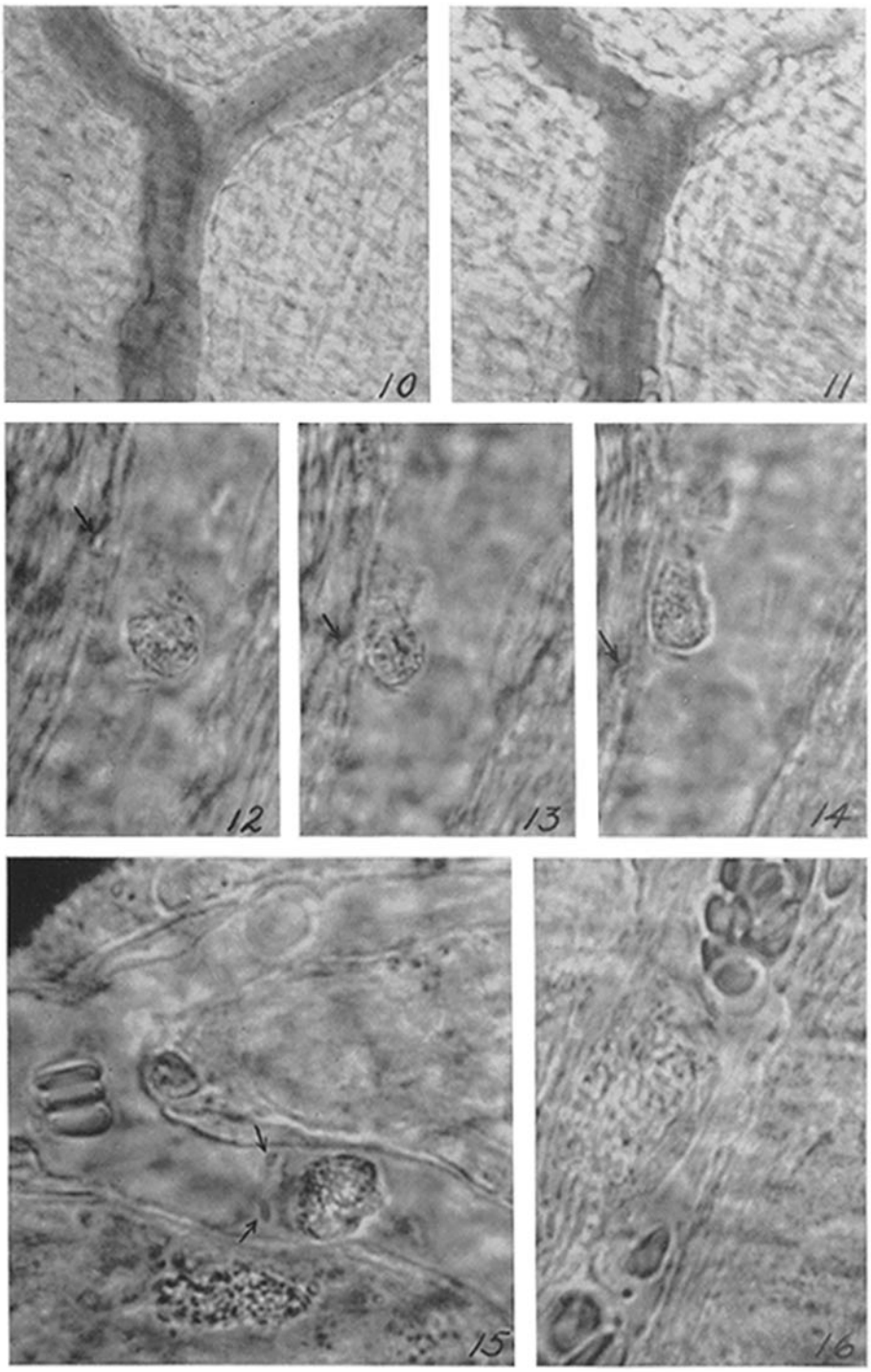

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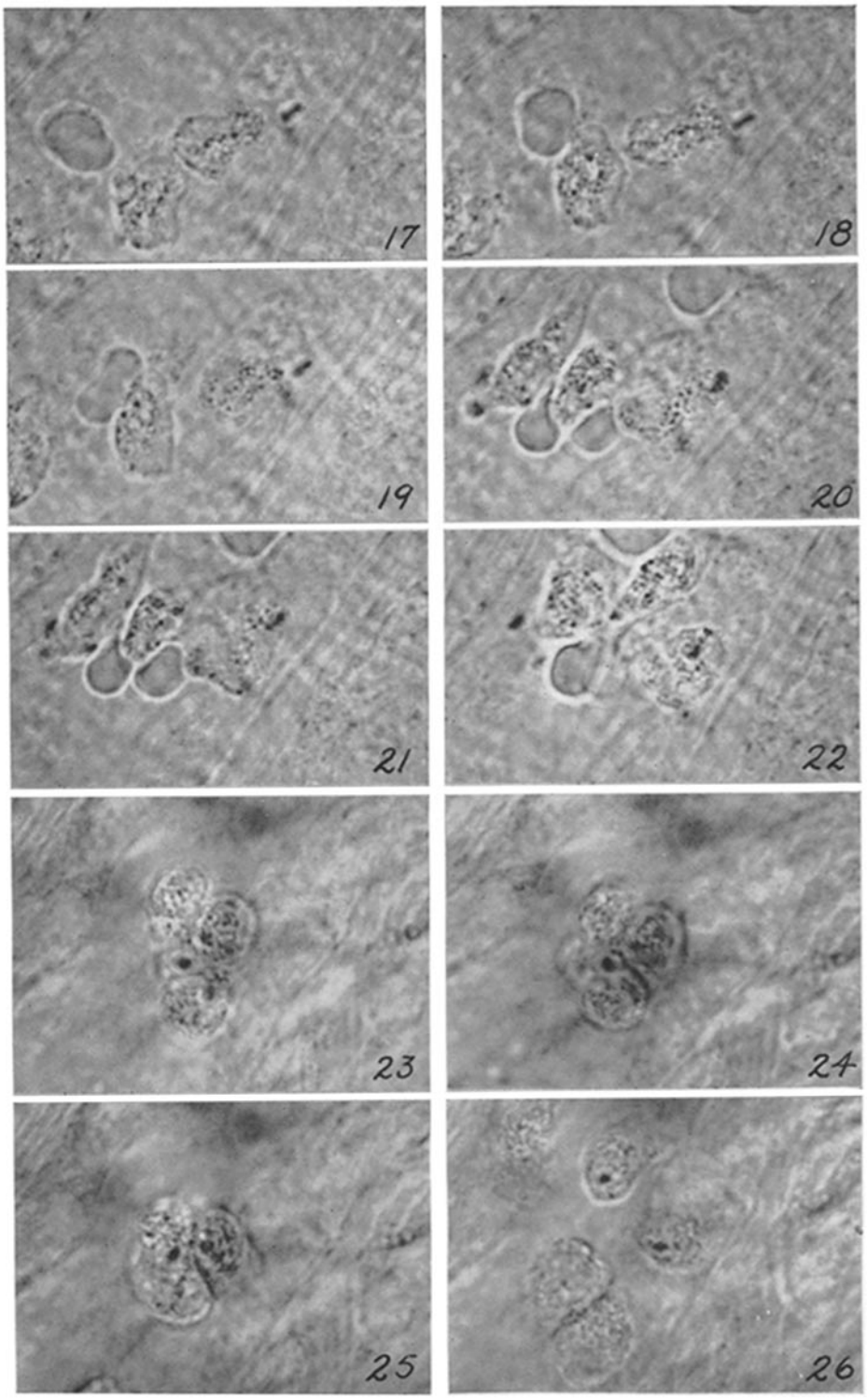
PLATE 35

FIGS. 17 to 22. Surface phagocytosis involving vascular endothelium. Motile leucocyte in stagnant capillary pushes Friedländer's' bacillus against endothelial spur on vessel wall (Fig. 17), traps the organism against the spur (Figs. 18 and 19), and finally surrounds the bacillus (Fig. 20) and engulfs it (Figs. 21 and 22). The elapsed time between the first and last photographs was $3\frac{1}{2}$ minutes. × 1380.

FIGS. 23 to 26. Surface phagocytosis resulting from organisms being trapped between two or more motile leucocytes in a stagnant capillary. Bacilli caught between surfaces of adjacent leucocytes (Figs. 23 and 24) are seen to be phagocyted by the two upper cells in the group (Figs. 25 and 26). Elapsed time: 90 seconds. The mechanism of phagocytosis was essentially the same as that depicted in Figs. 17 to 22, except that the bacilli were trapped against the surfaces of the adjacent leucocytes rather than against the capillary wall. × 1380.



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