STRUCTURAL BASIS FOR BLOOD STREAM INVASION BY *Pseudomonas aeruginosa* FROM THE PRIMARY SEPTIC FOCUS

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Clinical observations show that one of the leading places among pathogenic agents both of local inflammatory and suppurative processes and of generalized infection, i.e., of septicemia, is occupied by *Pseudomonas aeruginosa* [1, 2, 5-7], and this accounts for the unremitting interest in pyocyanic infection in general and in *Ps. aeruginosa* septicemia in particular.

The least studied aspect of septicemia is the problem of how and under what conditions do microorganisms penetrate from their habitat in a wound or other local focus of inflammation into the blood stream. In other words, the conditions under which a focus of inflammation is converted from a barrier in the path of penetration of microorganisms into the blood stream into a portal of entry of sepsis, are not clear.

The aim of the present investigation was, using a model of experimental *Ps. aeruginosa* septicemia, to elucidate the structural principles in a primary septic focus which can be regarded as the essential conditions for development of uncompensated bacteriemia and septicemia.

EXPERIMENTAL METHOD

The technique used to model experimental *Ps. aeruginosa* septicemia was described previously [3, 4]. Experiments were carried out on 13 male albino rats weighing 180-200 g. The soft tissues were investigated around a gauze swab, soaked with a suspension of a culture of *Ps. aeruginosa*, implanted subcutaneously: the subcutaneous and intermuscular fatty areolar tissue, skeletal muscles, and blood vessels. Material for histologic investigation was fixed in neutral formalin, cut into sections on a freezing microtome, or embedded in paraffin wax. Sections were stained with hematoxylin and eosin, with cresyl violet and counterstained with picrofuchsine, with toluidine blue, and by Goldman's and Levaditi's methods. Pieces of tissue for electron-microscopic investigation were fixed in 1% glutaraldehyde, postfixed in OsO_4, dehydrated in alcohols, and embedded in Epon. Ultrathin sections were stained by Reynolds' method and examined in the JEM 100B electron microscope.

Material for histologic investigation and electron microscopy was taken 5 h after infection.

EXPERIMENTAL RESULTS

The subcutaneous canal into which the swab soaked with a culture of *Ps. aeruginosa* was introduced contained a turbid serous fluid. The tissues of the canal walls were highly edematous and hyperemic.

On histologic investigation the canal surface was covered with a layer of exudate, consisting of a little fibrin and many polymorphonuclear leukocytes (PNL). From this superficial leukocytic barrier, groups of PNL apparently spread along the intermuscular areolar tissue into the depth of the soft tissue. Besides PNL, the exudate on the canal surface and in the thickness of the soft tissues frequently contained erythrocytes. Stasis of erythrocytes and leukocytes and thrombi was observed in the venules, capillaries, and arterioles,

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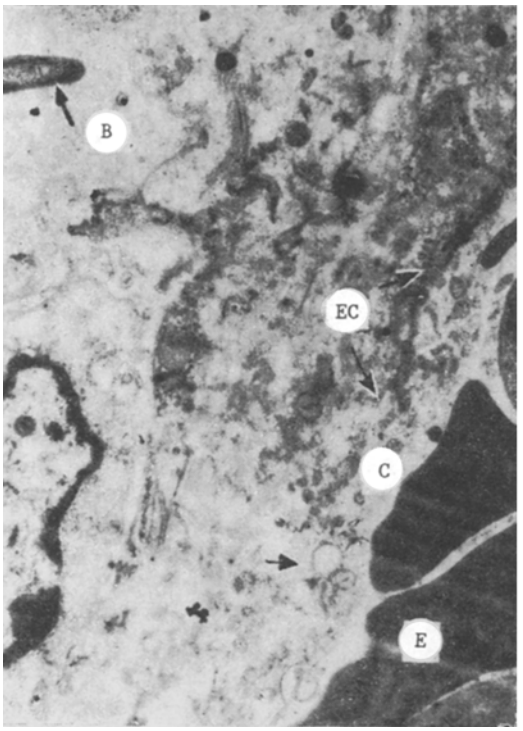

Fig. 1

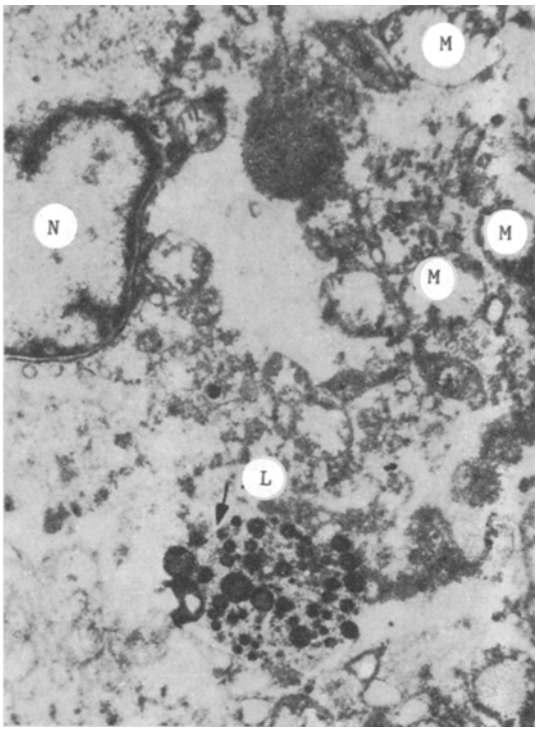

Fig. 2

Fig. 1. Stasis of erythrocytes and disturbance of integrity of capillary wall (C) in places where bacteria (B) are found in subcutaneous areolar tissue of a rat. E) Erythrocyte; EC) endothelial cell. 18,000×.

Fig. 2. Destructive changes in cytoplasm of a macrophage: lysis of plasma membrane, damage to mitochondria (M), lysosomes (L), and nucleus (N). 20,000×.

and the microcirculatory bed was congested with blood. Against this background of inflammation invasion of the soft tissues by bacteria was observed. Their greatest concentration was found in the strip of tissue directly adjacent to the swab. Confluent growth of microorganisms was observed in this position, and bacterial cell bodies were mixed with disintegrating PNL. In general, the PNL were distinctive markers of the location of bacteria. Microorganisms spread into the depth of the tissue along the course of the perivascular spaces around venules, capillaries, and arterioles. The reason is because of the presence of lymphatic spaces and the formation of places of least resistance for the exudate to escape into the perivascular spaces, giving rise to inflammatory edema in them. Although the number of bacteria in the perivascular space is small, usually leukocytic stasis is bound in the lumen of the vessel together with cutting of the vessel by PNL. In places where the barrier function of PNL proves inadequate the number of bacteria increases; they not only surround the blood vessel but also invade the structures of its wall and also the thrombi of agglutinated erythrocytes. With an increase in the number of bacteria, death of the leukocytes is observed.

The results of the histologic investigation show that concentrations of bacteria in the fatty areolar tissue are apparently located between fat cells. However, as the electron-microscopic investigation showed, the impression of absence of injury to fat cells by bacteria was deceptive. At sites of growth of bacteria, lysis of collagen fibers could also be observed.

Electron-microscopic investigation of the subcutaneous areolar tissue 5 h after implantation of the swab soaked in a suspension of bacterial cells revealed marked changes in the blood vessels. The capillaries of the subcutaneous areolar tissue were dilated and congested with blood, in many places stasis of erythrocytes was observed, and solitary neutrophils could be seen among the dense mass of erythrocytes. Marked changes in the capillary walls were observed, with the formation of wide openings and fragmentation of the processes of the endothelial cells, which came to resemble small vesicles. Continued destruction of the basal layer and lysis of the plasma membranes converted the capillary wall into an amorphous layer of finely granular material, small vacuoles, and remnants of membranes. In the pericapillary

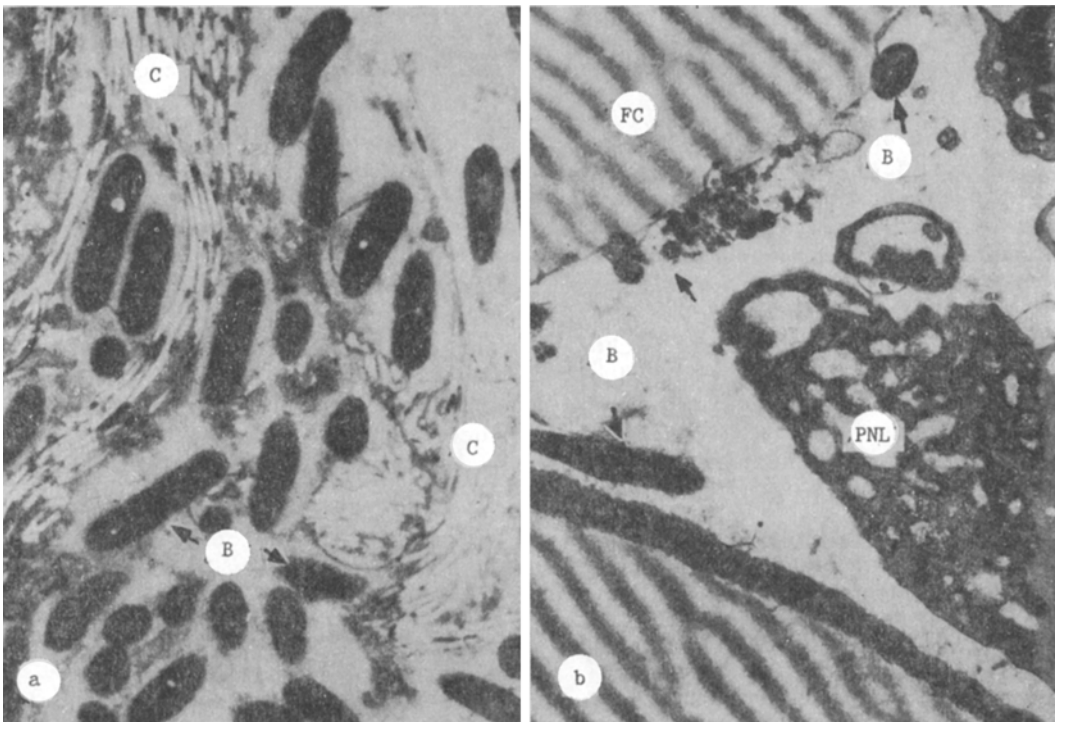

Fig. 3. a) Destructive changes in collagen (C); b) structural changes in fat cells (FC) at sites of contact with bacteria (B) and PNL. 20,000×.

space groups of argyrophilic fibers, small granules, and fibrous formations were observed. In fibroblasts located near the capillaries, destructive changes also were visible in the form of swelling of the mitochondria, and translucency of the cytoplasm and nucleus (Fig. 1). Signs of destruction also were observed in the macrophages and PNL: distinct translucency of the nucleoplasm, widening of the perinuclear space, destruction of mitochondria, and a considerable decrease in the number of vacuoles and cytogranules. As a result of lysis of the plasma membranes the contents of the cell filled the intercellular space and the intervals between collagen fibers with small granules, swollen mitochondria with a translucent matrix, and amorphous aggregates. Macrophages showed destruction of the cytoplasmic membrane and damaged, half-destroyed organelles (Fig. 2). Where bacteria were present destructive changes were observed in collagen. Bundles of collagen and argyrophilic fibers in these areas were disconnected and disoriented. Individual collagen bundles consisted of translucent fibers, along the length of which there were dark bands at varied distances from each other. Under the influence of bacteria the collagen bundles underwent lysis with the formation of zones of translucency of different sizes, in which groups of bacteria appeared to be swimming (Fig. 3a).

A considerable area of the subcutaneous areolar tissue was occupied by fat cells or adipocytes. Mature adipocytes contained one large vacuole of neutral fat, occupying the whole of the central part of the cell and surrounded by a rim of cytoplasm, in the thickened part of which were present the nucleus and a few mitochondria. Leukocytes, capillaries, and argyrophilic fibers and also many bacteria were located between the adipocytes. Where bacteria were present, lysis of the plasma membranes and destruction of the intracellular organelles took place, with exposure of the fat vacuole. The ultrastructures of the cell in these areas were fragmented and, in the form of concentrations of vacuoles and granular material, they lay next to a freely lying lipid vacuole (Fig. 3b). Consequently, not all the fat cell was apparently subjected to lysis by the bacterial enzymes, as might be supposed on the basis of light-optic investigation, but only the fat vacuole.

According to the electron-microscopic data, 5 h after subcutaneous implantation of a suspension of *Ps. aeruginosa* considerable changes were thus found in the blood capillaries, PNL, macrophages, and fat cells of the subcutaneous areolar tissue in the form of irreversible disturbances of the plasma membranes, lysis of the organelles and nuclear chromatin, ending with necrosis of the cells. The cause of these disturbances is evidently the direct action of bacteria, for pictures of cell necrosis were visible in places where there were many bac-

terial cells. The discovery of bacteria in the cytoplasm of macrophages and PNL evidently does not always reflect phagocytosis (they are all outside phagosomes). The most probable explanation is penetration of bacteria into the cytoplasm as a result of destruction of cell membranes.

Failure of the phagocytic barrier due to a large dose of bacteria with increased virulence as a result of passage through rats [4], destruction of cells of the tissue-blood barrier, and invasion by microorganisms of the lumen of blood vessels with stasis of the blood flow are the basic conditions for conversion of a local focus of inflammation into portals of entry of sepsis. As early as 5 h after infection invasion of the lumen of the blood vessels with stasis of the blood flow by bacteria was observed. This suggests that penetration of bacteria into the general circulation from a local septic focus takes place as the result of flushing out of the microorganisms where regions of the vascular system in which stasis and bacterial growth are taking place join regions where the blood flow has not ceased. In other words, entry of the microorganisms into the general circulation by a mechanism of microembolism is postulated. The inflammatory focus loses its barrier role since the PNL barrier, as the principal barrier mechanism, is unable to withstand invasion of blood vessels in which the blood flow has ceased by bacteria.

The main pathways along which bacteria in a primary septic focus invade and spread are the lymphatics, perivascular spaces, small blood vessels with stasis of the blood flow (mainly venules and capillaries), and the fatty areolar tissue.

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