As regards the idea of the unequal efficiency of formation of group- and type-specific antibodies to p26 gag, this may be a question of differences in regulation of the immune response in the patient who was the source of the 8H serum. In this case it would be fortuitous to consider only matching of the antigenic determinants as subjects of regulation of the immune response to their separation into group- and type-specific.

The practical importance of the phenomenon described above is that it demonstrates the fundamental impossibility in this and similar cases of establishing a correct serologic diagnosis of HIV infection if the immunologic types of the virus (or its analog) used in the test system are not identical with those of the virus that is the source of infection.

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EXPERIMENTAL STUDY OF THE ROLE OF THE LOCAL INFECTIOUS FOCUS IN DEVELOPMENT OF *Pseudomonas aeruginosa* SEPTICEMIA

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Ideas on the link between the local infectious process and the development of septicemia are still debated. These views have varied from accepting that pathological changes in the body at a certain stage of a disease are independent of the state of the primary focus [1, 3] to concluding that the view that the local and generalized processes are separate [4, 7] and that the disease is reversible after removal of the primary focus [6] are unjustified.

The solution of problems of the etiology and pathogenesis of septicemia extends far beyond the bounds of theoretical polemic, for it plays an exceptionally important role in the determination of the therapeutic tactics in relation to patients with infected wounds. The aim of this investigation was to study how the development of septicemia and the appearance of metastatic pyemic foci in an experimental model depend on the state of the primary septic focus and the time of its removal.

EXPERIMENTAL METHOD

Experiments were carried out on 156 noninbred male albino rats weighing 180-200 g. A local infectious process was produced by a single intramuscular injection of 0.3 ml of a suspension of a 24-h culture of microorganisms in a 10% solution of $CaCl_2$ containing $8 \cdot 10^9$ bacterial cells in 1 ml. The strain of *Pseudomonas aeruginosa* No. 453 which was used was obtained from the Culture Museum of the L. A. Tarasevich Research Institute of Standardization and Control of Medical and Biological Preparations. The use of this strain in a method of obtaining an experimental model of septicemia developed previously, leads to

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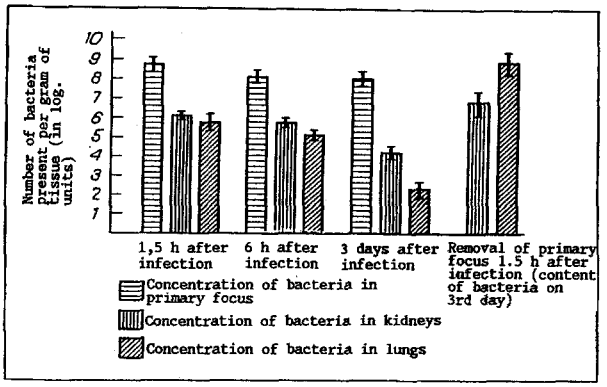

Fig. 1. Time course of contamination of tissues in the early stages of development of experimental *Ps. aeruginosa* septicemia.

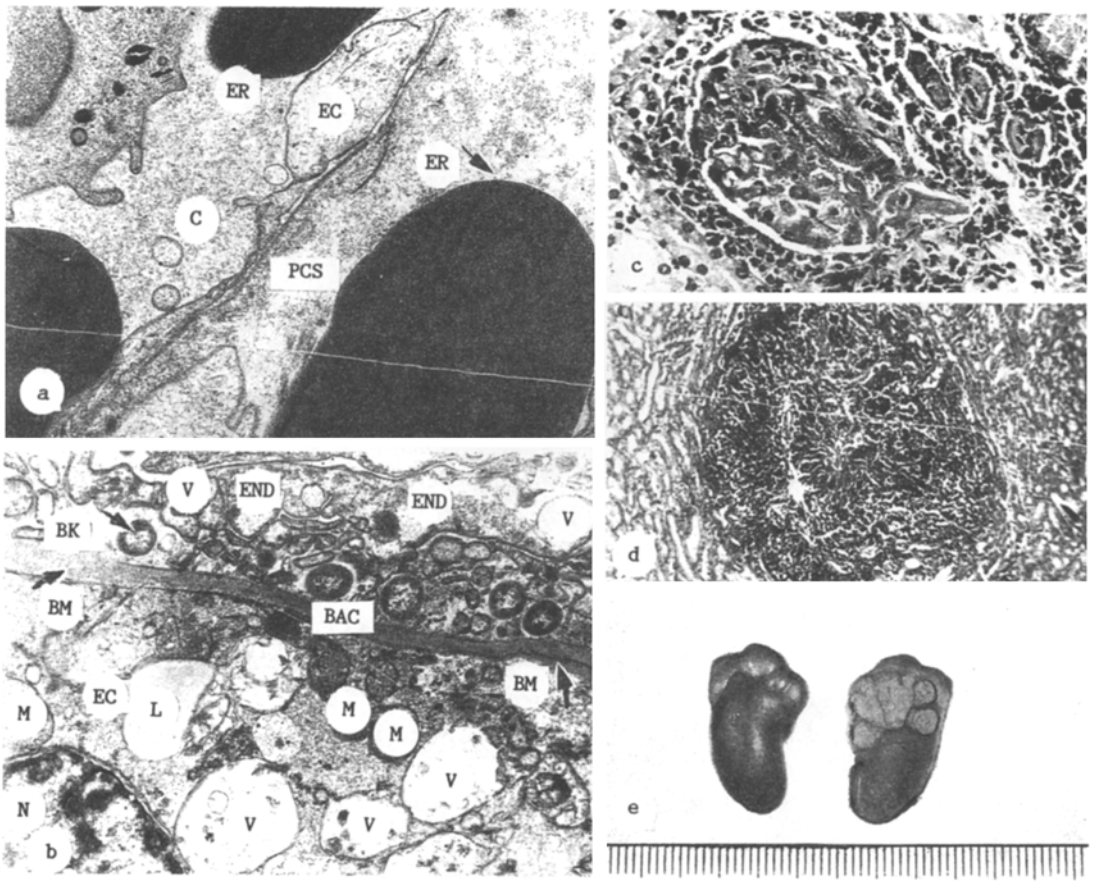

Fig. 2. Dynamics of development of metastatic pyemic foci in kidneys in experimental *Ps. aeruginosa* septicemia. a) Swelling and translucency of processes of epithelial cells (EC) in a blood capillary. Erythrocytes (ER) escaped into pericapillary space. 1 Day. 12,000×; b) Concentration of bacteria (BAC) near basement membrane (BM) of EC. Destruction of intracellular organelles and formation of vacuoles (V) and lipid inclusions (L) in EC. Fragmentation of processes of endothelial cells (END) and appearance of vacuoles (V). N) Nucleus, M) mitochondria. 1 Day, 12,000×. c) Septic emboli in capillaries of glomerulus, leukocytic infiltration around glomerulus. 2 Days. Hematoxylin and eosin. 250×. d) Septic vasculitis. Intensive leukocytic infiltration around blood vessel undergoing lysis. 3 Days. Hematoxylin and eosin. 250×. e) Metastatic pyemic foci. 14 Days.

the formation of metastatic pynemic foci in the kidneys only, and is accompanied by definite mortality [8]. The primary septic focus formed in the region of the thigh was excised 1.5, 6, 24, and 72 h after infection. Infected rats not undergoing an operation were used as the control. The qualitative and quantitative composition of the microflora was studied on material from 139 biopsies of tissues of the primary focus, kidneys, and lungs and seedings of blood taken in the initial period of infection and at the times indicated above. The level of contamination of 1 g of tissue was determined separately for *Ps. aeruginosa* and other microorganisms, and expressed as logarithms (to base 10) and subjected to statistical analysis.

For the histological study of the kidneys and lungs animals of both groups were killed between 24 h and 14 days after infection. Animals dying at various times after infection also were autopsied. Material was fixed in 10% neutral formalin and embedded in paraffin wax. Sections were stained with hematoxylin and eosin and by Leishman's method. Histochemical reactions were carried out for glycosaminoglycans with toluidine blue and the PAS reaction. Material for electron microscopy was fixed in 1% glutaraldehyde and 2% OsO_4 solutions and embedded in a mixture of Epon 812 and Araldite. Ultrathin sections were studied in the IEM 100B microscope.

EXPERIMENTAL RESULTS

The animals studied 1.5 h after infection had developed bacteriemia and positive cultures were obtained from the blood and internal organs. The concentration of microorganisms in tissues of the local focus, kidneys, and lungs, at this period reached their peak values, fell a little after 6 h, and showed a statistically significant decrease after 72 h (Fig. 1). *Ps. aeruginosa* was seeded in monoculture from tissues of the primary focus and internal organs in 43% of cases. In seedings from 41% of all biopsy specimens studied, besides *Ps. aeruginosa,* accounting for most of the microflora, other microorganisms that were cultured included those of the *E. coli* family, *Enterobacter* spp., *Proteus* spp., and the group of nonfermenting Gram-negative bacteria, i.e., representatives of the intestinal microflora. The concentration of these bacteria as a whole was very small, and averaged 0.34 ± 0.47. In the remaining cases only the intestinal microflora could be cultured. Blood tests also revealed a heterogeneous picture: a pure culture of *Ps. aeruginosa* was obtained from 39% of the animals, and in 31% mainly bacteria of the Enterobacteriaceae family were detected. Unlike internal organs, positive cultures from which were obtained in all the animals, the blood of 30% of the rats tested proved to be sterile.

The development of septicemia was accompanied by a marked vascular reaction, appearing 24 h after infection in the form of mild but abrupt dilatation of the vessels in the kidneys and tiny focal hemorrhages. Intensification of the PAS reaction was observed in the basement membranes of the vessel walls, with swelling of the endothelium. Separation of the walls of the arterioles, venules, and larger vessels into layers and loosening of their fibrous structure were observed. Perivascular and moderate interstitial edema reflected the increased vascular permeability. Degranulation and a decrease in the concentration of highly sulfated glycosaminoglycans in the mast cells were conspicuous.

Electron-microscopic examination of the kidney cells at this time revealed changes in the blood capillaries. Processes of the endothelial cells were swollen, their cytoplasm was translucent, and the number of cytogranules and pinocytotic vacuoles was appreciably reduced. The basal layer was intact, but separation of the intercellular junctions and escape of erythrocytes into the pericapillary space (Fig. 2a, PCS) were observed. In the zone of fixation of the bacteria, fragmentation of processes of the endothelial cells, their irregular swelling into the lumen of the capillary and vacuolation of the endothelial cells were observed. By penetrating through the disturbed membranes of the endothelium into the pericapillary space, the bacteria were located near the basement membrane of the epithelium. Under these circumstances, destruction of the intracellular organelles with the formation of large vacuoles and lipid inclusions were noted in the cytoplasm of the epithelium (Fig. 2b).

Consequently, the initial period of development of septicemia is characterized by endotoxemic shock, accompanied by a combination of vascular changes, increased vascular permeability, contamination of the internal organs with low concentrations of intestinal microflora, and a fluctuating course of bacteriemia, reflected in the periodic nature of positive blood cultures of the microflora.

A characteristic feature of the subsequent development of the septic process was localization of increasingly severe vascular disturbances in the kidney tissue. On the 2nd-3rd days, in the lumen of capillaries of individual glomeruli, and also in some blood vessels of larger caliber, bacterial emboli were present (Fig. 2c). The microorganisms accumulated in the perivascular spaces. Destruction of the vesse walls and of the surrounding parenchyma was intensified and accompanied by intensive leukocytic infiltration, with the appearance of spetic vasculitis (Fig. 2d). Involvement of neighboring tissue in the infectious inflammatory process led to the formation of pyemic foci, which by the 14th-15th day attained a diameter of 3-5 mm (Fig. 2e). In 77% of cases the septic process was accompanied by mortality; in 60% of the dying animals, metastatic abscesses could be seen in the kidneys.

Removal of the primary focus at various times after infection had a variable effect on the septic process depending on the phase of its development. The operation on the 3rd day caused virtually no change in the mortality level compared with the control, or in the incidence of secondary abscesses (62%). Excision of infected tissue after 24 h was accompanied by a marked decrease in mortality (31%) and the appearance of metastatic abscesses (23%). Fundamental differences were detected in the early stages of the process. The operation 6 h after injection of the dose of bacteria caused a sharp decrease in the number of animals which later developed signs of septicemia (mortality in 12% of cases, pyemic foci in 7% of rats). In the case of removal of muscle tissue 1.5 h after infection, no metastatic pyemic foci could be seen to have been formed, and none of the animals died.

The microbiological investigation showed that bacterial contamination of the internal organs and blood followed a different pattern depending on the time of removal of the primary focus and the time of formation of metastatic abscesses. At autopsy on the killed animals, in whose kidneys abscesses could be seen, revealed only slight contamination of the other tissues. The microflora discovered belonged mainly to the Enterobacteriaceae family, and seedings from tissue and blood were often sterile. Conversely, in tissue containing abscesses the concentration of Ps. aeruginosa was considered, and averaged 8.1 ± 0.52. The degree of contamination of tissue of the internal organs when the primary focus was excised 1.5 h after infection increased during the next 3 days, and significantly exceeded the initial level.

Thus in the first few hours after infection rapid contamination of the internal organs of the animals takes place, with fixation of microorganisms in vessels of the microcirculatory bed and their penetration into the perivascular space. This leads to the early (on the 2nd-4th days) formation of metastatic abscesses. Removal of the primary focus in the early stages of its formation (after 1.5 h) causes multiplication of the bacteria, probably associated with negative leukotaxis, leading to the subsequent development of bacteriemia, in the absence of septicopyemia. This is evidence that after the formation of metastatic abscesses the septic process becomes autonomous and irreversible. Bacteriemia is an essential but insufficient factor for the development of sepsis. For secondary abscesses to form, besides bacteriemia, the influence of other factors initiated by the primary focus also is evidently necessary. These factors may be proteases, with their effect on vascular permeability and the functional state of the immunocompetent cells, especially those of the macrophagal system [2, 3, 5]. Bearing in mind the reaction of the mast cells observed during the development of sepsis, it is logical to suggest the existence of a cascade mechanism, determining the transition of the infectious process from bacteriemia into the phase of septicopyemia.

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