MICROCIRCULATORY CHANGES IN THE LUNGS IN EXPERIMENTAL TETANUS

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The state of the lung tissue in experimental tetanus (ascending and blood-borne) in albino rats and mice was investigated by light and electron microscopy. During the development of the disease microcirculatory changes were found in the lung tissue, consisting of marked dilatation of the capillaries and lymphatics, diapedetic hemorrhages, effusions of blood into the lumen of the bronchi, concentrations of leukocytes around veins and venules, increased tone of the large veins, the formation of endothelial vesicles discharging into the lumen of the capillaries, destruction of cytoplasmic membranes, ruptures of the air-blood barrier, and vesiculation and fragmentation of erythrocytes. No evidence of inflammation was found. Inflammation occurred only in animals sacrificed in the late stages after recovery from tetanus.

Lesions of the lungs are the commonest and among the severest complications of tetanus [1-6]. Many aspects of their nature and pathogenesis remain unknown. This paper describes a study of some of these aspects.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats (200-220 g) and mice (18-20 g). Tetanus toxin was injected in a lethal dose either into the muscles of the hind limb or intravenously. In the first case ascending tetanus developed, in the second the tetanus was blood-borne [2]. The first animals died on the 5th day. Material for examination was taken at various times after injection of the toxin, at different stages of development of the disease. Material for examination under the light microscope was fixed in 10% neutral formalin and sections were stained with fuchselin and counterstained by Van Gieson's method. Pieces of lung tissue for electron-microscopic investigation were fixed in buffered 1% osmic acid solution by Palade's method [7], dehydrated, and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

Results of Light Microscopy. At the height of development of the disease the pattern of changes found in the rats with both general ascending and blood-borne tetanus was similar in all cases. One of its special features was marked dilatation of the alveolar capillaries which contained many erythrocytes and showed signs of diapedetic hemorrhage, especially in the zones of atelectasis (Fig. 1c). In individual cases effusions of blood were present in the lumen of the bronchi. The lymphatics around the veins were dilated, and concentration of leukocytes was observed in this region (Fig. 1a, b). In all cases the smooth muscles of the

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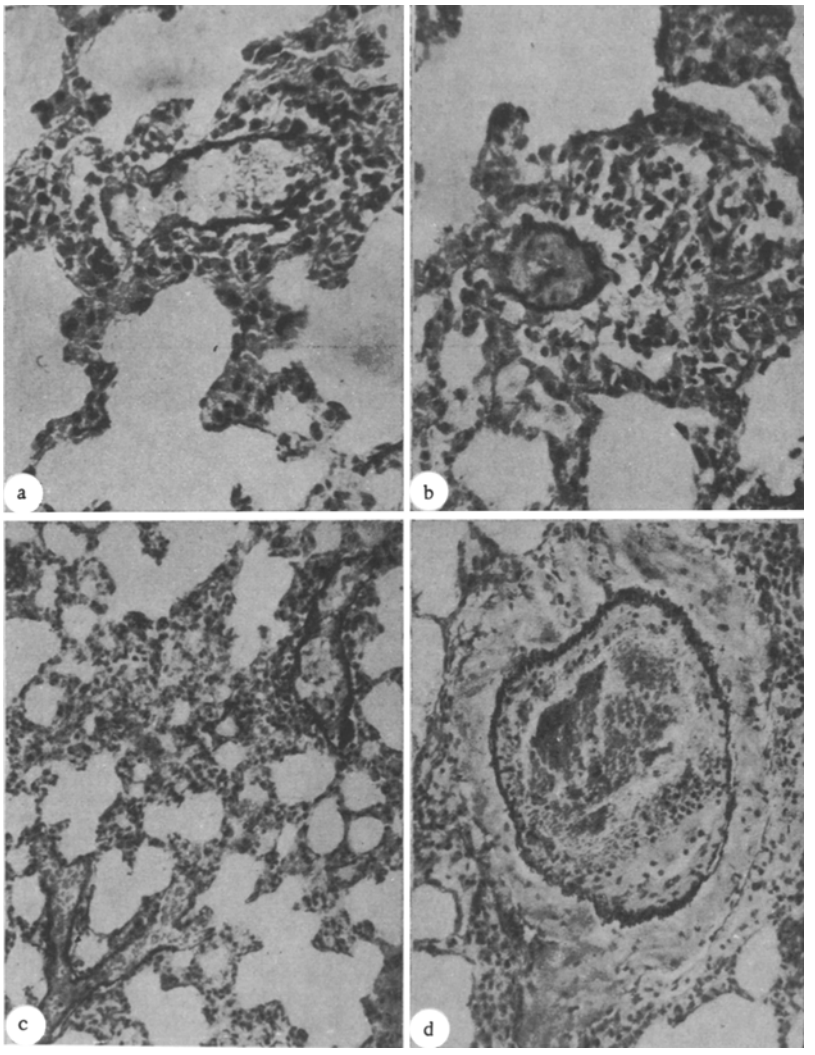

Fig. 1. State of the lung vessels in experimental tetanus: a) a small vein of the lung with peripheral arrangement and diapedesis of leukocytes (400×); b) small vein of rat lung in a state of spasm with cuffing of leukocytes around it (400×); c) small vein and artery of the lung, diapedesis of leukocytes marked only in the vein (200×); d) large vein of the rat lung in a state of increased tone; folding of layers of myocardial muscle in the wall of the vein (200×).

small veins were contracted, so that their lumen was considerably constricted (Fig. 1b), while the myocardial musculature of rodent type also was contracted; as a result it became crinkled in character (Fig. 1d). Foci of incomplete atelectasis alternated with foci of emphysema (Fig. 1c). These phenomena were more marked in the animals with ascending tetanus. An increased quantity of secretion and desquamation of the epithelium were observed in the bronchi.

These changes were accompanied by disturbances of the peripheral circulation of the venous crises type, as well as by state of pre- and post-stasis, resolved by diapedetic hemorrhages. Hypersecretion and desquamation of the epithelium developed in the bronchi against this background. Meanwhile no signs of inflammation were found either in the bronchi or in the alveolar tissue. Isolated foci of stasis of the leukocytes around the veins corresponded to venous crises [8]. In this connection it will be noted that in two animals which were treated with antitetanus serum and sacrificed much later, serohemorrhagic pneumonia and bronchitus were found.

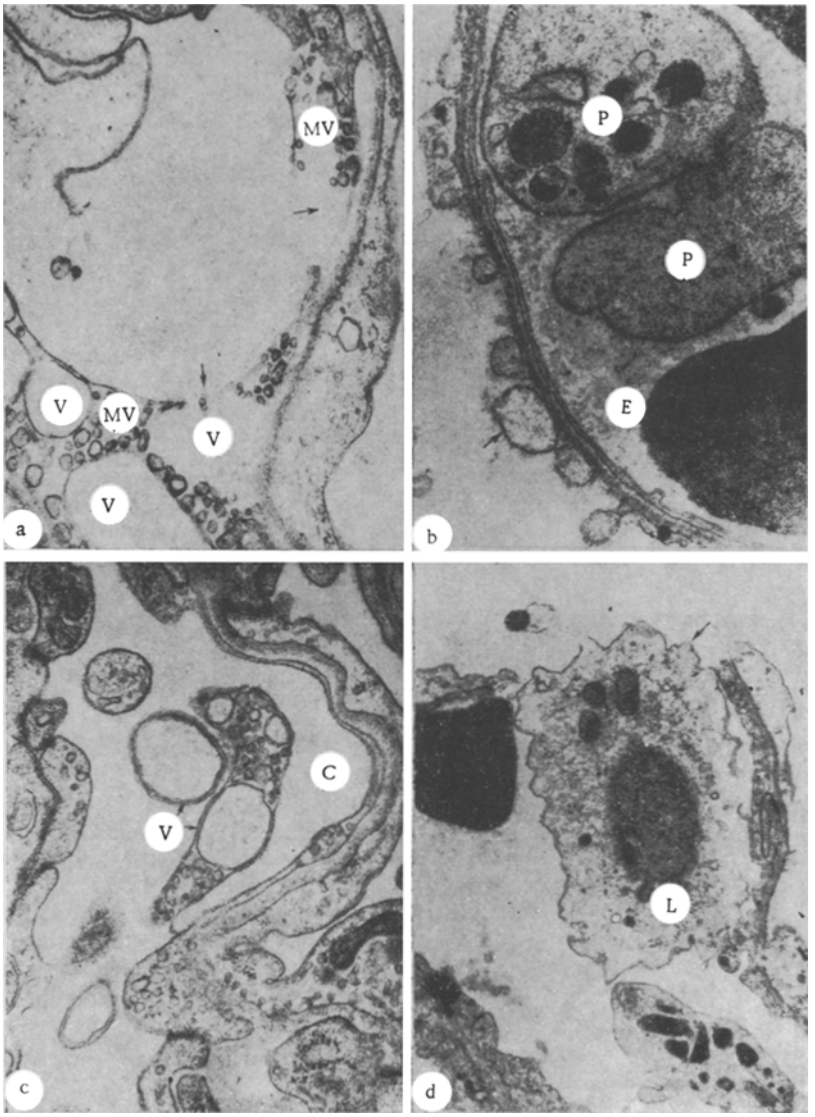

Fig. 2. Changes in ultrastructure of air-blood barrier in experimental tetanus: a) destruction of cytoplasmic membrane (marked by arrows) of endothelial cell and membranes of vesicles (V) on its surface with liberation of microvesicles (34,850×); b) destruction of cytoplasmic process of small alveolar cell into fragments, two platelets (P) and an erythrocyte (E) in the lumen of a capillary (34,850×); c) endothelial vesicles (V) discharging into lumen of alveolar capillary (C) (34,850×); d) rupture of air-blood barrier (marked by arrow) with lymphocyte (L) at the opening (12,750×).

Results of Electron Microscopy. At all stages of the disease the predominant changes in the lungs of the albino mice affected the ultrastructure of the alveolar capillaries and blood cells, while the epithelium was less affected. These changes reached their maximum at the height of development of the disease (the 4th day after injection of the toxin).

The most general change in the ultrastructure of the air-blood barrier and in the blood cells in the capillary lumen was local destruction of the outer cytoplasmic membranes of the endothelial and small alveolar cells, erythrocytes, leukocytes, and platelets (Fig. 2a, b).

Besides local destruction of the outer membrane and the adjacent zones of the cytoplasm in the endothelial cells of the alveolar capillaries, increased formation and destruction of vesicles were observed on

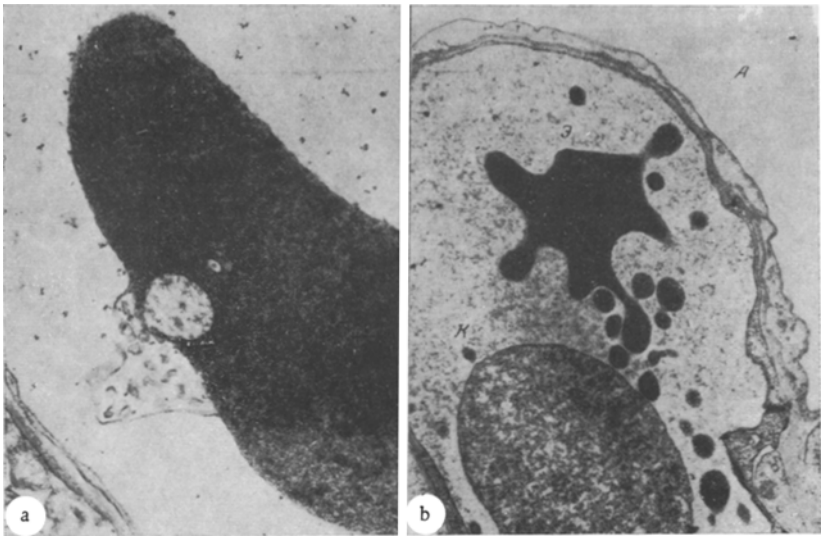

Fig. 3. Changes in erythrocytes within capillaries of the lungs in experimental tetanus: a) vacuolation of an erythrocyte (20,500×); b) disintegration of erythrocyte in the lumen of a capillary into spherical fragments (19,450×).

the surface of these cells (Fig. 2a) with intensive discharge of the vesicles into the lumen of the capillaries (Fig. 2c). By contrast with the vesicles still connected with the surface of the endothelium cells, other vesicles projecting into the lumen of the capillary were still intact and were approximately two-three times larger in size (1-1.5 μ). These structures contained short, twisted threads in their interior, and numerous microvesicles (500-100 Å) in their walls. Cytoplasmic veils, up to 1 μ in length, were frequently seen on the surface of the endothelial cells.

Most of the capillaries were greatly dilated and projected into the alveolar spaces. Complete ruptures of the air-blood barrier were frequently observed (Fig. 2d). The cytoplasmic processes of the small alveolar cells forming the air-blood barrier were in some cases broken up into spherical fragments (Fig. 2b). The cytoplasmic processes which remained intact were frequently highly edematous. Many alveolar spaces contained erythrocytes, leukocytes, platelets, and single fibrin threads. Some of the disturbances observed in the ultrastructure of the alveolar capillaries, such as local destruction of the endothelial cell and leukocyte membranes, appeared actually during the incubation period of the disease; these changes increased in intensity later and were joined by the other phenomena described above.

Changes in the ultrastructure of the erythrocytes within the lumen of the capillaries also were seen in many cases. One type of change consisted of the appearance of vacuoles located chiefly at the periphery of the erythrocytes. The size of the vacuoles varied within narrow limits (0.3-0.4 μ). Some of them communicated with the lumen of the capillary; in that case detachment of the erythrocyte membrane was often seen, and some sort of material, evidently its contents, was found next to the opening into the vacuole (Fig. 3a). This phenomenon can be regarded as local destruction of the erythro matrix. Another type of change in the ultrastructure of the erythrocytes consisted of the gradual or immediate breaking up of the cells into spherical fragments of about equal size (measured in tens of microns, Fig. 3b). This type of destruction of the erythrocytes was found only within the capillaries and did not occur in erythrocytes which had migrated into the alveolar spaces. Vacuolation of the matrix was not found in any of these fragmented erythrocytes. Vacuolation was observed in the earlier stages of the disease than fragmentation. Vacuolation and fragmentation are presumably two different and unconnected types of pathological change in erythrocyte ultrastructure due to different causes.

The results of these investigations show that in experimental tetanus marked disturbances of the microcirculation and changes in the ultrastructures take place in the lung tissue. It is important to note that some of the changes described occurred actually in the incubation period of the disease, although their intensity at this time was slight. The changes described reached their maximal degree at the height of

development of the disease. The fact that even at this period no signs of pneumonia were found is very important. In fact, permeability for neutrophils was disturbed only in the neighborhood of the veins and venules, while for erythrocytes it was disturbed mainly in the zone of the air-blood barrier. Signs of pneumonia likewise were not observed in those cases in which material was taken from hyperemic zones of the lung which appeared changed on external examination. Bronchopneumonia is thus not a primary pulmonary complication. The microcirculatory disturbances observed led to the development of hemorrhages into the lung tissue. Such hemorrhages are found in tetanus in both man and animals. Inflammatory lesions (bronchopneumonia, pneumonia, etc.) may arise as secondary complications of the primary microcirculatory disorders and hemorrhages. This is clear from the results of the observations described above showing that pneumonia developed in animals cured of tetanus and sacrificed later.

The cause of the microcirculatory and ultrastructural changes in the lungs in experimental tetanus is not yet clear. The possible role of the toxin itself cannot be ruled out, although a role has been postulated for neuroendocrine disturbances as a pathogenetic factor in the development of these changes. In the later stages of the disease, several secondary pathological processes may also become involved [2]. The results of the present investigation emphasize the concept of the pathogenesis of tetanus as a polysystemic disease put forward previously [2, 3].

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