EFFECT OF THE TOXIN OF Clostridium perfringens TYPE A AND ITS LETHAL FACTOR (LECITHINASE) ON THE MICROCIRCULATION

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The effect of the toxin of Clostridium perfringens type A and its lethal factor (lecithinase) on the microcirculation was studied by intravital microscopy. Microcirculatory disorders were observed in the mesentery of the small intestine, the wall of the small intestine, and the cremaster muscle of albino rats after both local (intraperitoneal and intramuscular) and general (intravenous) action of the toxin and lecithinase. The first phase of the disorders of the microcirculation was due to the direct action of the Cl. perfringens toxin and lecithinase on the smooth-muscle cells of the blood vessels, leading to a persistent disturbance of the microcirculatory hemodynamics and to a general slowing of the blood flow, causing a decrease in the volume of blood perfusing the tissues. The second phase is a continuation of the hemodynamic changes which arose in the microcirculatory system. During the metabolic disturbances, and as a result of the direct action of the Cl. perfringens toxin and lecithinase on the blood, stasis accompanied by a disturbance of permeability of the tissue-blood barrier develops. The strong cytolytic action of lecithinase causes massive intravascular hemclysis of the erythrocytes.

Evidence of substantial disorders of the microcirculation produced by bacterial toxins has recently been published [17, 7]. This raises the question of the role of microcirculatory disturbances in the development of the pathomorphological changes in toxico-infections.

The object of the investigation described below was to study the action of the toxin of Clostridium perfringens, the agent of gas gangrene in man, and of its main component, the enzyme lecithinase, on the microcirculation in vivo.

EXPERIMENTAL

Highly purified lecithinase was isolated by Ispolatovskaya's method [1, 2]. The enzyme was free from catalytic activity of other enzymes of the toxic complex of Cl. perfringens.

The state of the microcirculation after local and general administration of the toxin and lecithinase was studied in vivo in the blood vessels of the mesentery of the albino rats by intravital microscopy [16] in Kozlov's modification [3], and also in the vessels of the small intestine and of the cremaster muscle [4].

The experimental animals were subdivided into 4 groups, with 6 albino rats in each group. The rats of group 1 received the toxin by intraperitoneal injection in a dose of 0.5 MLD, and the rats of group 2 received an injection of 1-2 MLD intravenously. The local action of lecithinase, injected intraperitoneally and intramuscularly in doses of 0.5 MLD, was studied on the rats of group 3. Lecithinase was injected intravenously into the animals of group 4 in a dose of 1-2 MLD. The reaction of the blood vessels of the microcirculation was studied for 6-20 h after injection of the toxin or lecithinase. The changes observed were recorded by photomicrography and by microfilming.

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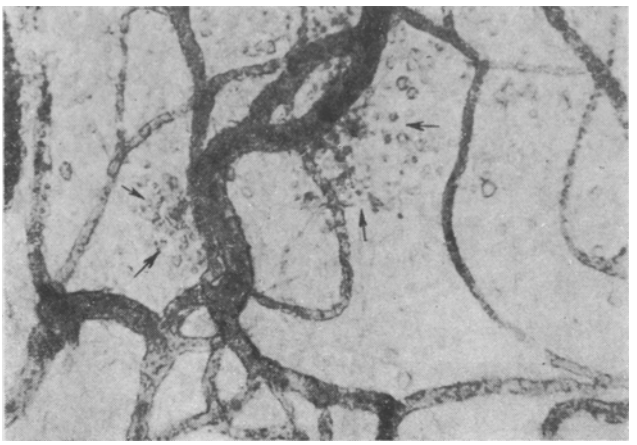

Fig. 1. Stasis in vessels of postcapillary-venular division of microcirculation of mesentery of albino rat 60 min after intraperitoneal injection of Cl. perfringens toxin. Arrows denote areas of diapedesis of erythrocytes. Intravital photomicrograph, 90 ×.

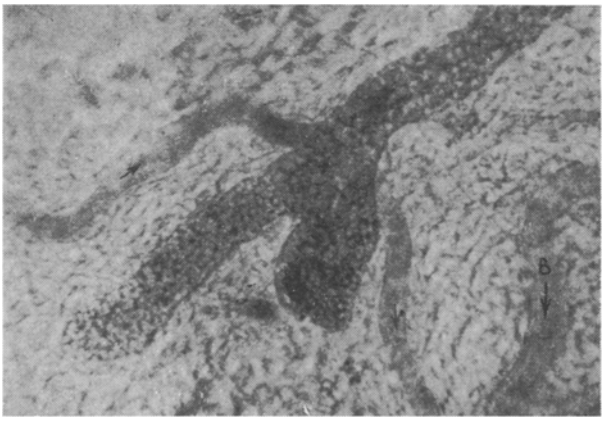

Fig. 2. Lymphatic capillaries in the mesentery of an albino rat filled with erythrocytes, 60 min after intravenous injection of Cl. perfringens. B) venule. Arrow shows direction of blood flow. Intravital photomicrograph, 140 ×.

EXPERIMENTAL RESULTS AND DISCUSSION

The reaction of the vessels of the microcirculation to the general and local action of Cl. perfringens toxin and lecithinase developed comparatively rapidly. Periodic contractions of the muscular components of the small arteries and arterioles, alternating with their relaxation, running in waves along the vessel were observed 3-5 min after injection of the toxin. The interval between the phases of contraction and relaxation of the smooth-muscle elements was 1.5-2 min. These transient rises and falls of capillary resistance modify the established rhythm of entry of blood into the microcirculation, as a result of which a general slowing of the peripheral blood flow takes place after 10-15 min. In the venous portion of the microcirculation there was slight dilatation of the venules and small veins.

A stable response of the vessels of the microcirculation developed after 30-60 min. The arterial vessels were sharply constricted, while the veins were dilated and congested. The mean diameter of the arteries was reduced from 68 to 53 μ, i.e., by 22%, while the diameter of the vein was increased from 110 to 152 μ, i.e., by 38%. The ratio between the lumen of the afferent arterial vessels and the lumen of the efferent venous vessels was reduced by almost half (from 0.625 to 0.345).

Parallel with the disturbance of perfusion of the tissues with blood, juxtamural stasis of leukocytes developed, first in the postcapillaries and venules, and later in the capillaries. The leukocytes protruded partly into the vessel wall, and partly formed juxtamural aggregates together with the platelets. Comparatively large aggregates consisting of platelets, leukocytes, and erythrocytes appeared in the peripheral blood 30-40 min after administration of Cl. perfringens toxin to the animal. By occluding the lumen of the postcapillaries and venules, these aggregates of blood cells caused arrest of the microcirculation.

During the local action of the toxin these phenomena were seen most intensively. By 60 min most of the capillary and postcapillary system (up to 70-80%) was excluded from the circulation as a result of progressively developing stasis (Fig. 1). Movement of blood continued only along the short and juxtacapillary pathways of the circulation which, as Kupriyanov and Kozlov [5] have shown, act as shunts for the blood flow.

In individual cases after local administration of Cl. perfringens toxin a massive outpouring of erythrocytes into the lymphatics and capillaries was observed (Fig. 2). The lymphatic capillaries filled with blood in a retrograde direction. The massive outflow of erythrocytes into the lymphatics is considered to be a manifestation of severe disturbance of the permeability of the tissue-blood barrier resulting from damage to the vessel wall caused by the toxin.

Disturbances of the microcirculation observed following local administration of lecithinase were largely similar to the changes produced by the toxin. The pathomorphological picture was dominated by the rapid development of extensive stasis with local diapedetic hemorrhages.

Following the general action of lecithinase on the animal, besides the circulatory disturbances of the intramural blood flow described above, the enzyme had a strong cytolytic action on the blood cells. The number of erythrocytes in the circulating blood fell sharply 1-2 h after intravenous injection of 1-2 MLD lecithinase, and "ghost" erythrocytes appeared. Within the blood vessels of the microcirculation hemolysis of erythrocytes took place rapidly. After lethal doses of lecithinase lysis of the erythrocytes was so rapid that the animal dies within a few hours of acute cardiovascular failure.

The early disturbances of the microcirculation produced by the action of Cl. perfringens type A toxin and its lethal factor (lecithinase) were directly connected with a disturbance of the hemodynamic function of the microcirculation. By producing spasm of the small arteries and arterioles and dilatation of the veins, the toxin and lecithinase led to a persistent slowing of the blood flow in the microcirculation and to a sharp decrease in the volume of blood perfusing the organs and tissues.

Similar changes in the microcirculatory hemodynamics have been demonstrated after the action of the endotoxin of E. coli on the animal [14, 9], and also during generalized poisoning by various toxins [15]. Most workers consider that the primary spasmogenic effect is due to the direct action of the bacterial toxins on the smooth-muscle cells of the blood vessels [10]. However, the possibility of accompanying disorders of the vasomotor innervation of the vessels cannot be ruled out [6, 8, 13].

The development of stasis, as the end-result of the microcirculatory disorders, is observed not only as a result of the action of bacterial toxins. Various forms of shock [11, 15] and mechanical trauma to vessels [12] lead ultimately to stasis. These facts suggest that not only the initial damage to elements of the microcirculation by the toxin but also a whole group of pathological changes connected with disturbances of tissue metabolism during infection of the tissues are concerned in the genesis of stasis.

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