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CHARACTER OF PHAGOCYTOSIS IN INTRAVITREAL HEMATOMA

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In the study of phagocytosis in hemophthalmia the problem of the ways of penetration of phagocytes into the vitreous body (VB) has been studied in more detail in recent years [6, 12-16]. However, the role of phagocytosis in the morphogenesis of intravitreal hematoma, the formation of which is inevitable in some forms of hemophthalmia, still remains unstudied [5].

Accordingly, the aim of this investigation was to determine the characteristics of phagocytosis of a hematoma in this situation, allowing for the specificity of its surrounding medium, viz. VB.

EXPERIMENTAL METHOD

Experiments to create a model of hemophthalmia were carried out on 74 rabbits (144 eyes) of the albino and Chinchilla breeds, of both sexes weighing 1.5-2.5 kg. Under local anesthesia (1% procaine solution), autologous blood (0.1 to 1.2 ml), taken from the auricular vein, was injected subconjuctivally into the VB by the retrobulbar route. The sclera was punctured in the flat part of the ciliary body after preliminary paracentesis. The animals were decapitated at various times (from 1 h to 3.5 years) after single or repeated (two to four times) injection of blood, and the eyes were quickly enucleated and fixed in 10% formalin solution, which was injected into VB. Before fixation the VB was removed and investigated cytologically by reactions for glycosaminoglycans (GAG, with toluidine blue) and lipids (with Nile blue). After fixation the eye was cut into three blocks and treated histologically in the usual way and then embedded in celloidin and paraffin wax. Sections were stained with hematoxylin and eosin, Sudan black, and Nile blue and the histochemical reactions of Perls, Hailey and Seitelberger, Brachet, and Gomori were used. Material for electron microscopy was fixed in a mixture of paraform and glutaraldehyde and then postfixed in 1% OsO_4 solution. Ultrathin sections were examined in the UMV-100K and Tesla BS-500 electron microscopes.

EXPERIMENTAL RESULTS

On the 2nd day after injection of the blood separate round mononuclear cells with bean-shaped nucleus appeared around the hematoma. On the 3rd-5th day the number of cells around the hematoma and in VB was increased somewhat. By this time single cells had already penetrated into the depth of the fibrin film of the hematoma. Zones of translucency of fibrin were visible around these cells (Fig. 1a), evidence of participation of phagocytes in fibrinolysis. Whole erythrocytes (Fig. 1b) or their derivatives were found in the cytoplasm of individual macrophages on the 3rd-6th day. Evidence of the phagocytic activity of these cells was given by their positive reactions for alkaline phosphatase and their marked pyroni-

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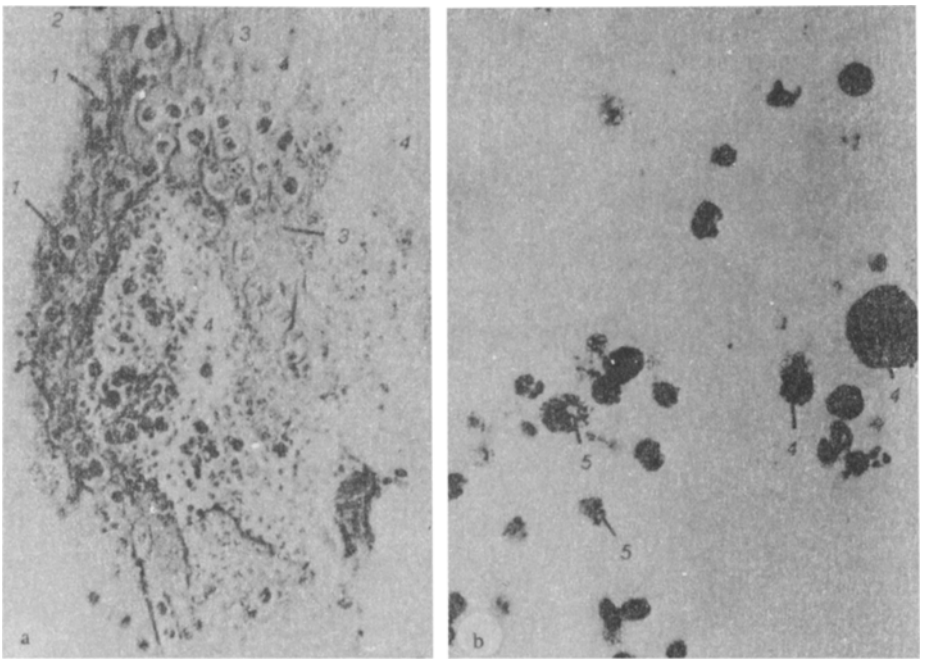

Fig. 1. Participation of phagocytes in hemolysis and fibrinolysis. a) Zones of translucency (1) around macrophages (2) in fibrin film (3) of hematoma (4) on 5th day of hemophthalmia (Van Gieson's stain, 100×). b) Erythrophagy of whole erythrocytes (5) on 12th day of hemophthalmia (Perls' reaction, 200×).

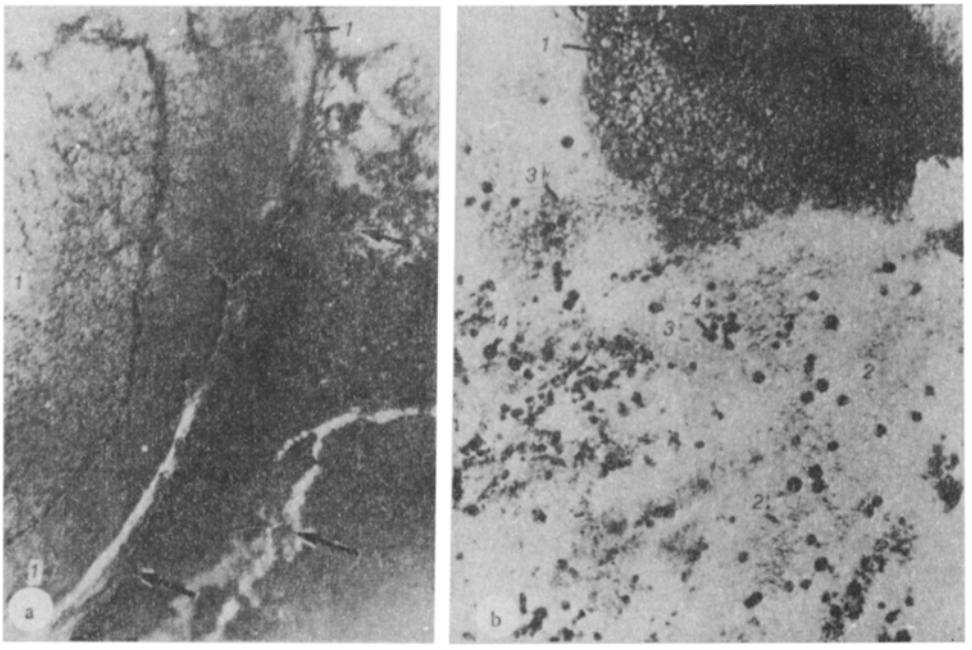

Fig. 2. Localization of phagocytes in VB in the presence of an intravitreal hematoma and of hemorrhagic infiltration of VB. a) Isolated hematoma with "white" clot (1) located in VB without phagocytes, but with marked leukocytic infiltration of VB (arrow) 2.5 months after injection of blood. b) Periretinal film in VB on 7th day of hemophthalmia: zone of hemorrhagic infiltration of VB with whole erythrocytes and their derivatives (3) and with macrophages (2), penetrating freely into zone of infiltration of VB. Hematoxylineosin, 100×.

nophilia. On the 3rd-6th day metachromasia was found cytochemically in the cytoplasm of V on staining with toluidine blue, and an abundance of phosphatides and cerebrosides was discovered. In addition, on the 4th day black granules could be observed in the cytoplasm of

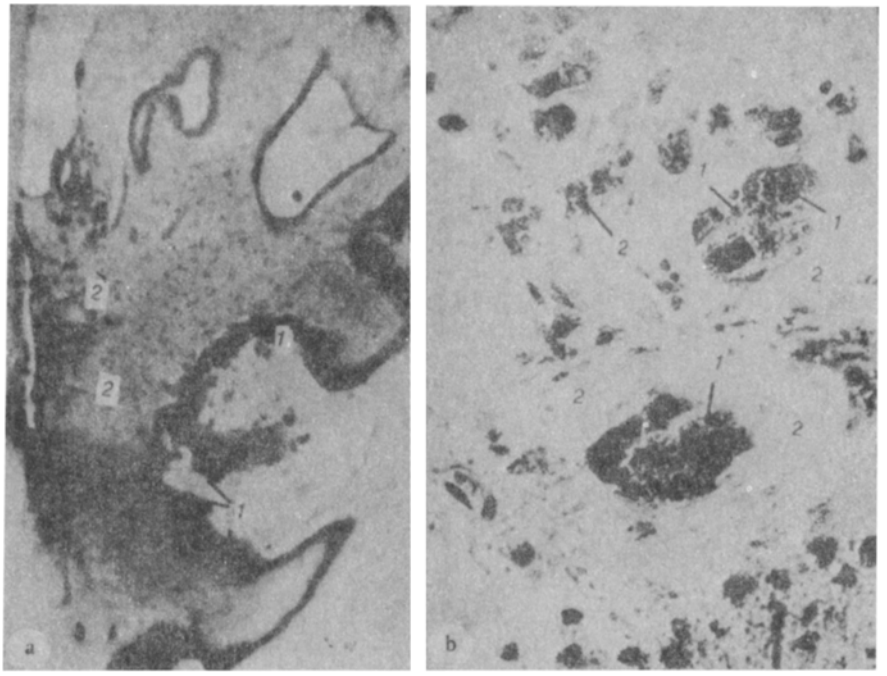

Fig. 3. Phagocytosis in early and late stages of hemophthalmia. a) Generalized phagocytosis with hemosiderosis of epithelial cells (1) of ciliary body, and also of endothelium of trabecular zone (2). b) Focal changes in hematoma on 31st day of hemophthalmia after injection of 0.3 ml of blood, with giant cells (1) surrounded by connective tissue (2). a) Perls' reaction; b) Hailey—Seitelberger reaction. 100×.

individual phagocytes close to the hematoma on staining with Sudan black. Single vacuoles could be seen electron-microscopically in macrophages of this same zone, and these empty spaces may perhaps reveal deposits of lipids, which dissolved after treatment of the sections. These results indicate the participation of phagocytes in lipid metabolism. By the same time (3rd-6th day) granular material giving a positive Perls' reaction appeared in the macrophages. Dynamic changes were noted in the location of siderophages, depending on the duration of hemophthalmia. For instance, they were seen soonest of all at the periphery of the hematoma (on the 3rd day), next in different zones of VB (5th-dth day), and on the 12th day they were actually seen preretinally, in the layers of the retina, and on the lens capsule (Fig. 1b).

Depending on the character of the focus of accumulation of blood, macrophages were distributed either along the periphery of the hematoma only (Fig. 2a) or diffusely, in the zone of hemorrhagic infiltration of VB, to form films with fibrils of VB, with separate erhtyrocytes, and with fibrin (Fig. 2b). Granular material appeared as early as on the 6th day not only in the free phagocytes, but also in other cells. For instance, hemosiderin granules were detected by Perls' reaction in the cytoplasm of whole layers of the nonpigmented and pigmented epithelium of the ciliary body (Fig. 3a), in the pigmented epithelium of the retina, and also in individual cells of its microglia and even in single ganglion cells. Marked granularity was observed in the cytoplasm of the stroma cells of the suprachoroid and perineuronally. In the later stages macrophages with material giving a positive Perls' reaction appeared in the corner of the anterior chamber and in its drainage zone (Fig. 3a). On the 12th day hemosiderin granules were found in the stroma of the vascular membrane, and on the 35th day in the stroma of the ciliary body. The hemosiderin granules in the cytoplasm of the different cells were round, loose or compact, and single or multiple. Sometimes they were perinuclear in distribution, but occasionally they covered the nucleus (Fig. 1b).

The intensity of the hemosiderosis correlated with the duration of hemophthalmia. After the 3rd-5th day the hemosiderosis gradually increased to reach a maximum by the 35th day. By the same time focal phagocytosis had developed in the zone of the hematoma. Whereas mainly mononuclear macrophages were observed until the 7th-9th day, around the hematoma or within

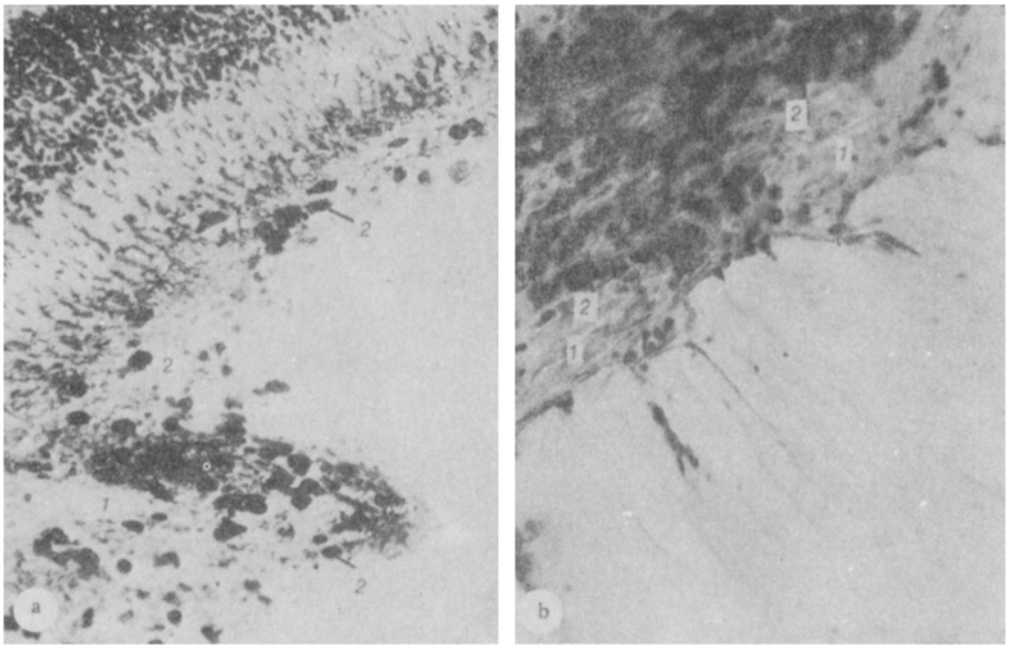

Fig. 4. Hemosiderosis in late phases of experimental hemophthalmia, with severe changes (bulky hematoma with recurrent small hematomas): a) 31st day of hemophthalmia: phagocytes (2) arranged haphazardly in layers of retina (1), with marked alkaline phosphatase activity; b) duration of hemophthalmia over 1 year: phagocytes (2) giving positive Perls' reaction in changed retina with proliferation of neuroglia (1). a) Gomori's reaction, 200×; b) Perls' reaction, 320×.

the zone of its film (Fig. 1a), giant cells with multiple nuclei, containing coarse Perls-positive granules in the cytoplasm and nucleus were found by the 12th-35th days. Macrophages were arranged in a lattice in the zone of the hematoma and were surrounded by newly formed connective tissue (Fig. 3b). During this period, however, the hemosiderosis was still widespread and becoming focal in the zone of the hematoma in the later stages (by 6-8 months).

The severity of the hemosiderosis was found to be dependent to some degree on the quantity of blood and also on the duration of hemophthalmia. After a single injection of a small quantity of blood (up to 0.3 ml) no siderosis was observed before the end of the second month. If larger volumes of blood were injected (more than 0.8 ml) or if small doses (0.2-0.3 ml) were injected frequently, hemosiderosis was observed for a long period and it had not disappeared after 3 h (Fig. 4). A high level of alkaline phosphatase activity was preserved in the cytoplasm of the phagocytic cells until 8 months or 1 year. During the same period, pyroninophilia was absent in the macrophages with brown pigment, or it was sharply depressed. A high level of alkaline phosphatase activity and absence of pyroninophilia are evidently indicative of weakness, but incompleteness, of phagocytosis in severe forms of hemophthalmia.

The results show that in intravitreal hematoma phagocytosis develops slowly, its intensity is weak, it remains incomplete for a long time with a mononuclear-cell and monomorphic response without the macrophages and the polymorphism observed in another investigation [15]. These features of phagocytosis can be connected with the fact that the hematoma, as an isolated, chemically inert focus, possesses weak chemotaxic properties during the first 2 days. The dense framework of its structure with an external film, possessing solid-state properties [4, 5], does not permit phagocytes to penetrate inside the hematoma for quite a long time. This explains the fact described by several workers [3, 7], and confirmed by the present investigation, that phagocytes are located on the surface of the blood clots. Accordingly, in intravitreal hematoma mainly extracellular hemolysis develops, whereas in hemorrhagic infiltration of VB intracellular hemolysis, connected with free access of the phagocytes to erythrocytes in the films of VB, is of principal importance (Fig. 2b).

The slow development of phagocytosis can also be linked with the specific features of the structure of VB, surrounding the hematome, which contains a few cells and possesses fairly high viscosity, thus impeding the rapid movement of blood phagocytes and other cells towards

the hematoma. Meanwhile the semisolid structure of VB facilitates rapid diffusion of chemical substances entering VB on destruction of the hematoma, with equalization of their concentration in different zones of VB. In this case the hemotaxic action of the hematoma during the period of its disintegration (2-3 days) also is disturbed, a fact that may be connected with the earlier development and predominance of generalized phagocytosis in intravitreal hematoma, at the expense of focal phagocytosis with involvement of whole layers of epithelial cells, connective-tissue histiocytes of the uveal tract, etc., which is analogous to phagocytosis by "fixed macrophages" [10]. Under these circumstances focal phagocytosis by free phagocytes in the zone of the hematoma (Fig. 3b) or blood clot, is late in developing [11, 17].

The features of phagocytosis in intravitreal hematoma are thus connected with its structure and the particular nature of its surrounding medium, namely VB, which determines the slow development of phagocytosis and the monomorphic response with predominance of generalized phagocytosis — by "fixed macrophages." The results of the investigation demonstrated the role in erythrocyte desturction, but also in processes of thrombocytolysis and fibrinolysis, as well as its marked detoxicating role — the earlier ingestion of lipids, GAG, and iron, with the subsequent formation of hemosiderosis. The latter becomes irreversible in the case of large or recurrent hematomas. The results are evidence that in hemophthalmia the character of the therapeutic tactics must be determined early (in the first 5 days), for by that time phagocytosis is becoming generalized.

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