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ROLE OF NEUTROPHIL-PRODUCED MYELOPEROXIDASE IN THE PATHOGENESIS OF CATARACT

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Polymorphonuclear leukocytes (neutrophils) are cells specialized morphologically and biochemically for undertaking defensive reactions by phagocytosis and also by secretion of biologically active substances into the pericellular space. However, stimulated neutrophils not only have a bactericidal effect, but they also induce nonspecific damage to nearby tissues and cells [1]. Antimicrobial factors of neutrophils are generally divided into oxygen-dependent and oxygen-independent [9]. The first group includes oxygen derivatives with varying degrees of reduction: the $^1O_2$-superoxide-radical, hydrogen peroxide (H_2O_2), the hydroxyl radical (OH), and its active form – singlet oxygen ($^1O_2$). The hydroxyl radical possesses the highest degree of oxidative capacity in living nature, enabling it to attack and destroy virtually all biomolecules. Singlet oxygen interacts particularly actively with unsaturated fatty acids of membrane phospholipids and induces their peroxidation. Singlet oxygen and the hydroxyl radical rupture peptide bonds in proteins, decarboxylate amino acids, induce peroxidation of membrane lipids, and degrade nucleic acids [5, 11]. Oxygen-independent antimicrobial factors of neutrophils include cationic proteins: myeloperoxidase, defensins, bactericidal permeability-increasing protein, cathepsin G, elastase, and lactoferrin. The action of the defensins [8], of bactericidal permeability-increasing protein [13], cathepsin G, and elastase [10] is associated with disturbance of permeability of biological membranes, whereas that of lactoferrin is dependent on production of the hydroxyl radical

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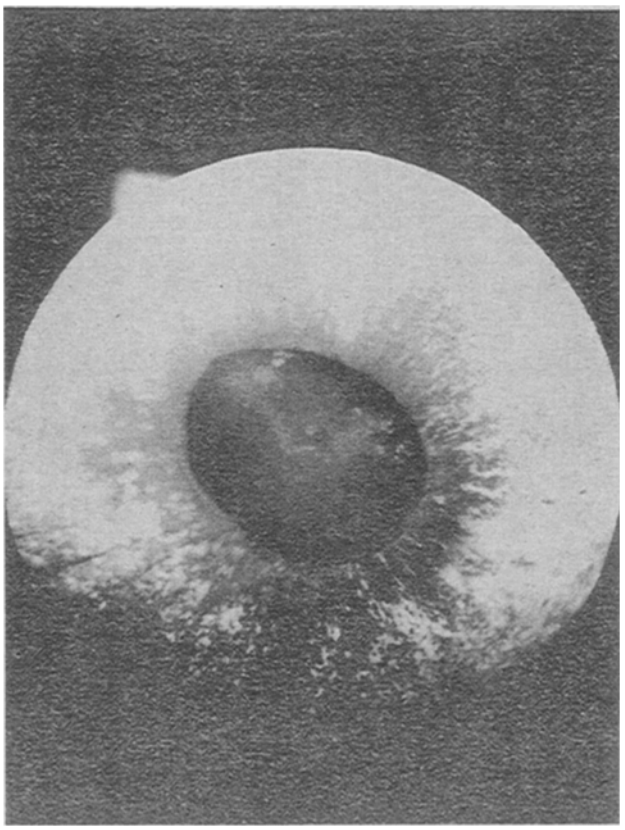

Fig. 1. Opacity of rabbit lens induced by injection of myeloperoxidase solution (315 μg/ml) into anterior chamber of the eye. Age of animal 3 years.

[6]. Myeloperoxidase is regarded as the leading antimicrobial factor. It acts as an enzyme in the composition of the myeloperoxidase system, which includes also hydrogen peroxide and one of three coenzymes (I^-, Br^-, Cl^-), and catalyzes oxidation of various biological compounds with hydrogen peroxide. The mechanism of the damaging action of the myeloperoxidase system is linked both with the generation of singlet oxygen [4], disturbing normal membrane permeability, and with production of the hypochlorite ion, which disturbs protein structure [14].

The aim of this investigation was to study the ability of myeloperoxidase to disturb transparency of the lens in experimental animals.

EXPERIMENTAL METHOD

Experiments were carried out on two groups of chinchilla rabbits. The 1st group consisted of healthy animals aged 2-3 years and weighing 3-4 kg. The 2nd group consisted of animals aged 3-4 months and weighing 1.5-2 kg. After general anesthesia with pentobarbital, 100 μl of an aqueous solution of myeloperoxidase, isolated from rabbit neutrophils, was injected through a fine needle into the anterior chamber of the animal's eye through paracentesis in the peripheral part of the cornea. Myeloperoxidase was isolated as described in [2]. We calculated that a concentration of myeloperoxidase of 0.315 μg/ml can develop in the fluid in the anterior chamber of the eye if 50 μl (one drop) of blood penetrates into it. Accordingly, the following concentrations of myeloperoxidase were used in the

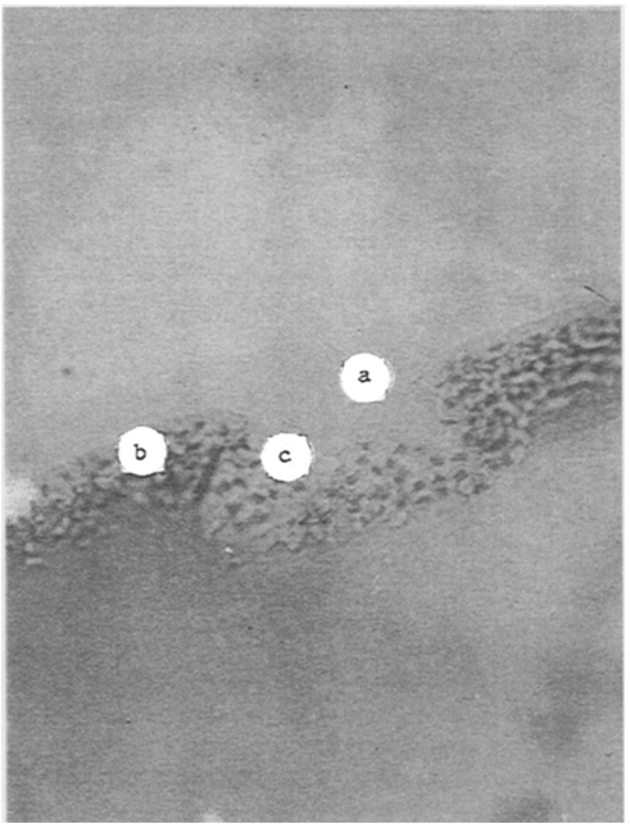

Fig. 2. Light microscopy of section through lens Local thickening of anterior capsule (a), formation of subepithelial vacuoles (b), and disturbance of regularity of anterior epithelium (c). Hematoxylin-eosin 250×.

experiments: 315, 31.5, and 3.15 μg/ml. In the control, a solution of fibrinogen of the corresponding concentration was injected into the anterior chamber of the other eye. Biomicroscopic observations were made and changes in the eyes of experimental animals recorded for 5 months after injection of myeloperoxidase by means of a Carl Zeiss photo-slit lamp. At the end of 5 months the experimental eyes intended for light microscopy were fixed in formalin and embedded in celloidin. Sections were stained with hematoxylin-eosin.

In the rabbits of the 1st group, dilatation of the vessels of the iris, constriction of the pupil, and the formation of a dense fixed exudative film on the anterior surface of the lens were observed on the 1st day after injection of myeloperoxidase. After absorption of the film and disappearance of the signs of iridocyclitis, which took 5-10 days, a local opacity of the anterior capsule and of the anterior subcapsular layers of the lens was found. In the course of time the opacity in five animals spread to the deeper layers of the lens, and in two animals it remained unchanged; no clear correlation could be found between the myeloperoxidase concentration and the size and intensity of the opacities (Fig. 1). The microscopic picture depended on the intensity of opacity. In areas with mild degrees of opacity, subepithelial vacuoles were found, but the structure of the epithelium was not disturbed. In areas of greater intensity of opacity, local thickening of the anterior capsule of the lens was found, with irregular accumulation of epithelial cells and the formation of subepithelial vacuoles (Fig. 2). In this group opacity of the lens was obtained in seven of 15 animals. Observations made during 5 months after injection of myeloperoxidase revealed irreversibility of the opacities. No changes were found in the lens in the control eyes.

In the animals of the 2nd group signs of iridocyclitosis with the formation of an exudative film also were observed after injection of the preparation. However, only in one case out of 10 was a mild degree of reversible opacity of the anterior capsule of the lens obtained.

Injection of myeloperoxidase into the anterior chamber of the experimental rabbit's eye can therefore induce opacity of the lens. Since hydrogen peroxide and chloride ions, necessary for myeloperoxidase activity, are contained both in the aqueous humor and in the lens itself [7], generation of free radicals and damage to the membranes of the lens by them would seem to be highly probable. The appearance of polymorphonuclear leukocytes in the tissues surrounding the lens and secretion of agents damaging membranes can take place in many different forms of ocular pathology. Tomoda found that polymorphs possess chemotactic activity toward components of the lens [12]. Consequently, even as a result of a temporary disturbance of permeability of the lens capsule and escape of its components into the surrounding tissues, oriented movement for polymorphs towards the lens and damage to its membranes by cationic proteins and free radicals can take place. Moreover, states exist when leukocytes are present in the intraocular fluid in large numbers. These may be various inflammatory diseases (iridocyclitis, keratitis) and hemorrhages from various causes (post-traumatic postoperative, and so on) into the cavity of the eye. In such cases damage to the membranes of the lens by products of neutrophils also seems very probable.

The formation of irreversible opacities of the lens only in adult animals is demonstrative. This fact may be associated with a decrease in efficacy of the antioxidant system of the lens with age. The absence of any definite relationship between the concentration of injected myeloperoxidase and the size and intensity of the opacities still awaits explanation.

It was thus shown experimentally that myeloperoxidase can cause damage to the tissue of the lens. This is of great importance in medical practice for the study of the pathogenesis of cataract and the development of measures preventing its development and progression.

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