# EFFECT OF EXPERIMENTAL HYPERTHYROIDISM ON LYSOSOMAL MEMBRANE FUNCTION AND STRUCTURAL ORGANIZATION OF THE RABBIT CORNEA

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Certain pathological states of the organ of vision are connected with a change in activity and localization of lysosomal enzymes and also with disturbance of the functional state of the lysosomal membranes [1, 2]. It has recently been shown that similar changes in tissues of the eye may arise under the influence of hormones of varied chemical nature [3]. At the same time many pathological processes developing against the background of changes in hormonal status of the body are known to be accompanied by disturbances of ocular functions [4]. However, the degree of participation of hormonal imbalance in the development of eye diseases still remains virtually unstudied.

The aim of the present investigation was to study the effect of experimental hyperthyroidism on lysosomal function and on the structural organization of the epithelium and endothelium of the rabbit cornea.

## EXPERIMENTAL METHOD

Experiments were carried out on 40 male chinchilla rabbits weighing 2-2.5 kg. Experimental hyperthyroidism was produced in rabbits by intraperitoneal injection of thyroxine in a dose of 100 μg/kg every 4 h. The animals were killed 8, 24, 48, and 72 h after the beginning of injection of the hormone. After sacrifice of the rabbits the cornea was removed (at 0°C) and used for subsequent biochemical and electron-microscopic investigation. The functional state of the lysosomal membranes was assessed from the ratio between free and total activity of the lysosomal glycosidases (β-glucosidase, β-galactosidase, and hyaluronidase), which hydrolyze glycosaminoglycans. The methods of determination of enzyme activity and of obtaining the homogenate and subcellular fractions of the cornea were described previously [5]. For investigation of its structural organization the cornea was immersed in 2.5% glutaraldehyde solution made up in 0.1M phosphate buffer (pH 7.4). After fixation the cornea was dehydrated in acetones and dried at the critical point over liquid $CO_2$ in a Hitachi NSP-1 apparatus. The specimens were then mounted with conductive glue on special object-holding stages, coated with a layer of gold on an "Eiko" apparatus, and studied in the Philips SEM-501 scanning electron microscope.

## EXPERIMENTAL RESULTS

Prolonged administration of thyroxine sharply reduced the activity of all glycosidases tested. However, total activity of β-glucosidase and β-galactosidase was reduced by a much lesser degree than their free activity. This indicates marked labilization of the lysosomal membranes. Changes in enzyme activity decreased toward 24 h of thyroxine administration. After 48 h of thyroxine administration total and free activity of the glycosidase rose sharply and subsequently remained at a steady level relative to normal. It was found that labilization of the lysosomal membranes is accompanied by considerable release of enzymes from lysosomes into the cytosol (Table 1), and this is probably accompanied also by their release into the extracellular space.

Investigation of the cornea of intact rabbits under the scanning electron microscope showed that on the outer side it is lined by flattened polygonal epithelial cells with clearly distinguishable cell boundaries and slightly prominent nuclei (Fig. 1a). Only solitary sites

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TABLE 1. Activity of Corneal Lysosomal Glycosidases during Thyroxine Administration (in mmoles/min/g protein)

| Enzyme | Normal | Time after beginning of thyroxine injection | |
|---|---|---|---|
| | | 24 h | 48 h |
| β-glucosidase: | | | |
| total activity | 0,68±0,01 | 0,45±0,03 | 0,80±0,05 |
| free activity | 0,40±0,05 | 0,35±0,03 | 0,65±0,06 |
| free activity/total activity, % | 57,1 | 77,8 | 81,2 |
| activity of enzyme in cytosol, % | 10,3±0,04 | 14,2±0,03 | 18,0±0,01 |
| β-galactosidase: | | | |
| total activity | 0,79±0,09 | 0,60±0,05 | 0,92±0,07 |
| free activity | 0,40±0,05 | 0,36±0,06 | 0,80±0,05 |
| free activity/total activity, % | 51,3 | 58,3 | 88,9 |
| activity of enzyme in cytosol, % | 15,2±0,01 | 16,0±0,05 | 20,2±0,07 |
| Hyaluronidase: | 38,2±3,2 | 35,1±2,0 | 46,0±2,5 |
| activity of enzyme in cytosol, % | 9,2±0,03 | 10,1±0,05 | 17,6±0,09 |

Legend. Mean results of 6-8 experiments are shown.

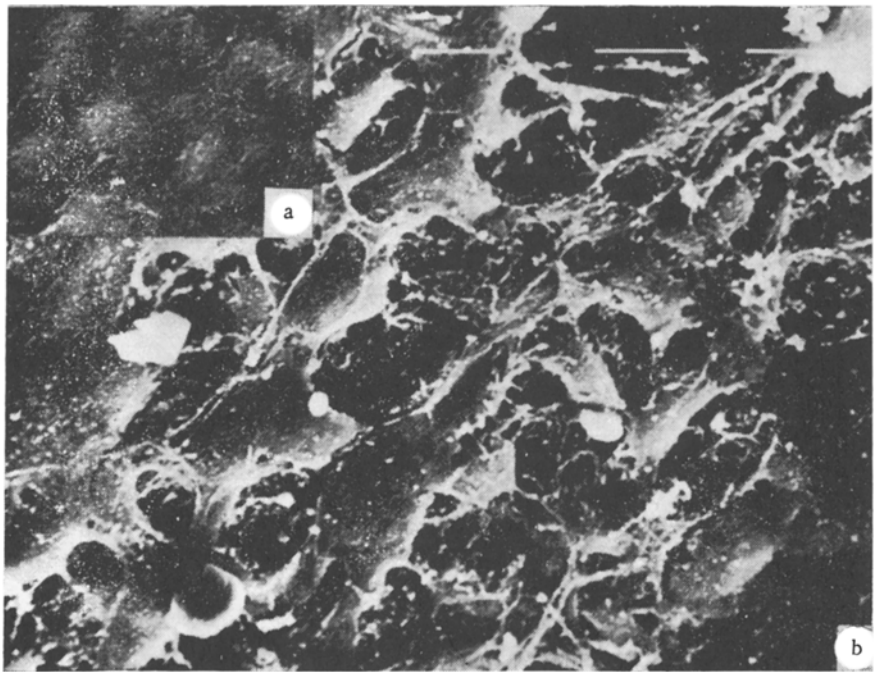


Fig. 1. Corneal epithelium under normal conditions (a) and in experimental hyperthyroidism (b). a) Flattened polygonal surface epithelial cells with distinct cell boundaries. 1250 ×; b) disintegration and desquamation of epithelial surface cells. 1500 ×.

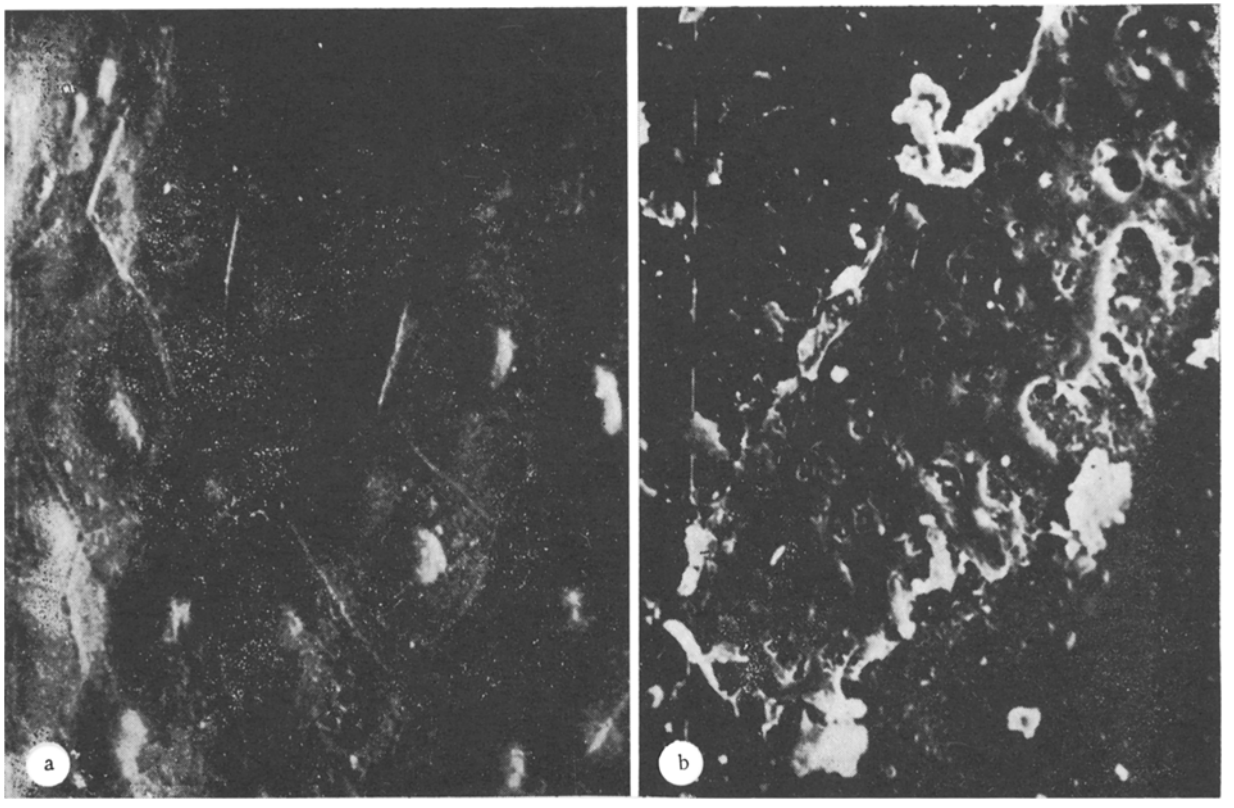


Fig. 2. Corneal endothelium under normal conditions (a) and in experimental hyperthyroidism (b). a) Flattened polygonal endothelial cells (numerous microvilli can be seen on the cell surface). 1500 ×; b) an island of endothelium can be seen against the background of the exposed Descemet's membrane. 700 ×.

of physiological desquamation of single cells were noted. On the inner side the cornea was lined with endothelial cells with numerous superficial microvilli, which evidently increased the area of exchange surface between the cornea and the humor in the anterior chamber of the eye. The largest number of microvilli was found in the region of the cell boundaries (Fig. 2a).

The picture of the surface organization of the epithelium and endothelium of the normal cornea is considerably modified in experimental hyperthyroidism (after administration of thyroxine for 72 h). For instance, well-marked disintegration and desquamation of the surface layers of epithelial cells are clearly visible in the epithelial layer (Fig. 1b) and are accompanied by exposure of the underlying layers, the appearance of circular intercellular openings, and considerable reduction in area of the cell surface in contact with the aqueous humor. Extensive zones of breakdown of cell complexes with disturbance of intercellular interaction also appear in the corneal endothelium, causing rounding of the endothelial cells and the formation of extensive circular spaces between them. In some cases the degree of disturbance of the cell complexes is so great that instead of a single continuous endothelial sheet of cells in the intact cornea only separate cell islets are found against the background of the denuded Descemet's membrane (Fig. 2b). This leads to a disturbance of the nutrition of the ground substance and the structural disorganization of the collagen and elastic fibers of the connective-tissue layer of the cornea.

The normal structural organization of the epithelial and endothelial layers of the cornea is known to depend on the character and degree of intercellular interaction, which also determines integration of single cells into multicellular layers and tissues. An important role in the maintenance of integrity of the cell junctions under these circumstances is played by the glycoprotein components of the cell surface – the glycocalyx, which consists mainly of glycosaminoglycans [6]. Accordingly, the disturbance of the corneal epithelium and endothelium is experimental hyperthyroidism described above may perhaps arise on account of the release of lysosomal enzymes into the extracellular space with the consequent breakdown of glycosaminoglycans, leading to cellular disintegration. This, in turn, leads to desquamation of the surface cells and to the appearance of defects in the epithelial and endothelial layers of the cornea. Disintegration of the cellular layers on account of the action of glycosidases and other lysosomal enzymes on the cytoskeletal complex of the cell, leading to its active contraction, which also causes the appearance of wide spaces between the cells, likewise cannot be ruled out. To verify the presence of the second mechanism of structural disturbances, it is necessary to study both the state of the cytoskeleton of the corneal epithelial and endothelial cells during labilization of the lysosomal membranes under hormonal influence and also the state of the system of cyclic nucleotides, known to be mediators of morphogenetic processes in the cell, in the cornea [7]. The hormonal imbalance may probably be the initial stage in the pathogenesis of several ophthalmopathies, and this is another matter for special study.

The results of the present investigation thus demonstrate a possible role of thyroxine in the development of some pathological processes that take place in the organ of vision against the background of increased thyroid function.

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