
The fine structural localization of peroxidase activity in goblet cells of the conjunctival epithelium of rats. Takeshi Iwata, Kin-ichi Ohkawa, and Masanobu Uyama.*

Cytochemical investigation of peroxidase was made on the conjunctival goblet cells, using the diaminobenzidine method. Many goblet cells in the conjunctiva demonstrated this reaction. The reaction was present in secretory granules as well as in the perinuclear cisterna, the rough endoplasmic reticulum, several Golgi sacules and adjacent small smooth-surfaced vesicles, and condensing vacuoles. Each secretory granule within a goblet cell showed a different degree of reaction, hence it is concluded that some goblet cells probably produce both mucous substance and peroxidase in one cell. The mode of production of peroxidase in goblet cells is concluded to be similar to that of secretory proteins in pancreatic exocrine cells. The enzyme, together with thiocyanate and endogenous hydrogen peroxide, is thought to serve as an anti-inflammatory agent in tears.

Endogenous peroxidases found in saliva, milk, and leukocytes are known to possess anti-infectious activities. Endogenous peroxidase activity has been witnessed in the cells of a variety of organs including the lacrimal gland and the Harder's gland. It is conceivable that peroxidase in tears from these glands plays an important role in preventing infection in the external eye.

On the other hand, goblet cells in the conjunctiva are known to have a mucous substance and this substance serves as a protecting lubricant and wetting agent in tears. Up to now, this has been the only known function of goblet cells in the conjunctiva. However, in the present experiment it was witnessed cytochemically that goblet cells in the conjunctiva of rats display, in addition, a kind of endogenous peroxidase activity and produce peroxidase in the same way as pancreatic exocrine cells produce secretory proteins.

Materials and methods. Normal male and female adult albino rats (Wistar strain) weighing 200 to 300 grams were used. Rats were anesthetized with nembutal. Lids were dissected keeping as much bulbar conjunctiva as possible. After dissection a subconjunctival injection of 4 per cent paraformaldehyde-6 per cent glutaraldehyde fixative buffered with 0.1 M sodium cacodylate (pH 7.4) was applied, then the specimen was cut into 1 mm. thick slices (vertical to the surface), and were put into the same fixative for 1 to 2 hours. 20 mm thick sections were cut for light microscopy, 50 mm thick sections for electron microscopy. These sections were incubated for 25 minutes at 37° C. in a reaction medium for peroxidase of 0.05 M Tris HCl buffer (pH 7.4) was applied, then the specimens were cut into 1 mm. thick slices (vertical to the surface), and were put into the same fixative for 1 to 2 hours. 20 mm thick sections were cut for light microscopy, 50 mm thick sections for electron microscopy. These sections were incubated for 25 minutes at 37° C. in a reaction medium for peroxidase of 0.05 M Tris HCl buffer (pH 8.5) containing 0.2 per cent diaminobenzidine tetrahydrochloride and 0.02 per cent H₂O₂. After being rinsed with the buffer, 50 mm thick sections were cut for 90 minutes with 1 per cent osmium tetroxide. They were dehydrated and embedded in Epon 812. Ultrathin sections were cut with an LKB Ultrotome. They were examined.
Fig. 2. The conjunctival epithelium. Goblet cells contain cytochemically reactive secretory granules, the contents of which have a fine granular appearance (SG). In the basal portion of the cells, the rough endoplasmic reticulum (Er) including the perinuclear cisternae, and condensing vacuoles (C) show peroxidase activity. In addition, they have strongly reactive granules (G,) which look rather uniformly stained. (x7,100.)

Control study. (1) Sections prepared as described above were incubated in the reaction medium without H,O2. To ensure elimination of endogenous H,O2, sections were also incubated in the reaction medium without H,O2, but containing 0.1 per cent catalase. (2) Sections were incubated in the reaction medium with 10-2 to 10-4 M potassium cyanide added, or 10-2 M 3-amino-1:2:4-triazole. (3) Sections were incubated in the reaction medium after 10 minutes' boiling at 100° C.

Results. Goblet cells were located randomly in the conjunctival epithelium and tended to be in groups of several cells. Light-microscopically, many goblet cells in the conjunctival epithelium demonstrated brown-colored cytochemical reaction of peroxidase (Fig. 1). Cytochemically reactive goblet cells seemed to be more numerous than nonreactive ones in many places in the conjunctiva. However, in the bulbar conjunctiva toward the corneal surface, and tarsal conjunctiva near the lid margin, fewer goblet cells reacted.

By electron microscopy, an electron-dense reaction product was revealed in secretory granules as well as in the corresponding perinuclear cisternae, the rough endoplasmic reticulum, the Golgi region, and condensing vacuoles (Figs. 2 and 4). Condensing vacuoles display a fine granular reaction similar to the reaction of secretory granules (Fig. 2). Secretory granules showed various degrees of osmiophilic electron-dense reaction, even in some individual cells, which had a fine granular appearance (Fig. 3). Frequently, goblet cells were found to contain both reactive and nonreactive secretory granules in one cell (Fig. 3). In the Golgi region, the reaction tended to be noticeable in several saccula, especially toward the concave face, and some small smooth-surfaced vesicles (Fig. 4). Condensing vacuoles display a fine granular reaction similar to the reaction of secretory granules (Figs. 2 and 4). In addition to these granules, there were very densely and rather uniformly stained granules (Fig. 1). They were located at the supranuclear region and at the peripheral portion of the cell. The basis of the reactivity of these granules is unknown.

Control study. Sections incubated in the same reaction medium not containing H,O2 showed a positive but weak cytochemical reaction. The distribution of the reaction was identical to that observed in sections in the presence of exogenous H,O2. This result is taken to indicate that endogenous H,O2 was present. In sections incubated in the reaction medium containing catalase in order
Fig. 3. Apical portion of goblet cells which are bulged with secretory granules. A, each secretory granule shows peroxidase activity to various degrees. B, one secretory granule shows strong peroxidase activity. (A, ×8,300; B, ×9,200.)

Fig. 4. Supranuclear region of a goblet cell. The Golgi complex (Gc) is well-developed. Peroxidase activity is revealed in the perinuclear cisterna, the rough endoplasmic reticulum (Er), several Golgi sacules and adjacent small smooth-surfaced vesicles (s), and condensing vacuoles (C). N: nucleus, SG: secretory granules. (×16,600.)
to eliminate endogenous $H_2O_2$, only erythrocytes were reactive. A $10^{-2}$ M solution of KCN did not inhibit the reaction, but a $10^{-1}$ M solution of KCN strongly inhibited it. Sodium azide ($10^{-2}$ M) completely inhibited the reaction. Aminotriazole ($10^{-3}$ M) considerably reduced the reaction.

**Discussion.** The control study which we conducted provided strong evidence that the cytochemical reaction product in goblet cells in the conjunctival epithelium demonstrates peroxidase activity, although one cannot absolutely certain that catalase is not present. The formation and intracellular transport of secretory proteins in pancreatic exocrine cells have been established by Palade. It is now believed that most secretory proteins are synthesized in the rough endoplasmic reticulum and transported via small smooth-surfaced vesicles, condensing vacuoles, and secretory granules. This fine structural pattern of localization of peroxidase activity is similar to that for other peroxidase producing cells.

The finding of the present experiment in goblet cells in the conjunctiva revealed peroxidase activity in the corresponding perinuclear cisterna, the rough endoplasmic reticulum, several Golgi sacules and adjacent small smooth-surfaced vesicles, condensing vacuoles, and secretory granules. This fine structural pattern of localization of peroxidase activity is similar to that for other peroxidase producing cells. The formation and intracellular transport of secretory proteins in pancreatic exocrine cells have been established by Palade. It is now believed that most secretory proteins are synthesized in the rough endoplasmic reticulum and transported via small smooth-surfaced vesicles to the Golgi complex, where the proteins are condensed and packed into secretory granules. Peroxidase in goblet cells of the conjunctiva probably is produced in the same way as secretory proteins in exocrine cells of the pancreas.

There are cytochemically reactive and non-reactive secretory granules in goblet cells. Each reactive granule showed the reaction to various degrees. It is known that goblet cells in the conjunctiva have mucous substance and it may be presumed that cytochemically nonreactive granules in the present experiment contain mucous substance. Therefore, it seems that one goblet cell may have both peroxidase and mucous substance.

Endogenous peroxidases such as lactoperoxidase in milk and saliva, and myeloperoxidase in leukocytes are known to have bactericidal action when in combination with thiocyanate and $H_2O_2$. Thiocyanate can be substituted for by halides, especially iodide. This peroxidase-thiocyanate or halides- $H_2O_2$ system has been extensively investigated and has been shown to have bactericidal, fungicidal, virucidal, and mycoplasmacidal activities. Thiocyanate is present in conjunctival secretions, and endogenous $H_2O_2$ was shown to be in the conjunctiva in the present experiment. These seem to provide an environment where peroxidase in tears act as an anti-infectious agent. The hitherto known function of goblet cells in the conjunctiva is that the cells produce mucous substance which serves as a protective lubricant and wetting agent in tears, and forms the deepest layer of the preconal tear film. There were many goblet cells containing peroxidase in the conjunctival epithelium. In other organs which are exposed to external insults, such as the colon and the larynx, mucous producing cells have peroxidase activity. These findings clearly imply that the production of peroxidase is not a vestigial function of goblet cells in the conjunctiva.

In the lacrimal gland and Harder's gland of cows and steers, and in the exorbital lacrimal gland of rats, peroxidase activity has been demonstrated. The peroxidase from goblet cells probably serves as an anti-infectious agent in combination with those from these glands.

We are grateful to Professor I. Uesaka, Department of Bacteriology, Chest Disease Research Institute, Kyoto University, for his encouragement. We also wish to thank Prof. I. Tsukahara, Department of Ophthalmology, Faculty of Medicine, Kyoritsu University, for his help in preparing this paper.

From the Department of Cytochemistry, Chest Disease Research Institute, and Department of Ophthalmology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan. Submitted for publication May 29, 1975. Reprint requests: Takeshi Iwata, Department of Cytochemistry, Chest Disease Research Institute, Kyoto University, Kyoto 606, Japan.

Key words: peroxidase, goblet cell, conjunctiva, rat, cytochemistry, electron microscopy, bactericidal action.

**REFERENCES**


After variation of the environmental lighting conditions, pupillary diameters of albino rabbits in response to progressively increasing doses of the sympathomimetics norepinephrine, epinephrine, and isoproterenol were measured. After maintenance of the rabbits under conditions of constant light for one week, an increased sensitivity of the iris dilator to the mydriatic effects of these adrenergic agents became evident. After maintenance of the rabbits in constant dark for one week, on the other hand, there was no change in the sensitivity of the iris upon administration of the same sympathomimetics. Norepinephrine levels measured in iris and ciliary processes after one week of either constant light or constant dark, moreover, did not differ significantly from those determined under normal lighting conditions. These results indicate that the sensitivity of the iris dilator can be altered by variation of the physiologic stimulus, light. In addition, the chronic interruption of contact between the normal neurotransmitter norepinephrine and its effector cells leading to supersensitivity in the dilator appears to be due to the removal of a trophic influence of norepinephrine, rather than its absolute loss.

The increased sensitivity of autonomically innervated structures to chemical mediators that follows denervation and the resulting loss of normal transmitter stores has been extensively studied in a variety of peripheral organs and tissues, including ocular structures. In man, supersensitivity of the pupils to methacholine has been documented in patients with familial dysautonomia, while supersensitivity to directly acting sympathomimetics has likewise been observed in some cases of Horner's syndrome. Increasing the amount of neurotransmitter in the iris sphincter, on the other hand, by topical or systemic long-term treatment of experimental animals with an inhibitor of cholinesterase, has been found to cause a diminished responsiveness, or subsensitivity, of the iris to cholinergic agents.

In a recent approach to the study of factors influencing the response of ocular tissues to drugs, Bito, Dawson, and Petrinovic have demonstrated that changes in the sensitivity of the iris sphincter could be produced by altering the intensity of the physiologic stimulus, light. By keeping cats in complete darkness for a period of one week, a condition which induces a temporary state of constant pupillary dilation and thereby reduces the cholinergic input to the iris, led to the development of subsensitivity to the miotic effect of pilocarpine. In the present experiments, changes in the sensitivity of the iris dilator to sympathomimetic drugs have been examined in rabbits maintained under conditions of constant light or constant dark. In addition, norepinephrine levels of the iris and ciliary processes after these variations of the environmental lighting conditions have been determined. Our data suggest that changes in the sensitivity of the iris dilator similar to those previously reported for the iris sphincter do occur as a consequence of variation of the physiologic stimulus, light.

Methods. Adult male albino rabbits weighing 2.5 to 3.5 kilograms were used in these experimental studies. Groups of five rabbits were housed in individual cages in a room in the animal quarters which provided timer regulation of the lighting conditions. Separate groups of animals were used for the study of each drug. Upon their arrival, each group was given a period of one week to adapt to the new environment. The rabbits were then maintained for consecutive 7-day periods under the following lighting conditions: (1) 12 hours light (300 lux) and 12 hours dark every 24 hours; (2) constant dark; (3) a 12-hour light-dark cycle again; (4) constant light (300 lux); and (5) a final 12-hour light-dark cycle.

On the last day of maintenance at each lighting condition, pupillary diameters in response to increasing doses of each of the sympathomimetic agents studied were determined. The pupil size was measured visually with a transparent millimeter ruler. All readings were taken in the horizontal meridian and recorded to the nearest half millimeter.

Solutions of the experimental drugs were dissolved in isotonic saline and applied topically to the left eyes with a Hamilton microliter syringe (Kontes Glass Co., Vineland, N. J.). Increasing...