Ocular changes in rabbits with corticosteroid-induced ocular hypertension

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SUMMARY Rabbit eyes with steroid-induced ocular hypertension were investigated in order to evaluate the histochemical abnormalities in the chamber angle region. The right eye of 14 rabbits was treated by dexamethasone 1% 3 times daily for 3 to 5 weeks. The eyes were stained by haematoxylin-eosin, periodic acid Schiff, fibrin, colloidal iron, and alcian blue with and without hyaluronidase. All treated eyes developed elevated intraocular pressure up to 4 weeks after treatment. These globes showed alcian-blue-positive hyaluronidase-sensitive staining in the amorphous material adjacent to Schlemm's canal and in the cytoplasmic granules of trabecular endothelial cells. There was no increase in incorporation of radioactive thymidine into nuclei of endothelial cells as seen by autoradiography. These results provide further support for the idea that there is abnormal accumulation of acid mucopolysaccharides in the chamber angle in steroid-induced ocular hypertension.

Steroid-induced ocular hypertension is a well-known clinical problem which at times leads to optic nerve damage and blindness (Armaly, 1963; Becker and Hahn, 1964). In contrast to open-angle glaucoma the existence of an animal model for this disease makes it possible to investigate its pathogenesis by various approaches (Podos, 1976). It has been previously shown that transient increase in intraocular pressure can be induced in rabbits by topical corticosteroid therapy (Tuovinen et al., 1966; Wood et al., 1967; Brown and Geeraets, 1972; Levene et al., 1974; Podos, 1976). However, the mechanism of this phenomenon has not been elucidated. Routine histopathological examination did not reveal any abnormalities in the anterior chamber angle (Levene et al., 1974), yet some biochemical changes in the aqueous humour were found (Virno et al., 1974; François and Victoria-Troncoso, 1974).

Recently much attention has been given to the possible role of biochemical abnormalities such as accumulation of mucopolysaccharides in the formation of steroid-induced glaucoma (François and Victoria-Troncoso, 1974; François, 1975). The purpose of the present study was to evaluate further the possible histochemical abnormalities in the chamber angle of rabbits with ocular hypertension induced by topical corticosteroids.

Materials and methods

ANIMALS
Fourteen age-matched female albino rabbits weighing approximately 2 kg were tested. They were kept in separate cages in animal room conditions. The rabbits were fed Purina and drank water freely. No ocular abnormalities could be found on external and slit-lamp examination prior to the beginning of the experiment.

TREATMENT
The right eye of the 14 rabbits was treated 3 times daily by 1% dexamethasone eye drops while the left eye served as control. Dexamethasone sodium phosphate (Merck Sharp & Dohme) was dissolved in phosphate buffer to a final concentration of 1% at pH 7.4. The intraocular pressure was measured at noon twice a week by both the Mackay-Marg and a modified Schiotz tonometers. The animals' weights were recorded simultaneously. Six animals were killed at 3 weeks, 6 animals at 4 weeks, and 2 more 5 weeks after the beginning of treatment.

HISTOLOGY
The eyes were enucleated and fixed in 10% buffered...
formalin. All eyes were processed for paraffin embedding and later 4 to 6 μm sections were cut. From each globe sections were stained by haematoxylin and eosin, periodic acid Schiff, fibrin, colloidal iron, and alcian blue stains with and without bovine testicular hyaluronidase. The intensity of the staining was graded as − when no reaction was seen; ± when only faint stain could be identified; + when definite positive stain of tissue was present, and ++ when a strong reaction was seen.

**Autoradiography**

In 3 animals of the first and second group and 2 of the third group paracentesis of both eyes was done and 0·1 ml of aqueous was drained. Through the same needle 0·1 ml of tritiated thymidine (KMG-Israeil) at final activity of 0·2 μCi was injected into the anterior chamber. The needle was withdrawn and the puncture site plugged. Three hours later the animals were killed, and the eyes were fixed in 10% buffered formalin and processed for paraffin embedding. These eyes were later deparaffinised, dipped in Kodak NTB 2 emulsion, and developed 2 to 4 weeks later for autoradiography.

**Results**

It is evident that in the present study all treated eyes developed raised intraocular pressure as compared to control eyes (Table 1). The pressure was equal in both eyes during the first week of treatment, in the second week increased an average of 4 mmHg, and in the third week further increased to 9 mmHg above control levels. In the fourth week of treatment the pressure levels began to decline. In the control eyes there was a non-significant rise of 1 to 2 mmHg on repeated measurements. A statistical analysis by the paired t test showed the results to be highly significant.

Weight loss continued during the treatment period, in particular during the second week after treatment (Table 2). Histopathological examination of sections stained by haematoxylin-eosin revealed vacuolisation and oedema of the trabecular endothelial cells more frequently in the treated eyes. Fibrin stain was faintly positive in 8 treated eyes and 2 untreated eyes. The fibrin stain was not consistent and was usually associated with a small haemorrhage in the area. Periodic acid Schiff positive material was present in 8 treated and 6 untreated eyes. It was usually located extracellularly in close association with trabecular endothelial cells. Colloidal iron stain showed faintly positive granules in 11 treated and 5 untreated eyes. This reaction was seen mainly in endothelial cells of the trabecular meshwork, the amorphous material of the interstitial space, and in close proximity to Schlemm’s canal.

Alcian-blue-positive staining, which partially disappeared after treatment with hyaluronidase, was present in the treated and untreated eyes. However, it was seen more often and was more intense in eye treated by steroids (Table 3). This staining was noted as amorphous material in the intertrabecular spaces and adjacent to Schlemm’s canal. In addition alcian-blue-positive intracellular granules were noted in the cytoplasm of endothelial cells (Figs. 1, 2). The most positive reaction in steroid-treated eyes was seen 3 to 4 weeks after the beginning of therapy. However, a faint reaction was present in untreated eyes as well (Fig. 3).

The autoradiographic sections showed almost no grains over the nuclei of the trabecular meshwork in either the treated or the untreated eyes. It is interesting to note that in the steroid-treated eyes larger numbers of labelled cells were seen in the conjunctival and corneal epithelium, the iris melanocytes, and within the endothelial cells of iris blood vessels.

**Discussion**

No good experimental model of primary open-angle glaucoma is yet available; consequently, investi-
Table 3  Histopathological findings

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N = no pathological findings.

previous studies, the rabbit eyes which were treated by topical steroids developed a significant pressure rise 2 to 3 weeks after the commencement of therapy accompanied by systemic weight loss (Wood et al., 1967; Levene et al., 1974). The mechanism which lies behind that rise in intraocular pressure is still debateable. Routine histopathological studies did not reveal any abnormalities (Wood et al., 1967). At times pressure-related vacuoles in the endo-

Fig. 1  Chamber angle of the steroid-treated right eye. Alcian-blue-positive hyaluronidase-sensitive material is seen as dark-stained granules in the filtration area and adjacent to Schlemm’s canal (SC). E = detached ciliary epithelium; P = posterior chamber. Alcian blue, ×40

Fig. 2  Higher magnification of the same area. The alcian-blue-positive material is seen intra- and extracellularly and in the juxtacanalicular tissue (arrows). SC = Schlemm’s canal. Alcian blue, ×360
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Fig. 3 Filtration region in an untreated eye. Only a few alcian-blue-positive granules are seen in the juxta-canalicular area (arrow). SC = Schlemm’s canal. Alcian blue, × 360

...theum of Schlemm’s canal could be seen (Tripathi, 1972; Johnstone and Grant, 1973), yet in the light microscopic study we had the impression that vacuoles or endothelial swelling could be identified more often in the treated eyes.

In an attempt to evaluate the cellular function in short-term experiments by thymidine uptake no connection between nuclear morphology or function and the moderate elevation of intraocular pressure was found. It seems as if the cells of the trabecular endothelium are rather stable, and therefore no short-term incorporation of tritiated thymidine into those nuclei could be observed with or without steroids.

In the present study intra- and extracellular accumulations of hyaluronidase-sensitive acid mucopolysaccharides were seen in both treated and untreated eyes. However, it was our impression that these were present to a greater degree in the treated eyes. Since it was first described, these substances have been repeatedly identified in the filtration area (Segawa, 1971; Armaly and Wang, 1975).

Some reports claim that trabecular endothelial cells in tissue culture secrete mucopolysaccharides. Further characterisation of this substance by histochemical methods indicates that the non-fibrillar material which corresponds to the glass membrane and elastic fibres in the corneoscleral meshwork contains chondroitin sulphate protein complex and glycoprotein. This material, which stains PAS positive, increases in thickness with age and is not related to increased intraocular pressure in man. On the other hand the amorphous material underneath the endothelial wall of Schlemm’s canal is hyaluronidase-sensitive and is present in large quantities when the outflow facility is reduced (Segawa, 1975). In addition physiological data support this observation, since perfusion experiments indicate that the outflow facility increases temporarily with hyaluronidase (Barany and Woodin, 1955; Berggren and Vrabec, 1957). Moreover, it was previously suggested that patients with primary open-angle glaucoma have increased sensitivity to topical steroids (Armaly, 1963; Becker and Mills, 1963; Becker, 1965). It can be speculated, therefore, that increased amount of acid mucopolysaccharides in an already susceptible eye may be responsible for an increase in outflow resistance which is induced by topical steroids (Armaly and Wang, 1975; François, 1975). The present study supports the observation that such accumulations are seen in the chamber angle affected by topical steroids. The exact mechanism of this accumulation is not clear, yet it may be related to the inhibition of lysosomal enzymes, which are responsible for the degradation of mucopolysaccharide-protein complexes (Dingle and Fell, 1969). These findings suggest that the abnormal metabolism of this material may be involved in some of the mechanisms which lead to increased intraocular pressure, even though a direct cause-and-effect relationship remains to be established.

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