

Ocular changes in rabbits with corticosteroid-induced ocular hypertension

U. TICHO, M. LAHAV, S. BERKOWITZ, AND P. YOFFE

From the Department of Ophthalmology, Hadassah Hospital, and the Hebrew University School of Medicine, Jerusalem, Israel

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

Address for reprints: Dr U. Ticho, Department of Ophthalmology, Mayer de Rothschild Hadassah University Hospital, Kiriyat Hadassah, Jerusalem, Israel

formalin. All eyes were processed for paraffin embedding and later 4 to 6 μ m 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; \pm 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-Israel) at final activity of 0.2 μ Ci 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

Table 1 Mean IOP of rabbits during 1% dexamethasone treatment

Treated right eye						
Weeks	Mean	SD	N	t	P value	
1	0	12.21	0.83	14	1:2	9.48 <0.001
2	1	17.46	1.80	14	2:3	5.95 <0.001
3	2	22.79	3.21	14		
Untreated left eye						
Weeks	Mean	SD	N	t	P value	
1	0	12.18	0.67	14	1:2	2.92 0.012
2	1	12.89	0.84	14	2:3	2.03 0.06
3	2	13.39	0.68	14		

Table 2 Mean weights of rabbits treated by 1% dexamethasone eye drops

Weeks	Mean	SD	N	t	P value	
1	0	2.385	0.568	14	1:2	4.05 0.001
2	1	2.268	0.536	14	2:3	3.54 0.004
3	2	2.175	0.518	14		

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*

No.	Week	H and E		PAS		Colloidal iron		Alcian blue hyaluronidase sensitive	
		R	L	R	L	R	L	R	L
1	2	N	N	±	±	±	-	±	-
2	2	Oedema	Vacuoles	±	±	±	-	+	±
3	2	N	N	±	±	+	±	±	±
4	2	Oedema	Vacuoles	+	+	±	±	-	±
5	2	Vacuoles	Vacuoles	±	±	-	-	+	+
6	2	N	N	-	-	±	-	+	-
7	3	Vacuoles	N	±	-	+	+	+	+
8	3	Oedema	N	-	-	±	-	±	±
9	3	N	N	-	-	-	-	+	±
10	3	Vacuoles	N	-	-	±	±	+	±
11	3	Vacuoles	N	±	-	±	+	+	±
12	3	N	N	+	-	±	-	±	-
13	4	N	N	-	-	-	-	±	±
14	4	Vacuoles	N	±	±	±	-	±	±

N = no pathological findings.

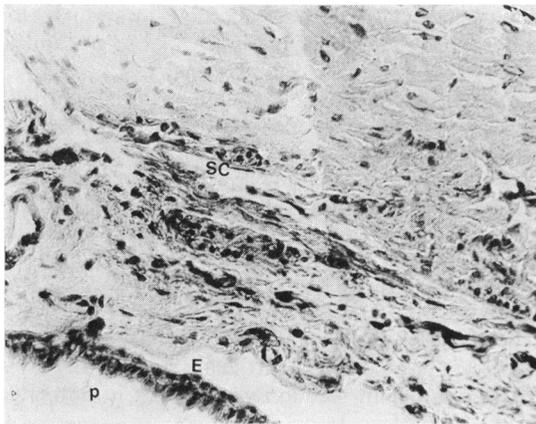


Fig. 1 Chamber angle of the steroid-treated right eye. Alcian-blue-positive hyaluronidase-sensitive material is seen as dark-stained granules in the filtration area and adjacent to Schlemm's canal (SC). E = detached ciliary epithelium; P = posterior chamber. Alcian blue, $\times 40$

gations into the pathogenesis of the disease are rather limited (Podos, 1976). However, steroid-induced elevation of intraocular pressure, which is a well-established clinical entity, can be reproduced to some extent in experimental animals (Armaly, 1964; Wood *et al.*, 1967; Brown and Geeraets, 1972; Levene *et al.*, 1974). In the present study, as in

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 debatable. Routine histopathological studies did not reveal any abnormalities (Wood *et al.*, 1967). At times pressure-related vacuoles in the endo-

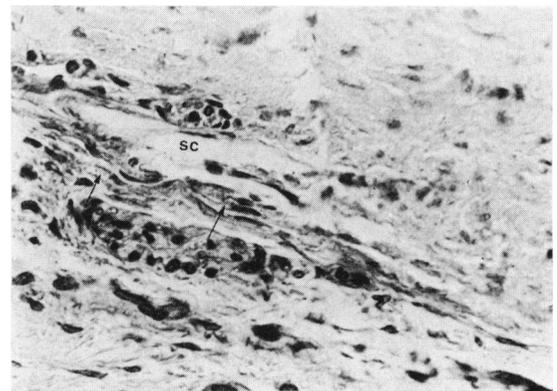


Fig. 2 Higher magnification of the same area. The alcian-blue-positive material is seen intra- and extracellularly and in the juxtacanalicular tissue (arrows). SC = Schlemm's canal. Alcian blue, $\times 360$

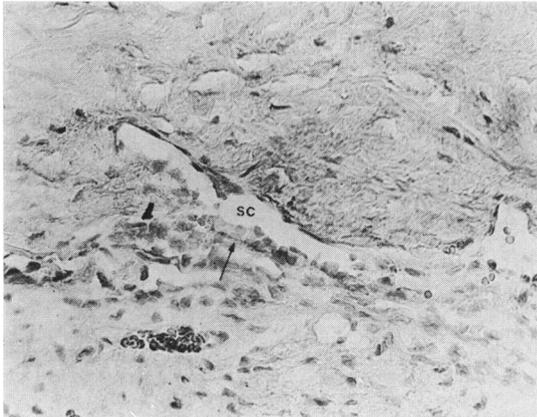


Fig. 3 Filtration region in an untreated eye. Only a few alcian-blue-positive granules are seen in the juxtacanalicular area (arrow). SC = Schlemm's canal. Alcian blue, $\times 360$

thelium 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.

Dexamethasone sodium phosphate was kindly donated by Merck Sharp & Dohme, West Point, Pennsylvania. The technical assistance of H. Genesin and A. Itzhak is gratefully acknowledged.

References

- Armaly, M. F. (1963). Effect of corticosteroids on intraocular pressure and fluid dynamics. *Archives of Ophthalmology*, **70**, 482-491.
- Armaly, M. F. (1963). Effect of corticosteroids on intraocular pressure and fluid dynamics. 2. The effect of dexamethasone in the glaucomatous eye. *Archives of Ophthalmology*, **70**, 492-499.
- Armaly, M. F. (1964). Aqueous outflow facility in monkeys and the effect of topical corticoids. *Investigative Ophthalmology*, **3**, 534-538.
- Armaly, M. F., and Wang, Y. (1975). Demonstration of acid mucopolysaccharides in the trabecular meshwork of the rhesus monkey. *Investigative Ophthalmology*, **14**, 173-176.
- Barany, E., and Woodin, A. (1955). Hyaluronic acid and hyaluronidase in the aqueous humour and the angle of the anterior chamber. *Acta Physiologica Scandinavica*, **33**, 257-290.
- Becker, B. (1965). Intraocular pressure response to topical corticosteroids. *Investigative Ophthalmology*, **4**, 198-205.
- Becker, B., and Hahn, K. (1964). Topical corticosteroids and heredity in primary open-angle glaucoma. *American Journal of Ophthalmology*, **57**, 543-551.
- Becker, B., and Mills, D. (1963). Corticosteroids and intraocular pressure. *Archives of Ophthalmology*, **70**, 500-507.
- Berggren, L., and Vrabec, F. (1957). Demonstration of a coating substance in the trabecular meshwork of the eye and its decrease after perfusion experiments with different

- kinds of hyaluronidase. *American Journal of Ophthalmology*, **44**, 200–208.
- Brown, J., and Geeraets, W. (1972). The effect of hyaluronidase on the facility of outflow in normal and buphthalmic rabbits. *Acta Ophthalmologica*, **44**, 901–905.
- Dingle, J. T. (1969). The extracellular secretion of lysosomal enzymes. In *Lysosomes in Biology and Pathology*, vol. 2, pp. 421–436. Edited by J. T. Dingle and H. B. Fell. North Holland Publishing Co.: Amsterdam/London.
- François, J. (1975). The importance of the mucopolysaccharides in intraocular pressure regulation. *Investigative Ophthalmology*, **14**, 173–176.
- François, J., and Victoria-Troncoso, V. (1974). Mucopolysaccharide et hypertension oculaire (pathogenie du glaucome cortisonique). *Annales d'Oculistique*, **207**, 625–641.
- Johnstone, M., and Grant, W. (1973). Pressure-dependent changes in structures of the aqueous outflow system of human and monkey eyes. *American Journal of Ophthalmology*, **75**, 365–383.
- Levene, R., Rothberger, M., and Rosenberg, S. (1974). Corticosteroid glaucoma in the rabbit. *American Journal of Ophthalmology*, **78**, 505–510.
- Podos, S. M. (1976). Animal model of human glaucoma. *Transactions of the American Academy of Ophthalmology and Otolaryngology*, **81**, 632–635.
- Segawa, K. (1975). Ultrastructural changes of the trabecular tissues in primary open angle glaucoma. *Japanese Journal of Ophthalmology*, **19**, 317–338.
- Segawa, R. (1971). Localization of acid mucopolysaccharide in the human trabecular meshwork. *Japanese Journal of Clinical Ophthalmology*, **24**, 363–367.
- Tripathi, R. (1972). Aqueous outflow pathway in normal and glaucomatous eyes. *British Journal of Ophthalmology*, **56**, 157–174.
- Tuovinen, E., Liesman, M., and Esila, R. (1966). The influence of corticosteroids on intraocular pressure in rabbits. *Acta Ophthalmologica*, **44**, 901–905.
- Virno, M., Schirru, A., Pecori-Giraldi, J., and Pellegrine, N. (1974). Aqueous humor alkalosis and marked reduction in ocular ascorbic acid content following long-term topical cortisone (9a-fluoro-16a-methylprednisolone). *Annals of Ophthalmology*, **6**, 983–992.
- Wood, D., Contaxis, I., Sweet, D., Smith, J., and Van Dolah, J. (1967). Response of rabbits to corticosteroids. *American Journal of Ophthalmology*, **63**, 841–856.