COMPOSITION OF MUCOPOLYSACCHARIDES IN THE CORNEA DURING NEUROGENIC DISORDERS

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Neurogenic disorders of the cornea were produced by intracranial division of the trigeminal nerve or by blocking the ciliary ganglion with alcohol and procaine. It was shown by ion-exchange column chromatography that in the course of neurogenic degeneration the content of glucosamine-containing (keratosulfate) mucopolysaccharides falls, while the content of galactosamine-containing mucopolysaccharides (chondroitin sulfate and chondroitin) increases. This is particularly marked in stages III (ulcer) and IV (scar formation). Following intracranial division of the trigeminal nerve the changes in composition of the corneal mucopolysaccharides were more severe than after alcohol-procaine blocking of the ciliary ganglion.

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During recent years considerable attention has been paid to the study of acid mucopolysaccharides in the cornea under normal conditions and in various pathological states [6, 7, 10, 11]. Acid mucopolysaccharides play an important role in maintenance of transparency of the cornea. So far, however, little attention has been paid to the study of these compounds during neurogenic disorders of the cornea. Neurogenic disorders of the cornea, a typical manifestation of which is neuroparalytic keratitis, are a widely used model for the study of general problems concerned with neurotropic disturbances. Our previous investigations revealed important changes in the content and distribution of mucopolysaccharides in the various stages of neurogenic keratitis [1, 2]. However, no attempt was made then to study the character of changes in individual mucopolysaccharides.

The object of the present investigation was to study the composition of the corneal mucopolysaccharides at various stages of neurogenic keratitis produced by different methods.

EXPERIMENTAL METHOD

Experiments were carried out on 48 rabbits. Neurogenic disorders of the cornea were produced in two ways: 1) by intracranial division of the trigeminal nerve and 2) by alcohol-procaine block of the ciliary ganglion. The rabbits were sacrificed at various stages of neurogenic keratitis and the corneas were dried. Acid hydrolysates of the corneas were dried in vacuo and the residue was dissolved in 0.3 N HCl and applied to Dowex 50 × 8 ion exchange resin, 200-400 mesh. Subsequent fractionation was carried out by Gardell's method [8] with slight modifications. Glucosamines and galactosamines were detected in the eluates as two peaks.

At the same stages mucopolysaccharides were detected histochemically by staining with alcian blue, toluidine blue, colloidal iron by Hale's method, and by the PAS reaction. The type of mucopolysaccharide was identified by the use of an enzyme control with hyaluronidase. In some cases contrast methods were combined: the PAS reaction and staining with alcian blue.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. In the normal cornea the relative content of glucosamine was twice that of galactosamine.

At the beginning of neurodegenerative keratitis (stage I) no appreciable changes were found in the qualitative composition of the corneal mucopolysaccharides. The onset of opacity (stage II) was accompanied by a decrease in the glucosamine/galactosamine ratio to 1.62 ±0.1 after intracranial division of the

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TABLE 1. Ratio between Glucosamine and Galactosamine in Cornea at Various Stages of Neurodegenerative Keratitis Produced by Different Methods

Stage of keratitis	Method	Glucosamine/ galactos-amine (M ±m)	P
Normal		2.04 ±0.07	
stage I. Initial changes (6-8 h)	intracranial division of trigeminal nerve	1.96 ±0.12	> 0.5
stage II. Opacity (1-2 days)	Ditto	1.62 ±0.10	< 0.05
	alcohol-procaine block of ciliary ganglion	1.81 ± 0.08	< 0.2
stage III. Ulcer, beginning of scar formation (7-20 days)	intracranial division of trigeminal nerve	0.82 ± 0.07	< 0.001
	alcohol-procaine block of ciliary ganglion	1.27 ± 0.09	< 0.01
stage IV. Scar formation (35-60 days)	intracranial division of trigeminal nerve	1.17 ± 0.11	< 0.01
	alcohol-procaine block of ciliary ganglion	1.66 ± 0.16	< 0.1

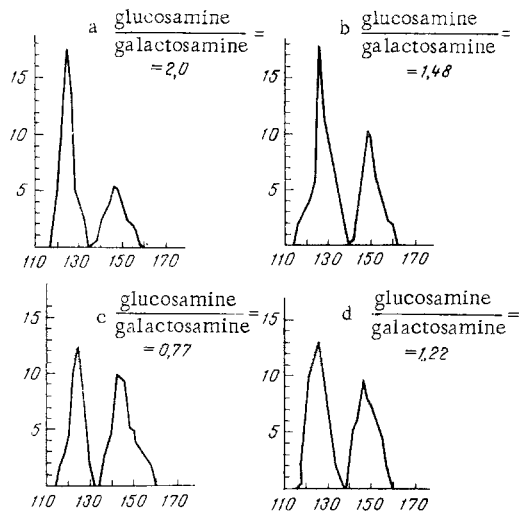

Fig. 1. Ratio between glucosamine and galactosamine in cornea at various stages of neuroparalytic keratitis produced by intracranial division of the trigeminal nerve. a) Normal cornea; b) stage II; c) stage III; d) stage IV. Ordinate, content of glucosamine-HCl (2 μg/ml); abscissa, volume of eluate (in ml).

trigeminal nerve. In stage III, when an ulcer formed and proliferative processes were well marked microscopically, sharp changes were found in the composition of the corneal mucopolysaccharides and this ratio fell to 0.82 ±0.07. In the last stages of scar formation (stage IV) the normal mucopolysaccharide composition was not restored. Fractionation of glucosamine and galactosamine in the cornea at various stages of neuroparalytic keratitis is illustrated graphically in Fig. 1.

Depending on the method used to produce neurogenic keratitis, differences were observed in the qualitative composition of the mucopolysaccharides. The changes were more marked after intracranial division of the trigeminal nerve. This was perhaps because alcohol-procaine block did not completely interrupt nerve conduction.

The results of the biochemical investigations agreed with the histochemical findings. In the early stages a decrease in acid mucopolysaccharides was found in the outer parts of the corneal stroma. In stage III, in regions surrounding the ulcer, acid mucopolysaccharides were almost completely absent. In the zones of proliferation an intensive accumulation of acid mucopolysaccharides of chondroitin sulfate type was found.

Hence, on the basis of the accepted morphological stages of neuroparalytic keratitis [3], characteristic changes in composition of the corneal mucopolysaccharides were found, consisting of a relative decrease in the glucosamine-containing (kerato-sulfate) and an increase in galactosamine-containing mucopolysaccharides (chondroitin sulfate and chondroitin).

Changes of this type are also characteristic of other pathological states of the cornea. An increase in content of chondroitin sulfate has also been described during healing of wounds and granulation tissue

formation [5, 11]. The relative increase in content of galactosamine-containing mucopolysaccharides, especially chondroitin sulfate, in various pathological states [4, 9] is evidently a common feature of the course of the pathological process in connective tissue.

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