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The Concentrations of Sugar Nucleotides in Bovine Corneal Epithelium and Endothelium

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The cornea is an example of a composite tissue made up of three distinct and separable cellular layers, the epithelium, the stroma and the endothelium. The epithelium is of ectodermal origin and is separated from the stroma by Bowman's membrane, which is a modified layer of the stroma. The stroma and the endothelium are of mesodermal origin and separated from each other by Descemet's membrane, a basement membrane laid down by the endothelium. In the course of experiments designed to elucidate the pathway of biosynthesis of keratan sulphate in the corneal stroma (Handley & Phelps, 1972*b*), we became interested in the differences that might be found in the nucleotide and nucleotide sugar concentrations in each of the three component layers. The present communication reports the concentrations of sugar nucleotides present in the epithelium and endothelium layers of the cornea.

The epithelium and the endothelium layers of the cornea were removed from the stroma by scraping with a stainless-steel scraper. The total water space of the epithelium and endothelium was determined by drying in an oven at 104°C for 24h and was found for both these tissues to be 74% of the wet wt. This value was taken as an estimate of the intracellular water and was used in the calculation of the cellular concentrations of the nucleotide sugars.

Samples of the endothelium (approx. 1.0g) and of the epithelium (approx. 0.7g) were powdered in a stainless-steel percussion mortar and cooled to −180°C. The powder was extracted with perchloric acid as described by Handley & Phelps (1972*a*). The neutralized extract of either the corneal epithelium or the endothelium was applied to the top of a column (4cm×1cm) of Dowex 1 (formate form; 200–400 mesh), prewashed with 8 bed volumes of 3M-ammonium formate and with distilled water until all traces of NH_4^+ ion had been removed. The eluent from the column was monitored with a LKB Uvicord II (LKB Produckter AB, Stockholm 12, Sweden), which measured the percentage transmission at 254nm. After application of the sample, the column was washed with distilled water until the percentage transmission returned to the value before the extract was added. The column was then eluted with an increasing formic acid–ammonium formate gradient at a rate of 20ml/h. Fractions of volume 1ml were collected. The gradient was formed by using a sealed mixing vessel of 27ml internal volume containing 20ml of distilled water fed from a single reservoir. The solution in the reservoir was changed in the following manner: fractions 1–10, 1M-formic acid; fractions 11–50, 4M-formic acid; fractions 51–70, 4M-formic acid and 0.25M-ammonium formate; fractions 71–81, 4M-formic acid and 0.5M-ammonium formate; fractions 81–130, 4M-formic acid and 0.8M-ammonium formate.

The elution profiles obtained for extracts of the epithelium and endothelium showed a similar distribution to that of bovine corneal stroma (Handley & Phelps, 1972*b*) and that reported by Duda & Pogell (1958) for rat lens and bovine corneal epithelium.

Fractions corresponding to the nucleotide sugars were pooled, deionized and freeze-dried as described by Hardingham & Phelps (1968). This fraction was then analysed for glucose, galactose, glucosamine, galactosamine, xylose and mannose by g.l.c. of the trimethylsilyl ethers (Clamp *et al.*, 1967). Table 1 reports the concentrations of nucleotide sugars observed in bovine corneal epithelium and endothelium and those reported by Handley & Phelps (1927*b*) for corneal stroma.

The proportions of the epimers of UDP-hexose, UDP-*N*-acetylhexosamine, UDP-*N*-acetylglucosamine and UDP-*N*-acetylgalactosamine are similar to the free equilibrium ratios reported for the two epimerases, UDP-glucose 4-epimerase (EC 5.1.3.2) and UDP-*N*-acetylglucosamine 4-epimerase (EC 5.1.3.7) (Leloir, 1951; Glaser, 1959).

The endothelium, as well as maintaining the hydration of the stroma, synthesizes a basement membrane, Descemet's membrane. The chemical analysis of the component sugars of the glycoproteins has been reported for the Descemet's membrane of the dog (Kefalides, 1970) and the calf (M. Moczar & E. Moczar, personal communication). The molar proportions of the sugars of the basement membrane compare well, considering any age or species difference, with the molar proportions of the nucleotide sugars observed in the endothelium of bovine cornea.

The epithelium, like the endothelium, maintains the hydration of the stroma. The epithelium contains high concentrations of UDP-xylose that are similar to the values obtained for the corneal stroma (Handley & Phelps, 1972*b*). The enzyme UDP-glucose dehydrogenase (EC 1.1.1.22), which converts UDP-glucose into UDP-glucuronic acid, is present (Gainey & Phelps, 1972). Neufeld & Hall (1965) demonstrated that this enzyme is inhibited by UDP-

Table 1. *Cellular concentrations of sugar nucleotides in bovine corneal epithelium, endothelium and stroma*

For experimental details see the text. Values are given as means±S.D. for three or four experiments.

	Corneal epithelium		Corneal endothelium		Corneal stroma (Handley & Phelps, 1972*b*)	
Nucleotide sugar	Tissue content (nmol/g wet wt.)	Cellular concn. (mM)	Tissue content (nmol/g wet wt.)	Cellular concn. (mM)	Tissue content (nmol/g wet wt.)	Cellular concn. (mM)
UDP-*N*-acetyl-glucosamine	274.5±10.0	0.368±0.013	127.2±25.5	0.172±0.034	10.5±1.3	0.211±0.022
UDP-*N*-acetyl-galactosamine	102.7± 3.0	0.138±0.004	60.9±11.7	0.082±0.012	4.8±0.9	0.095±0.018
UDP-glucose	208.2± 8.0	0.279±0.011	64.6±12.7	0.087±0.017	18.2±1.1	0.364±0.022
UDP-galactose	85.5± 1.0	0.115±0.001	20.4± 3.8	0.028±0.005	4.3±0.1	0.085±0.002
UDP-xylose	63.6± 2.0	0.085±0.003	6.7± 0.9	0.009±0.001	3.6±0.1	0.072±0.002
GDP-mannose	35.4± 3.0	0.048±0.004	24.0± 4.0	0.032±0.005	—	—

xylose. Balduini *et al.* (1970) showed that, when UDP-xylose was added to cornea incubated *in vitro*, the chondroitin sulphate content was significantly decreased. It seems probable that the high concentrations of UDP-xylose present in the epithelium and the stroma regulate the spectrum of glycosaminoglycans produced in the corneal tissue. But why such high concentrations of UDP-xylose should be present in these tissues is at present unexplained.

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