Glycoproteins of the lens in relation to age and cataract formation

III. Differences in composition and distribution of the carbohydrate of glycoprotein of lens fibers in different regions of the lens

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The equatorial region of lens fibers of beef and rabbit shows a higher concentration of the carbohydrate in the albuminoid fraction when calculated either per gram lens or per gram albuminoid.

The ratio of sialic acid to hexosamine and fucose is significantly lower in the polar than in the equatorial region of the cortex.

The content in glycoprotein carbohydrate and hexosamine of the nucleus of the lens in both species is significantly lower than that of either of the two cortical regions.

It has been reported in the two preceding communications of this series[1, 2] that the decapsulated lens of beef and rabbit contain significant amounts of a glycoprotein fraction with a glycan, which essentially consists of galactose, some mannose, glucosamine, fucose, and derivatives of neuraminic acid. In fractionation by centrifugation, this glycoprotein could be sedimented with the albuminoid. It had been pointed out that this glycoprotein material does not form a prosthetic group with the albuminoid itself, as the ratio of carbohydrate to albuminoid varied significantly at different ages of the animal. This ratio in the nuclear albuminoid was much lower than the cortical one. It has been suggested that this glycoprotein fraction may be identical with the material demonstrated in the boundary regions of the lens fibers of rabbit by Permutt and Johnson,[3] which stained with a modified Hotchkiss reagent and was not attacked by amylase. Most recent electron microscopic studies of the lens structure seemed to indicate[4] that there are no free spaces between adjacent lens fibers except at the sutures located in the vicinity of the two poles of the lens. These observations raised the question of whether the glycoprotein fraction may not be predominantly localized only in these interfibrillary spaces in the suture region. To answer this question, it appeared promising to determine the amount and composition of the carbohydrate of this fraction separately in the equatorial region of the lens cortex and in the polar region. It also seemed possible that if the glycoprotein

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This work was supported by Grant B 1142 from the Institute of Neurological Diseases and Blindness of the National Institutes of Health, United States Public Health Service.

fraction were found to be present in the equatorial part of the lens, information could be obtained about a possible relation or significance of this glycoprotein fraction to the process of differentiation of the lens epithelium into lens fibers. Such an interrelation appeared not improbable in view of the findings, reported in the second communication of this series,[2] that not only the concentration but also the composition of the carbohydrate of this fraction show significant changes with the age of the whole animal as well as with the age of individual lens fibers, as the nuclear part of the lens differs significantly in these two respects from the cortical part.

Experimental

Preparation of the glycoprotein fraction. Batches of 100 lenses of beef (ice-cooled from the slaughter house) and 200 lenses of adult rabbits weighing about 500 milligrams each were used for the experiments. The rabbit lenses were sent frozen by the Pel-Freeze Company, Arkansas. The capsules were stripped off after thawing the superficial layers of the lenses, and the remaining lens fibers were frozen again and subdivided into an equatorial cortical, polar cortical, and a nuclear part in the following way. The frozen lenses were first put on a frozen glass plate with the lens axis in perpendicular position. A peripheral ring was cut with a cork borer of an appropriate diameter from beef lenses and a smaller trephine from rabbit lenses. This equatorial part corresponded to about 35 to 40 per cent of the total wet weight of the lens. The remaining part of the lens was then tilted by 90 degrees, and the anterior and posterior part around the poles cut off again by trephines of appropriate diameter. The remaining central block of lens fibers represented about 25 per cent of beef lenses and about 45 per cent of rabbit lenses. The three parts of the lenses were homogenized and fractionated as previously described.[1, 2] Only the albuminoid fraction, centrifuged in 30 minutes at 600 × G, was used for these experiments. As had been previously pointed out, one part of the nuclear albuminoid required a higher speed of centrifugation for sedimenting, but this part of the nuclear albuminoid was not investigated because of the possibility of contamination with mitochondrial elements. The washed albuminoid fractions were freed from nucleotides, as described,[1] and then washed with 10 per cent trichloroacetic acid, resuspended in it, and extracted for four consecutive time intervals of 30 minutes, 2 hours, 4 hours, and 12 hours each, to bring the total carbohydrate of the glycoprotein fraction into solution. In one of the experiments with beef lenses, the equatorial part was divided into three parts, and one of the three parts was treated in the same manner as the other parts of the lens. The albuminoid of the two other equatorial parts, after having been washed with water and saline, was either directly pre-extracted for half an hour at room temperature with a 3:1 mixture of methanol and chloroform, or was first dehydrated by several acetone extractions at 4° C. and then also extracted with a methanol $CHCl_3$ mixture. This extraction was carried out to determine whether the lens fibers contain gangliosides in addition to glycoprotein. The results were negative.

Analytical procedures. The determination of hexose, fucose, hexosamine, and neuraminic acid was carried out in exactly the same way as described.[1, 2] Because the hexose fraction in the glycoprotein carbohydrate of the lens was shown to contain predominantly galactose and only traces of glucose, and no evidence could be obtained that the polar and equatorial and nuclear

Table I. Molar ratios of constituents of the carbohydrate of the lens fibers glycoprotein of beef in equatorial and polar cortex and nucleus of the lens

Experiment	*Part of lens*	*Wet weight in % of total*	$\frac{H^\circ}{Ham}$	$\frac{F}{Ham}$	$\frac{S}{Ham}$	$\frac{S}{F}$
I	Equatorial cortex	38.9	1.36	0.085	0.304	3.57
	Polar cortex	32.4	1.68	0.060	0.230	2.94
	Nucleus	28.7	1.36	0.055	0.205	3.67
II	Equatorial cortex	49.6	2.03	0.087	0.290	3.33
	Polar cortex	27.7	1.95	0.079	0.230	3.00
	Nucleus	22.7	1.48	0.050	0.207	4.12 (2.5)
III	Equatorial cortex	44.7	1.36	0.069	0.222	3.19
	Polar cortex	21.5	1.34	0.056	0.161	2.90
	Nucleus	33.6	1.23	0.052	0.143	3.02

°H = hexose; Ham = hexosamine; F = fucose; S = sialic acid.

regions differ in this respect from each other, the total hexose was calculated, for the purpose of these experiments, in terms of galactose. Since glucose was found to be present only in very small amounts, the error in the calculation of the total hexose under this procedure could be barely significant.

Results

Differences in composition of the carbohydrate of the lenticular glycoprotein between different regions of the lens.

Beef lenses. In Table I are listed the ratios of constituents of the carbohydrate of the lenticular glycoprotein in the equatorial, polar, and nuclear regions. The determinations were carried out on three different batches of beef lenses which all show nearly identical results. In all three experiments, the ratio of fucose (F) to hexosamine was significantly higher in the equatorial than in the polar region and in the latter was again significantly higher than in the nuclear region. The same relation prevails for the ratio of sialic acid (S) to hexosamine. Altogether, therefore, the sum of the two end group constituents and the ratio $\frac{S}{F}$ are significantly lower in the polar than in the equatorial region, the difference in the three experiments varying between 7 and 21 per cent. In the nuclear region, on the other hand, in all three experiments $\frac{S}{F}$ was significantly higher than even in the equatorial part.[1] As can be seen from Table II, the equatorial regions of the lens contain significantly more hexosamine and total carbohydrate calculated per gram wet weight than the two other regions. These differences become even more pronounced, particularly between the equatorial and polar cortex, when they are calculated per milligram protein, as the albuminoid content of the polar cortex was found to be about three times as high as that in the equatorial part.

Rabbit lenses. As can be seen in Table IV, the differences between the three regions of the lens as far as the content of hexosamine and total carbohydrate is concerned are even more marked than those of beef. When calculated per milligram protein, the polar region of the rabbit lens has only about one fourth of hexosamine and total carbohydrate as compared with the equatorial part, and in the nucleus this ratio is only about one third of that in the equatorial part. The ratio of sialic acid to fucose is significantly lower in the polar than in the equatorial part, and this difference is more pronounced here than with the beef. This difference, however, in rabbits is mainly the result of a sharp decrease in the ratio of sialic acid to hexosamine. Whereas the ratio of fucose to hexosamine in beef shows also a decrease in the polar region and in the nuclear region, in the rabbit, on the contrary, a significant in-

Table II. Content in hexosamine and total carbohydrate of the lens fibers glycoprotein of beef in equatorial and polar cortex and nucleus of the lens

Experiment	*Part of lens*	*Wet weight in % of total*	*Hexosamine*		*TCH**	
			(μg/Gm. lens fibers)	*(μg/Gm. protein)*	*(μg/Gm. lens fibers)*	*(μg/Gm. protein)*
I	Equatorial cortex	38.9	57.5	7.9	166.5	23.0
	Polar cortex	32.4	51.6	4.3	152.4	12.7
	Nucleus	28.7	39.2	3.95	106.1	10.7
II	Equatorial cortex	49.6	50.8		188.5	
	Polar cortex	27.7	47.0		166.2	
	Nucleus	22.7	39.0		112.5	
III	Equatorial cortex	44.7	69.0		183.2	
	Polar cortex	21.5	62.3		164.2	
	Nucleus	33.6	61.9		164.8	

*TCH, total carbohydrate = sum of anhydro residues of sugars + 1 acetyl per 1 hexosamine.

Table III. Molar ratios of constituents of the carbohydrate of the lens fibers glycoprotein of rabbit in equatorial and polar cortex and nucleus of the lens

Experiment	*Part of lens*	*Wet weight in % of total*	$\frac{H°}{Ham}$	$\frac{F}{Ham}$	$\frac{S}{Ham}$	$\frac{S}{F}$
I	Equatorial cortex	30.6	1.60	0.097	0.099	1.02
	Polar cortex	30.6	1.40	0.151	0.078	0.49
	Nucleus	38.8	1.75	0.285	0.239	0.84
II	Equatorial cortex	48.4	1.51	0.109	0.0830	0.76
	Polar cortex	18.8	1.65	0.125	0.0670	0.54
	Nucleus	32.8	1.30	0.150	0.0690	0.46

°H = hexose; Ham = hexosamine; F = fucose; S = sialic acid.

Table IV. Content in hexosamine and total carbohydrate of the lens fibers glycoprotein of rabbit in equatorial and polar cortex and nucleus of the lens

			Hexosamine		*TCH°*	
Experiment	*Part of lens*	*Wet weight in % of total*	*(µg/Gm. lens fibers)*	*(µg/Gm. protein)*	*(µg/Gm. lens fibers)*	*(µg/Gm. protein)*
I	Equatorial cortex	30.6	97.0	9.1	302.0	28.3
	Polar cortex	30.6	69.4	2.2	196.5	6.2
	Nucleus	38.8	18.6	0.24	69.8	0.84
II	Equatorial cortex	48.4	135.3		403.0	
	Polar cortex	18.8	79.6		224.5	
	Nucleus	32.8	41.1		106.0	

°TCH, total carbohydrate = sum of anhydro residues of sugars + 1 acetyl per 1 hexosamine.

crease appears in the two last parts of the lens (Table III).

Discussion

The results obtained on lenses of beef and rabbit concerning differences in the content and composition of the carbohydrate moiety of glycoprotein of the lens fibers are in agreement in two respects. First, there is a significant decrease in the content in the total carbohydrate as well as in hexosamine, calculated per gram wet weight, in the polar region and nuclear region as compared with the equatorial region. The results in the beef and rabbit lenses also agree as far as the ratio of sialic acid to hexosamine and hexose is concerned, as this ratio was found throughout to be lower in both the polar region and nucleus than in the equatorial region. With respect to the ratio of fucose to hexosamine, however, the beef and rabbit lenses yielded completely different results. This ratio is lower in the polar and nuclear region in beef lenses, but is significantly higher in rabbit lenses. Nevertheless, the ratio of sialic acid to fucose is significantly lower in the polar region than in the equatorial region, even in beef lenses. On the other hand, this ratio was higher in the nuclear than in the polar region in the rabbit.

The glycoprotein fraction of the lens is not exclusively localized in the interfibrillary spaces in the region of sutures. The polar part in which these sutures are located shows much lower content in the carbohydrate of this glycoprotein. It is assumed that the decrease in the carbohydrate at the poles is associated with the process of differentiation of the lens fibers. The polar part of the cortex cannot be considered of higher age than the equatorial part since it is constituted of those parts of fibers which grew out of the equatorial lens epithelium during its differentiation to fibers.

The much lower values of the total carbohydrate of the glycoprotein in the nuclear part could be due either to the influence of the aging process itself or it could be considered a result of further differentiation of cortical fibers.

The equatorial sectors of lens fibers contain the nuclei and show a much higher concentration of ATP and pyridine nucleotides than the rest of the cortex. It seemed, therefore, that this part of the fibers is the site of metabolic and directive functions controlling the process of differentiation. The higher level of glycoprotein glycans in the equatorial region may have a role in the organization and development of lens fibers from lenticular epithelium.

In the first article of this series, it was reported that the ratio $\frac{S}{F}$ in the nucleus of the beef lens is not lower than in the cortex. This finding was probably due to the fact that in this first series of experiments the nuclear part cut out of the lens represented about 50 per cent of the total weight, whereas in experiments I and II of this series it represents only 22 and 28 per cent, respectively, and obviously corresponds to the more centrally situated part of the lens.

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