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GLYCOSYLATION IN VIVO OF HUMAN LENS CAPSULE (BASEMENT MEMBRANE) AND DIABETES MELLITUS

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Glycosylation represents a nonenzymatic posttranslational modification of some proteins in vivo. We have investigated possible glycosylation, in vivo, of human lens capsule (basement membrane of lens epithelium) using a colorimetric method. Our study reveals, for the first time, in vivo glycosylation of human lens capsule. Furthermore, the glycosylation of the lens capsule in the diabetics (57.30 ± 11.26 n moles/mg. protein) is found to be significantly greater than that in their nondiabetic counterparts (29.11 ± 4.90 n moles/mg. protein) ($p < 0.0005$). The present observation represents the first example of increased glycosylation of a basement membrane in the diabetic patients as compared to the nondiabetic controls.

Hemoglobin A_{1c}, the most abundant minor hemoglobin component in human red blood cells, represents a glycosylated hemoglobin with the glycosyl moiety attached to the NH_2-terminal amino group of the β-chain by a ketoamine linkage (1-9). Glycosylation of hemoglobin is nonenzymatic (4,6,7) and occurs continuously in vivo throughout the 120 day lifespan of the red blood cell (4). Further study on glycosylation of hemoglobin has shown that such a process can occur in vitro dependent on temperature and duration of incubation in the presence of glucose (6). Thus nonenzymatic glycosylation is likely to occur to proteins with long enough life-span especially in the presence of high levels of glucose. Indeed, red blood cell membrane proteins (10,11), plasma proteins including albumin (12-16), lens crystallins (17-22), collagen (23-26), and basic nerve myelin protein (27) have been found to undergo glycosylation. Of interest and importance, glycosylation of hemoglobin and albumin is several-fold higher in the diabetic patients than their nondiabetic counterparts (2,3,5,8,13-16), and measurement of glycosylated hemoglobin provides an

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index of average levels of blood glucose over the preceding two or three months (2,3,5,8).

We have investigated possible glycosylation in vivo of human lens capsule which represents the basement membrane of the lens epithelium. The lens capsule, we thought, is likely to be glycosylated due to its long life-span. We have also looked into a possible difference in degree of glycosylation between the diabetic and nondiabetic lens capsules.

Materials and Methods

The lens capsules were isolated from surgically removed cataractous lenses obtained at the time of intracapsular cataract extraction in 17 diabetic and 29 nondiabetic patients with senile cataract. Eleven (65%) of the diabetic and 17 (59%) of the nondiabetic cataract patients were male. The average age of the diabetic and the nondiabetic patients was 67 and 71 years, respectively. Thus, there was no significant difference in sex and age between the diabetic and the nondiabetic cataract patients. The lens capsules were further purified according to the method of Carlson, et al. (28), using solutions devoid of glucose. Several specimens thus isolated and purified were studied by electron microscopy and were confirmed to be morphologically intact basement membrane free of debris. Determination of glycosylation of the lens capsule (basement membrane) was by the colorimetric test of Flückiger and Winterhalter (6), whereby the glucosyl moiety in ketoamine linkage is released as 5-hydroxymethylfurfural, which is detected in color reaction with 2-thiobarbituric acid. The colorimetric test has been shown to be accurate and to correspond closely to ion-exchange chromatographic measurement of glycosylated hemoglobin (9,13). Protein was determined by using the method of Lowry et al. (29) with modification for micro-assay using bovine albumin as standard. The lens capsules from the 17 diabetic patients were divided into five approximately equal portions for five separate assays for glycosylation. The lens capsules from the 29 nondiabetic subjects were divided into six approximately equal portions. Assay for glycosylation of the lens capsules

from the nondiabetic cataracts was carried out at the same time as that for the diabetic except in the sixth experiment as shown in Table I.

Results

Glycosylation in vivo of the lens capsule was detected in both diabetics and their nondiabetic counterparts, and ranged in value from 22.92 to 70.68 n moles/mg. protein. Moreover, glycosylation of the diabetic capsule (57.30 ± 11.26 n moles/mg. protein) was significantly greater than that of the nondiabetic lens capsule (29.11 ± 4.90 n moles/mg. protein) ($t = 5.572$; $p < 0.0005$) (Table I). A scattergram (Fig. 1) shows no overlap in glycosylation of the lens capsule between the diabetic and nondiabetic groups.

Discussion

The lens epithelial basement membrane is formed very early during the embryonal development and stays intact as lens capsule throughout life. Considering that glycosylation affects even less long-lived proteins such as hemoglobin, red blood cell membrane proteins, and plasma proteins, it is not surprising to observe substantial glycosylation of the lens capsule which represents the basement membrane of the lens epithelium. Of most interest, however, the glycosylation of the diabetic lens capsules is observed to be

TABLE 1
Glycosylation of Lens Capsules
in Diabetics and Nondiabetic Controls

Experiment	Diabetics (n moles/mg. protein)	Nondiabetic Controls (n moles/mg. protein)
1	58.82	26.24
2	51.13	35.80
3	41.75	22.92
4	70.68	31.90
5	64.14	32.16
6	---	25.64
Mean ± Standard Deviation	57.30 ± 11.26*	29.11 ± 4.90*

*$t = 5.572$, $p < 0.0005$

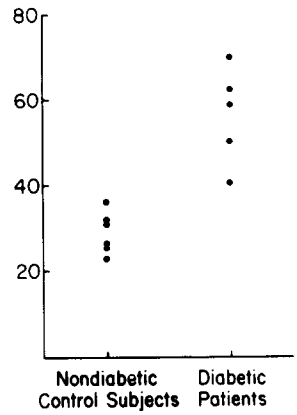

Fig. 1. Scattergram of levels of glycosylation (n moles/mg. protein) in diabetics and nondiabetic controls.

significantly higher than that of their nondiabetic counterparts ($p < 0.0005$). Considering that the diabetic and the nondiabetic patients from which lens capsules were obtained, were comparable with respect to age and sex, the difference is independent of age-related change and sex. Whether or not there might have been substantial or significant difference in degree of cataracts, and some metabolic conditions other than diabetes mellitus between the diabetics and the nondiabetics, had not been looked into, and therefore is not certain.

Although nonenzymatic glycosylation, in vitro, of purified rat lens capsule has been demonstrated (30), the exact site of glycosylation is not known at present. Available evidence from studies on hemoglobin and other proteins (4,6,18,19,21,13) indicates that the glycosylation site is ε-amino group of lysine residues and/or the NH_2-terminal amino group.

Glycosylation of hemoglobin affects the iso-electric point (1) and, in the presence of 2,3-diphosphoglycerate, the oxygen affinity of the hemoglobin (31). Glycosylation of the lens capsule is also expected to influence its physical and functional properties. The increased thickness of the lens capsule of the diabetics as compared to that of the nondiabetics has been reported (32), and it may possibly relate to the increased glycosylation of the diabetic lens capsule.

Initial studies (17,18) on the lens crystallins from diabetic and nondiabetic animals indicated that increased glycosylation and secondary sulfhydryl oxidation might contribute to the development of "sugar" cataract (33,34). Subsequent investigations (19-22), however, did not reveal significant differences in glycosylation of lens crystallins between diabetics and nondiabetics in both animals and humans. On the other hand, a study on nonenzymatic glycosylation of bovine lens crystallins has indicated an age-dependent increase (21) indicating that, at least to a certain degree, glycosylation of the lens crystallins may represent a normal part of the aging process. Whether glycosylation of lens capsule can be considered a degenerative process is uncertain. Nor can we state that the markedly increased glycosylation that occurs in diabetes causes any alteration of function; specifically, hastening the process of senile cataractogenesis. There is, however, some evidence that senile cataract has an earlier onset and higher prevalence in diabetics than nondiabetics (35-37). Although experimental diabetic cataracts in animals, and quite possibly, the rapidly progressive cataracts in young human diabetic patients, are caused by intracellular accumulation of sorbitol with osmotic decompensation of the lens epithelial cells (33,34), the situation in senile cataracts in diabetic patients is less clear. Increased sorbitol levels have been reported in the lenses of such individuals (38), but these levels may or may not be sufficient to cause osmotic damage. Furthermore, any osmotic damage that results may not be entirely responsible for increased onset and higher prevalence of senile cataracts in diabetic patients. In this respect, increased glycosylation of lens capsule seen in older diabetic patients with senile cataracts may, at least in part, be causally related to their cataractogenesis

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