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Research Article

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Biochemical Properties of Human Glomerular Basement Membrane in Normal and Diabetic Kidneys

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ABSTRACT To determine the presence of any significant structural abnormalities in the glomerular basement membrane (GBM) of diabetic individuals, GBM from normal and diabetic human kidneys were isolated and analyzed chemically and structurally. The amino acid composition of the normal GBM revealed the presence of significant amounts of hydroxyproline, hydroxylysine, glycine, and carbohydrate suggesting the presence of a collagen-like protein. There was no significant increase in the amount of hydroxylysine, hydroxyproline, or in the hydroxylysine-linked glycoside glucosyl-galactose in the diabetic kidneys. There was, however, a significant decrease in the cystine and sialic acid content of GBM from diabetic kidneys. It was further shown that the α-chains isolated from the collagens of normal and diabetic basement membranes had similar amino acid and carbohydrate compositions. The hydroxylysine, hydroxyproline, glycine, and hexose contents were higher by 82, 56, 74, and 94%, respectively in the α-chains compared with the intact basement membranes from both the normal and diabetic kidneys. The results indicate that the slight increases in hydroxylysine and hexose content observed occasionally in diabetic GBM preparations are of no statistical significance and cannot be attributed to increases in the activities of enzymes which hydroxylate lysine or glycosylate hydroxylysine, respectively.

INTRODUCTION

Capillary basement membranes undergo morphologic changes in diabetes mellitus characterized primarily by thickening of these structures (1–5). In addition to the thickening, deposits of basement membrane-like material have been described in the subendothelial and mesangial regions of renal glomeruli (2, 3). The possibility has been raised that the widespread thickening of capillary basement membranes in diabetes may be the result of a single lesion leading to the functional abnormalities seen in this disease (6–8).

At present, it is not known whether the morphologic changes in basement membrane are a consequence of the carbohydrate abnormalities seen in diabetes or the result of some other metabolic derangement. There are conflicting reports regarding the biochemical properties of glomerular basement membrane in diabetes. Lazarow and Speidel (9) reported an increase in the total amount of glomerular basement membrane (GBM)[1] isolated from kidneys of patients with diabetes without any significant change in the amino acid or carbohydrate composition. Recently, Beisswenger and Spiro (10) reported that there was an increase in the hydroxylysine and hexose contents of GBM isolated from diabetic kidneys. The authors attributed these changes to increased hydroxylation of lysine and subsequent increased glycosylation of hydroxylysine. While this manuscript was in preparation, Westberg and Michael (11) reported that they were unable to confirm the observations of Beisswenger and Spiro (10) of an increased hydroxylation of lysine in GBM from diabetic kidneys.

Studies which deal with the structural characterization of basement membranes indicate that one of the protein components in basement membranes is a collagen (12–15). Basement membrane collagen contains twice as much hydroxylysine and hexose, 40% more hydroxyproline, and 30% more glycine than intact basement membrane. Recently, it was demonstrated that the hydroxylysine-linked disaccharide units are distributed along the entire length of the collagen polypeptide chain which in turn is linked to a noncollagen polypeptide containing oligosaccharide units composed of mannose

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[1] *Abbreviation used in this paper:* GBM, glomerular basement membrane.

TABLE I
Amino Acid Composition of Human GBM from Normal and Diabetic Kidneys

	Residues/1,000 residues		
	Normal		
	Sonicated 1X*	Sonicated 2X	Diabetic‡ sonicated 1X
Hydroxylysine	24.5±0.75	35.0	25.6±1.35
Lysine	26.0±0.53	20.0	30.0±1.77
Histidine	18.7±1.07	13.4	18.6±2.59
Arginine	48.3±1.84	44.2	50.0±0.77
3-Hydroxyproline	7.0±1.0§	9.9§	8.0±2.0§
4-Hydroxyproline	66.0±3.05	90.0	71.0±3.0
Aspartic	65.0±1.89	59.0	70.0±0.79
Threonine	40.0±1.51	40.0	38.3±1.53
Serine	60.0±2.06	44.6	51.0±0.93
Glutamic	103.0±1.14	89.2	101.2±0.59
Proline	62.0±1.39	69.8	63.2±1.94
Glycine	227.0±7.48	270.0	242.0±10.65
Alanine	58.0±0.44	59.8	57.7±3.98
Valine	36.0±1.27	29.7	40.6±1.14
Half-cystine	23.0±0.82	22.7	18.0±0.87
Methionine	7.0±0.63	6.0	8.5±1.64
Isoleucine	28.0±1.34	24.8	34.8±0.09
Leucine	66.0±2.08	54.2	72.7±1.206
Tyrosine	14.5±0.55	12.0	17.1±0.13
Phenylalanine	28.0±0.82	26.4	31.6±1.32

* Mean values ±SEM from six normal individuals.
‡ Mean values ±SEM from six patients.
§ 3-Hydroxyproline values recalculated using the color value for proline.

and *N*-acetylglucosamine (16). Unlike most interstitial collagens, but like the collagen of cartilage (17), the basement membrane collagen molecule is composed of three identical α-1 chains. If the reason for the increased hydroxylysine content in diabetic basement membranes is due to increased hydroxylation of lysine, then the isolated α-1 chain from the collagen of the diabetic basement membrane should be richer in hydroxylysine and hydroxylysine-linked disaccharide than control α-1 chains.

The present study was designed to examine the chemical properties of normal and diabetic GBM and to compare the composition of α-1 chains of basement membrane collagen isolated from normal and diabetic kidneys.

METHODS

Human kidneys were obtained at autopsy from six normal and six diabetic individuals matched for age. The age of the normal and diabetic individuals ranged between 42 and 56 yr. The disease was known to be present for more than 10 yr. GBM was prepared essentially by the method of Krakower and Greenspon (18) with some modifications. For the separation of glomeruli, the cortex was pressed through a 150 mesh wire screen. The glomeruli were separated from tubules by differential gravitational settling in buffered physiological saline (0.14 M NaCl, 0.02 M Na_2HPO_4). For the sonication of glomeruli, we used a more powerful sonic oscillator (Raytheon Co., Microwave & Power Tube Div., Waltham, Mass., model DF-101, 10 kcycle) than the one originally described by the above authors. This permits a more thorough disruption of cellular elements. 2–3 mg from each preparation of basement membrane was set aside for amino acid and carbohydrate determinations. The remainder of the basement membrane preparations were combined into control and diabetic pooled samples. A portion of control pooled basement membrane was resonicated for 20 min and chemical analyses were performed. Although the average yield of GBM from the diabetic kidneys was higher (29 vs. 23 mg/kidney), the difference was not statistically significant.

The collagen component of the basement membrane was prepared with minor modifications of a previous method (13). The basement membranes were incubated with pepsin (pepsin: basement membrane, 1:10) in the presence of 0.5 M acetic acid at 10°C for 16 h. At the end of this period, the undigested membranes were separated by centrifugation at 34,800 *g* for 45 min at 4°C. The pH of the supernate was adjusted to 8.5 by the dropwise addition of 7.5 N NH_4OH. Following this, the supernate was dialyzed for 18 h against 0.05% acetic acid containing 0.02 M Na_2HPO_4. The collagen was then precipitated in the supernatant solution by the addition of solid KCl to a final concentration of 15%. The collagen was separated by centrifugation and reprecipitated twice more as described above. The undigested residue was treated with pepsin four more times and the collagen precipitated as described above.

Preparation of α-chains from the basement membrane collagen was accomplished after denaturation of the protein and chromatography on CM-cellulose as described previously (15).

Amino acid and carbohydrate analyses were performed according to previously published methods (13, 19, 20). The amount of 3-hydroxyproline was determined in a JLC-6AH automatic amino acid analyzer (Jeol U. S A., Cranford, N. J.) using a color value equal to that of proline. Neutral hexoses were measured by the Technicon automatic sugar analyzer (Technicon Instruments Corp., Tarrytown, N. Y.). The isolation and characterization of the hydroxylysine-linked disaccharide, glucosyl-galactose,

TABLE II
Carbohydrate Composition of Human GBM from Normal and Diabetic Kidneys

	Normal	Diabetic*
	g/100 g	
Hexose	6.8±0.3	6.4±0.28
Glucose	2.5±0.09	2.4±0.09
Galactose	2.6±0.09	2.6±0.1
Mannose	1.7±0.1	1.4±0.1
Glucosamine	1.7±0.1	1.5±0.1
Galactosamine	0.3±0.06	0.2±0.05
Fucose	0.7±0.1	0.5±0.1
Sialic acid	1.5±0.04	1.2±0.03

* Mean values ±SEM from six patients.

and the monosaccharide, galactose was accomplished on the isolated collagen component according to a previous method (21).

RESULTS

The amino acid composition of GBM isolated from control and diabetic human kidneys is shown in Table I. The high content of hydroxyproline, hydroxylysine, and glycine is characteristic; the values, however, do not differ significantly between the control and diabetic membranes. There were instances where the content of these amino acids was higher in the diabetic group but there was never an isolated increase in hydroxylysine alone or in any other amino acid alone. Analysis of the normal basement membrane that was sonicated twice revealed higher concentrations for hydroxylysine, hydroxyproline, and glycine. The parallel increase in the content of these three amino acids is noted. Differences which were of no statistical significance occurred in the aspartic and valine content. Significant increases occurred in the amounts of isoleucine and tyrosine content of the diabetic GBM, whereas significant decreases appeared in the serine and half-cystine content.

The carbohydrate composition of normal and diabetic human GBM appears in Table II. The presence of large amounts of hexose and the almost equimolar distribution of glucose and galactose is evident. No significant differences were noted in the content of the above sugars. The mannose content was lower in the diabetic GBM but the difference was not significant. A statistically significant decrease was noted in the amount of sialic acid in the diabetic GBM.

The amount of collagen isolated from the pooled control GBM accounted for 18% by weight of the starting material, while the amount isolated from the pooled diabetic GBM represented 19.2% by weight of the starting material.

Compositional studies on the isolated collagens from the pooled normal and diabetic GBM appear in Tables III and IV. The amino acid composition of the intact collagens is very similar. There is a parallel increase in the content of hydroxylysine, hydroxyproline, and glycine with a concomitant decrease in the lysine concentration in both groups. Further evidence that the collagens from the two types of basement membranes do not differ significantly comes from the analyses of their respective α-chains (Tables III and IV). The α-chains from the control and diabetic basement membrane collagens have a very similar amino acid composition. The carbohydrate content is similar in both. The hexose is accounted for by almost equimolar amounts of glucose and galactose. Determination of the hydroxylysine-bound disaccharide glucosyl-galactose and the monosaccharide galactose revealed again the similar distribution of these carbohydrate units between the normal and diabetic basement membranes.

TABLE III

Amino Acid Composition of the Collagen Isolated from Human GBM of Normal and Diabetic Kidneys

	Residues/1,000 residues*			
	Normal		Diabetic	
	Collagen	α-Chain	Collagen	α-Chain
Hydroxylysine	43.0	44.6	42.0	45.0
Lysine	9.5	10.0	10.6	11.0
Histidine	9.3	10.4	9.5	11.0
Arginine	36.0	33.0	34.0	38.0
3-Hydroxyproline	10.0	11.0	9.8	9.0
4-Hydroxyproline	118.0	130.0	125.0	127.0
Aspartic	52.0	51.0	55.0	50.0
Threonine	26.0	23.0	25.0	23.0
Serine	36.0	37.0	33.0	35.0
Glutamic	84.0	84.0	83.0	81.0
Proline	60.0	61.0	62.5	60.0
Glycine	315.0	310.0	320.0	322.0
Alanine	35.0	33.0	30.0	31.0
Valine	29.0	29.0	28.0	28.0
Half-cystine	8.5	8.0	7.0	7.5
Methionine	9.6	10.0	9.0	9.5
Isoleucine	32.0	30.0	29.0	30.0
Leucine	55.6	54.0	55.0	52.0
Tyrosine	5.0	6.0	6.0	5.0
Phenylalanine	27.5	27.0	28.0	25.0

* Duplicate analyses.

TABLE IV

Carbohydrate Composition of the Collagen Isolated from Human GBM of Normal and Diabetic Kidneys

	Normal	Diabetic
	*g/100 g**	
Hexose	12.0	13.0
Glucose	5.5	6.0
Galactose	6.0	6.3
Mannose	Trace	Trace
Hexosamine	0	0
Fucose	0	0
Sialic acid	0	0
	μmol/μmol α-chain	
Glc-Gal-Holy‡	34.0	34.5
Gal-Holy§	2.0	1.9

* Duplicate analyses.
‡ Glucosyl-galactosyl-hydroxylysine.
§ Galactosyl-hydroxylysine.

DISCUSSION

The morphologic changes observed in the GBM of diabetic kidneys have generated a great deal of speculation with respect at the probable role they may play in the functional alterations of the kidney. Since one of the fundamental defects in diabetes mellitus involves glucose metabolism and since this sugar is one of the carbohydrate components found in GBM (22, 23), it became tempting to search for structural defects in the molecule which contained the glucose.

Although the early studies of Lazarow and Speidel (9) showed an increase in the basement membrane content of glomeruli from diabetic kidneys, they did not demonstrate any significant differences in the amino acid or carbohydrate composition between normal and diabetic basement membranes. A later study by Beisswenger and Spiro (10, 24) demonstrated some increase in the hydroxylysine and disaccharide content of diabetic GBM. They interpreted their data to mean that in diabetes there is an increase in the degree of hydroxylation of lysine followed by an increase in glycosylation, without any changes in the content of the other amino acids. The data presented here and those reported by Westberg and Michael (11) do not support the observations of Beisswenger and Spiro (10) nor the concept that in diabetes mellitus there is increased hydroxylation of lysine and increased glycosylation of hydroxylysine in GBM. Analyses of the collagen component, which contains all the hydroxylysine present in GBM, indicate that the hydroxylysine and the glucosyl-galactose contents do not differ between the control and diabetic GBM. This is further supported by the demonstration that the α-chains isolated from the GBM collagens had very similar amino acid and carbohydrate composition. There is adequate evidence (12–14, 25, 26) to indicate that basement membranes from various sources are complexes of dissimilar protein subunits and that one of them is a collagen. This is further supported by the studies of Grant, Kefalides, and Prockop (27, 28) who demonstrated that cells from chick embryo lenses synthesize a collagen precursor in a fashion analogous to that synthesized by chick tendon cells (29) and chick embryo calvaria (30). The newly synthesized basement membrane collagen has the features of the mature protein which include the high 3-hydroxyproline content, the high degree of hydroxylation of lysine, and glycosylation of hydroxylysine, as well as the predominance of the disaccharide over the monosaccharide unit (16, 27, 28).

The changes seen in the amounts of other amino acids not characteristic of the collagen component are difficult to explain. Since disulfide bonds are involved in the cross-linking of the protein molecules in GBM (14, 16), the observation made by Westberg and Michael (11) and substantiated by the present study that the diabetic GBM contains lower amounts of half-cystine could be of potential importance. It would require the isolation of the protein component which is rich in half-cystine and demonstrates a decrease in the content of this amino acid in diabetes mellitus. The half-cystine content of the collagen from the control and diabetic GBM was lower than that of the intact basement membrane but did not differ between the two groups. The decrease in sialic acid again is difficult to interpret since GBM preparations are not very "pure" and could very easily be contaminated by protein components having amino acid and carbohydrate compositions different from those of the "normal" GBM. At any rate, it would be difficult to prove with the available data that such changes have resulted from modifications in the protein components of the GBM proper rather than from contamination by cellular or other interstitial proteins.

It would appear that in diabetes mellitus there is not only thickening of the basement membrane proper, but also accumulation of basement membrane-like material in the mesangial region. At present, we do not know the chemical and physical properties of the portion of the basement membrane which accumulates in the mesangium. It is possible that diabetic glomeruli with an excess of basement membrane-like material in the mesangium may yield GBM preparations with less contamination from the epithelial or endothelial cells surrounding the capillary GBM. This may result in preparations of basement membrane with higher amounts of hydroxyproline and hydroxylysine, amino acids which characterize the collagen component. From these studies, we would have to conclude that the cause for the thickening of the GBM in diabetes mellitus is still unknown.

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