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# Biochemistry of glomerular basement membrane of the normal and diabetic human

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The histological appearance of kidneys from patients with diabetic nephropathy is characterized by a variety of findings. The most characteristic finding is a diffuse and/or nodular glomerulosclerosis, consisting of thickening of the peripheral basement membranes and an increase of the mesangial matrix. The nodular transformation of the mesangium leads to the classical Kimmelstiel-Wilson lesion. These typical morphological findings have directed attention to the process and nature of basement membrane thickening. Also, it has been recognized for a long time that basement membrane thickening is dependent on the duration and metabolic control of diabetes mellitus [1–4]. The question of how basement membrane thickening and diabetes mellitus are related biochemically was less clear. This lack of clarity was mainly due to the fact that the chemical structure of the basement membrane was not known. Earlier chemical analyses were beset by methodological drawbacks limiting the value of the results [5, 6], whereas recent studies on the chemical composition of normal and diabetic human glomerular basement membranes (HGBM) produced conflicting results [7–13]. The isolation procedure and the source of the kidneys, as well as other factors, have been considered responsible for the different results obtained by various investigators. This prompted us to carry out further biochemical analyses on normal and diabetic HGBM under more carefully controlled conditions. Special attention has been paid to the source of kidneys and the evaluation of contamination of the isolated basement membranes.

## Methods

Human kidneys have been obtained at autopsy within 12 hr following the death of diabetic and nondiabetic subjects. The kidneys were processed immediately; freezing was avoided. The 22 diabetic patients, aged 26 to 80 years (median, 66.5) had a duration of diagnosed diabetes mellitus between 3 and 22 years (median, 10 years). Clinical data on the diabetic patients are given in Table 1.

The nature and degree of renal changes was evaluated in each individual patient by histological examination using light microscopy on 200 randomly selected glomeruli (100 per kidney). Measurements of mesangial width have been done on periodic acid Schiff's reagent (PAS)-stained sections at a magnification of 420×. In kidneys with nodular glomerulosclerosis the percentage of glomeruli containing noduli was estimated in a total of 200 glomeruli. All except one diabetic patient had diffuse and/or nodular glomerulosclerosis of differing degrees of severity. Classification of the histological alterations was done according to Ditscherlein [14] (Table 2). About 50% of all the kidneys from the diabetic patients showed a mesangium width of more than 20 μm; the remainder showed even more severe lesions.

The 32 nondiabetic subjects, between 33 to 87 years of age (median 60.1), died from diseases unrelated to diabetes mellitus or kidney diseases; they had no history of diabetes mellitus. With microscopic examination, the most common histological finding was a few hyaline glomeruli. There was no difference in sex and age distributions between the two groups.

*Preparation of glomeruli.* The isolation procedure followed was the technique of Krakower and Greenspon [15] as modified by Spiro [16] and Westberg and Michael [17]. Using this procedure, the kidney cortex was minced and pressed through a 115-mesh stainless sieve. The bigger tissue fragments were removed during a passage through an 80-mesh sieve; the glomeruli are collected on a 150-mesh sieve and washed extensively with cold saline. The absence of tubular remnants and other tissue fragments in this glomerular preparation was checked generally by phase contrast microscopy.

*Preparation of HGBM.* For isolation of basement membranes, glomeruli were disrupted with a Branson sonifier (S 125) at number 6 power setting. The sonication period was standardized at 7 bursts of 1-min durations with a cooling period between the bursts. The glomeruli were suspended in 1 M saline at a concentration equivalent to 2 g cortex per milliliter. The HGBM was collected by centrifugation in 1 M sodium chloride at a very low speed (121 g/15 min) and washed repeatedly three times in 1 M sodium chloride and three times in water. Undisrupted glomeruli were removed by filtration over a 200-mesh sieve. Random samples of the isolated HGBM (15%) were checked by transmission and scanning electronmicroscopy for impurities. HGBM were then lyophilized and stored under sealed conditions at −15° C.

*Amino acids.* For amino acid analysis HGBM was submitted to a 24-hr hydrolysis in constant boiling hydrochloric acid at a concentration of 1 mg HGBM dry weight/1 ml at 110° C in sealed evacuated glass tubes. The hydrochloric acid was evaporated over potassium hydroxide and phosphorus pentoxide.

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**Table 1.** Clinical data on diabetic patients

| Patient no. | Age/Sex | Duration of diabetes mellitus *years* | Therapy | Serum creatinine *mg/dl* | Cause of death | Type of glomerulosclerosis | See Table 2 |
|---|---|---|---|---|---|---|---|
| 1 | 45 M | 21 | Insulin | 2.3 | Myocardial infarction | Nodular | 3 |
| 2 | 67 M | 8 | Oral agents | 2.5 | Myocardial infarction | Diffuse | 3 |
| 3 | 30 F | 20 | Insulin | 9.2 | Uremia | Diffuse | 5 |
| 4 | 76 M | 10 | Insulin | 1.9 | Apoplexia | Diffuse | 3 |
| 5 | 52 F | 10 | Insulin | 1.4 | Myocardial infarction | Diffuse | 1 to 2 |
| 6 | 80 F | 3 | Diet | 1.2 | Myocardial infarction | Diffuse | 1 to 2 |
| 7 | 65 M | 3 | Oral agents | 2.0 | Uremia | Diffuse | 4 |
| 8 | 79 F | 10 | Oral agents | 1.8 | Pneumonia | Diffuse | 3 |
| 9 | 78 F | 15 | Insulin | 1.5 | Myocardial infarction | Diffuse | 4 |
| 10 | 78 M | 10 | Oral agents | 1.7 | Pneumonia | Diffuse | 1 to 2 |
| 11 | 78 M | 3 | Diet | 2.4 | Pulmonary embolism | Diffuse | 1 to 2 |
| 12 | 70 M | 10 | Insulin | 2.2 | Apoplexia | Nodular | 1 to 2 |
| 13 | 76 F | 15 | Insulin | 1.1 | Pulmonary embolism | Diffuse | 1 to 2 |
| 14 | 63 M | 10 | Oral agents | 1.8 | Sepsis | Diffuse | 1 to 2 |
| 15 | 68 F | 3 | Insulin | 1.2 | Cancer of esophagus | None | |
| 16 | 65 F | 10 | Insulin | 11.6 | Uremia | Diffuse | 5 |
| 17 | 66 F | 19 | Oral agents | 1.2 | Diabetic coma | Nodular | 1 to 2 |
| 18 | 26 F | 16 | Insulin | 4.5 | Myocardial infarction | Nodular | 5 |
| 19 | 43 M | 25 | Insulin | 1.5 | Pneumonia | Nodular | 1 to 2 |
| 20 | 66 F | 46 | Insulin | 2.0 | Myocardial infarction | Nodular | 3 |
| 21 | 66 M | 16 | Oral agents | 4.0 | Uremia | Diffuse | 5 |
| 22 | 66 F | 3 | Diet | 1.2 | Breast cancer | Diffuse | 1 to 2 |

**Table 2.** Classification of diffuse and nodular glomerulosclerosis

| Stage | Diffuse glomerulosclerosis | Stage | Nodular glomerulosclerosis |
|---|---|---|---|
| 0 | Normal histology | 1 to 2 | Less than 50% of glomeruli contain noduli |
| 1 to 2 | Mesangium width less than 20 μm | 3 | More than 50% of glomeruli contain noduli |
| 3 | Mesangium width more than 20 μm | 4 | Like 3 with 20 to 40% of glomeruli hyalinized |
| 4 | Like 3 with 20 to 40% of glomeruli hyalinized | 5 | Like 3 with more than 40% of glomeruli hyalinized |
| 5 | Like 3 with more than 40% of glomeruli hyalinized | | |

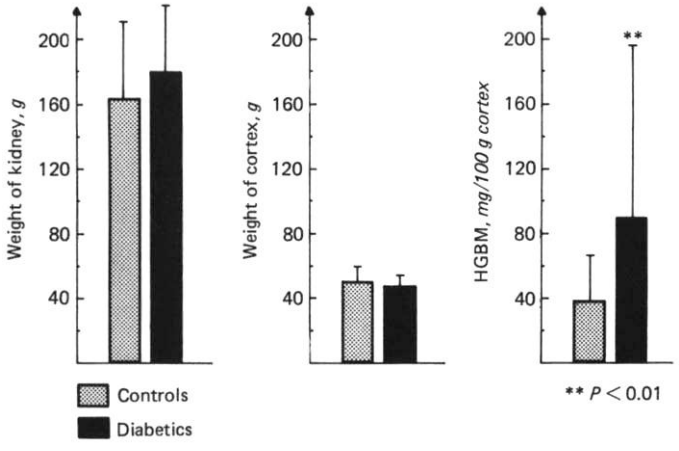


**Fig. 1.** *Weights of kidneys, cortices and HGBM in diabetics* (N = *19*) *and normals* (N = *32*), ($\bar{x} \pm s$).

Aminex A6 (15.5 to 19.5 μm) was used on a 0.9 × 54 cm column for the acidic amino acids. The sample was eluted with 0.2 N sodium citrate buffer first at pH 2.93 and then at pH 3.3 and 4.0 at 55° C. The basic amino acids were separated on a 0.9 × 15 cm column of Aminex A5 (11.5 to 15.5 μm) with a 0.4 N sodium citrate buffer at pH 5.3 at 50° C. Methionine and cystine were analyzed according to the method described by Moore [18]. All determinations were run in duplicate on a BC 200 Biocal Instruments analyser. The average percent differences in individual amino acids from the mean was 8% except for histidine and arginine (27.3 and 25.3%, respectively). Losses during the analyses were corrected using norleucine as an internal standard; the mean recovery rate was 97.4%.

*Carbohydrates.* Glucose, galactose, and mannose were liberated from HGBM by hydrolysis in sulphuric acid at 100° C over 4 hr in sealed glass tubes. The determination was made in the neutralized supernatant by commercially available test kits (Boehringer Mannheim). For the determination of fucose, the Dische-Shettles cystine reaction was used [19]. Sialic acids were evaluated according to the method described by Warren [20].

*Other chemical analyses.* DNA analyses were performed by the method of Burton [21]. Total phosphorus was determined by the method described by Bartlett [22].

*Microscopic examination.* Transmission and scanning electronmicroscopic examinations were done as reported previously [23, 24].

*Statistical methods.* Except otherwise stated, all values are given as mean ± SD ($\bar{x} \pm s$). Statistical significance of group differences was tested by the nonparametric two-tailed Wilcoxon test for random samples. Correlations were calculated using linear regression analysis [25].

**Fig. 2.** *Scanning electronmicroscopic view of isolated normal HGBM.*

## Results

*Preparations of glomeruli and HGBM.* The amount of kidney cortex isolated from 32 normal kidneys was 50.8/100 g kidney ± 8.4/100 g kidney. The cortex-weight was recorded in 19 kidneys with diabetic glomerulosclerosis and amounted to 48.2 g/100 g of kidney ± 6.1 g/100 g of kidney. The difference was not statistically significant. The amount of basement membrane isolated from 32 normal kidneys was 39.4 mg/100 g of cortex ± 27.3 mg/100 g of cortex; the median was 31.3 mg/100 g of cortex. The average yield of basement membranes in the diabetic group ($N$ = 19) was 90 mg/100 g of cortex ± 107 mg/100 g of cortex. The positive skewness of the distribution of the basement membrane weights was due to the irregular distribution of the different grades of severity of diabetic glomerulosclerosis; the median was 59.1 mg/100 g of cortex. The yield of HGBM in the diabetic group was significantly higher ($P < 0.01$, Fig. 1) than the yield of the nondiabetic group.

*Microscopic examinations.* About 15% of the isolated glomeruli retained their capsules. When viewed by transmission electronmicroscopy, the basement membrane appeared as intact dense ribbons with occasional cell remnants attached to it, devoid of intact collagen fibers. Figure 2 shows a scanning electronmicroscopic view of isolated normal HGBM.

### *Chemical composition*

*DNA and phosphorus contents.* The DNA and phosphorus contents of normal and diabetic HGBM are given in Table 3; no statistical difference between the two groups was found. When calculated on the basis of 4-hydroxyproline, the phosphorus content of diabetic basement membranes was slightly lower than the normal basement membranes. The difference did not reach any level of statistical significance.

*Amino acid composition.* The amino acid composition of normal and diabetic basement membranes is given in μM/100 mg HGBM in Table 4 and in residues per 1000 amino acid residues in Table 5. When compared on the basis of μM/100 mg

**Table 3.** DNA and phosphorus content of diabetic and normal HGBM[a]

| Composition | Diabetic $\bar{x} \pm SD$ | Normal $\bar{x} \pm SD$ |
|---|---|---|
| DNA, *μg/100 mg* HGBM | 136.54 ± 42.65 ($N$ = 11) | 134.7 ± 44.5 ($N$ = 17) |
| Phosphorus, *phosphate/g* HGBM | 16.23 ± 4.72 ($N$ = 15) | 18.46 ± 7.52 ($N$ = 27) |

[a] The $N$ in parentheses represents the number of samples analyzed.

**Table 4.** Amino acid composition of HGBM, μM/100 mg HGBM[a]

| Amino acid | Normal, $N$ = 16 $\bar{X}$ | ± SD | Diabetic, $N$ = 22 $\bar{X}$ | ± SD |
|---|---|---|---|---|
| 3-hydroxyproline | 8.93 | 2.26 | 8.17 | 2.23 |
| 4-hydroxyproline | 60.78 | 6.49 | 61.74 | 10.33 |
| Aspartic acid | 48.96 | 2.81 | 49.49 | 3.45 |
| Threonine | 24.94 | 1.94 | 24.62 | 2.35 |
| Serine | 36.12 | 2.22 | 36.24 | 2.67 |
| Glutamic acid | 74.56 | 5.66 | 75.54 | 5.41 |
| Proline | 47.03 | 2.88 | 46.31 | 3.79 |
| Glycine | 203.85 | 17.44 | 200.14 | 25.31 |
| Alanine | 41.52 | 3.26 | 41.02 | 4.98 |
| Half-cystine | 23.05 | 4.52 | 18.66[b] | 6.23 |
| Valine | 26.59 | 1.97 | 26.77 | 1.70 |
| Methionine | 6.02 | 3.23 | 5.46 | 3.23 |
| Isoleucine | 23.46 | 1.20 | 24.19 | 1.61 |
| Leucine | 47.74 | 3.02 | 47.12 | 3.56 |
| Tyrosine | 11.56 | 0.91 | 11.60 | 1.51 |
| Phenylalanine | 24.07 | 2.05 | 24.67 | 2.68 |
| | $N$ = 31 | | $N$ = 17 | |
| Hydroxylysine | 20.69 | 2.56 | 19.45 | 3.06 |
| Lysine | 12.38 | 1.96 | 13.24 | 2.70 |
| Histidine | 9.35 | 1.33 | 8.92 | 1.86 |
| Arginine | 33.34 | 3.89 | 32.21 | 6.18 |

[a] $N$ represents the number of HGBM preparations analyzed.
[b] $P < 0.05$

HGBM, the only amino acid behaving differently was half cystine; when compared on the basis of residues per 1000 amino acid residues, the diabetic basement membrane showed a smaller hydroxylysine content. The latter result could have been influenced by a smaller sample size.

*Carbohydrate composition.* Carbohydrate composition of normal diabetic basement membranes is given in Table 6. The glucose content of the 22 diabetic basement membranes was slightly lower than in the normal basement membranes ($P < 0.05$). This significance could also be shown for the mannose content ($P < 0.01$). The sialic acid content showed a tendency toward lower values; the difference between the two groups, nevertheless, did not reach any level of statistical significance.

*Chemical findings in a selected subgroup.* The great variation of basement membrane weights, possibly reflecting different compositions [26], prompted a separate analysis of a group of eight diabetic basement membrane preparations. Their weight was double the amount of normal basement membrane. The mean duration of diabetes mellitus in the group was 14 years; all kidneys except one showed extremely severe alterations of diffuse and/or nodular diabetic glomerulosclerosis. The mean basement membrane yield was 170 mg/100 g of cortex (median

**Table 5.** Amino acid composition of HGBM, residues/1000 amino acid residues[a]

| Amino acid | Normal, $N$ = 16 $\bar{X}$ | ± SD | Diabetic, $N$ = 17 $\bar{X}$ | ± SD |
|---|---|---|---|---|
| 3-hydroxyproline | 11.24 | 2.42 | 10.32 | 2.45 |
| 4-hydroxyproline | 76.98 | 6.62 | 78.85 | 9.59 |
| Aspartic acids | 62.08 | 2.37 | 63.85 | 3.84 |
| Threonine | 31.63 | 2.14 | 31.62 | 3.20 |
| Serine | 45.78 | 1.52 | 46.85 | 2.82 |
| Glutamic acid | 94.43 | 3.84 | 97.84 | 4.51 |
| Proline | 59.63 | 2.42 | 59.66 | 3.47 |
| Glycine | 258.12 | 12.89 | 255.81 | 17.64 |
| Alanine | 52.68 | 4.02 | 53.11 | 6.69 |
| Half-cystine | 29.21 | 5.66 | 23.84[b] | 7.36 |
| Valine | 33.75 | 2.56 | 34.64 | 2.46 |
| Methionine | 7.60 | 4.10 | 7.07 | 3.95 |
| Isoleucine | 29.76 | 1.37 | 31.10 | 2.59 |
| Leucine | 60.52 | 2.34 | 60.61 | 3.78 |
| Tyrosine | 14.67 | 1.22 | 14.87 | 1.89 |
| Phenylalanine | 30.51 | 2.04 | 32.19 | 3.61 |
| Hydroxylysine | 28.19 | 2.66 | 25.68[b] | 3.86 |
| Lysine | 15.73 | 2.96 | 17.57 | 3.94 |
| Histidine | 11.64 | 1.91 | 11.80 | 2.51 |
| Arginine | 45.84 | 5.40 | 42.70[b] | 8.94 |

[a] $N$ represents the number of HGBM preparations analyzed.
[b] $P < 0.05$

113) in the diabetic group and 47 mg/100 mg of cortex (median 43) in the normal group. The latter group, comprising nine basement membrane preparations, was chosen to match age and sex. Except for half cystine there was no difference in the amino acid and carbohydrate composition between the two groups, irrespective of the calculation mode.

## Discussion

The conflicting results concerning the composition of normal and diabetic HGBM suggest a substantial influence of the method of isolation and source of basement membranes on the results obtained. Spiro suggested the use of only those kidneys which show unequivocal changes characteristic of diabetic glomerulosclerosis [26]. This study agrees completely with him on that point. With one exception, all kidneys analyzed in this study showed severe alterations corresponding to the histological picture of diabetic glomerulosclerosis. As expected, the yield of isolated HGBM depended on the severity of the histological alteration ($r = 0.55$, $P < 0.01$). Contrary to the contention of other reports [8], it is believed that this yield is proof for our assumption that the morphological substrate of diabetic glomerulosclerosis has been isolated and analyzed. This statement holds true only for the insoluble backbone of the HGBM due to the inherent limitations of the isolation technique. Information regarding constituents such as glycosaminoglycans [27, 28], P-component [29], and laminin [30, 31] cannot be obtained. The weights of diabetic kidneys and renal cortices did not differ from the nondiabetic control subjects; this finding agrees with evidence shown previously by pathologists [14]. The basement membrane yield of diabetic kidneys, however, was significantly higher ($P < 0.01$) than the yield of nondiabetic kidneys (90.0 vs. 39.4/100 mg HGBM). To our knowledge, similar findings have only been reported elsewhere by Westberg and Michael [9] and Cruz and Moreau-Lalande [12].

**Table 6.** Carbohydrate composition of HGBM, μM/g HGBM

| Carbohydrate | Normal | | | Diabetic | | |
|---|---|---|---|---|---|---|
| | $N^a$ | $\bar{x}$ | ±SD | $N^a$ | $\bar{x}$ | ±SD |
| Glucose | 30 | 154.38 | 19.12 | 22 | 142.29[b] | 24.90 |
| Galactose | 30 | 160.06 | 19.97 | 22 | 149.57 | 22.33 |
| Mannose | 29 | 40.53 | 10.03 | 22 | 32.44[c] | 8.31 |
| Fucose | 21 | 38.31 | 9.59 | 16 | 37.65 | 7.30 |
| Sialic acids | 29 | 23.29 | 3.74 | 21 | 20.87[d] | 4.69 |

[a] $N$ represents the number of HGBM preparations analyzed.
[b] $P < 0.05$
[c] $P < 0.01$
[d] $P = 0.062$

**Table 7.** Comparison of several studies on the composition of the diabetic HGBM

| Composition | Beisswenger and Spiro [8] | Westberg and Michael [9] | Kefalides [10] | Cruz et al [12] | Wahl et al (present observations) |
|---|---|---|---|---|---|
| Hydroxyproline | + | N | N | N | N |
| Glycine | + | N | N | + | N |
| Half-cystine | N | — | — | — | — |
| Hydroxylysine | + | N | N | + | N |
| Lysine | — | — | N | N | N |
| Glucose | + | + | N | + | — |
| Galactose | + | N | N | + | N |
| Mannose | N | N | (—) | N | — |
| Sialic acids | n.g. | — | — | — | (—) |

Symbols are: n.g., not given; +, greater than normal; −, less than normal; N, not changed from normal ($P < 0.05$); (−), statistically not significant.

To compare analytical results with other published reports, an identical degree of purity of the isolated HGBM is required. The phosphorus and DNA content of our HGBM preparations falls in the lower range of the values reported thus far. There was no significant difference between the degree of purity of diabetic and nondiabetic HGBM. Calculated as μM/100 mg HGBM, the amino acid composition differed only in one amino acid between diabetic and nondiabetic HGBM. With 19 μM/100 mg HGBM the half-cystine concentration in diabetic HGBM was significantly lower compared with nondiabetic HGBM. Calculated as residues/1000 residues, hydroxylysine and arginine contents were lower in the diabetic HGBM. The lower half-cystine content agrees with previous studies. Contrary to Beisswenger and Spiro [8] and Cruz and Moreau-Lalande [12], this study could not find an increase in the hydroxylysine or a decrease in lysine content (Table 5). The carbohydrate composition was slightly different from the composition reported thus far for normal and diabetic basement membranes. A slight decrease of the glucose and mannose content was found. The lower sialic acid content in diabetic HGBM was not significant.

Our data cannot confirm the finding of a specific alteration of diabetic HGBM as proposed by Beisswenger [32]. They are in full accordance with those of Westberg and Michael [9], Kefalides [10], and partly with Cruz and Moreau-Lalande's [12] results (Table 7). The results of Sato et al [11] were not taken into account because only two patients of their diabetic group showed histological alterations of glomerulosclerosis. The results of Canivet, Cruz, and Moreau-Lalande [13] were published previously by Cruz and Moreau-Lalande [12]. Differences in the composition that eludes recognition due to the applied method of isolation cannot be excluded. It is unclear why most of the published results vary from those of Beisswenger and Spiro [7]. Differences in the source of diabetic kidneys and the isolation procedure could contribute substantially to these different results. This study has tried to exclude all possible sources of error alluded to by Brownlee and Spiro [33] without confirming their findings.

*Summary.* HGBM were isolated from kidneys of 22 diabetic patients and 32 normal control persons. All diabetic kidneys showed severe alterations of glomerulosclerosis. The amount of basement membranes isolated from diabetic kidneys was significantly higher. The HGBM preparations were individually analyzed for phosphorus, DNA, amino acids, and carbohydrates. The cystine concentration was found to be lower in the diabetic than in normal HGBM. Contrary to other reports, there was no increase in the amounts of hydroxylysine, glucose, and galactose, nor was there a decrease in the lysine content. Glucose and mannose were shown to be significantly lower in the diabetic HGBM. The mean value for sialic acid content was lower in diabetic HGBM; the value did not reach statistical significance. This study's data could not confirm the finding of a specific alteration of diabetic HGBM as proposed previously.

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