# Increased Concentration of Albumin in Kidney Basement Membranes in Diabetes Mellitus

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**SUMMARY**

**The concentration of albumin was measured by a double-antibody radioimmunoassay in the glomerular basement membrane (GBM) and tubular basement membrane (TBM) of patients with diabetic nephropathy, other kidney diseases, and normal kidneys. The TBM from diabetics contained significantly more albumin (m ± SD; 2.75 ± 0.34 μg/mg) than the TBM from other diseases (1.00 ± 0.24 μg/mg) or normals (1.21 ± 0.26 μg/mg) ($P < 0.0001$). Similarly, diabetic GBM contained more albumin (2.25 ± 0.59 μg/mg) than other diseases (1.22 ± 0.55) or normals (1.31 ± 0.36 μg/mg) ($P < 0.01$–$0.001$). No differences were observed between the normal and other disease groups. Although there were no differences in hydroxyproline content, there was a highly significant correlation between the concentration of hydroxyproline and albumin in the diabetic TBM ($r = 0.82$), diabetic GBM ($r = 0.70$), and normal TBM ($r = 0.79$). Elution studies with different buffers on frozen sections of diabetic kidneys suggest that the albumin is relatively firmly bound. Although albumin is present in extracellular membranes of normal and diseased human kidneys, the concentration is higher in diabetes mellitus. DIABETES *30*:843–846, October 1981.**

The relationship between the metabolic derangement of diabetes mellitus and the pathogenesis of microangiopathy remains obscure. In spite of numerous studies demonstrating an increase in thickness of muscle capillary basement membranes, the mechanism and relevance of these observations have been controversial and unexplained. Diabetic nephropathy, which develops after a number of years in patients with insulin-dependent diabetes, is characterized by progressive thickening of the glomerular and tubular basement membranes (GBM and TBM). Immunofluorescence studies of diabetic kidneys have demonstrated intense linear staining for human albumin and IgG in GBM, TBM, and Bowman's capsule.[1–3] This staining is greater than that observed in normal kidneys or kidneys with nondiabetic diseases. In addition, increased immunofluorescence for albumin has also been observed in the capillary basement membranes of muscle and skin, as well as in the sarcolemmal basement membrane, a noncapillary structure.[4,5] Similar immunofluorescence changes develop in the basement membranes of normal kidneys transplanted into patients with diabetic vascular disease.[6] We report here that the concentration of albumin in diabetic GBM and TBM when measured by a double-antibody radioimmunoassay is higher than that observed in basement membranes obtained from normal kidneys or those injured by nondiabetic diseases. This finding appears to be specific for diabetes mellitus.

## MATERIALS AND METHODS

Kidneys were obtained at the time of nephrectomy for renal transplantation from eight patients with diabetic nephropathy and seven patients with other glomerular or vascular diseases (i.e., membranous glomerulopathy, arteriolar nephrosclerosis with malignant hypertension, membranoproliferative glomerulonephritis, polyarteritis, systemic lupus erythematosus, steroid-resistant nephrotic syndrome, and noncategorized chronic glomerulonephritis). Five of the seven patients with other diseases manifested nephrotic syndrome during the course of their disease. Normal kidneys were obtained from seven patients within 6 h of death (caused by trauma). The mean age (range) for patients with diabetic nephropathy was 28.9 (22–42) yr, for patients with other diseases 28.8 (12–47) yr, and for patients with normal kidneys 39.5 (29–51) yr. The kidneys were maintained at −20°C until thawed for isolation and preparation of GBM and TBM.

Glomeruli and tubules were isolated from the cortex of the kidney by sieving sequentially through stainless-steel screens (100-, 150-, 200-, 250-, and 325-mesh) according to

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previously described methods.[7] Glomeruli were trapped on the surface of the 100- and 150-mesh screens whereas tubules were present on the 250- and 325-mesh screens. The isolation procedures for preparation of glomeruli and tubules, as well as for GBM and TBM, were monitored by phase-contrast microscopy. Sonication of glomerular and tubular preparations was carried out at 4°C in 1.0 M sodium chloride. The GBM and TBM were recovered by centrifugation at 121 g, washed in 0.15 M sodium chloride and distilled water, and lyophilized.[7]

The amount of albumin in GBM and TBM was determined in triplicate by a double-antibody radioimmunoassay[8–10] on collagenase-digested basement membrane. The immunoassay, carried out in ovalbumin buffer (0.15 M sodium chloride, 0.01 M phosphate, pH 7.40; 5.0 g/L ovalbumin), utilized goat anti-human albumin in a dilution of 1:7600, and rabbit anti-goat IgG in a dilution of 1:50. Human albumin (Miles Laboratory, Elkhart, Indiana) was chromatographed on agarose (Bio-Gel A 1.5, Bio-Rad Laboratories, Richmond, California). The albumin peak was then concentrated and labeled with $Na^{125}I$, using lactoperoxidase bound to sepharose beads in a molar ratio of albumin/iodine/lactoperoxidase/hydrogen peroxide of 1/3/.01/3.6.[11,12]

Precisely weighed aliquots (approximately 200 μg) of TBM and GBM or known amounts of human albumin were incubated at 37°C in 0.6 ml ovalbumin buffer (0.1 M Tris, pH 7.40, 0.005 M calcium acetate, 5.0 g/L ovalbumin) containing 12.5 U of chromatographically pure collagenase (Advance Biofactures Corporation, Lynwood, New York) for 24 h. Additional collagenase (12.5 U in 0.1 ml) was added at 24 and 48 h and the incubation was terminated at 72 h. Sodium azide (0.04%) and toluene (0.01 ml/10 ml) were added to each incubation tube to discourage bacterial growth. When 500 ng of albumin was subjected to this enzymatic digestion on each of nine separate occasions, the recovery of human albumin was 514 ± 35.2 ng (m ± SD). This demonstrates the reproducibility of the assay system, as well as the lack of significant breakdown of albumin. Hydroxyproline (HYP) was measured according to a previously described method.[13]

Immunofluorescence studies were carried out on 4-μm frozen sections of a diabetic kidney after incubation at 37°C for 4 and 16 h in these solutions: 0.15 M sodium chloride containing 0.02 M phosphate, pH 7.40 (16.1 mS); 0.015 M sodium chloride containing 0.0002 M phosphate, pH 7.40 (1.75 mS); 1.0 M or 3.0 M sodium chloride (pH 7.40); 2.5 M potassium thiocyanate (pH 7.40); citric acid-disodium phosphate buffers at pH 7.40 (19.5 or 2.6 mS) or at pH 2.8 and 3.6 (0.79–7.1 mS).

## RESULTS

The concentration of albumin in diabetic TBM and GBM was significantly greater than that found in TBM and GBM from normal kidneys or kidneys with other diseases (Table 1 and Figure 1). No differences were observed in the concentrations of albumin in TBM and GBM between the normal and other disease group. The HYP content of GBM in the three groups was similar (normal 86.4 ± 11.6 μg/mg; diabetic 85.0 ± 19.4 μg/mg; other diseases 88 ± 12.5 μg/mg). The HYP content of TBM in diabetics (92.5 ± 26.9 μg/mg) was not significantly different from that observed in normal kidneys (90.4 ± 20.7 μg/mg) or those with other diseases (80.3 ± 16.8). Linear regression analysis demonstrated a significantly positive correlation between the concentration of albumin and that of HYP in diabetic TBM ($r = 0.82$), diabetic GBM ($r = 0.70$), and normal TBM ($r = 0.79$).

No elution of albumin as detected by immunofluorescence was observed after incubation of kidney sections in hypotonic or hypertonic sodium chloride buffers at 7.40 or in potassium thiocyanate. A decrease in albumin in TBM and GBM was observed only after incubation at pH 2.8 and 3.6.

## DISCUSSION

These studies show conclusively that renal diabetic basement membranes contain higher amounts of albumin than do extracellular membranes derived from normal kidneys or kidneys with other diseases, and reconfirm the immunohistochemical observations previously reported. Furthermore, these differences are apparent when expressed per unit basement membrane weight of HYP content. The relevance of the highly significant correlation between albumin and HYP concentrations in diabetic GBM and TBM and normal TBM is unknown but may indicate a specific interaction with a particular glycoprotein or collagen moiety. The recent demonstration of an array of glycoprotein and collagen antigens within renal extracellular basement membranes indi-

TABLE 1
Concentration of albumin in tubular basement membrane (TBM) and glomerular basement membrane (GBM)

| | Albumin in TBM | | Albumin in GBM | |
|---|---|---|---|---|
| Patient groups | μg/mg TBM (m ± SD)† | μg/mg HYP* (m ± SD) | μg/mg GBM (m ± SD) | μg/mg HYP (m ± SD) |
| Diabetic nephropathy | 2.75 ± 0.34 (8)‡ | 4.07 ± 0.71 (8) | 2.25 ± 0.59 (7) | 3.51 ± 0.77 (7) |
| Other diseases | 1.00 ± 0.24 (7) | 1.82 ± 0.67 (6) | 1.22 ± 0.55 (6) | 1.88 ± 0.95 (6) |
| Normal | 1.21 ± 0.26 (7) | 1.79 ± 0.28 (7) | 1.31 ± 0.36 (7) | 1.98 ± 0.59 (7) |
| Statistical analysis (Student's *t* test) | | | | |
| Diabetic vs. other diseases | $P < 0.0001$ | $P < 0.0001$ | $P < 0.005$ | $P < 0.005$ |
| Diabetic vs. normal | $P < 0.0001$ | $P < 0.0001$ | $P < 0.01$ | $P < 0.001$ |
| Other diseases vs. normal | NS§ | NS | NS | NS |

* HYP, hydroxyproline.
† m ± SD, mean ± standard deviation.
‡ Number of patients indicated in parenthesis.
§ NS, not significant.

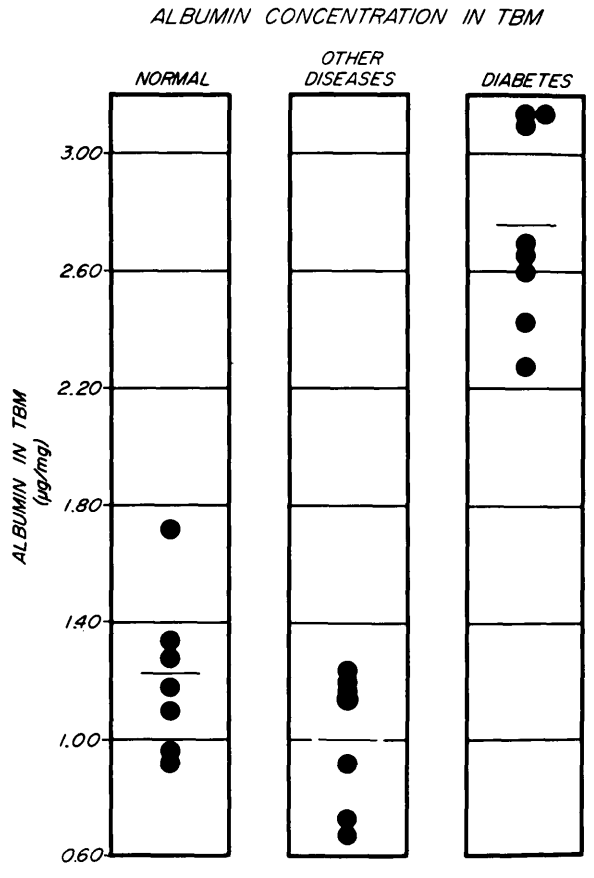


**FIGURE 1. Distribution of values for the concentration of albumin in TBM in normal individuals and in patients with diabetic nephropathy and other diseases.**

cates a striking antigenic heterogeneity not previously appreciated.[14–16] The basement membrane preparations were derived by sonic disruption of glomeruli and tubules or exposing these tissues to significant disruptive forces. Whether or not this technique results in loss of more weakly bound albumin or alters the content of albumin is not known. Comparative studies on basement membranes isolated by detergents would be of interest, although the effect of this procedure on the detection of trapped or bound protein antigens such as albumin is unknown.

The basement membranes obtained from normal kidneys or kidneys with other diseases also contain albumin, but in a concentration that was significantly less than that observed in diabetes mellitus. It is unknown whether the mechanism of albumin binding to these membranes is similar but simply exaggerated in diabetes mellitus. It is unlikely that albuminuria, as a consequence of increased glomerular permeability, played a role in these observations. First, the basement membrane preparations from seven patients with other diseases were intentionally selected because of glomerular disease. All seven had proteinuria and in five the nephrotic syndrome was a prominent part of the clinical history. Second, the changes described were more marked in diabetic TBM, a structure not known to be primarily involved in diseases associated with albuminuria and the nephrotic state. Third, immunohistochemical studies in a variety of other diseases associated with the nephrotic syndrome, including membranoproliferative glomerulonephritis and idiopathic nephrotic syndrome, have failed to demonstrate the characteristic changes in GBM and TBM observed in diabetic nephropathy.[2] And fourth, a number of nonrenal basement membrane structures, including those of skeletal muscle and skin, have demonstrated similar immunohistochemical changes in diabetes mellitus.[4,5]

The nature of the highly specific increase in albumin in diabetic GBM and TBM is unknown. The affinity is relatively strong, and this is suggested by the persistence of albumin after the rigorous isolation technique described above as well as the elution studies carried out on 4-$\mu$m unfixed, frozen sections of diabetic kidney. The decrease in immunofluorescence for albumin observed only after its exposure to low pH buffers must be interpreted carefully since incubation of kidney sections at low pH has resulted in loss of normal components, including the glomerular polyanion and epithelial cell C3b receptor.[17] The significance and specificity of this pH-dependent loss must await further investigation.

Previous studies on the biochemical composition of GBM from diabetic kidneys compared to normal ones have led to conflicting results, including an increase in hydroxylysine and glucosylgalatosyl hydroxylysine,[18,22] a decrease in cystine,[19–22] a decrease in sialic acid,[19–22] and an increase in glucose.[18,19,22] It is unknown whether the binding of albumin is a consequence of an intrinsic abnormality in a component of the basement membrane or a change in albumin itself. Recently, higher than normal concentrations of nonenzymatic-glycosylated albumin has been found in the plasma of diabetic patients.[23,24] In addition, there is preliminary evidence that the administration of glycosylated proteins to mice over a period of time leads to thickening of the GBM, although there is no evidence that the albumin became incorporated into the basement membrane in this model.[25] The recent demonstration of a slight increase in muscle membrane immunofluorescence for albumin in nondiabetic HLA-identical siblings of diabetic patients suggests that genetic factors may play a role in this phenomenon.[26] Further studies are needed to resolve the nature of the interaction between albumin and basement membranes, and the relationship of this phenomenon to the metabolic abnormality and the genetics of diabetes mellitus.

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