Glomerulopathy in Spontaneously Diabetic Rat

Impact of Glycemic Control

ANDREW J. COHEN, PAUL D. McGILL, RONALD G. ROSSETTI, DENNIS L. GUBERSKI, AND ARTHUR A. LIKE

SUMMARY

The spontaneously diabetic BioBreeding/Worcester (BB/W) rat was used to examine the role of glycemic control in the pathogenesis of diabetic glomerulopathy and proteinuria. Nondiabetic BB/W rats (group 1) were compared with moderately (group 2) and severely (group 3) hyperglycemic diabetic animals of similar age. Urinary protein excretion and morphometric measurements of glomerular basement membrane (GBM) width and mesangial area were performed after 4, 8, and 12 mo of study.

At 4 mo, urinary protein excretion both in group 2 (9.3 ± 0.7 mg/24 h) and group 3 (24.8 ± 1.98 mg/24 h) exceeded that in group 1 (5.4 ± 0.6 mg/24 h; $P < .05$). Moreover, proteinuria in group 3 was significantly greater than in group 2 ($P < .05$). In addition, proteinuria increased in group 3 animals between 4 and 12 mo of study but did not advance in groups 1 or 2.

GBM width in both diabetic groups (168.8 ± 2.4 and 165.7 ± 2.2 nm, groups 2 and 3, respectively) exceeded that in group 1 (148.3 ± 3.8 nm; $P < .01$) by 4 mo. At 12 mo, severely hyperglycemic group 3 animals had significantly greater GBM thickening than group 2. GBM width increased in all three groups over the course of study, but the rate of growth did not differ between groups 1 and 2. However, the rate of growth in group 3 was greater than in either group 1 or group 2. Urinary protein excretion correlated significantly with GBM width in diabetic rats. No differences in proportional mesangial area or the proportional area made up of mesangial matrix or cytoplasm could be found among the three groups.

In the spontaneously diabetic BB/W rat, poor glycemic control contributes to both proteinuria and GBM widening but apparently does not cause mesangial expansion. Proteinuria and GBM widening may either be interdependent or related to a common third factor in the diabetic milieu. *Diabetes* 36:944–51, 1987

From the Departments of Medicine and Pathology, University of Massachusetts Medical School, Worcester, Massachusetts.

Address correspondence and reprint requests to Andrew J. Cohen, MD, Division of Renal Medicine, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01605.

Received for publication 29 May 1986 and accepted in revised form 11 February 1987.

Nephropathy in insulin-dependent diabetes mellitus (IDDM) is signaled by the appearance of proteinuria attended by morphological changes including mesangial expansion and glomerular basement membrane (GBM) thickening. Pathogenetic mechanisms linked to diabetic glomerulopathy include a direct effect of hyperglycemia (1) and renal vasodilation associated with intraglomerular hypertension (2,3).

Most studies of experimental diabetic nephropathy have, however, relied on the islet cell toxins alloxan or streptozocin (STZ). These agents are also nephrotoxins (4,5) and therefore have the potential to confound subsequent nephropathological changes.

The BioBreeding/Worcester (BB/W) rat develops spontaneous autoimmune insulitis, insulinopenia, and hyperglycemia, mimicking IDDM (6,7) and thus is a potential model of diabetic nephropathy free of these confounding factors. Our study examines the impact of glycemic control on the occurrence of proteinuria and morphometrically measured glomerular changes in this model of spontaneous IDDM.

MATERIALS AND METHODS

Only BB/W rats bred and housed at the University of Massachusetts Medical School were used. BB/W rats after age 60 days were tested for glycosuria three times weekly. The appearance of glycosuria, as indicated by a 2+ result by Testape (Lilly, Indianapolis, IN), and hyperglycemia, with plasma glucose >180 mg/dl, signaled the onset of diabetes. A total of 54 animals entered the study between 75 and 85 days of age and were housed in metabolic cages. All animals were fed standard Purina rat chow.

Eighteen nondiabetic animals (group 1) were normoglycemic BB/W rats, including those selected from a "dia-

betes-resistant" (incidence of diabetes <1%) strain. Diabetic animals were randomly divided into two groups. Group 2 (n = 18), a moderately hyperglycemic group, received daily subcutaneous injections of 2.0–3.2 U of protamine zinc insulin (PZI, Lilly) and had 24-h urinary volumes <25 ml. Group 3 (n = 18), severely hyperglycemic animals, received less daily PZI (0.4–2.0 U s.c.), sufficient to prevent ketonuria but with extreme polyuria (>25 mg/24 h).

At intervals of 4, 8, and 12 mo of study, blood glucose, 24-h urinary glucose, and protein excretion were determined for at least 3 wk consecutively and averaged for each animal. Protein was measured in trichloroacetate precipitates of urine by the dye-binding method (8). Agarose gel electrophoresis was performed on selected urine samples. Urinary and blood glucose levels were determined with a Beckman glucose analyzer.

At 4, 8, and 12 mo of study, animals were randomly selected from each group to be killed. Kidneys were fixed by perfusion with paraformaldehyde in phosphate buffer at systemic arterial pressure (mean pressure 90–100 mmHg) via left ventricular puncture. Kidneys were quickly removed, and the cortices were minced and fixed for 2 h. After postfixation in osmium tetroxide and dehydration, tissues were embedded in Epon-araldite. One-micrometer sections were examined for the selection of glomeruli for thin sectioning. Ultrastructure was studied in a JEM 100S electron microscope without knowledge of the age or physiologic status of the animal (9,10).

Morphometric determinations were performed as described by others (9,10). Briefly, sections were photographed at magnifications between ×19,000 and ×21,000 and standardized with a calibration grid. GBM width was determined by measurement of intersections with grid lines. At least 100 measurements were performed in each of two glomeruli per kidney. The true mean was calculated from the harmonic mean of the determinations and multiplied by the magnification factor to generate the true basement membrane width.

Fractional volumes of mesangial components were determined by use of a 120-point grid, counting the proportion of points that superimposed on either mesangial matrix or mesangial cytoplasm. The reference volume included the glomerular tuft but not the urinary space. At least 1900 points were counted on each of two glomeruli per kidney. The mean of the resulting determinations expresses the percentage of mesangial components and a proportion of total glomerular volume (excluding urinary space).

Statistical comparisons between groups were carried out by two-way analysis of variance, and individual pairs were compared by Newman-Keuls and Bonferroni's least significant difference tests with adjustment to compensate for additive type I errors. Linear regression analysis was applied where appropriate.

RESULTS

Metabolic parameters. Group 1 rats demonstrated mean blood glucose between 94 and 112 mg/dl as shown in Fig. 1. Both diabetic groups were hyperglycemic. However, group 2 animals were moderately hyperglycemic (mean blood glucose range 228–337 mg/dl), and group 3 animals were severely hyperglycemic (mean blood glucose range 391–463 mg/dl) (Fig. 1).

Nondiabetic BB/W rats excreted small daily urinary volumes and negligible glucose (Table 1). Both diabetic groups were polyuric; however, group 2 animals exhibited a 3-fold increase over group 1, whereas group 3 animals showed a 15-fold increase in urinary volume.

Similarly, both diabetic groups displayed glycosuria. Group 3 animals, however, had six times as much glycosuria as group 2, indicating considerably less daily glycemic control.

As demonstrated in Fig. 2, both groups of diabetic rats achieved similar growth rates but lower steady-state body weights than group 1. Body weights of group 3 animals were significantly lower than those of group 1 or group 2 (F = 14.6, P < .02).

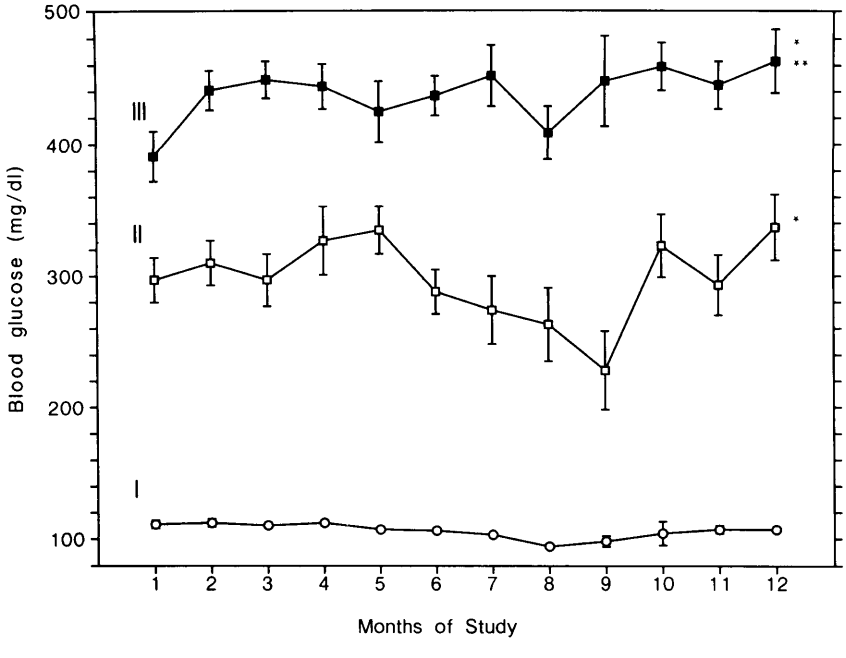

FIG. 1. Monthly blood glucose levels in 3 groups of BB/W rats. Group 1, nondiabetic rats; group 2, well-controlled diabetic rats; group 3, poorly controlled diabetic rats. *P < .02 vs. group 1 at each month studied; **P < .02 vs. group 2 at each month studied.

TABLE 1
Urinary volume and glucose in BB/W rats

	Nondiabetic (group 1)	Diabetic	
		Well controlled (group 2)	Poorly controlled (group 3)
Volume (ml/day)	4.2 ± 0.4	12.8 ± 1.2	62.7 ± 6.0
Range	2.1–6.3	5.3–20.1	34.8–51.0
Glucose (mg/day)	5 ± 1	1149 ± 86	6731 ± 672
Range	4–7	818–1668	3863–8754

n = 18 in each group.

Urinary protein excretion. By 4 mo of study, increased urinary protein excretion was evident in group 2 (9.3 ± 0.7 mg/24 h) and group 3 (24.8 ± 1.8 mg/24 h) compared with group 1 (5.4 ± 0.6 mg/24 h) (Fig. 3). Furthermore, Fig. 1 also reveals that group 2 and group 3 had significantly greater urinary protein excretion than group 1 ($P < .02$), and group 3 had significantly greater proteinuria than group 2 ($P < .05$). Similarly, at 8 and 12 mo of study, both diabetic groups displayed significant proteinuria, and group 3 demonstrated greater proteinuria than group 2 in each instance.

Figure 3 also reveals that, although the level of urinary protein excretion increased progressively in group 3 rats during the course of the study, this parameter did not increase in groups 1 and 2. Analysis of variance showed a significant effect of duration of study on urinary protein excretion in the group 3 rats ($F = 3.37$, $P = .04$).

Agarose gel electrophoresis of the urinary protein excreted by diabetic animals revealed a variable electrophoretic mobility. Between 9 and 20% of the excreted protein had the electrophoretic pattern of albumin. The remainder was made up of a broad range of both α- and β-globulins. Nondiabetic animals showed negligible urinary albumin.

Morphometric studies of GBM thickness. Diabetes caused a substantial effect on GBM morphology in BB/W rats. As shown in Fig. 4, at 4 mo of study, GBM thickness was significantly greater in diabetic groups 2 (168.8 ± 2.4 nm) and 3 (165.7 ± 2.2 nm) than in group 1 (148.3 ± 3.8 nm). At 8 mo of study, a continuation of the same pattern was observed: both diabetic groups displayed greater GBM thickness than group 1. Although mean GBM thickness in group 3 exceeded that of group 2 by 8 mo, this difference did not achieve statistical significance. By 12 mo, however, GBM thickness of both diabetic groups exceeded that of group 1, and group 3 animals displayed significantly greater GBM thickening than group 2 animals ($P < .001$).

Linear regression analysis (data not shown) showed a significant correlation between age and GBM thickness in group 1 ($r = .81$, $P = .00004$), group 2 ($r = .86$, $P < .00001$), and group 3 ($r = .93$, $P < .00001$) animals. Hence, increasing age led to increasing GBM width in all three groups ($F = 4.29$, $P < .00001$).

Figure 5 shows the relationship between the duration of study and GBM width in the three groups of BB/W rats. GBM thickness increased in all groups. The slopes of groups 1 and 2, however, were identical, indicating that the rate of GBM thickening was similar in these two groups. Group 3 animals, on the other hand, displayed a faster rate of thickening than either of the other groups ($P < .0001$).

The relationship between GBM width and urinary protein excretion in diabetic animals is shown in Fig. 6. Pooled data from groups 2 and 3 demonstrate that GBM thickness correlated significantly with the level of proteinuria. There was

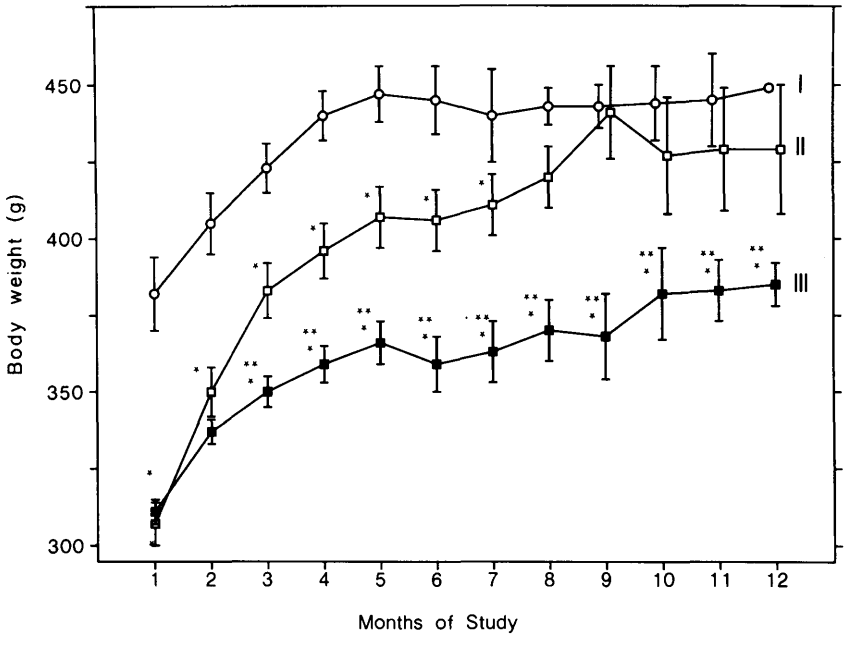

FIG. 2. Monthly body weights in 3 groups of BB/W rats. See Fig. 1 for explanation of groups. *$P <$ .02 vs. group 1; **$P <$ .02 vs. group 2.

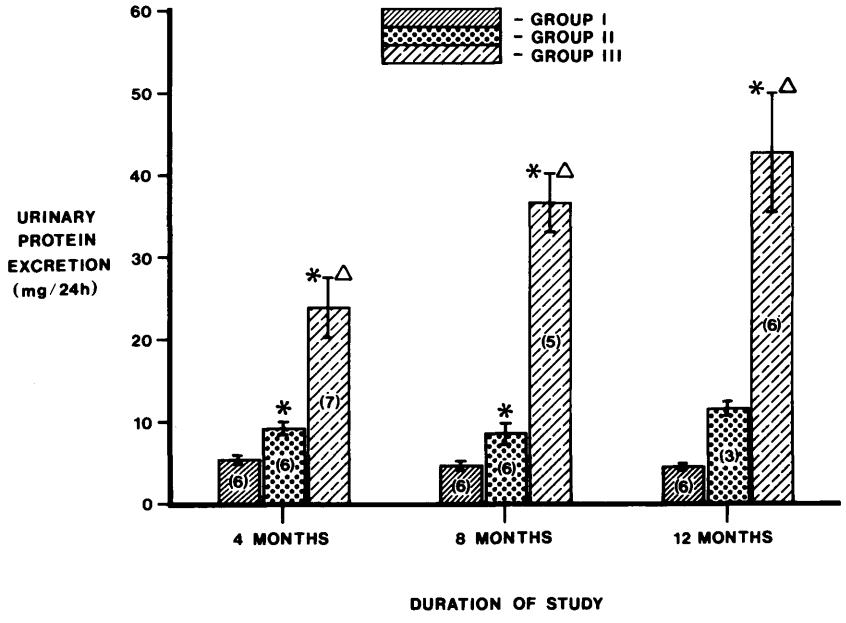

FIG. 3. Influence of glycemic control on urinary protein excretion in BB/W rats. Significant effects of both glycemic control ($F = 9.79$, $P < .0001$) and duration of study ($F = 5.01$, $P = .01$) were found by ANOVA. See Fig. 1 for explanation of groups. *$P < .02$ vs. group 1; $\Delta P < .05$ vs. group 2.

no significant correlation between GBM width and urinary protein excretion in group 1 animals (data not shown).

Mesangial components. There were no differences in total mesangial area among the experimental groups at the three observation time points (Fig. 7). Furthermore, there were no differences in the mesangial components, mesangial cytoplasmic area, or mesangial matrix (data not shown). Finally, there was no correlation between the proportion of any mesangial component and urinary protein excretion.

DISCUSSION

Experimental diabetic glomerulopathy has been characterized by GBM thickening and mesangial widening, both features shared by human diabetic glomerular disease. In previous experimental studies, diabetes was induced by STZ or alloxan. Both drugs have been associated with renal lesions, including those glomerular changes described above (4,5). STZ also produces characteristic functional alterations of early diabetes, including glomerular hyperperfusion, glomerular hyperfiltration (11), and proteinuria (12). Both agents also produce tubular lesions (4,5). STZ in particular induces proximal tubular vacuolization, glycogen accumulation, mitochondrial disruption, and myeloid body accumulation (5). In human studies, subdiabetogenic doses of STZ cause tubular dysfunction and renal failure (13). The described renal functional abnormalities and some of the glomerular morphological changes may be secondary to tubular aberrations that are not part of the diabetic syndrome. Alterations in tubular function, for example, might lead to perturbations in tubuloglomerular feedback, with subsequent renal vasodilation, intraglomerular hypertension, and hyperfiltration. Intraglomerular hemodynamic disturbances,

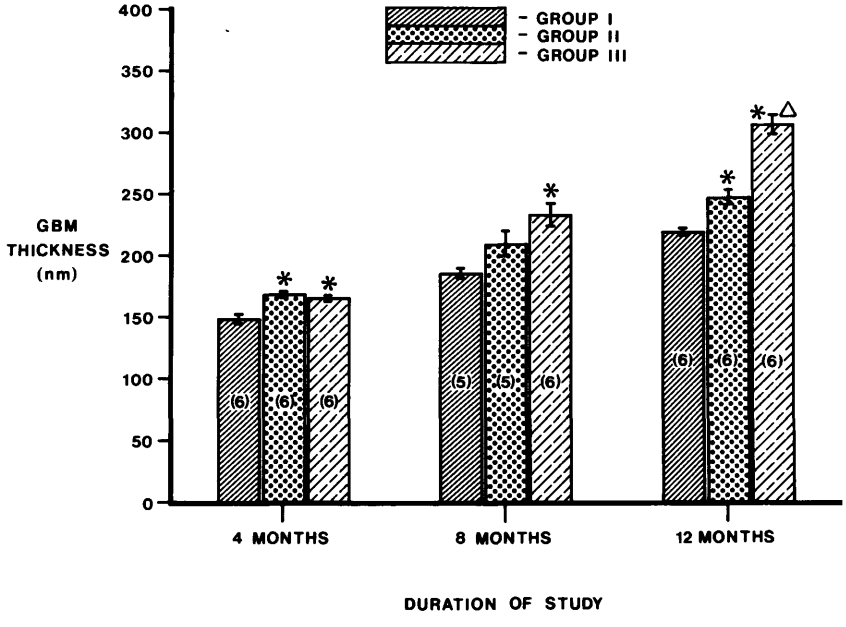

FIG. 4. Influence of glycemic control on glomerular basement membrane (GBM) thickness in BB/W rats. Significant effect of glycemic control ($F = 4.74$, $P = .01$) was seen by 2-way ANOVA. See Fig. 1 for explanation of groups. *$P < .01$ vs. group 1; $\Delta P < .001$ vs. group 2.

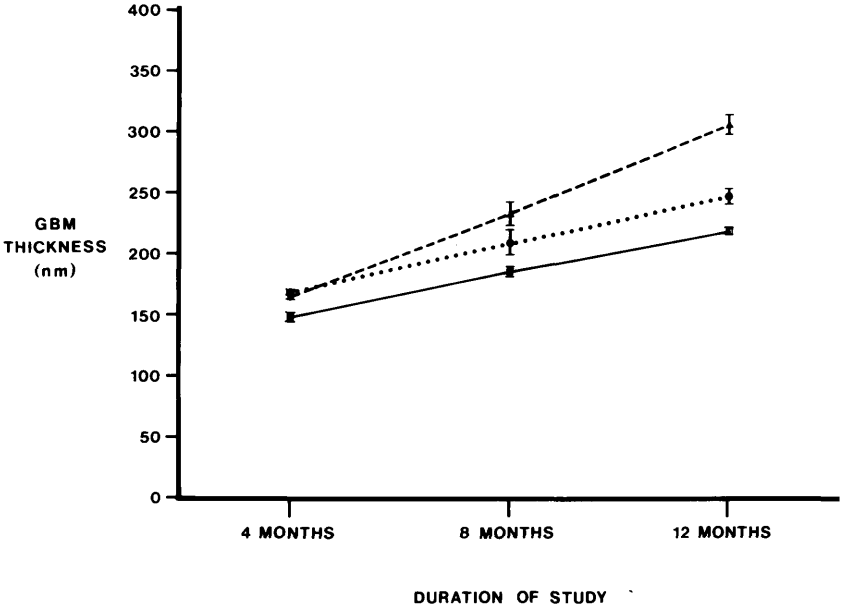

FIG. 5. Influence of glycemic control on glomerular basement membrane (GBM) growth in BB/W rats. Growth rate (slope of line) was significantly different in group 3 (*dashed line*) animals from either group 1 (*solid line*) or group 2 (*dotted line*) animals ($P < .0001$). See Fig. 1 for explanation of groups.

particularly elevated capillary hydraulic pressure, may be the predominant etiologic factor in glomerulosclerosis (14). Hence, alterations in nephron structure and function caused by STZ (but not diabetes) may synergize with the diabetic milieu to cause the characteristic lesions of experimental diabetic nephropathy.

This study was designed to examine the functional and morphological changes of diabetes in a spontaneously diabetic model, the BB/W rat. Because this model closely resembles IDDM, it is postulated that the morphology and pathogenesis of the renal lesions in this animal might resemble that observed in human disease. Moreover, the avoidance of STZ or alloxan obviates their potential direct effects on renal function and histology. The impact of glycemic control was studied by examining two groups of insulin-treated diabetic animals with moderate and severe hyperglycemia and glycosuria.

Our findings confirm earlier studies that proteinuria corresponds to the degree of glycemic control (16–18). Previously published work in STZ-induced diabetes (STZ-D) showed that protein excretion increased within 5 days of the onset of hyperglycemia and glycosuria and that insulin therapy promptly reduced urinary protein excretion (18). In our study, both groups of diabetic animals were insulin treated,

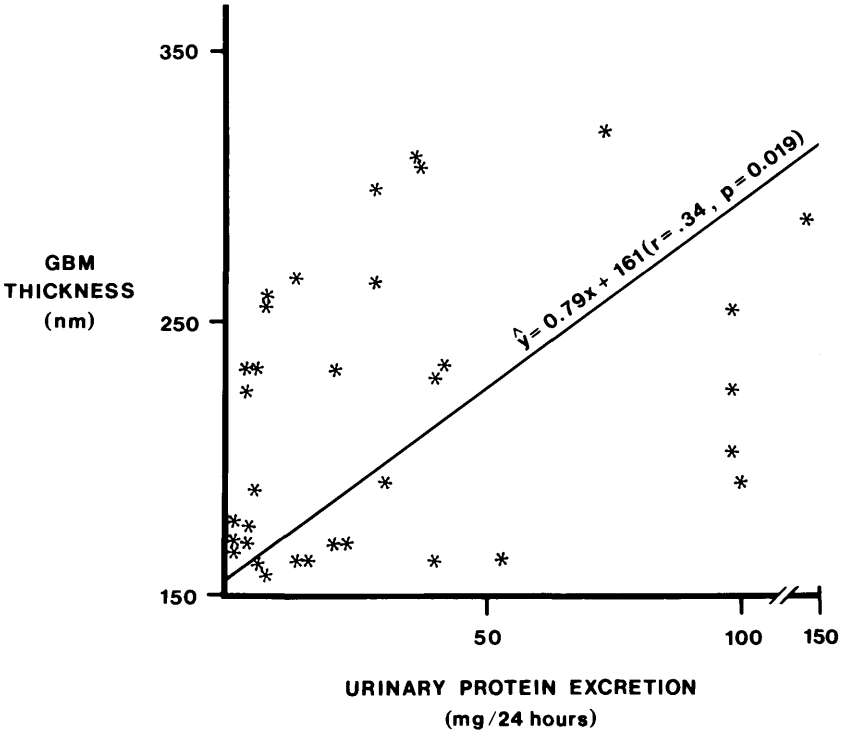

FIG. 6. Relationship of glomerular basement membrane (GBM) thickness and urinary protein excretion in diabetic BB/W rats. SEs for linear regression were: *y*, ±0.2; *a*, ±8; and *b*, ±30.

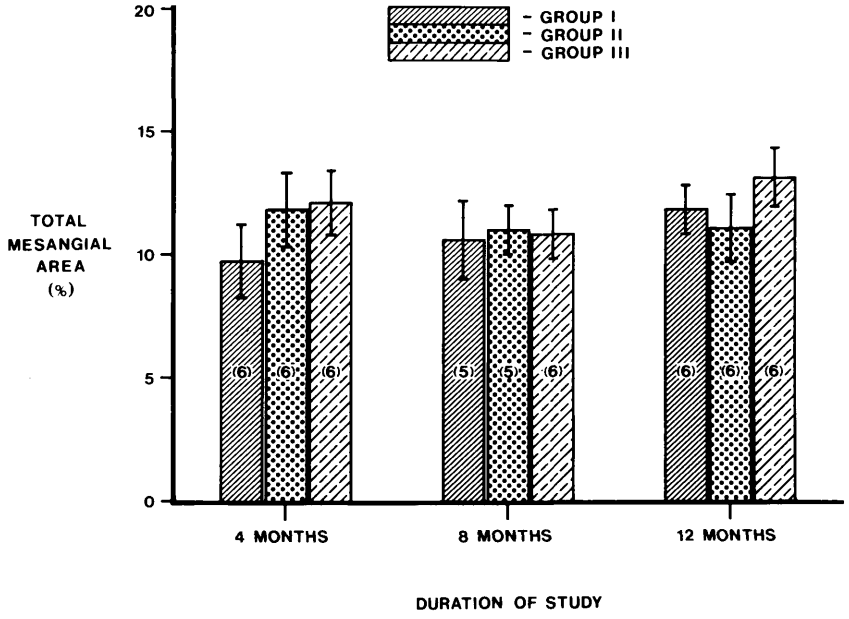

FIG. 7. Influence of glycemic control on total mesangial area in BB/W rats. See Fig. 1 for explanation of groups.

but those receiving larger doses had less urinary volume and glycosuria and diminished proteinuria.

It is perhaps surprising that group 1 rats showed negligible proteinuria, because many nondiabetic rodent species exhibit progressive protein excretion (19–21). We cannot explain the absence of proteinuria in the nondiabetic BB/W rat. However, we postulate that this strain may be genetically predisposed to resist those environmental factors, e.g., dietary protein (2) and immune complex formation (21), which apparently contribute to both age-associated proteinuria and glomerulosclerosis in the rodent. An alternate explanation is that the BB/W rat is housed behind a pathogen-free barrier that may lessen the likelihood of environmental exposure to antigens and the subsequent formation of immune deposits.

Considerable attention has been focused on the nature of the urinary protein excretion in experimental diabetes. Pennell and Meinking (22) have demonstrated that after induction of diabetes, a small increase in albuminuria is seen, accompanied by the appearance of numerous new protein species with a broad range in molecular weight. We noted similar findings by agarose gel electrophoresis. These urinary proteins may be excreted as a result of either alterations in the glomerular barrier to macromolecules or changes in tubular reabsorption of proteins. A recent study indicates that osmotic diuresis per se does not lead to proteinuria. Whereas the urinary excretion of the lysosomal hydrolase *N*-acetylglucosaminidase (NAG) has been noted to increase in diabetes and during artificially induced hyperglycemia, an equipotent osmotic diuresis induced by mannitol failed to elicit an increase in urinary NAG excretion (23). Hence, an alteration in either glomerular sieving or tubular handling of macromolecules is apparently induced by hyperglycemia and/or glycosuria. It has been postulated that glycosylation of plasma proteins (24) or GBM proteins might account for these phenomena (25). Because we did not measure urinary albumin excretion, a more precise marker of glomerular macromolecule sieving, the source(s) of the urinary proteins is not clear. However, because 10–20% of the urinary protein has the electrophoretic mobility of albumin, we postulate that at least part of the proteinuria is glomerular in origin (negligible albumin was found in nondiabetic rat urine).

Brown et al. (26) have previously shown that the diabetic BB rat develops GBM widening. Our study confirms and extends these findings by demonstrating that GBM width was a function of the degree of hyperglycemia. Differences between moderately and severely hyperglycemic groups were apparent by 12 mo of study. An increase in GBM width in group 1 over the course of study suggests that some GBM growth is due to aging in the BB/W rat. However, the rate of GBM growth in moderately hyperglycemic animals paralleled that of group 1, whereas severely hyperglycemic rats showed accelerated GBM widening. Hence, more aggressive insulin therapy may restore GBM growth rates toward that seen in nondiabetic animals. Other studies of STZ-D demonstrated that insulin therapy (27) or treatment with an oral hypoglycemic agent (28) ameliorates or prevents the development of GBM thickening. In contrast, islet cell transplantation into STZ-D rats fails to reduce GBM thickening, although it does reverse mesangial widening.

A significant correlation between urinary protein excretion and GBM width was observed in this study. This suggests the two are either interdependent or are related to a common third factor in the diabetic milieu.

Mesangial cell and matrix volumes were not increased in diabetic BB/W rats. This finding contrasts with STZ-D rats in which mesangiopathy is usually observed (5,9). In their study of diabetic BB rats, Brown et al. (26) also found no evidence of mesangial widening, even after 30 wk of diabetes. Because the diabetic BB rats did not exhibit albuminuria in their study, the authors postulated a linkage between excessive urinary albumin excretion and mesangial widening. They also suggested that because GBM thickening appeared in the absence of albuminuria, independent mechanisms were responsible for the mesangial and GBM alterations (26).

Other work by these authors suggests an alternative ex-

planation: physiologic factors may be responsible for mesangial expansion. Uninephrectomy (29) or two-kidney Goldblatt hypertension (in the unclipped kidney) (30) accelerates both mesangial widening and albuminuria in STZ-D rats. Brenner and co-workers (2,3,24) have advanced the theory that after reductions in nephron mass, subsequent renal vasodilation and intraglomerular hypertension and hyperfiltration lead to mesangial widening, albuminuria, and glomerulosclerosis. Hence, mesangial expansion is a final common pathway in many renal diseases and is not a pathological change unique to diabetic glomerulopathy.

Why do diabetic BB rats lack mesangial expansion? The answer is not immediately apparent but may be related to hemodynamic factors. The BB rat may not develop the degree of intraglomerular hyperperfusion, hyperfiltration, and hypertension seen in other diabetic models. Previous whole-animal studies of diabetic BB rats have reported moderate (26) or no (32) glomerular hyperfiltration.

Brown et al. (26) found that both glomerular filtration rate and renal blood flow increased in diabetic BB rats compared with nondiabetic controls. However, these authors normalized GFR and renal blood flow for body weight, which, as in our study, was lower in the diabetic group. Had they normalized these parameters for kidney weight, comparable values would be found in both groups, as was seen in our study (32). In contrast, Hostetter et al. (11) found elevated whole-kidney GFR (factored for kidney weight) in STZ-D rats.

Combined, these data suggest that severe intraglomerular hypertension, as seen in the STZ-D rat, correlates with severe "mesangiopathy." This is also observed indirectly in studies of human diabetes. Mauer et al. (33) reported a significant relationship between systemic hypertension and mesangial expansion in renal biopsy specimens from human diabetics. By being transmitted to the glomerular capillary, systemic hypertension may contribute to intraglomerular hypertension and thereby lead to mesangial expansion and glomerulosclerosis (14). The absence of mesangial expansion might therefore imply the absence of intraglomerular hypertension in the BB/W diabetic rat.

Direct measurement of microcirculatory hydraulic pressures by micropuncture has not been performed in the BB/W rat. This information would help determine whether this experimental model of diabetes exhibits a pattern of glomerular capillary hydraulics similar to or different from that seen in STZ-D.

Because glomeruli from STZ-D rats demonstrate increased prostaglandin synthesis, it has been presumed that the vasodilation and glomerular hypertension result from this abnormality (34). A recent study measuring glomerular prostaglandin production from these two models of diabetes, however, demonstrated enhanced synthesis from STZ-D but not BB diabetic rats (35). Hence, BB diabetic rats may not possess the same autocoid and hemodynamic abnormalities seen in STZ-D.

ACKNOWLEDGMENTS

We gratefully acknowledge the secretarial help of Judith Konan and Joan Michael.

This study was supported by NIH Grants AM-19155 and 1P30-AM-32520.

REFERENCES

1. Mauer SM, Steffes MW, Brown DM: The kidney in diabetes. *Am J Med* 70:603–12, 1981
2. Hostetter TH, Rennke HG, Brenner BM: The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. *Am J Med* 72:375–80, 1982
3. Zatz R, Meyer PW, Rennke HG, Brenner BM: Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. *Proc Natl Acad Sci USA* 82:5963–67, 1985
4. Bailey CC, Bailey OT, Leech RS: Alloxan diabetes with diabetic complications. *N Engl J Med* 230:533–36, 1944
5. Arison RN, Ciaccio EI, Glitzer MS, Cassaro JA, Pruss MP: Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. *Diabetes* 16:51–56, 1967
6. Nakhooda AF, Like AA, Chappel CI, Murray FT, Marliss EB: The spontaneously diabetic Wistar rat: metabolic and morphologic studies. *Diabetes* 26:100–12, 1977
7. Rossini AA, Faustman D, Woda BA, Like AA, Szymanski I, Mordes JP: Lymphocyte transfusions prevent diabetes in the Bio-Breeding/Worcester rat. *J Clin Invest* 74:39–46, 1984
8. Bradford M: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–54, 1976
9. Steffes M, Barbosa J, Basgen JM, Sutherland DER, Najarian JS, Mauer SM: Quantitative glomerular morphology of the normal human kidney. *Lab Invest* 49:82–86, 1983
10. Osterby R: Early phases in the development of diabetic glomerulosclerosis. *Acta Med Scand Suppl* 574:1–80, 1975
11. Hostetter TH, Troy JL, Brenner BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19:410–15, 1981
12. Weil R, Nozawa M, Koss M, Weber C, Reemstma K, McIntosh R: The kidney in streptozotocin diabetic rats. *Arch Pathol Lab Med* 100:37–49, 1976
13. Sadoff L: Nephrotoxicity of streptozotocin. *Cancer Chemother* 54:457–59, 1970
14. Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke H, Brenner BM: Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J Clin Invest* 77:1925–30, 1986
15. Mauer SM, Brown DM, Matas AJ, Steffes MW: Effects of pancreatic islet transplantation on the increased urinary albumin excretion rates in intact and uninephrectomized rats with diabetes mellitus. *Diabetes* 27:959–64, 1978
16. Selawry HP, Pennell JP, Pardo V, Alzamora DB, Millard MM: The effects of cultured maintained pancreatic islets on metabolic parameters, renal function, and glomerular lesions in the diabetic rat. *Metabolism* 29:261–69, 1980
17. Weber CJ, Silva FG, Hardy MA, Pirani CC, Reemstma K: Effects of islet transplantation on renal function and morphology of short-and long-term diabetic rats. *Transplant Proc* 9:549–56, 1979
18. Pennell JP, Millard MM, Ashby MH: Proteinuria in the acutely diabetic rat and its response to insulin treatment. *Diabetologia* 21:54–57, 1981
19. Couser WG, Stilmant MM: Mesangial lesions and focal glomerular sclerosis in the aging rat. *Lab Invest* 33:491–501, 1975
20. Elema JD, Arends A: Focal and segmental glomerular hyalinosis and sclerosis in the rat. *Lab Invest* 33:545–61, 1975
21. Linder E, Pasternack A, Edgington TS: Pathology and immunology of age-associated disease of mice and evidence of analogous immune complex pathogenesis of the associated renal disease. *Clin Immunol Immunopathol* 1:104–21, 1972
22. Pennell JP, Meinking TL: Pattern of urinary proteins in experimental diabetes. *Kidney Int* 21:709–13, 1982
23. Rowe JR, Brouhard BH, Dunn JK, LaGrone L: Response of urinary *n*-acetyl-beta-D-glucosaminidase to two osmotic diuretics in the dog (Abstract). *Kidney Int* 27:151, 1985
24. Cohen MP, Urdanivia E, Surma M, Wu VY: Increased glycosylation of glomerular basement membrane collagen in diabetes. *Biochem Biophys Res Commun* 91:498–501, 1979
25. Day JR, Thorpe SR, Baynes JW: Nonenzymatically glycosylated albumin: in vitro preparation and isolation from normal human serum. *J Biol Chem* 254:595–97, 1979
26. Brown DM, Steffes MW, Thibert P, Azar S, Mauer SM: Glomerular manifestations of diabetes in the BB rat. *Metabolism* 32:131–35, 1983
27. Rasch R: Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment: glomerular basement membrane thickness. *Diabetologia* 16:319–24, 1979
28. Reddi AS, Oppermann W, Patel DG, Ehrenreich T, Camarini-Davalos RA: Diabetic microangiopathy in KK mice. III. Effect of prolonged glyburide treatment on glomerulosclerosis. *Exp Mol Pathol* 29:92–101, 1978
29. Steffes MW, Brown DM, Mauer SM: Diabetic glomerulopathy following unilateral nephrectomy in the rat. *Diabetes* 27:35–41, 1978
30. Mauer SM, Steffes MW, Azar S, Sandberg SK, Brown DM: The effects of Goldblatt hypertension on the development of the glomerular lesions of diabetes mellitus in the rat. *Diabetes* 27:738–44, 1978

31. Brenner BM, Meyer TW, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging renal ablation, and intrinsic renal disease. *N Engl J Med* 307:652–59, 1982
32. Cohen AJ, McCarthy DM, Rossetti RR: Renin secretion by the spontaneously diabetic rat. *Diabetes* 35:341–46, 1986
33. Mauer SN, Steffes MW, Ellis EN, Sunderland DER, Brown DM, Goetz FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1145–55, 1984
34. Schambelan M, Blake S, Sraer J, Bens M, Nivez M-P, Wahbe F: Increased prostaglandin production by glomeruli isolated from rats with streptozotocin-induced diabetes mellitus. *J Clin Invest* 75:404–12, 1985
35. Barnett R, Scharschmidt L, Schlondorff D: Comparison of glomerular and mesangial PGE_2 synthesis and glomerular contraction in two models of diabetes (Abstract). *Proc Am Soc Nephrol* 19:103A, 1986