The Effect of Chronic α-Glycosidase Inhibition on Diabetic Nephropathy in the *db/db* Mouse

STANLEY M. LEE

SUMMARY

Acarbose, a complex oligosaccharide, is a potent competitive inhibitor of sucrase and decreases postprandial hyperglycemia when administered with food. To evaluate its potential for metabolic control and prevention of diabetic nephropathy, groups of genetically diabetic mice (C57 BLKsJ *db/db*) were treated with Acarbose for 10 wk. Control mice received normal chow and experimental groups were given Acarbose prepared as a drug-food mixture in doses of 10, 20, and 40 mg/100 g of food. Acarbose did not influence fasting blood glucose, food intake, or the normal development of obesity in the mice. Urinary glucose excretion and glycosylated hemoglobin was significantly reduced in animals receiving high-dose Acarbose (40 mg/100 g food). Immunopathologic examination of the kidneys showed a dose-dependent decrease in glomerular mesangial immunoglobulin deposition. By light microscopy, glomerular mesangial thickening was significantly reduced in the group receiving high-dose Acarbose (40 mg/100 g food). To the extent that Acarbose improves metabolic control in the *db/db* mouse, chronic treatment with this agent produces a dose-dependent amelioration of diabetic nephropathy. Alphaglycosidase inhibition may be a useful adjunctive therapy for blood glucose control in diabetes mellitus. DIABETES 31:249–254, March 1982.

The *db/db* mouse provides a useful experimental model to study the complications of hyperinsulinemic diabetes mellitus.[1] These genetically diabetic animals are recognizable at the age of 1 mo by the appearance of obesity. Hyperphagia, enhanced metabolic efficiency, and progressive weight gain are characteristic features during the first 4 mo of life. Increased insulin secretion occurring at 10 days is the earliest identifiable metabolic abnormality,[2] and persists until 5–6 mo of age, at which time insulin levels decline in association with degenerative changes in the pancreatic islets.[3]

As a result of the diabetic state, these mice develop widespread pathologic abnormalities including a well-defined nephropathy.[4,5] In a previous study we showed that blood glucose could be controlled, and the renal lesions prevented, by carefully modulated diet restriction.[6] Pharmacologic control of the diabetic state has been difficult in these animals. Hyperinsulinemia and its resulting downregulation of insulin receptors makes them relatively resistant to the effects of exogenous insulin.[7,8] In many respects this mouse model resembles type II diabetes mellitus in humans.

It was therefore of interest to test the effects of Acarbose in the *db/db* mouse. This compound is a complex oligosaccharide (mol. wt. 645) originally discovered in culture broths of Actinomycetes. It is an alpha-glucosidase inhibitor with a special affinity for sucrase.[9] When administered orally with starch or sucrose, it effectively prevents sucrose digestion and decreases the postprandial rise of blood glucose.[10–12]

The present study was designed to evaluate the effectiveness of Acarbose in the *db/db* mouse during a 10-wk treatment period, with specific regard to blood glucose control and prevention of diabetic nephropathy.

MATERIALS AND METHODS

Animal model. C57 BL KsJ *db/db* mice were obtained from a breeding colony maintained at the University of Arizona Health Sciences Center. Animals were entered into the study at the age of 5–6 wk, a time when their characteristic early obesity was clearly recognizable. Equal numbers of both sexes were used.

Acarbose administration. Normal mouse chow (Lab-Blox, Wayne Laboratories) was pulverized to the consistency of a fine powder. Acarbose (provided by Miles Laboratories, New Haven, Connecticut) as a lyophilized powder, was thoroughly mixed with this mouse chow in several different concentrations, namely, 10, 20, and 40 mg/100 g. Control mouse chow was prepared in a similar fashion but without addition of Acarbose. The drug-chow powder

From the Department of Internal Medicine, University of Arizona, Tucson, Arizona 85724.
Address reprint requests to Stanley M. Lee, M.D., at the above address.
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was reconstituted into normal appearing pellets. The drug-food preparation was undertaken by ICN Nutritional Biochemicals (Cleveland, Ohio). Efficacy of the drug was assured under these preparative conditions for 6 wk. Fresh drug-food mixture was prepared every 6 wk. Because of its photosensitivity, the drug-food mixture was shielded from direct light during animal feeding by covering with aluminum foil.

Experimental protocol. Four groups of animals (three males and three females) were assigned. Group 1(C) controls received normal mouse chow without drug (N = 6). Group 2(A-10) received food containing Acarbose in a concentration of 10 mg/100 g (N = 6). Group 3(A-20) received food containing Acarbose in a concentration of 20 mg/100 g (N = 7). Group 4(A-40) received food containing Acarbose in a concentration of 40 mg/100 g (N = 6).

All groups were permitted free access to the food and water throughout the study. The duration of the study period was 10 wk. Animals were weighed on a weekly basis, and weekly food and water consumption was measured.

Fasting blood glucose (overnight fast) was obtained at weeks 4 and 8, and at the conclusion of the study. Animals were bled by capillary puncture of the retro-orbital venous plexus without anesthesia. Glucose was measured on a Beckman glucose analyzer (Beckman Instruments, Fullerton, California).

At the conclusion of the study, the heart, liver, and kidneys were weighed, and the kidney examined by light and immunofluorescent microscopy. All histologic samples were examined in a single-blind fashion, with the identity of tissues unknown to the observer.

To provide additional data regarding metabolic control, a separate experiment was performed using two groups of six animals. A control group was given normal chow and an experimental group received Acarbose (40 mg/100 g food). These mice were observed during a 10-wk period. Every week, a 24-h urine sample was collected for measurement of total glucose excretion during the latter 6 wk of study. Blood was obtained on weeks 8 and 10 for estimation of glycosylated hemoglobin concentration. Glycosylated hemoglobin was measured by ion-exchange chromatography using a kit supplied by Isolab Inc. (Akron, Ohio). Before this procedure, the red blood cells were washed in normal saline for 48 h at 4°C to remove labile hemoglobin fractions that may reflect acute changes in blood glucose.

Histologic procedures. For light microscopy, the kidney was fixed in 10% buffered formalin, sectioned at 5 μm and stained with periodic-Schiff reagent. Sections were evaluated blindly. The earliest pathologic changes in experimental diabetic nephropathy are mainly confined to the mesangial region of the glomerulus. Thickening of mesangial matrix provides an index to evaluate the severity of diabetic change. To evaluate this change, a modified stereologic technique was used.[13] Sections were viewed at × 1000 under oil immersion, and using a camera lucida drawing tube, the image of the glomerulus, as defined by the parietal epithelium of Bowman capsule, was outlined. The mesangium, including matrix and cells, as defined by PAS stain was drawn within the same glomerulus. The area of glomerulus occupied by the mesangium was established by overlaying a transparent sheet with intersecting lines, and counting the number of intersections occupied by the total glomerulus and mesangium. At least 10 glomeruli were analyzed from each animal, and the results expressed as percentage area of the glomerulus occupied by mesangium. Those glomeruli offering a profile through the axial pole were preferentially counted. The process of formalin fixation and paraffin embedding of tissues results in a certain degree of shrinkage. This method tends to overestimate the true mesangial area (or volume), as compared with stereologic methods using electron microscopy. Differences between the various experimental groups were compared using Student's *t* test for unpaired samples.

Immunopathology. Small pieces of renal cortex were snap-frozen in isopentane that was precooled in liquid nitrogen. Cryostat sections, 6 μm, were stained for the presence of endogenous mouse IgG, IgM, IgA, and albumin. The specificity of the antisera was confirmed by methods previously described.[5] Sections were viewed on a Nikon microscope equipped with epifluorescent optics using F420-490, OM505, and 520W filters. The methodology for grading of immunofluorescent staining has been detailed previously[5] and is outlined below. The intensity and distribution of glomerular immunofluorescent staining was graded from 0 through 4+, trace indicating fluorescence just above background and 1+ through 4+ indicating increasing degrees of positivity. At least 10 glomeruli were examined from each section.

Comparison of immunofluorescent staining between groups was assessed using the Mann-Whitney test for nonparametric data.[14]

RESULTS

No adverse effects were encountered due to Acarbose administration, and no animals died during the study. The growth curves and final weight achieved by control and drug-treated animals was similar (Figure 1). Food intake was not influenced by Acarbose, and all animals consumed approximately 35–45 g/wk. The controls and low-dose Acarbose group (A-10) exhibited a progressive increase in water intake throughout the study, while the other two drug-treated groups (A-20 and A-40) had a substantially lower water consumption (Figure 2).

There were no differences in fasting blood glucose levels between control and drug-treated animals when measured at 4, 8, and 10 wk (Table 1). Glycosylated hemoglobin was significantly reduced in the high-dose Acarbose group (A-40) at 8 ($P < 0.01$) and 10 wk ($P < 0.02$), when compared with control animals (Table 2). Urinary glucose excretion was consistently decreased in drug-treated animals (A-40) throughout the latter part of the study (Table 3). The weights of heart, liver, and kidneys were not affected by Acarbose treatment.

Immunopathologic examination of the kidney from control animals revealed diffuse immunoglobulin staining throughout the glomerular mesangial region, with 3+ intensity (Figure 3a). When stained for IgG and IgM, the drug-treated group receiving low-dose Acarbose (A-10) resembled the controls in both intensity and distribution of immunoglobulin staining. The A-20 group differed from controls in respect to IgM and IgA staining, and the high-dose Acarbose (A-40) group had significantly less immunofluorescent staining than the controls with each immunoglobulin

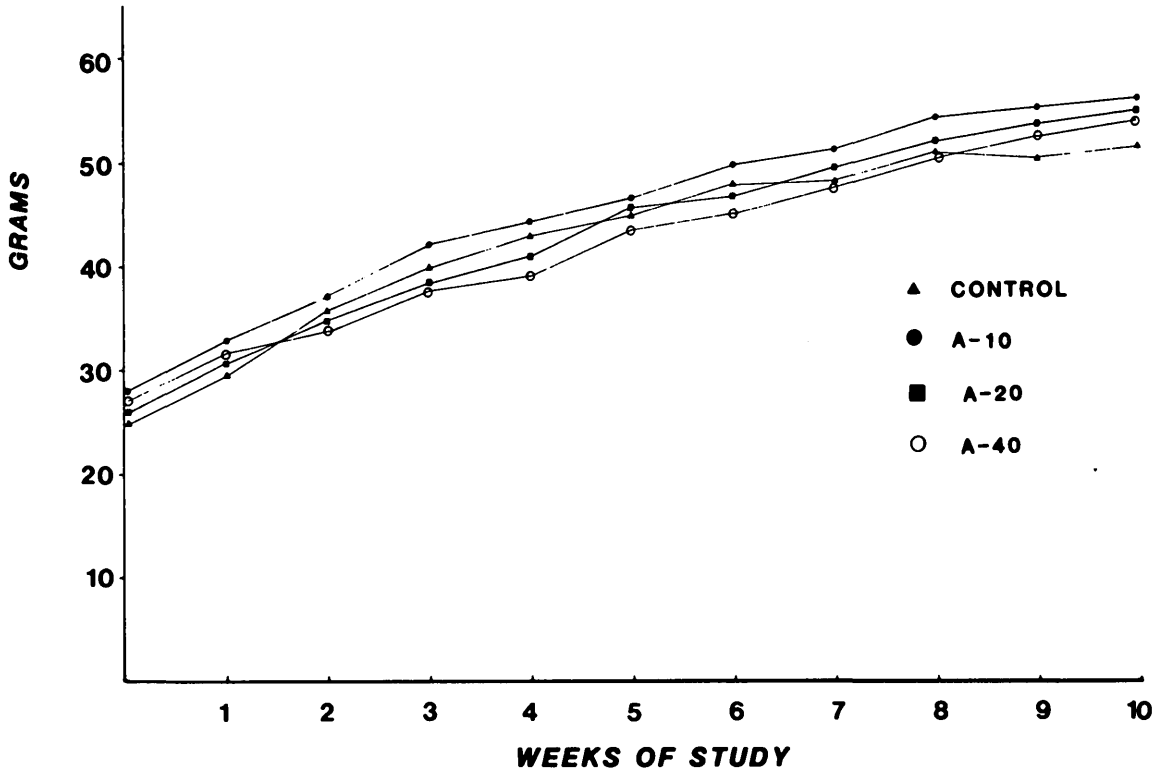


FIGURE 1. Body weight of animals throughout the study, showing no differences between the control and drug-treated groups. A-10, A-20, and A-40 indicate Acarbose-dosed groups.

(Figure 3b). This included both a decreased intensity and distribution of the fluorescent staining. The glomerular immunopathology is depicted schematically in Figure 4.

When examined by light microscopy, the control animals displayed generalized and diffuse glomerular mesangial widening (Figure 5). There was no evidence of increased mesangial cellularity, and glomerular and tubular basement membranes appeared normal. Qualitative evaluation of the light microscopic sections suggested decreased mesangial widening in the high-dose group (A-40), and this was con-

FIGURE 2. Weekly water consumption during the 10-wk study. Control and low-dose Acarbose (A-10) display a progressive rise in water intake throughout. The other Acarbose groups (A-20 and A-40) drink less water, especially during the latter 6 wk of study. (Nondiabetic mice consume 30–40 ml/wk.)

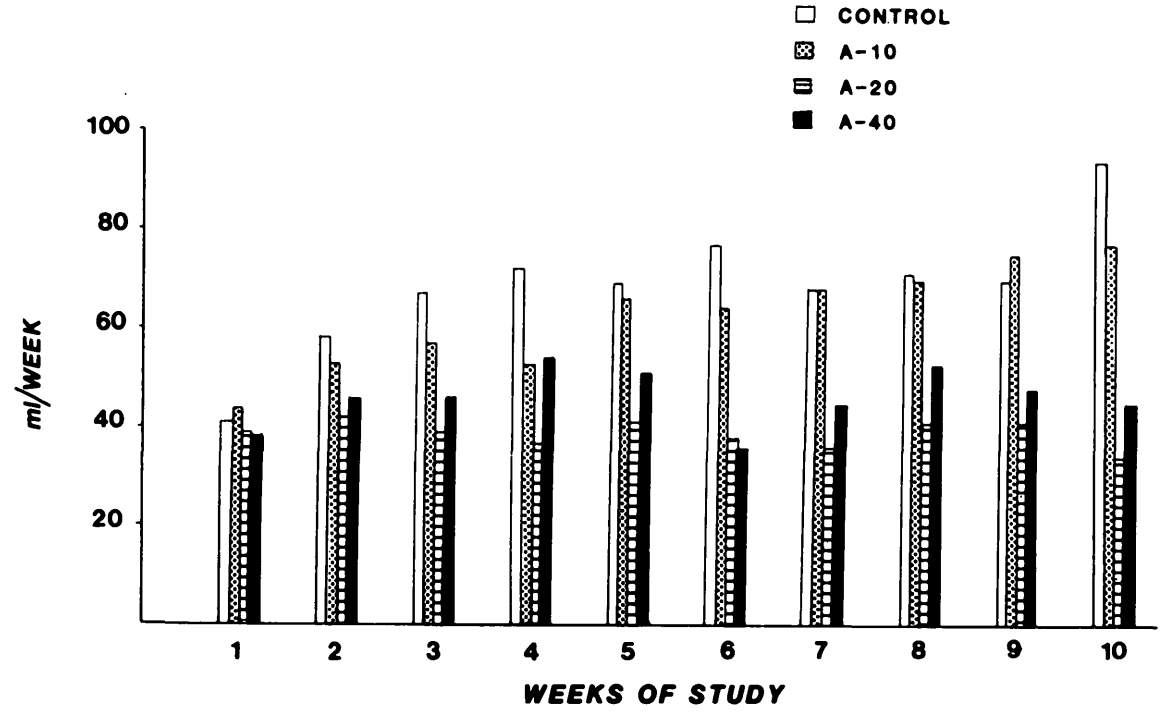


TABLE 1
Blood glucose (mg/dl)

	N	Week 4	Week 8	Week 10
Control	(6)	153 ± 32	176 ± 30	282 ± 78
Acarbose-10	(6)	167 ± 28	189 ± 22	348 ± 87
Acarbose-20	(7)	148 ± 17	134 ± 16	184 ± 25
Acarbose-40	(6)	165 ± 19	232 ± 78	280 ± 63

Results are expressed as mean ± SEM.

TABLE 2
Glycosylated hemoglobin (%)

		Week 8		Week 10
Control	(6)	7.1 ± 0.48	$P < 0.01$	6.6 ± 0.32
Acarbose-40	(6)	5.1 ± 0.10	$P < 0.02$	5.1 ± 0.34

Results are mean ± SEM.
Nondiabetic heterozygotes (+/*db*) of this strain have values of 3.1 ± 0.1 (N = 22).

TABLE 3
Urinary glucose excretion (g/24 h)

		Week 6	Week 8	Week 10
Control	(6)	1.51 ± 0.11	1.40 ± 0.16	1.62 ± 0.10
		$P < 0.01$	$P < 0.05$	$P < 0.02$
Acarbose-40	(6)	0.94 ± 0.13	0.99 ± 0.08	1.29 ± 0.06

Values are mean ± SEM.

firmed by morphometric analysis which revealed a significantly decreased mesangial area in this group. The other experimental groups (A-10, A-20) exhibited a dose-dependent trend toward decreased mesangial area, but these groups were not significantly different from control at the $P < 0.05$ level (Figure 6).

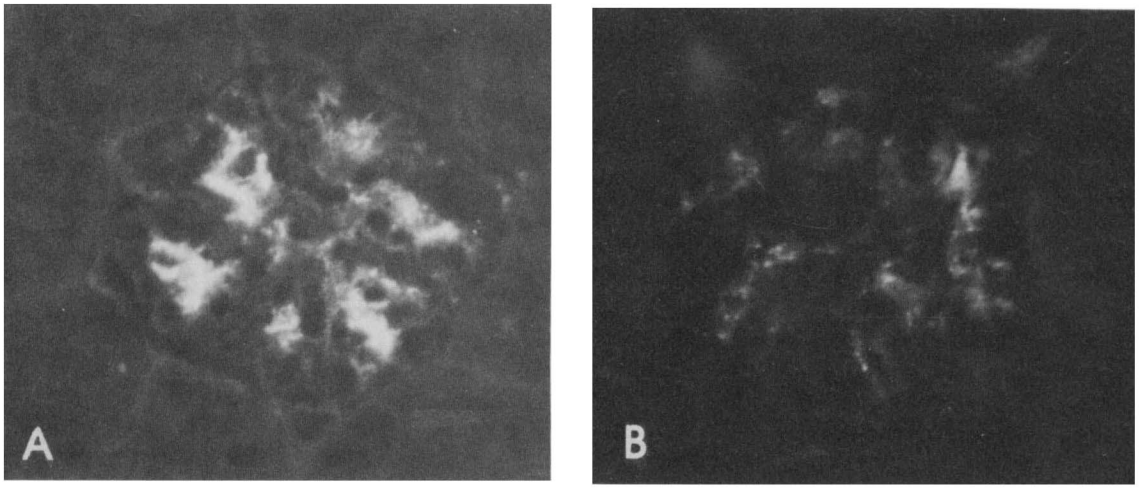
DISCUSSION

This study has shown that chronic treatment of genetically diabetic mice (db/db) with Acarbose improves glycemic control and exerts a preventive effect on the development of experimental diabetic nephropathy. Acarbose, a complex oligosaccharide, is a competitive inhibitor of intestinal α-glucosidases including sucrase, dextrinase, maltase, and glucoamylase. It is not absorbed to a significant extent and is most effective when given immediately before or with a carbohydrate-containing meal when it inhibits starch and sucrose digestion. The unabsorbed carbohydrate is subsequently degraded by microorganisms in the large bowel with formation of hydrogen and short-chain fatty acids. The protocol used in this study ensured that the animals received the drug each time they consumed food.

The efficacy of Acarbose in achieving improved blood glucose control was apparent from the decrease in glycosylated hemoglobin concentration and the lowered urinary glucose excretion. It was of interest, but not unexpected, to observe that the drug did not influence fasting blood glucose levels. In these animals, the hypoglycemic effect of Acarbose did not extend beyond the immediate postprandial period, and due to the nocturnal feeding habits of rodents, overnight food deprivation may be equivalent to a 24-h fast.

It was noted that Acarbose did not influence the food consumption or growth rate of the *db/db* mice. This contrasts with observations in the genetically obese rat (Zucker) where the drug exerted a dose-dependent decrease in food intake, resulting in a lower body weight and total body lipid content.[15] The sucrose malabsorption that follows Acarbose administration results in disaccharide fermentation in the large bowel with gas formation. The symptoms of flatulence and meteorism that occur in individuals receiving Acarbose could exert a negative influence on the food intake of laboratory animals, although this phenomenon was not observed in the present study. The failure to observe weight loss in the mice during this study could be explained by absorption of short-chain fatty acids in the colon which would overcome the influence of sucrose malabsorption on net energy balance. In addition, the *db/db* mouse possesses a markedly enhanced metabolic efficiency[6,18] that permits them to maintain their usual growth and weight gain in the face of modest intake reduction. A substantial decrease in food intake is necessary to alter their growth curve.

FIGURE 3. Representative examples of glomeruli stained for the presence of endogenous IgG. A-Control; B-Acarbose 40. Control shows 3+ immunofluorescent staining confined to the glomerular mesangium, while Acarbose treated exhibits 1+ intensity. (×200.)



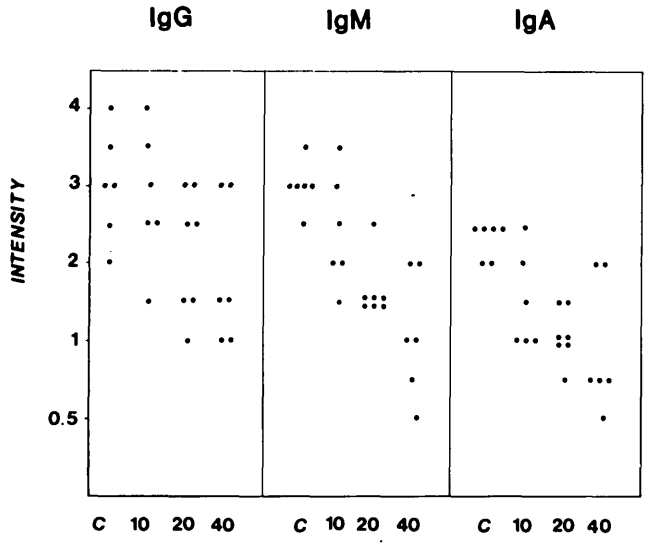


FIGURE 4. Schematic representation of glomerular fluorescent staining. C-Controls; 10, 20, and 40 are the different Acarbose-treated groups. With IgG staining, controls and A-40 differ significantly (P = 0.05). With IgM, both A-40 (P = 0.001) and A-20 (P = 0.002) differ from controls. With IgA, A-40 (P = 0.01), A-20 (P = 0.001), and A-10 (P = 0.047) all differ significantly from controls.

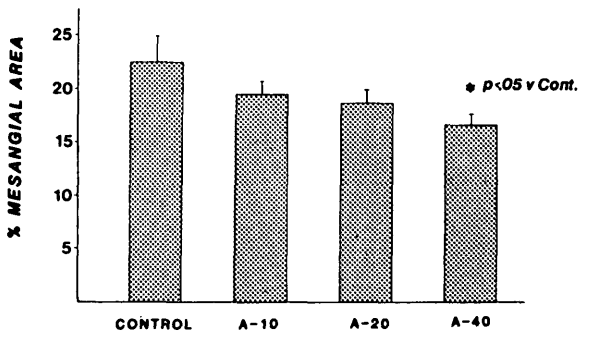


FIGURE 6. Morphometric analysis of glomerular mesangial thickening. The bars represent mesangial area expressed as a percentage of total glomerular area. Results are expressed as mean ± SEM. The high-dose Acarbose-treated group (A-40) showed significantly less mesangial area when compared with controls (P < 0.05).

This study provides evidence for the relationship between glycemic control and the prevention of diabetic pathologic complications. The rapidity with which nephropathy develops in the *db/db* mouse makes it an ideal model in which to evaluate the consequences of short-term therapeutic intervention. The renal lesion in these mice is first recognizable by the occurrence of endogenous immunoglobulin deposition in the glomerular mesangium. This is followed by progressive widening of the glomerular mesangial matrix, which is apparent by 3 mo of age.[5]

We have shown that blood glucose control can be achieved by diet restriction in these mice, and this completely prevents the development of renal lesions.[6] In this study, Acarbose administration produced a dose-dependent amelioration in the diabetic renal lesions. However, even in the high-dose Acarbose group (A-40) the appearance of the kidney was not normal. This observation was consistent with the fact that these animals were not euglycemic.

Short-term studies (7 days) in humans with type I and type II diabetes mellitus have shown that Acarbose treatment attenuates the rise in postprandial hyperglycemia and decreases urinary glucose excretion.[12] Prolonged administration of the drug has been associated with symptoms of carbohydrate malabsorption, including diarrhea and flatulence. While Acarbose therapy was effective throughout a 10-wk period in this study, the long-term adaption of the intestinal mucosa to glycosidase inhibition remains to be evaluated. This form of pharmacologic intervention may prove to be a useful adjunctive therapy in patients with diabetes mellitus.

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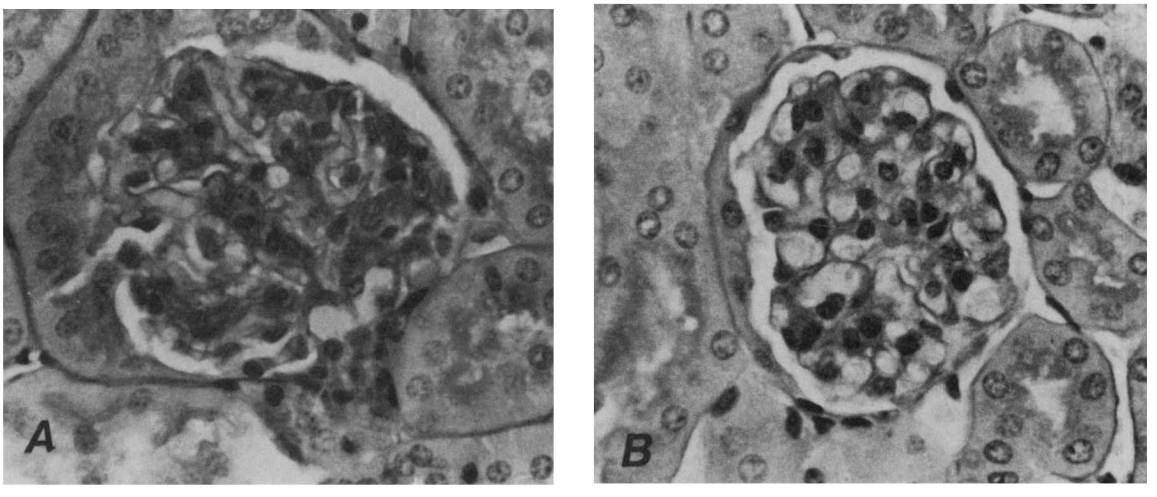
FIGURE 5. Photomicrographs of glomeruli from Control-A and Acarbose (A-40)-B animals. Thickening of the glomerular mesangial matrix is apparent in the control. There is minimal mesangial widening in the drug-treated animal. (PAS × 200.)



kindly supplied by Miles Laboratories, a division of the Bayer Company.

REFERENCES

[1] Hummel, K. P., Dickie, M. M., and Coleman, D. L.: Diabetes: a new mutation in the mouse. Science *153*:1127–28, 1966.

[2] Coleman, D. L., and Hummel, K. P.: Hyperinsulinemia in pre-weanling diabetic mice. Diabetologia *10*:607–10, 1974.

[3] Baetens, D., Stefan, Y., and Ravazzola, M., et al.: Alteration of islet cell populations in spontaneously diabetic mice. Diabetes *27*:1–7, 1978.

[4] Like, A. A., Lavine, R. L., Poffenberger, P. L., and Chick, W. L.: Studies in the diabetic mutant mouse. Am. J. Pathol. *66*:193–204, 1972.

[5] Lee, S. M., and Graham, A.: Early immunopathologic events in experimental diabetic nephropathy: a study in db/db mice. Exp. Mol. Pathol. 33:323–32, 1980.

[6] Lee, S. M., and Bressler, R.: Prevention of diabetic nephropathy by diet control in the *db/db* mouse. Diabetes *30*:106–111, 1981.

[7] Sol, A. H., Kahn, C. R., Neville, D. M., and Roth, J.: Insulin receptor deficiency in genetic and acquired obesity. J. Clin. Invest. *56*:769–80, 1975.

[8] Coleman, D. L., and Hummel, K. P.: Studies with the mutation diabetes in the mouse. Diabetologia *3*:238–48, 1967.

[9] Puls, W., Kemp, U., Krause, H. P., Thomas, G., and Hoffmeister, F.: Glucosidase inhibition: a new approach to the treatment of diabetes, obesity and hyperlipoproteinemia. Naturwissenschaften *64*:536–37, 1977.

[10] Walton, R. J., and Sherif, I. T.: Improved metabolic profiles in insulin-treated diabetic patients given an alpha-glusoside hydrolase inhibitor. Br. Med. J. *1*:220–21, 1979.

[11] Sachse, G., and Willems, B.: Effect of the alpha-glusosidase-inhibitor BAY-g-5421 on blood glucose control of sulphonylurea-treated diabetics and insulin-treated diabetics. Diabetologia *17*:287–90, 1979.

[12] Schoffling, K., Hillebrand, I., and Berchtold, P.: Treatment of diabetes mellitus with the glycoside hydralase inhibitor acarbose (BAY g 5421). Front. Horm. Res. (Basel) *7*:248–57, 1980.

[13] Kawano, K., McCoy, J., Wenzl, J., Porch, J., Howard, C., Goddard, M., and Kimmelstiel, P.: Quantitation of glomerular structure: a study of methodology. Lab. Invest. *25*:343–48, 1971.

[14] Siegel S.: Nonparametric Statistics for the Behavioral Sciences. New York, McGraw-Hill Book Co., 1956, pp. 95–156.

[15] Puls, W., Kemp, U., Krause, H. P., Muller, L., Schmidt, D. D., Thomas, G., and Truscheit, E.: Pharmacology of a glucosidase inhibitor. Front. Horm. Res. (Basel) *7*:235–47, 1980.

[16] Cox, J. E., and Powley, T. L.: Development of obesity in diabetic mice pair-fed with lean siblings. J. Comp. Physiol. Psychol. *91*:347–58, 1977.