# Altered Lysosomal Glycohydrolase Activities in Juvenile Diabetes Mellitus

*Fredric M. Bomback, M.D., Sachiko Nakagawa, Ph.D., Selma Kumin, M.S., and Harold M. Nitowsky, M.D., Bronx, N.Y.*



SUMMARY

**Studies have been carried out on activities of lysosomal β-N-acetylhexosaminidase (hex), β-galactosidase (β-gal), α-glucosidase (α-glu), and acid phosphatase (AP) in serum and urine from patients with juvenile diabetes and matched controls. There is a large increase in blood and urinary hex activity (the former presenting three distinct patterns of abnormality), a moderate increase in urinary β-gal, and a small increase in urinary α-glu activity, but no elevation of blood or urinary AP in the diabetics. Urinary α-glu activity in the diabetics shows striking inhibition by glucose, and this may reflect a similar phenomenon in vivo.**

**Although glycohydrolase activities are elevated in patients with no detectable microangiopathy, more striking changes may be observed in patients with severe small-vessel disease. These alterations may be associated with increased glycoprotein catabolism in the diabetic, an area in need of further studies in the human and experimental diabetic animal. DIABETES *25*:420-27, May, 1976.**

Previous investigators have demonstrated increased activity of several lysosomal hydrolases (β-N-acetylhexosaminidase, β-glucuronidase, and acid phosphatase) in the serum of patients with diabetes mellitus.[1-4] In addition, urinary excretion of hexosaminidase has been shown to be increased in the diabetic.[5] The significance of these findings has not, however, been defined.

Much effort has been expended in studies on the chemical composition and biosynthesis of carbohydrate-protein macromolecules in both experimental animal[6,7] and human[8-12] diabetes. In contrast, there has been relatively little work on the turnover or catabolism of these substances.

Lysosomes have been shown to be the sites of intracellular glycoprotein catabolism.[13-15] They apparently contain a full complement of the requisite glycohydrolases for virtually every sugar and type of linkage found in glycoproteins. The purpose of our studies has been to define the changes in serum and urine activities of several lysosomal hydrolases in patients with diabetes mellitus as a preliminary to more definitive investigations of glycoprotein metabolism in this disorder.

Attention has been focused on four lysosomal enzymes, two of which (β-N-acetylhexosaminidase and β-galactosidase) have previously[14] been shown to be operative in glycoprotein catabolism, one of which (α-1, 4 glucosidase) is active in glycogen catabolism and may also have a role in glycoprotein catabolism,[16] and one of which (acid phosphatase) has not been implicated in glycoprotein breakdown, although its physiologic role is still obscure.

## SUBJECTS

The subjects for these studies included a group of 28 patients (ages 13 to 24 years) with juvenile diabetes and 28 healthy age- and sex-matched controls. The duration of overt diabetes ranged from one to 18 years. In general, the patients' treatment regimen consists of a single morning injection of an intermediate-acting insulin (dosage range 0.5 U./kg. to 1.5 U./kg. per 24 hours), and the consumption of a balanced diet divided into three regular meals and two or three snacks a day. With one exception (*vide infra*), all of the patients are asymptomatic and excrete 5 to 15 per cent of their daily oral carbohydrate intake in

From the Departments of Pediatrics and Genetics and the Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, New York.

Accepted for publication January 21, 1976.

their urine. None of the control subjects had a history of diabetes in first-degree relatives, nor did they manifest hyperglycemia on fasting and two-hour postprandial glucose determinations.

The diabetic patients were assayed for the severity of microangiopathy, if any, by establishing the presence and degree of retinopathy (by ophthalmologic examination and, in selected patients, by fluorescein angiography), nephropathy (by urine examination and renal function studies as well as renal biopsy where indicated), and neuropathy (by clinical examination and studies of motor-nerve-conduction velocities in upper and lower extremities).

The diabetic patients, with one exception, show minimal or no detectable evidence of small-vessel disease. Patient R.F., a 19-year-old Puerto Rican male with a 12-year history of overt diabetes, has severe microangiopathy affecting the kidneys, retina, and peripheral nervous system. All patients, except R.F., had a 12-hour urinary protein excretion of less than 50 mg. and had normal values for BUN, creatinine, creatinine clearance, and Addis count. R.F.'s renal disease is characterized by massive proteinuria, decreased creatinine clearance, and arteriolonephrosclerosis and glomerulosclerosis on renal biopsy.

## METHODS

The initial studies dealt with various properties of selected lysosomal glycohydrolases in serum and urine, including their stability to freezing and storage and the effects of glucosuria on enzyme activity and stability. For this purpose, patients with diabetes mellitus were admitted for short-term observation to the Clinical Research Unit, where consecutive 24-hour-urine samples were collected and serum for enzyme assay was obtained from clotted venous blood specimens drawn before breakfast and prior to insulin administration. Additional blood specimens were obtained during the course of the day, before and after meals. There was no significant diurnal fluctuation in the activity of the serum enzymes studied. Moreover, no differences were noted in urinary enzyme activities in samples collected during hospitalization when patients were relatively sedentary from urine samples collected at home, where there was less restriction in daily activities and food intake.

The findings in these initial studies, which are summarized below, permitted standardization of the procedures for collection and storage of samples and aided in establishing optimal conditions for enzyme assay. Because of these preliminary studies, assay of each of the lysosomal enzymes selected for investigation in serum and urine of controls and patients with diabetes mellitus was carried out in a majority but not all the subjects. Since the variations between patients and controls were quantitatively consistent, there is no apparent selection bias that can account for the differences observed.

The activities of β-N-acetylhexosaminidase (hex), β-galactosidase (β-gal) and α-glucosidase (α-glu) were assayed with the appropriate 4-methylumbelliferyl (4-MU) glycoside as fluorogenic substrate.[17] The 4-MU liberated in the reactions was converted to its highly fluorescent anionic form by addition of alkaline buffer, and the reaction product was measured by fluorometry. DEAE-cellulose chromatography was employed for the separation of hex isozymes and for characterization of the other lysosomal hydrolases.[18] The assay for acid phosphatase (AP) used p-nitrophenyl phosphate as substrate, and the amount of p-nitrophenol released was measured spectrophotometrically.

The following are the details of the individual assay procedures adopted after the initial studies noted previously:

1. *Hexosaminidase.* Serum (1.5-ml.) or urine (3.0-ml.) samples were dialyzed against 10 mM phosphate buffer (pH 6.0) for five to six hours at 3° C. and then centrifuged at 4,000 rpm for 10 minutes. The clear supernatant fluid was applied to a DEAE-cellulose column (1.3 × 6.5 cm.) that had previously been equilibrated with the 10-mM phosphate buffer (pH 6.0). The elution was carried out first with 20 ml. of the buffer, followed by 30 ml. of a linear gradient of 0 to 0.25 M NaCl in the buffer and then 20 ml. of 0.25 M NaCl in the buffer. One-ml. fractions were collected and assayed for hex activity. Effluents were also monitored at 280 nm for protein concentration. The hex activity was assayed in a Technicon AutoAnalyzer II attached to a fluorometer with a primary filter of 365 nm and a secondary filter of 450 nm. The substrate 4-MU-2-acetamido-2-deoxy-β-D-glucopyranoside (Koch-Light) was used for the assay at a concentration of 1.38 mM dissolved in citrate (6 mM)-phosphate (10 mM) buffer, pH 4.4. The reaction was terminated by adding glycine (0.17 M)-carbonate (0.17 M) buffer pH 10.0. To determine the total (unfractionated) hex activity, urine and serum samples were first diluted with the citrate-phosphate buffer to an appropriate concentration and assayed in the AutoAnalyzer.

2. *β-galactosidase.* (a) Urine—100 μl. of 24-hour

urine collection properly diluted in citrate (48.6 mM)-phosphate (102 mM) buffer, pH 5.0, was added to 400 μl. of 1.03 mM 4-MU-β-D-galactoside (Pierce) in distilled water and incubated for 20 minutes in the dark at 37° C. The reaction was terminated by adding 4 ml. of glycine (0.17 M)-carbonate (0.17 M) buffer, pH 10.0. Fluorescence was read in a Turner fluorometer with a primary filter of 365 nm and a secondary filter of 450 nm.

(b) Serum—as above, except that dilution was carried out in citrate (69.8 mM)-phosphate (60.4 mM) buffer, pH 3.5, and 200 μl. of the diluted serum was added to 300 μl. of substrate.

In this and in all other assay procedures, each enzymic determination was performed in triplicate and the results of several determinations were averaged. Appropriate blanks and standards were employed. Conditions for each assay showed a linearity of reaction with time and amount of serum or urine added.

3. *α-glucosidase in urine.* Urine samples were dialyzed overnight in 0.10 M sodium acetate buffer, pH 4.8, at 3° C. to remove the glucose present in diabetic urine. One hundred μl. of dialyzed urine diluted in 0.2 M acetate buffer, pH 4.8 was added to 100 μl. of 5.0 mM 4-MU-α-D-glucoside (Pierce) in 0.2 M acetate buffer and incubated in the dark for 60 minutes at 37° C. The reaction was terminated by adding 3.8 ml. of 1.0 M glycine-sodium hydroxide, pH 10.5, and results were read in a fluorometer.

4. *Acid phosphatase.* (a) Urine—50 μl. of undiluted urine was added to 0.5 ml. of 5.5 mM sodium-p-nitrophenyl phosphate (Sigma 104) in 50 mM citrate buffer, pH 4.9, and incubated for 30 minutes at 37° C. The reaction was stopped with 4 ml. of 40 mM sodium hydroxide, and the amount of p-nitrophenol released was read in a Gilford spectrophotometer at 400 nm.

(b) Serum—as above, undiluted serum except that the incubation time was 60 minutes.

## RESULTS

1. *Hexosaminidase.* The diabetic group has significantly higher serum and urinary total hex activity than the controls (table 1). The diabetics can be subdivided into three well-defined groups (table 2) on the

TABLE 1

Total hexosaminidase activity

| *In serum* Subjects (number) | Enzyme activity (nmoles 4-MU/hr./ml. serum*) | Comparison between† |
|---|---|---|
| 1. Diabetic males (12) | 1,278 ± 398 | 1 and 2 $p < 0.01$ |
| 2. Normal males (11) | 833 ± 120 | |
| 3. Diabetic females (16) | 1,313 ± 352 | 3 and 4 $p < 0.01$ |
| 4. Normal females (10) | 835 ± 190 | |
| *In urine* Subjects (number) | Enzyme activity (μmoles 4-MU/hr./gm. creatinine*) | Comparison between† |
| 1. Diabetic males (9) | 256 ± 104 | 1 and 2 $p < 0.01$ |
| 2. Normal males (20) | 82 ± 20 | |
| 3. Diabetic females (6) | 324 ± 145 | 3 and 4 $p < 0.01$ |
| 4. Normal females (9) | 97 ± 15 | |

*Mean ± 1 S.D. Creatinine determined by Jaffe reaction with Lloyd's reagent.
†Student's *t*-test analysis.

TABLE 2

Activities of hexosaminidase isozymes in serum (males and females combined)

| Subjects (number) | Hex A (n moles 4-MU/hr./ml. serum) | Hex B | Hex I |
|---|---|---|---|
| 1. Controls (21) | 675 ± 118 | 33 ± 15 | 124 ± 56 |
| 2. Diabetic group I (9) | 1,060 ± 154 | 203 ± 44 | 169 ± 51 |
| 3. Diabetic group II (11) | 1,177 ± 303 | 103 ± 23 | 192 ± 30 |
| 4. Diabetic group III (8) | 695 ± 64 | 67 ± 27 | 146 ± 95 |

$p < 0.01$ for comparison between group I and group III or group II and group III for hex A and hex B; group I and group II for hex B; group I or II or III and controls for hex B. There is no significant difference between controls and group II for hex A and hex I.

basis of their serum hex isozyme-distribution pattern. Patients in group I show high A and high B. Those in group II show a significant elevation, mainly in hex A, with a moderate increase in hex B activity. Hex A in group III is similar to the control group, but there is a small but significant elevation in hex B. Hex I is not significantly elevated in any of these groups. The increase in total urinary hex is due to both elevated hex A and hex B; however, no grouping in terms of isozyme patterns is evident. (Hex I is not detected in the urine.)

Four separate serum and urine specimens were obtained from patient R.F. The results of his enzyme determinations are not included in the tables because of his clinical dissimilarity to the other diabetics in the study.

While the mean serum hex activity of R.F. is within the range of values for the diabetic group, his mean total urinary hex is 1,251±325, a value three-to-five-fold greater than in the other male diabetics. This value reflects markedly increased urinary hex A and hex B activities, the former threefold and the latter tenfold greater than those of the other diabetic males.

2. *β-galactosidase (table 3).* No significant difference in serum levels is noted among the groups studied. There is, however, a highly significant increase in the 24-hour urinary excretion of this enzyme in the diabetics. Again, patient R.F. falls well outside the range of values for the diabetic group. His mean urinary β-gal activity of 394±91 is two-to-three-fold greater than the other male diabetics.

3. *α-glucosidase (table 4).* As has been previously shown,[19] there is no measurable activity of lysosomal α-1,4 glucosidase in serum of either normals or diabetics. However, urinary activity is easily detected. Diabetic males and diabetic females have significantly elevated α-glu activities above those of their respective controls. Diabetic females have significantly higher activities than do diabetic males. These conclusions become apparent only after studies of dialyzed urine specimens. The α-glu activity is essentially unchanged when urine from control subjects is analyzed before or after dialysis. However, in the diabetics, the α-glu activity in urine assayed after dialysis is twice as great as that in nondialyzed urine. This is due to a direct inhibitory effect of glucose on enzyme activity, as measured in our assay system. Figure 1 shows that

TABLE 3

β-galactosidase activity

| *In serum* Subjects (number) | Enzyme activity (μmoles 4-MU/hr./ml. serum) | Comparison between |
|---|---|---|
| 1. Diabetic males (6) | 12.5 ± 2.0 | 1 and 2* |
| 2. Normal males (11) | 11.4 ± 7.5 | |
| 3. Diabetic females (4) | 7.9 ± 3.0 | 3 and 4* |
| 4. Normal females (5) | 6.3 ± 1.0 | |
| *In urine* Subjects (number) | Enzyme activity (μmoles 4-MU/hr./gm. creatinine) | Comparison between |
| 1. Diabetic males (8) | 107 ± 47 | 1 and 2 $p < 0.01$ |
| 2. Normal males (19) | 39 ± 23 | |
| 3. Diabetic females (5) | 142 ± 25 | 3 and 4 $p < 0.01$ |
| 4. Normal females (7) | 28 ± 16 | |

*Not statistically significant.

TABLE 4

α-glucosidase activity in urine*

| Subjects (number) | Enzyme activity (μmoles 4-MU/hr./gm. creatinine) | Comparison between |
|---|---|---|
| 1. Diabetic males (9) | 61 ± 13 | 1 and 2 $p = 0.01$ |
| 2. Normal males (12) | 44 ± 13 | |
| 3. Diabetic females (6) | 111 ± 25 | 3 and 4 $p < 0.01$ |
| 4. Normal females (9) | 37 ± 15 | |

*Dialyzed urine samples.

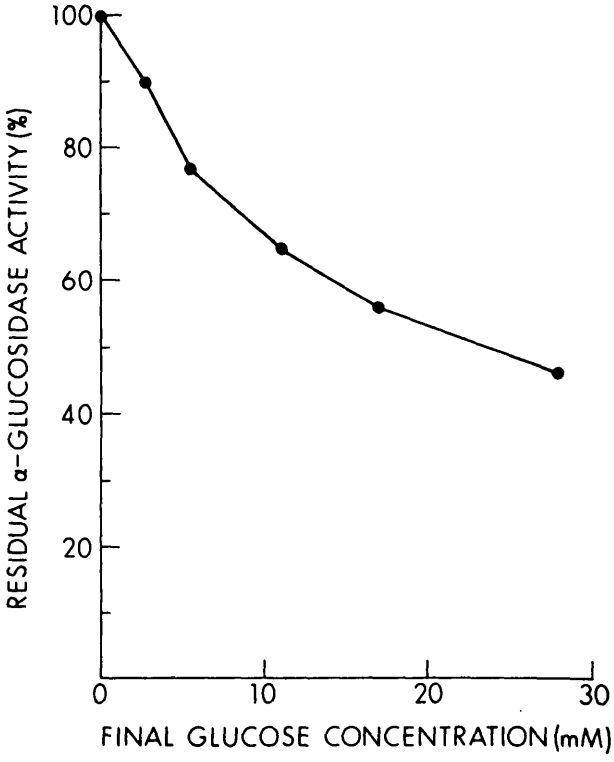


**FIG. 1. Effect of added glucose on urinary α-glucosidase activity.**

this effect can be reproduced by addition of glucose, and inhibition of α-glu in urine from normal subjects is evident at a final concentration of added glucose as low as 5.5 mM. At 10 mM glucose, which is not unusual for blood glucose levels in diabetics, there is 35 per cent inhibition of enzyme activity.

4. *Acid phosphatase.* The results of our studies on acid phosphatase are summarized in table 5. We are unable to confirm a previous report[4] of increased AP activity in serum of diabetics over that of healthy subjects. Moreover, no statistically significant difference in AP activity is demonstrable between urines of diabetics and controls when assays are performed on freshly collected unfrozen urines. When urine samples from nondiabetic subjects are frozen and stored at −20° C., AP activity is progressively lost. Storage of urine of diabetics in the frozen state, on the other hand, usually results in loss of only a small fraction (less than 5 per cent) of the initial activity. It was found that glucose in urine protects AP from inactivation with freezing. There is, in fact, a direct relationship between urine glucose concentration and the amount of protection against loss of AP activity with

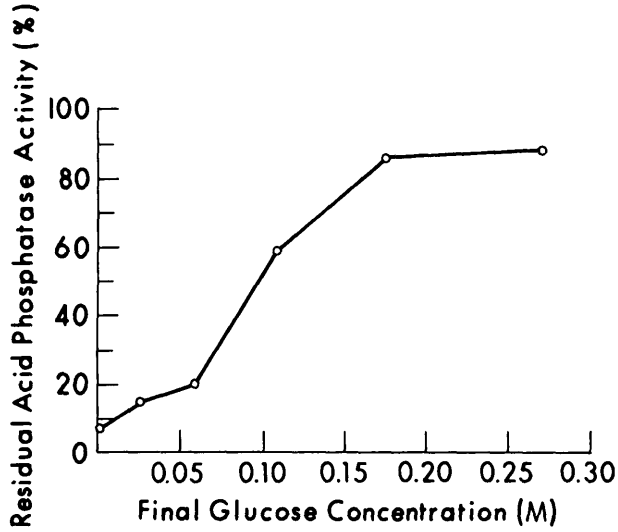


**FIG. 2. Effect of added glucose on stability of urinary acid phosphates during freezing.**

TABLE 5

Acid phosphatase activity

| | | |
|---|---|---|
| *In serum* | | |
| Subjects (number) | Enzyme activity (μmoles p-nitrophenol/hr./ml.) | Comparison between |
| 1. Diabetic males (6) | 516 ± 98 | 1 and 2* |
| 2. Normal males (14) | 511 ± 109 | |
| 3. Diabetic females (4) | 537 ± 43 | 3 and 4* |
| 4. Normal females (6) | 569 ± 222 | |
| *In urine*† | | |
| Subjects (number) | Enzyme activity (μmoles p-nitrophenol/hr./gm. creatinine) | Comparison between |
| 5. Diabetic males (9) | 9,962 ± 6,459 | 5 and 6* |
| 6. Normal males (7) | 4,086 ± 2,195 | |
| 7. Diabetic females (6) | 2,036 ± 617 | 7 and 8* |
| 8. Normal females (5) | 2,107 ± 506 | |

*Not statistically significant.

†Unfrozen (refrigerated) 24-hour urine specimens.

freezing. Figure 2 shows that with increasing concentration of glucose in urine of controls before freezing, there is a decreasing loss of enzyme activity until a plateau is reached at about 200 mM glucose. A similar effect is noted with equimolar concentrations of sucrose, but the effect is much less with equiosmotic concentrations of sodium chloride. Addition of 1 per cent bovine serum albumin also does not protect the enzyme from inactivation with freezing.

Studies of elution patterns of urinary hex, $\beta$-gal, $\alpha$-glu, and AP from DEAE-cellulose columns showed no qualitative differences between diabetics and controls. These findings, together with studies of enzyme patterns in serum, will be reported elsewhere.

There is no apparent correlation between the serum and urine activities of any of the enzymes studied and the age of the patients (all postpubescent), the duration of their disease, the degree of control, or their insulin dosage.

## DISCUSSION

Studies have been carried out on the activities of various lysosomal acid glycohydrolases in serum and urine from patients with diabetes mellitus and matched controls. This investigation was prompted by previous reports of increased activities of hexosaminidase in serum and urine and of increased serum $\beta$-glucuronidase in diabetics.[2-5] Moreover, previous studies in our laboratory have demonstrated altered patterns of serum hexosaminidase isozymes in juvenile diabetes.[20,21]

In addition to a large increase in urinary hexosaminidase, we have found a moderate elevation of $\beta$-galactosidase, a small but statistically significant increase in $\alpha$-1,4 glucosidase, and no change in acid phosphatase. In contrast to the enzyme changes in urine, hexosaminidase is the only enzyme that shows increase in serum activity. The quantitative relationship among the hexosaminidase isozymes is altered in serum from diabetes, with a consistent increase in the B, or heat-stable isozyme, and a variable increase in the heat-labile, or A, isozyme.

The finding of increased activity of various lysosomal acid hydrolases in patients with diabetes may reflect one of a variety of pathologic alterations, including accelerated cell death or turnover, or cell injury with resultant lysosomal instability. However, the demonstration of a selective alteration of lysosomal enzyme activities suggests that the basis for these changes is more specific and, indeed, may be related in some direct manner to the pathophysiologic disturbances observed in this disorder. It is of interest that the most striking changes in urine-enzyme activities were observed in a patient with severe microangiopathy, as manifest by an advanced degree of proliferative vascular changes in the retina and signs of nephropathy with renal insufficiency.

The elevated urinary $\beta$-galactosidase in the face of normal serum activity and the elevated urinary lysosomal $\alpha$-glucosidase in the face of undetectable activity in the serum suggests a renal origin of these enzymes. A similar source may account for urinary hexosaminidase, although in this instance serum enzyme activity is also increased. However, the I isozyme, which is easily identified in serum, does not appear in urine. If, in fact, the urinary acid glycohydrolases are of renal parenchymal origin and in some way reflect cellular injury, then studies of enzyme activity in urine may provide a useful method to monitor the course of glomerular basement-membrane thickening and other pathologic changes in diabetic nephropathy. Indeed, it has been shown in recent studies[22] that urinary hexosaminidase activity is increased in patients with other types of renal disease and may serve as a sensitive indicator of renal damage. It is of interest, in this regard, that, with the exception of patient R.F., none of the diabetics in our study had evidence of renal-function abnormalities.

Alterations in both the quantity and composition of the glomerular basement membrane (a glycoprotein structure) in diabetes mellitus have been correlated with changes in activities of biosynthetic enzymes.[23,24] There is little if any information, however, concerning the catabolic pathways of glycoproteins in diabetes. The results of our studies on urinary $\alpha$-glucosidase activities in the diabetic group may be of particular significance in this regard. If lysosomal $\alpha$-glu is a factor in glycoprotein degradation, then the inhibition of $\alpha$-glu activity by glucose in vitro may reflect a similar response in vivo in the diabetic. If so, the accumulation of the $\alpha$-glucosyl-$\beta$-galactosyl disaccharide subunit in diabetic glomerular basement-membrane glycoprotein that has been reported by some investigators[25] may be explained not only by an increase in biosynthesis[23,24] but also by a decrease in catabolism. The therapeutic implications of this are clear, for maintenance of blood glucose at normoglycemic levels may minimize the inhibition of $\alpha$-glucosidase and hence enhance the catabolic turnover of the glycoprotein disaccharide subunit. However, it should be stated that some other studies were

unable to confirm the aforementioned alterations of basement membrane components.[26,27]

Our studies, and previous reports, of altered acid glycohydrolase activities in serum or urine of patients with diabetes are based on assays with synthetic chromogenic or fluorogenic substrates. Thus, it remains unclear whether the changes observed have any relevance to alterations of glycoprotein catabolism or other changes in vivo. Indeed, it has been shown[28] that mutations can occur resulting in absence of activity of hexosaminidase A against synthetic substrate (4-methylumbelliferyl-β-N-acetylglucosaminide) with retention of activity against the natural substrate, $GM_2$-ganglioside. Unfortunately, the natural substrates have not been defined for many of the lysosomal glycohydrolases, and the role postulated for these enzymes in the normal turnover of glycoproteins is based on indirect or tenuous evidence. It has been reported that both fetuin and orosomucoid can be hydrolyzed by rat liver and kidney lysosomal extracts.[14] In addition to excessive accumulation of glycoproteins in capillary basement membranes,[29] more generalized abnormalities in glycoprotein metabolism have been observed in both naturally occurring and experimental diabetes mellitus. These include elevated levels of protein-bound carbohydrates (hexoses, fucose, hexosamines, and sialic acid) in blood of diabetic patients[9] and significant chemical changes in glycoproteins and mucopolysaccharides in human diabetic kidneys.[8,10] Streptozotocin-diabetic rats also exhibited significant modification in their connective-tissue macromolecules.[7] Thickening of the capillary basement membrane is only one aspect of this abnormality, yet it is responsible for the severe vascular lesions, with their well-known morbidity and mortality.[30]

Clearly, further studies are required using natural substrates, including glycopeptides, other glycoprotein derivatives, and basement-membrane glycoproteins, to determine the significance of the observed alterations of glycohydrolase activities with synthetic substrates. Moreover, our preliminary findings point to a need for further studies of synthesis as well as degradation or turnover of gycoproteins in vivo in the experimental animal with diabetes. Since it has been shown that fibroblast cell cultures from patients with diabetes have a shortened replicative life span in vitro,[31] this system may serve as a useful experimental model for similar studies of glycoprotein metabolism.

## ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health (GM 19100, 2-M01-RR00500, and IF 22-AM 00396) and the New York Diabetes Association.

## REFERENCES

[1]Belfiore, F., Napoli, E., and LoVecchio, L.: Increased activity of some enzymes in serum in cases of severely decompensated diabetes, with and without ketoacidosis. Clin. Chem. *18*:1403-06, 1972.

[2]Belfiore, F., Napoli, E., and LoVecchio, L.: Serum N-acetyl-beta glucosaminidase activity in diabetic patients. Diabetes *21*:1167-72, 1972.

[3]Miller, B.F., Keyes, F.P., and Curreri, P.W.: Increase of serum beta-glucuronidase activity in human diabetes mellitus. J.A.M.A. *195*:189-92, 1966.

[4]Belfiore, F., LoVecchio, L., and Napoli, E.: Serum enzymes in diabetes mellitus. Clin. Chem. *19*:447-52, 1973.

[5]Belfiore, F., LoVecchio, L., Napoli, E., and Borzi, V.: Increased β-N-acetyl-glucosaminidase activity in diabetes mellitus. Clin. Chem. *20*:1229-30, 1974.

[6]Schiller, S., and Dorfman, A.: The metabolism of mucopolysaccharides in animals. IV. The influence of insulin. J. Biol. Chem. *227*:625-32, 1957.

[7]Berenson, G.S., Radhakrishnamurthy, B., Dalferes, E.R., Jr., Ruiz, H., Srinivasan, S.R., Plavidal, F., and Brechman, F.: Connective tissue macromolecular changes in rats with experimentally induced diabetes and hyperinsulinism. Diabetes *21*:733-43, 1972.

[8]Berenson, G.S., Ruiz, H., Dalferes, E.R., Jr., Dugan, F.A., and Radhakrishnamurthy, B.: Acid mucopolysaccharide changes in diabetic kidneys. Diabetes *19*:161-70, 1970.

[9]McMillan, D.E.: Changes in serum proteins and protein-bound carbohydrates in diabetes mellitus. Diabetologia *6*:597-604, 1970.

[10]Srinivasan, S.R., Berenson, G.S., and Radharkrishnamurthy, B.: Glycoprotein changes in diabetic kidneys. Diabetes *19*:171-75, 1970.

[11]Spiro, R.G., and Spiro, M.J.: Effect of diabetes on the biosynthesis of the renal glomerular basement membrane. Studies on the glycosyltransferase. Diabetes *20*:641-48, 1971.

[12]McMillan, D.E.: Elevation of glycoprotein fucose in diabetes mellitus. Diabetes *21*:863-71, 1972.

[13]Aronson, N.N., Jr., and DeDuve, C.: Digestive activity of lysosomes. II. The degradation of macromolecular carbohydrates by extracts of rat liver lysosomes. J. Biol. Chem. *243*:4564-73, 1968.

[14]Mahadevan, S., Dillard, C.J., and Tappel, A.L.: Degradation of polysaccharides, mucopolysaccharides, and glycoproteins by lysosomal glycosidases. Archiv. Biochem. Biophys. *129*:525-33, 1969.

[15]Gregoriades, G., Morell, A.G., Sternlieb, I., and Scheinberg, H.: Catabolism of desialylated ceruloplasmin in the liver. J. Biol. Chem. *245*:5833-37, 1970.

[16]Patel, V., and Tappel, A.L.: Glycosidases in glycoprotein catabolism. Chapter VIII. *In* Glycoproteins of Bllod Cells and Plasma. Jamieson, G.A., and Greenwalt, T.J., Eds., Philadelphia, J.B. Lippincott Co., 1971, p. 133.

[17]Leaback, D.H., and Walker, P.G.: Studies on glucosaminidase. The fluorometric assay of N-acetyl-β-glycosaminidase. Biochem. J. *78*:151-56, 1961.

[18]Price, R.G., and Dance, N.: The demonstration of multiple heat stable forms of N-acetyl-β-glucosaminidase in normal human serum. Biochim. Biophys. Acta *271*:145-53, 1972.

[19]Cox, R.P., Douglas, G., Hutzler, J., Lynfield, J., and Dancis, J.: In-utero detection of Pompe's disease. Lancet *1*:893, 1970.

[20]Nakagawa, S., Kumin, S., and Nitowsky, H.M.: Studies on human β-D-N-acetyl-hexosaminidase isozymes. Fed. Proc. *33*:1593, 1974 (abst.)

[21]Bomback, F., Nakagawa, S., Kumin, S., and Nitowsky, H.M.: Serum and urine activities of lysosomal hydrolases in juvenile diabetes mellitus. Ped. Res. *9*:348, 1975 (abst.).

[22]Wellwood, J.M., Ellis, B.G., Price, R.G., Hammond, K., Thompson, A.E., and Jones, N.F.: Urinary N-acetyl-β-D-glucosaminidase activities in patients with renal disease. Br. Med. J. *3*:408-11, 1975.

[23]Spiro, R.G., and Spiro, M.J.: Studies on the biosynthesis of the hydroxylysine-linked disaccharide unit of basement membranes and collagens. I. Kidney glucosyltransferase. J. Biol. Chem. *246*:4899-4909, 1971.

[24]Spiro, R.G., and Spiro, M.J.: Studies on the biosynthesis of the hydroxylysine-linked disaccharide unit of basement membranes and collagens. II. Kidney galactosyltransferase. J. Biol. Chem. *246*:4910-18, 1971.

[25]Beisswenger, P.J., and Spiro, R.G.: Studies on the human glomerular basement membrane. Composition, nature of the carbohydrate units and chemical changes in diabetes mellitus. Diabetes *22*:180-93, 1973.

[26]Kefalides, N.A.: Biochemical properties of human glomerular basement membrane in normal and diabetic kidneys. J. Clin. Invest. *53*:403-07, 1974.

[27]Westberg, N.G., and Michael, A.F.: Human glomerular basement membrane: Chemical composition in diabetes mellitus. Acta Med. Scand. *194*:39-47, 1973.

[28]Tallman, J.F., Brady, R.O., Navon, R., and Padeh, B.: Ganglioside catabolism in hexosaminidase A deficient adults. Nature *252*:254-55, 1974.

[29]Spiro, R.G.: Biochemistry of the renal glomerular basement membrane and its alterations in diabetes mellitus. N. Engl. J. Med. *288*:1337-42, 1973.

[30]Osterby, R., and Lundbaeck, K.: The basement membrane morphology in diabetes mellitus. *In* Diabetes Mellitus: Theory and Practice. Ellenberg, M., and Rifkin, H., Eds. New York, McGraw-Hill, 1970, p. 178.

[31]Martin, G.M., Sprague, C.A., and Epstein, C.J.: Replicative life-span of cultivated human cells: Effects of donor's age, tissue, and genotype. Lab. Invest. *23*:86-92, 1970.