Glycoprotein Changes in Diabetic Kidneys

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SUMMARY

A study of glycoproteins from renal cortical tissue from seventeen individuals with diabetes mellitus and four controls was performed. The diabetic tissues were divided, based on observations of the acid mucopolysaccharide composition from an earlier study, into three groups: seven showed a milder clinical course of diabetes and minimal histologic changes; five had severe clinical diabetes and predominantly nodular Kimmelstiel-Wilson lesions; and the remaining five had moderate to severe clinical diabetes with predominantly diffuse intercapillary glomerulosclerosis. In the latter two groups, a marked increase of neutral sugars was noted, particularly suggesting an increase in galactose-containing glycoprotein. Similarly, shifts to electrophoretically faster-moving glycoproteins and more intense PAS-staining were noted in severe diabetic changes. These observations suggest that there may be significant chemical changes in the nature of glycoproteins asociated with diabetes which may reflect on an uncontrolled metabolic defect of this disease. DIABETES *19*:171-75, March, 1970.

The vascular changes occurring in diabetes mellitus are of considerable importance, yet the nature of these changes is still not entirely clear. For example, the microangiopathy which contributes greatly to the disability of patients with long-standing diabetes mellitus is attributed to alterations of macromolecules such as glycoproteins of the small blood vessels.[1,2] Evidence that glycoprotein is involved comes entirely from histochemical data, since no chemical characterizations of the soluble glycoprotein composition of the kidney, normal or in disease states, are yet available. A residual, rather insoluble, carbohydrate-protein, fibrous material has been characterized from basement membrane of glomeruli,[3] but the more soluble glycoproteins, sialoprotein type, have not been studied. The complexity of the problem is further indicated by recent studies from this laboratory which indicate that changes in the acid mucopolysaccharides (MPS) also occur in diabetic kidneys,[4] an observation which is contradictory to current impressions.[5] Recent studies from this laboratory have indicated that a family of glycoproteins, quite soluble, can be obtained from the kidney, and this group has been suggested to be different from similar material obtained from other organs and serum.[6] Interestingly, the glycoproteins of this type readily accept a periodic acid-Schiff stain,[6] occur as a heterogeneous mixture,[7] are antigenic,[6,7] and, for some, enzymatic activities have been found.[8] Application of similar studies[6,7] of the soluble glycoprotein material from kidneys involved in diabetes seem important to the elucidation of pathogenesis of vascular disease occurring in diabetes.

Although the intricate structure of the kidney is appreciated, this initial survey of kidney glycoproteins was carried out on tissue from the renal cortex without further subdivision into other structures. These glycoproteins are water-soluble and the saline solution used in the usual procedure for separation of renal components dissolves material under consideration.

MATERIAL AND METHODS

Materials

Tissues from kidneys used in this investigation were available in the laboratory from a previous investigation.[4] Detailed description of the clinical data concerning diabetes and related illness of the patients and histologic findings on kidney sections were reported earlier.[4] A total of seventeen diabetic and four nondiabetic sets of kidneys were used in this investigation. The tissues were stored at -20 ° C. until used for isolation of glycoproteins. The kidneys were arbitrarily arranged into three groups based on MPS composition, which correlated to some extent with the histologic changes.[4] Group A represented seven patients with mild diabetes, of short duration, and histologic changes that were minimal; Group B consisted of five patients with severe diabetes of long duration, often with a clinical diagnosis of Kimmelstiel-Wilson syndrome and with ad-

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vanced renal disease, uremia and edema, and upon histologic study nodular changes of glomeruli were prominent; Group C was composed of five patients with diabetes of more intermediate severity and duration than Group B, although one of these patients had a clinical diagnosis of the Kimmelstiel-Wilson syndrome, and upon histologic studies these individuals showed changes primarily of diffuse intercapillary glomerulosclerosis. The division into the three groups is not entirely distinct, since a certain overlap was apparent and this observation is similar to the extensive clinical and pathological study by Gellman et al.[9] and others.[10] Yet, based upon the studies of MPS changes, this sort of grouping seemed reasonable to investigate the glycoprotein chemistry. There are little data which offer a basis for orientation of the chemical studies of glycoprotein materials in tissue in relationship to disease, i.e., diabetes or even categories within diabetes.

Isolation of glycoproteins from kidney cortex

The kidney cortex was removed by careful gross dissection and glycoproteins were isolated by the same method as described earlier.[7] Briefly, this consisted of extraction of minced kidney cortex (approximately 20 gm.) with 180 ml. 0.15 M NaCl. The extract was dialyzed exhaustively against distilled water followed by adjustment to pH 4.0 and fractional precipitation with $(NH_4)_2\ SO_4$ at 40, 60, and 100 per cent saturations. The fraction precipitated between 60 and 100 per cent salt saturation represented the glycoprotein preparation. This material was dissolved in water, dialyzed against distilled water, and concentrated by pervaporation for analysis.

Analyses

The glycoprotein materials were analyzed for protein concentration by a biuret method,[11] sialic acid by the diphenylamine reaction,[12] total hexosamine after hydrolysis by 4 N HCl for fourteen hours in sealed tubes by the method of Boas omitting the use of resin,[13] and total neutral carbohydrate (hexoses) by the phenol-sulfuric acid method.[14] Differential determination of hexosamines was performed by a gas-liquid chromatographic method.[15] After hydrolysis of the glycoprotein with 3 N HCl for six hours at 100° C., glucose was determined by glucose oxidase (Glucostat, Worthington Biochemical Corp., Freehold, N.J.) and galactose by galactose oxidase (Galactostat, Worthington Biochemical Corp., Freehold, N.J.). Mannose was calculated by subtracting galactose from the total neutral carbohydrate values (hexoses). Fucose was determined by the method of Dische and Shettles.[16] The conditions for analyses were found optimal in detailed studies of similar glycoproteins from bovine aorta.[7,17]

Dry weight

Aliquots of tissue from kidney cortex were dried in vacuo over H_2SO_4 until constant weight was obtained and percentage dry weight calculated.

Electrophoresis in polyacrylamide gel

Electrophoresis of 0.03 ml. of samples containing 800 to 900 μg. of glycoprotein was carried out in polyacrylamide gels (5 per cent) with borate buffer, pH 8.6, $\mu = 0.03$, volts 300, employing the methods described previously.[6,18] E.C. No. 470 vertical gel electrophoresis cell (E.C. Apparatus Corp.) was used. After electrophoresis, the gels were stained with amidoblack and with periodic acid-Schiff reagent (PAS).[6] Bovine serum albumin (Nutritional Biochemical Corp., Cleveland, Ohio) was used as a marker.

Paper chromatography

Glycoprotein samples were hydrolyzed with 3 N HCl for four to six hours and chromatography was performed on Whatman No. 52 in solvents; ethyl acetate: pyridine: water (12:5:4). A good resolution of glucose, galactose, mannose and fucose can be achieved in this solvent system.[7] A silver nitrate stain was used to detect the reducing sugars.[19]

RESULTS

The results are presented in figures 1 and 2 and table 1.

Crude glycoprotein materials isolated from the cortex of individual kidneys were analyzed for protein and carbohydrate composition and the values for each group are shown in figure 1. The total amounts of soluble glycoproteins obtained from each kidney varied from individual to individual. Although there is no significant difference in the average total protein values between nondiabetic and two of the diabetic groups, A and C, there is a marked increase (about 55 per cent) in Group B over the normals. The values shown in the figure may also include small amounts of noncarbohydrate-containing proteins, as traces of other proteins are likely to be present in these preparations, exact amount of which cannot be determined. Carbohydrate analyses of the glycoprotein material revealed further variations within the groups.

The diabetic samples show an increase in all of the sugar constituents of glycoproteins over nondiabetic samples (figure 1). The extent of this increase is less in Group A but is very high in Group B, Group C being intermediate. Such findings may reflect either the incorporation of more sugar units into the glycoprotein

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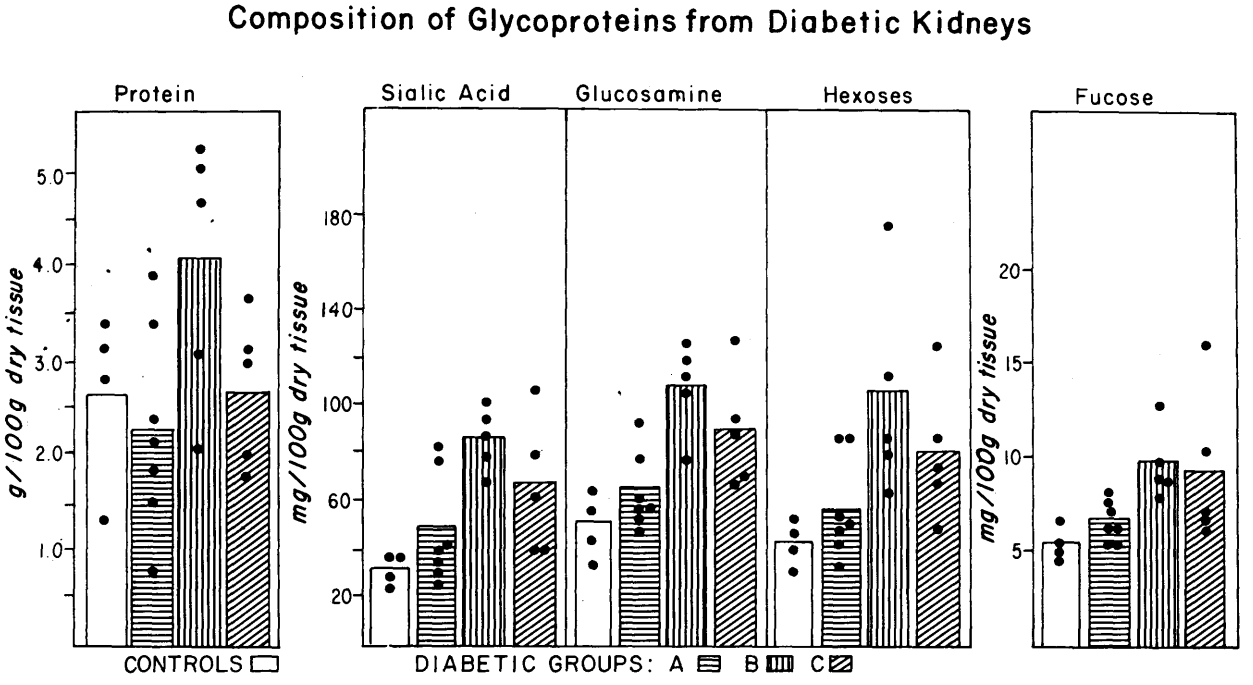

FIG. 1. Composition of glycoproteins from normal and diabetic kidneys. The values are expressed based on the dry weight of tissue. Details of the analytical methods are described in the text. Glycoproteins from diabetic kidneys showed marked increases in all the sugar constituents over controls. A remarkable increase in protein content of Group B over normals is apparent.

molecule or production of more glycoprotein, which also may result in an overall increase in sugar constituents.

Paper chromatography of neutral sugars showed the presence of galactose, mannose and fucose in normal as well as in the diabetic tissues, and GLC analyses indicated that glucosamine was the only hexosamine detected in all the glycoprotein preparations. These findings are consistent with earlier studies of the chemical composition of glycoproteins from cardiovascular connective tissue.[7] In order to find the relative proportion of individual sugars in the glycoprotein material, the constituent carbohydrate units were calculated on the basis of moles per mole of fucose. These results are presented in table 1. There was only slight change in molar ratios with respect to sialic acid, hexosamine, and total CHO between control and diabetic Groups A and C kidney glycoprotein fractions, but diabetic Group B showed a considerable increase in these values. A marked contrast with respect to molar ratios of galactose was observed between normals and diabetic kidney glycoprotein of all

TABLE 1

Carbohydrate composition of glycoproteins from diabetic kidneys

	Sialic acid	Hexosamine	Total CHO	Galactose	Mannose
	Moles/mole of fucose average (ranges)				
Normal (4)*	3.2 (2.4-3.8)	7.0 (5.6-8.8)	7.0 (5.9-7.7)	1.4 (1.2-1.7)	5.6 (4.7-6.0)
Diabetics:					
Group A (7)	3.9 (2.2-7.7)	7.7 (5.6-13.5)	8.0 (5.3-14.6)	3.4 (1.3-6.6)	4.6 (3.1-9.4)
Group B (5)	4.9 (3.9-6.1)	8.8 (6.7-10.2)	10.3 (7.1-17.9)	5.5 (2.5-10.9)	4.8 (3.0-7.0)
Group C (5)	3.9 (3.1-5.0)	7.6 (6.1-10.0)	8.3 (6.5-10.7)	4.7 (2.5-5.8)	3.7 (1.1-8.2)

*Number of samples.

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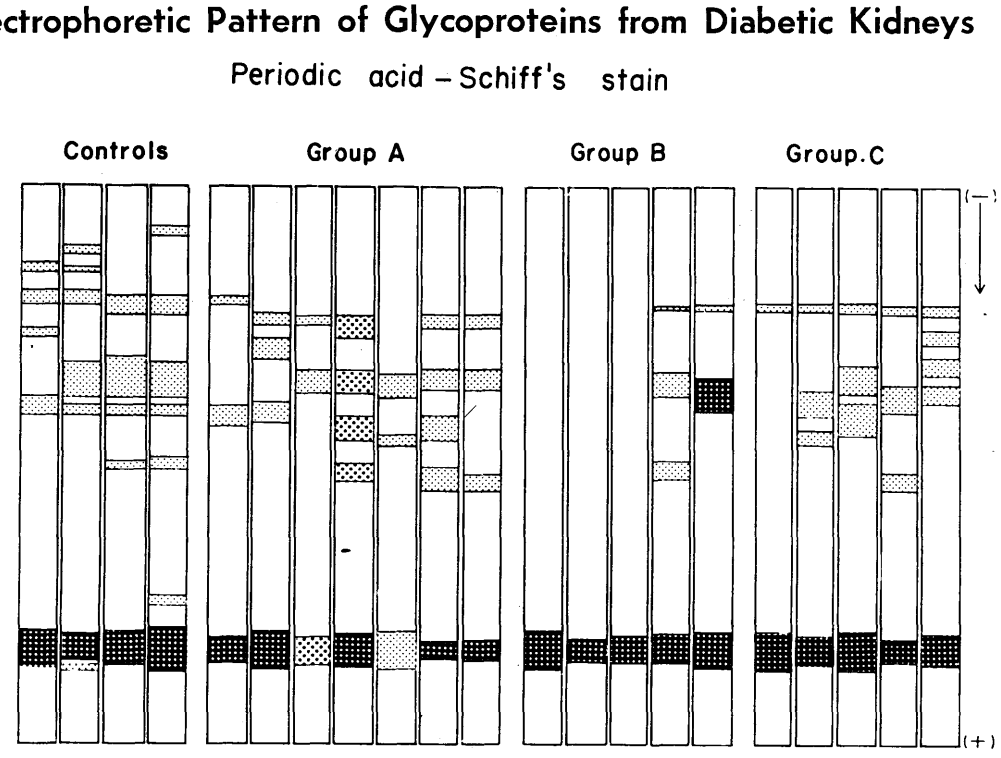

FIG. 2. Composite illustration of electrophoretic patterns of glycoproteins from normal and diabetic kidneys. Electrophoresis was carried out in borate buffer, pH 8.6μ = .03. The gels after electrophoresis were stained by PAS.[6] Certain distinct group differences in the patterns of glycoproteins from diabetic kidneys are apparent, although individual variations within the same group are observed. For details, refer to text.

groups. The increase in values are again of the same order as observed for the other sugars, namely diabetic Group B > Group C > Group A. In contrast, a slight decrease in molar ratios of mannose was found in preparations from the diabetic kidneys when compared to the control glycoprotein fractions. These observations suggest that incorporation of more constituent sugar units, except mannose and especially galactose, into the glycoprotein molecule may occur in the diabetic state. The extent of incorporation appears to vary with the nature and extent of involvement of renal disease.

The variation in types of proteins between control and diabetic kidney glycoproteins was studied by polyacrylamide gel electrophoresis, employing both PAS and amidoblack stains. A composite illustration of electrophoretic patterns of glycoprotein bands (PAS) is shown in figure 2. The patterns of glycoproteins indicate variation from person to person within normal as well as within the diabetic groups. This individual variation was anticipated, since similar observation noted earlier on glycoproteins from human aortas revealed distinct electrophoretic patterns that differed from individual to individual.[6] However, the over-all electrophoretic pattern, as well as number of bands, shows a distinct difference among the different diabetic groups. Electrophoretic patterns of Group A resemble those of kidney glycoprotein material from control tissue in frequency and heterogeneity of the glycoprotein bands. Although there are fewer bands in the case of diabetic Groups B and C, the fastest moving bands, particularly in Group B, were more intensely stained than the corresponding bands for controls. Similar differences were also observed in electrophoretic patterns stained with amidoblack, although certain slow-moving bands which were not stained by PAS were faintly stained by amidoblack, indicating the presence of small amounts of proteins with no bound carbohydrate in some isolates. The results of the electrophoretic analyses concur with the chemical analytical findings of increased content of sugar constituents (see figure 1) in diabetic kidney glycoproteins and, furthermore, suggest there are definite changes in the nature of glycoproteins associated with diabetes.

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DISCUSSION

It is evident from the results of this investigation that a family of glycoproteins are important to pathogenesis of tissue changes occurring in diabetes mellitus, although histochemical studies in diabetes[1,2] have for some time shown that glycoprotein material accumulates in vascular tissue. Chemical and electrophoretic analyses of glycoproteins isolated from kidneys involved with diabetes clearly illustrate changes in total glycoprotein content as well as types of glycoproteins. Elevations of protein-bound sialic acid, glucosamine, fucose, and particularly galactose in diabetes would suggest that the biosynthetic pathways of these sugars are not dependent on insulin. Spiro has demonstrated at least the biosynthesis of one of these sugars, glucosamine, from glucose is not regulated by insulin.[20] In diabetes, as the major pathway of glucose to glycogen is blocked, more glucose is available for metabolic pathways which are independent of insulin and this can result in elevated synthesis of sugar constituents of glycoproteins.

The occurrence of a family of glycoproteins with differences from one individual to another observed in cardiovascular tissue was also found in this study of renal tissue. The observation is interesting from a genetic standpoint but introduces difficulty in unraveling specific changes of this heterogeneous glycoprotein material in diabetes mellitus. The electrophoretic patterns of glycoproteins and shifts to faster moving fractions with more intense PAS staining are consistent with the results of chemical studies mentioned above. The disappearance of slower moving proteins might represent a destruction of renal tissue including certain enzyme fractions. It is also conceivable that depression of protein synthesis may be a factor in glycoprotein changes. The biological role of these diversely occurring glycoproteins is not yet clear, although a number of interesting proteins, heretofore recognized for genetic or certain biologic properties, e.g., transferrins, esterase, Hageman factor, have subsequently been shown to be sialic acid and neutral sugar-containing proteins. These studies point out only a facet of the complexity of glycoprotein chemistry of tissue involved in disease states.

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