SERUM AND URINARY PROTEINS IN

Diabetic Glomerulosclerosis

RESULTS OF ELECTROPHORETIC ANALYSIS

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Since the original description of diabetic intercapillary glomerulosclerosis by Kimmelstiel and Wilson[4] in 1936, numerous reports have confirmed its existence as a specific clinical and pathologic entity. Although the fully established syndrome is well known to the trained clinician, one must be constantly on the alert for variants in the clinical picture. The presence of doubly refractile lipoid elements in the urinary sediment is an important aid in the diagnosis, provided it is properly related to the clinical data.[11] Study of the serum proteins may furnish additional laboratory evidence.

Although the clinical and pathologic findings in diabetic glomerulosclerosis are now well delineated, the pathogenesis of the lesion is poorly understood. McManus has demonstrated, by means of the Periodic acid-Schiff reagent, that the hyalin material in the glomeruli in diabetic glomerulosclerosis is a carbohydrate-containing protein.[9] It has also been postulated that increased circulating serum polysaccharide may be casually related to this renal lesion of diabetes mellitus.

In this study an electrophoretic analysis of the serum and urinary proteins has been undertaken. A special emphasis has been placed upon the alpha-2 globulin fraction since this has the highest polysaccharide content in normal serum[16] and has been reported[15] as varying directly with the serum polysaccharide content.

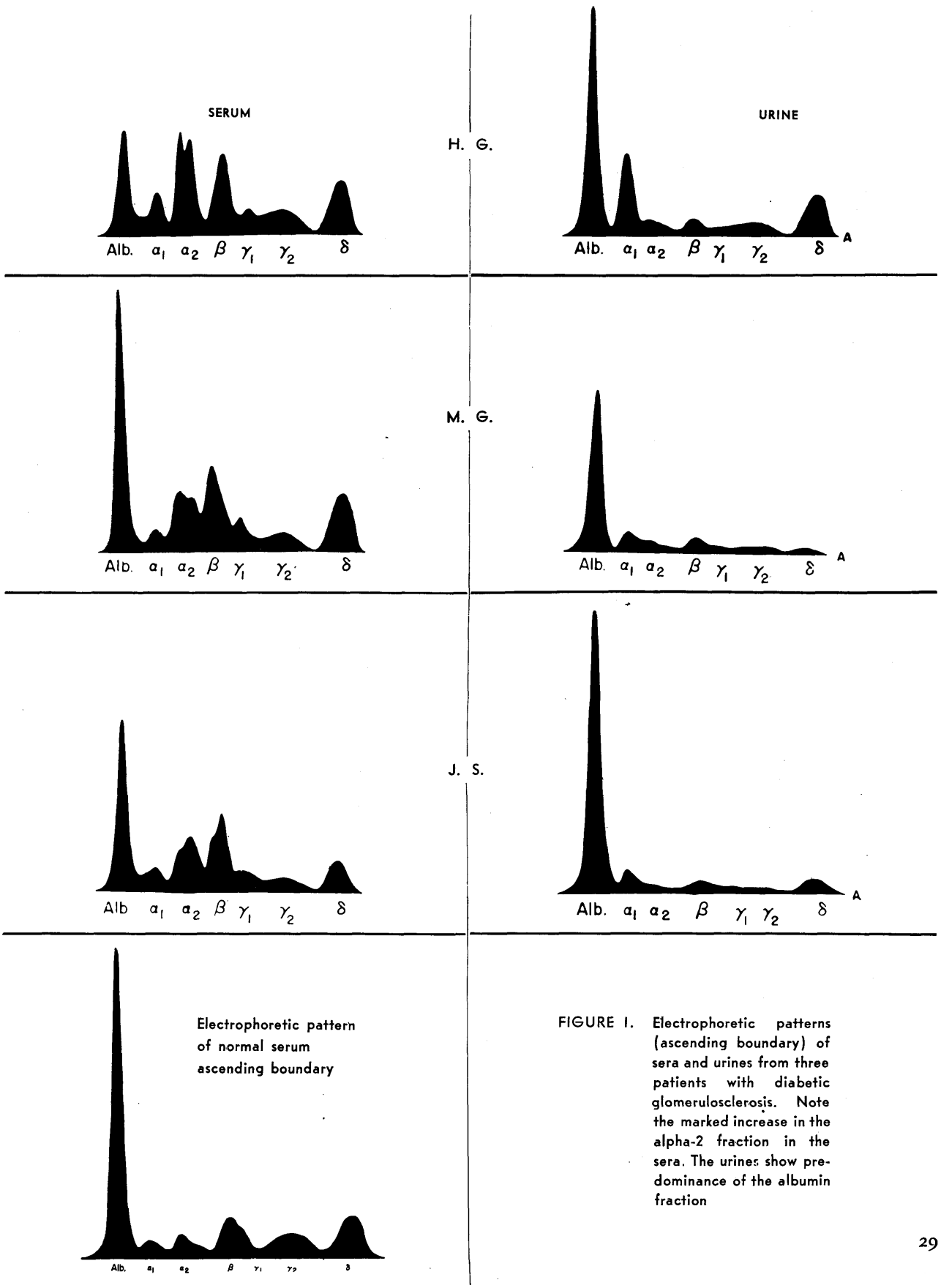
MATERIAL AND METHODS

Electrophoretic analyses were carried out on the sera of 10 patients and the urines of 3 patients with the fully established clinical syndrome. The analyses were done in veronal buffer, at pH 8.6 and ionic strength 0.10. The serum samples were diluted to 2 per cent protein before dialysis. The urine samples were not diluted, but were dialyzed extensively against saline prior to dialysis against the veronal buffer.

RESULTS

The electrophoretic patterns obtained on the sera and the urines of 3 representative patients are shown in Figure 1, and the analytical results on these same cases

Read at the Annual Meeting of the American Diabetes Association, Atlantic City, June 10, 1951.



Electrophoretic pattern of normal serum ascending boundary

FIGURE 1. Electrophoretic patterns (ascending boundary) of sera and urines from three patients with diabetic glomerulosclerosis. Note the marked increase in the alpha-2 fraction in the sera. The urines show predominance of the albumin fraction

TABLE 1 Analytical results of the serum and urinary protein from three representative cases of diabetic glomerulosclerosis. Compare these results with the values obtained in normal healthy subjects

	Total Protein	Albumin	Globulin				
			$Alpha_1$	$Alpha_2$	Beta	$Gamma_1$	$Gamma_2$
	GRAMS PER 100 c.c.						
Normal subjects Range (serum)	6.31-7.97	3.35-4.35	0.28-0.62	0.53-0.81	0.63-1.18	0.20	0.49-0.87
Patients with diabetic glomerulosclerosis							
H.G.—Serum	5.1	1.11	0.56	1.45	0.99	0.30	0.69
Urine	1.9	0.99	0.42	0.17	0.11	0.05	0.17
M.G.—Serum	6.6	2.52	0.38	1.38	1.21	0.51	0.60
Urine	0.8	0.52	0.08	0.07	0.06	0.02	0.05
J.S.—Serum	6.0	2.24	0.46	1.19	1.25	0.41	0.47
Urine	0.9	0.67	0.08	0.05	0.05	0.02	0.03

are given in Table 1. The sera from all 10 patients revealed a decrease in albumin, with a marked elevation of the alpha-2 globulins. An extra boundary between the beta and gamma globulins was noted in the serum of 3 patients, the mobility of which was close to that reported for gamma-1 globulin (sometimes called beta-2 globulin). Alpha-1 and gamma-2 globulins were within the normal range. The beta-globulins were either within normal limits or slightly elevated, as might be expected in diabetes mellitus.[5, 8, 15]

The urinary proteins consisted chiefly of albumin; in contrast to the results of the serum analyses the distribution of urinary globulins showed a predominance of the alpha-1 type.

Six of the 10 patients have now come to autopsy and the diagnosis of diabetic glomerulosclerosis has been confirmed. The 3 patients whose electrophoretic patterns are noted in Figure 1 are included in this group. As a control group, the sera of 5 patients with diabetes mellitus and hypertensive cardiovascular disease, but without the other clinical features suggestive of diabetic glomerulosclerosis, were studied. Electrophoretic analysis revealed either a normal or slight decrease in the albumin fraction with normal alpha-2 globulins and a slight increase in the beta globulin, which is the usual picture in uncomplicated diabetes mellitus. Postmortem examination of 3 of these patients revealed no evidence of the specific renal lesion.

TABLE 2 Contrast the electrophoretic analysis of the serum protein in the nephrotic phase of glomerulonephritis and in diabetic glomerulosclerosis. Although the $alpha_2$ globulin is elevated in both groups, the gamma globulin in contrast to the normal concentration in diabetic glomerulosclerosis is markedly reduced in nephrotic glomerulonephritis

Case	Cholesterol**	Urinary Protein***	Total Serum Protein*	Serum Albumin*	Serum Globulin			
					$Alpha_1$*	$Alpha_2$*	Beta*	Gamma*
Normal subjects (Range)			6.31-7.97	3.35-4.35	0.28-0.62	0.53-0.81	0.63-1.18	0.49-0.87
Nephrotic nephritis								
Case 1	985	14.5	5.3	1.38	0.42	1.16	1.59	0.26
Case 2	430	8.1	5.6	2.06	0.62	0.84	1.23	0.16
Diabetic glomerulosclerosis								
Case 1	620	13.5	5.1	1.11	0.56	1.45	0.99	0.69
Case 2	390	7.0	6.0	2.24	0.46	1.19	1.20	0.52

*Grams/100 ml. **Mg./100 ml. ***Grams/24 hours

DISCUSSION

The findings noted here differ significantly from those reported in diabetes mellitus without the specific renal complication. Electrophoretic patterns of sera from patients with mild untreated diabetes[14] reveal a normal distribution of protein although the total proteins are slightly decreased. Following therapy on a standard diet with not more than 80 Gm. of protein, the plasma proteins revert to normal. In patients with uncomplicated diabetic acidosis, an elevation in beta globulin occurs,[14] which is probably due to an increase in beta lipoproteins.[1] Electrophoretic analysis of the plasma proteins of patients with diabetic retinitis has been reported to show a low plasma albumin with a high beta globulin.[14] Although proteinuria and hypertension were noted in a number of these patients, it is not possible to determine from the data whether any of the patients had a true Kimmelstiel-Wilson syndroms. This is an important point since the electrophoretic data have to be interpreted in the light of the exact diagnosis. It should be recalled that all of our patients presented a composite picture of diabetes, edema, hypertension, combined diabetic and hypertensive retinopathy, severe proteinuria, and doubly refractile lipoid cells in the urinary sediment.

The electrophoretic pattern described for diabetic glomerulosclerosis differs from that obtained in most acute and chronic disease states, which show an increase in both alpha-1 and alpha-2 globulins with a decrease in the serum albumin. [3, 5, 8, 17] In our cases, the alpha-1 globulins were within the normal range.

The results appear somewhat similar to the electrophoretic analysis of the serum proteins in the well-developed nephrotic phase of glomerulonephritis.[5, 6, 8, 18] Here, too, there is a marked decrease in serum albumin, associated with an increase in the alpha-2 and beta globulins. The gamma globulin, however, in contrast to the normal concentration in diabetic glomerulosclerosis, *is markedly reduced in nephrotic glomerulonephritis* (Table 2). It is possible that the elevation of the alpha-2 globulin noted in both of these renal diseases is the result of a mobilization of protein reserves as in starvation,[17] and not due to the disease process.

Our patients were all in the sixth and seventh decades of life. It is important, therefore, that electrophoretic studies be repeated on young diabetic patients with the specific renal lesion. There is hope that further observations may prove of value both in the diagnosis of early cases of diabetic glomerulosclerosis, and in the differential diagnosis from coexisting diabetes mellitus and hypertensive cardiovascular disease.

SUMMARY AND CONCLUSIONS

1. In each of 10 patients with diabetic glomerulosclerosis, there was a significant elevation in the serum alpha-2 globulin.

2. Since in diabetes mellitus, uncomplicated by the specific renal lesion, the alpha-2 globulin is within normal limits, the possibility is suggested that the determination of the alpha-2 globulin may prove to be of value in the diagnosis of diabetic intercapillary glomerulosclerosis.

ACKNOWLEDGMENTS

We express appreciation to Dr. Louis Leiter, Chief of the Medical Division, and Dr. Harry M. Zimmerman, Chief of the Division of Laboratories, Montefiore Hospital, for their helpful suggestions and criticisms; Dr. James I. Berkman, associate pathologist, Montefiore Hospital, for his constant stimulation and study of the autopsy protocols; Mr. George Ross and Miss Ann Solomon for the serum and urinary protein determinations; Mrs. Mary G. Hamilton for the electrophoretic analyses.

The authors wish to acknowledge the assistance of the National Cancer Institute of the U.S. Public Health Service and the Atomic Energy Commission (Contract AT (30-1)-910) to the Sloan-Kettering Laboratory.

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DISCUSSION

DR. LOUIS LEITER *(New York):* The great interest aroused in recent years by the so-called degenerative vascular lesions of prolonged diabetes has coincided with a revival of careful clinical and pathological studies of the renal complications of diabetes. As a result, it is now clear that the diabetic patient in the course of years faces a number of renal hazards, of which diabetic glomerulosclerosis may be the most serious. Its clinical manifestations, including the specific urinary findings which Dr. Rifkin has mentioned, readily distinguish it from the acute glomerulonephritis or pyelonephritis likely to occur in the younger diabetic subjects, and from chronic pyelonephritis and the hypertensive renal vascular disease so common in the diabetics of the older age groups. However, difficulties in the differential diagnosis of diabetic glomerulosclerosis do arise: first, in younger age diabetics who may have nephrotic or hypertensive stages of chronic glomerulonephritis; second, in the older age diabetics who have proteinuria and renal insufficiency associated with congestive heart failure and renal vascular disease.

The increase in the serum alpha-2 globulin described by Dr. Rifkin will probably help in the differential diagnosis of diabetic glomerulosclerosis in this older age group from renal vascular disease in patients who have proteinuria and congestive heart failure. Unfortunately, alpha-2 globulin is also elevated in cases of nephrotic glomerulonephritis.

It should also be noted that along with the increase in beta globulin and fibrinogen, the alpha globulins are elevated in normal pregnancy, usually by the third trimester and continuing on through the first two weeks of the postpartum period. Whether this rise has implications as to the site of protein regeneration or is related to the endocrine disturbances in pregnancy is not known. Whether the alpha-2 globulins of nephrotic nephritis and in diabetic glomerulosclerosis are different or distinguishable from the normal alpha-2 globulins, electrophoretically or otherwise, remains to be determined.

An important question arises: Does the increase in alpha-2 globulin merely follow prolonged proteinuria or does it represent an early, perhaps primary, change in the serum protein pattern of the patient with diabetic glomerulosclerosis? If the latter alternative holds, then we may have a valuable tool for an early diagnosis of the specific diabetic renal lesion.

At present the evidence is too tenuous to warrant any conclusion as to a pathogenic relationship between increases in alpha-2 globulin and serum polysaccharides and the deposition of the polysaccharide containing hyalin material in the glomeruli of diabetic glomerulosclerosis.

DR. HOWARD F. ROOT *(Boston, Mass.):* The idea long established, that the so-called Kimmelstiel-Wilson lesion is peculiar to mild diabetes in middle and late life and unrelated to severity of diabetes, is not supported by observation of patients whose diabetes began early in life. Those of us who see diabetic children and young patients after twenty years of diabetes recognize the diabetic nephropathy, which includes the Kimmelstiel-Wilson lesion, arteriolarsclerosis and chronic pyelonephritis as the most common single cause of death. I would offer the conclusion that this lesion is really directly related to the severity of the diabetes, its duration and the character of its control.

DR. HAROLD RIFKIN (Closing): This renal lesion may certainly be associated with diabetes in young people, and we urge you to obtain electrophoretic analyses in these cases.