Kidney International, Vol. 33 (1988), pp. 100–106

Glomerular size and charge selectivity in insulin-dependent diabetes mellitus

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Glomerular size and charge selectivity in insulin-dependent diabetes mellitus. The pathogenesis of clinical nephropathy in Type 1 (insulin-dependent) diabetes was investigated by measuring renal fractional clearances of albumin, total IgG, IgG_4 and β_2-microglobulin, four plasma proteins which differ in size and charge. Seventy patients and eleven control subjects were studied. In diabetic patients with normal urinary albumin excretion (<30 mg/24 hr), fractional IgG clearance was two to three times higher than in control subjects, whereas fractional clearance of the anionic plasma proteins IgG_4 and albumin was similar to that of control subjects. These alterations indicate an increase in anionic pore charge within the glomerular basement membrane concomitant with an increase in either pore size or impairment of tubular reabsorption. Diabetic patients, whose urinary albumin excretion has started to rise (30 to 100 mg/24 hr), had unchanged fractional IgG compared to patients with normal albumin excretion, while fractional IgG_4 and albumin clearances were increased three- to fourfold; indicating unchanged glomerular pore size, but a decrease in anionic pore charge. In patients demonstrating urinary albumin excretion of greater than 100 mg/24 hr fractional IgG clearance increased to the same extent as fractional albumin clearance, indicating an increase in large pore area. Fractional β_2-microglobulin clearances were similar to that of control subjects in the different patient groups indicating unchanged tubular reabsorption of proteins. Thus, the increase in large pore area seen in patients with clinical nephropathy is preceded by loss of anionic charge in the glomerular basement membrane. It is likely that this loss of anionic charge is due to loss of heparan sulphate-proteoglycan.

Patients with Type 1 (insulin-dependent) diabetes mellitus and urinary albumin excretion ($U_{alb}V$) of more than 300 mg/24 hr have a poor prognosis [1]. The pathogenesis of albuminuria is, however, unknown. Neither is it known why only 30 to 40% of the patients develop this serious complication [2], while the majority of patients have a normal $U_{alb}V$ below 30 mg/24 hr after more than 40 years of diabetes in spite of poor metabolic regulation [3] and rather severe glomerulosclerosis [4].

In patients with persistently increased $U_{alb}V$ greater than 300 mg/24 hr (equivalent to persistent proteinuria), changes in glomerular pore size have been found simultaneously with decreasing filtration surface [5, 6] and widened and loosened epithelial foot processes [7]. Intraglomerular pressure is assumed to be increased since filtration fraction is unchanged in these patients [8] in spite of decreasing filtration surface [6] and lower oncotic pressure [9]. Loss of sialic acid and heparan sulphate [10] in glomerular basement membranes (GBM) of these patients has, together with a preferential urinary excretion rate of albumin over IgG [11], suggested changes in charge selectivity as well. These functional and structural alterations together with the rise in systemic blood pressure [12] might explain the fiftyfold increase of $U_{alb}V$ in patients with persistent proteinuria. However, in the very early phase of clinical nephropathy. that is, in Albustix negative patients with elevated glomerular filtration rate (GFR) and $U_{alb}V$ in the borderline between normal and clearly elevated $U_{alb}V$, very little is known about changes in glomerular permselectivity. Since more knowledge about these patients might give some insight into the pathogenesis of clinical nephropathy, we studied the renal fractional clearance of four endogenous plasma-proteins in patients with Type 1 (insulin-dependent) diabetes and normal serum creatinine, who demonstrated a wide range of $U_{alb}V$. The four plasma proteins differed in size and charge and were albumin (molecular weight [MW] 69,000, Stokes radius 36 Å, pI 4.7 to 5.5), total IgG (MW 156,000, Stokes radius 55 Å, pI 5.8 to 7.3), IgG_4 (MW 156,000, Stokes radius 55 Å, pI 5.5 to 6.0) and β_2-microglobulin (MW 11,800, Stokes radius 16 Å, pI 5.8).

Subjects

The patients were selected from the outpatient clinic at the Steno Memorial Hospital, where most of them have been followed for many years. They had no history of non-diabetic renal or cardiac disease and all had a negative bacterial culture of the urine. No drugs other than insulin were given. All subjects gave informed consent for participation and the study was approved by The Regional Scientific Ethical Committee. The diabetic patients were subdivided into four groups according to level of albuminuria identified on the basis of the median albumin excretion in three 24-hour urine collections performed at home during periods of normal physical activity. This was done because of the high (50%) day to day variation of the 24-hour urinary albumin excretion [13]. Eleven healthy non-diabetic subjects served as controls. These subjects only delivered one 24-hour urine sample. All subjects were below the age of 50 and all patients had had diabetes for five years or more. Any ongoing antihypertensive treatment had been discontinued for eight weeks. The four groups were defined as follows:

Group 1. Eighteen patients with normal urinary albumin

Received for publication February 13, 1987
and in revised forms June 29 and August 24, 1987

Table 1. Clinical data

	$U_{alb}V$ *mg/24 h*	Sex *M/F*	Age *years*	Duration of diabetes *years*	HbA_{1c} %	$U_{alb}V$ *ml/24 hr*	Serum creatinine *μmol/l*	GFR *ml/min per 1.73m²*	BP *mm Hg*		Retinopathy *O/B/P*
Controls *N* = 11	<30	5/6	31 ± 1.9	—	5.2 ± 0.1	12 ± 2.1	80 ± 3.0	101 ± 3.6	124 ± 2.4	77 ± 2.4	0
Group 1 IDDM *N* = 18	<30	10/8	35 ± 2.1	19 ± 1.9	8.3 ± 0.3	16 ± 2.1	76 ± 2.4	111 ± 3.8	131 ± 2.6	84 ± 2.4	15/3/0
Group 2 IDDM *N* = 22	30–100	12/10	32 ± 2.0	17 ± 1.2	9.2 ± 0.4	46 ± 4.7	80 ± 2.4	117 ± 4.5	127 ± 2.6	81 ± 1.5	11/11/0
Group 3 IDDM *N* = 16	101–300	11/5	33 ± 2.5	17 ± 1.4	9.4 ± 0.4	230 ± 30	89 ± 3.3	102 ± 4.0	132 ± 2.8	86 ± 1.5	5/9/2
Group 4 IDDM *N* = 14	>300	9/5	31 ± 1.9	19 ± 2.1	9.8 ± 0.4	2932 ± 581	94 ± 8.0	86 ± 8.3	159 ± 5.6	102 ± 2.4	2/5/7

Mean ± SEM are indicated.
Retinopathy is graded in O, minimal changes or less; B, background retinopathy; P, proliferative retinopathy.

excretion, that is, less than 30 mg/24 hr and with blood pressure less than 160/95 mm Hg.

Group 2. Twenty-two patients with slightly elevated urinary albumin excretion in the range of 30 to 100 mg/24 hr, that is, patients with persistent microalbuminuria. Patients with blood pressure readings more than 160/95 mm Hg were excluded in order to avoid contamination with hypertension-induced hyperalbuminuria [14].

Group 3. Sixteen patients with moderately elevated urinary albumin excretion in the range of 100 to 300 mg/24 hr. Patients with blood pressure readings greater than 160/95 mm Hg were excluded.

Group 4. Fourteen patients with diabetic nephropathy ($U_{alb}V$ >300 mg/24 hr).

The clinical characteristics of patients and control subjects are shown in Table 1.

Methods

The daily excretion of proteins was calculated on the basis of a 24-hour urine sample collected at home. Urinary albumin concentrations, urinary total IgG concentration and urinary β_2-microglobulin concentration were measured by an ELISA assay, interassay variation 8.3%, 9.0%, and 9.2%, respectively [15–17]. Serum albumin, total IgG and β_2-microglobulin were determined by the same ELISA method after proper dilution of the sample. Urinary and serum IgG_4 were measured by an immunoradiometric assay in which Maxisorb test tubes (Nunc, Roskilde, Denmark) were coated with 0.25 ml affinity-purified antihuman IgG_4 ($aIgG_4$) (Janssen Biochemica, 2340 Beerse, Belgium; list No.: SH164-01-S1), diluted 1:3,000 with 0.01 M phosphate buffered saline, pH 7.5 (PBS) with 0.01% (wt/vol) sodium azide. After overnight incubation, the tubes were washed five times with PBS containing 0.1% (wt/vol) Tween 20 (PBS/tw). The assay was calibrated by use of appropriate dilutions (1 to 200 ng/ml) of a human reference serum from Central Laboratory of the Laboratory, Red Cross Blood Transfusion Service (list No.: HOO-2). Reference serum, patients' sera, and urines were diluted in an incubation buffer (IB) containing 0.5% (wt/vol) bovine gamma globulin, 5% (wt/vol) heat inactivated (½ hr; 56°C) normal sheep serum, and 0.5% (wt/vol) Tween 20 in 0.1 M potassium phosphate buffered saline, pH 7.5. The $aIgG_4$ coated tubes were incubated for four hours at room temperature with 0.25 ml diluted serum or urine, washed five times with PBS/Tw, and then incubated overnight at room temperature with about 0.1 μCi ^{125}I-labelled $aIgG_4$ (SA about 7 Ci/g) in 0.25 ml IB. After washing five times with PBS/Tw the tubes were counted for one minute in a gamma-counter. Sensitivity of the assay was 1 ng IgG_4/ml (in diluted solutions). Specificity and reactivity were evaluated by incubation with 100 ng/ml, highly-purified IgG myeloma proteins [18], one of each subclass. No IgG_4 was detectable by incubation with IgG_1, IgG_2, or IgG_3 proteins, indicating that cross-reactivity was less than 1%. Furthermore, the specificity of the $aIgG_4$ applied in this assay has been found by others [19] to be comparable to that of a highly specific monoclonal anti-IgG_4 [18]. Recovery of the IgG_4 myeloma protein was 97%. Interassay coefficient of variation was 7.6%.

Hemoglobin A_{1c} was measured by a chromatographic technique [20]. The normal range was 4.1 to 6.4%. Blood pressure was measured with a standard sphygmomanometer on the right arm after 20 minutes of supine rest on several occasions. The diastolic blood pressure was recorded when the Korotkoff sounds disappeared (phase V). Serum creatinine was measured by a reaction rate method modified to eliminate pseudocreatinines. The interassay coefficient of variations was 2.5% [21]. The glomerular filtration rate was measured after a single intravenous injection of 51-^{Cr}EDTA (at 9 a.m.) by observation of the plasma disappearance for four hours [22]. The fractional protein clearance was calculated as Urinary protein excretion/ Serum protein concentration × glomerular filtration rate:

$$\frac{\text{Urinary protein excretion}}{\text{Serum protein concentration} \times \text{glomerular filtration rate}}$$

All results were corrected for variation in body surface. The patients eyes were examined by routine ophthalmoscopy through the dilated pupil by a trained observer.

Standard parametric statistics were applied except for protein clearances which are not normally distributed, unless logarithmically transformed [15]. For these calculations we therefore

Table 2. Fractional clearance, mean (± SEM, log. normal distribution)

	$U_{alb}V$ *mg/24 hr*	^{C}alb/GFR $\times 10^{-6}$	^{C}IgG/GFR $\times 10^{-6}$	$^{C}IgG_4$/GFR$^{\times}$ $\times 10^{-6}$	$^{C}\beta_2$-microglobulin/ GFR $\times 10^{-4}$	SI (^{C}IgG/^{C}alb)
Controls	<30	1.63^{NS}	0.65^{c}	0.80^{NS}	3.38^{NS}	0.41 ± 0.10^{b}
N = 11		(1.39–1.90)	(0.56–0.74)	(0.66–0.96)	(2.86–4.00)	
IDDM	<30	1.80^{c}	1.53^{NS}	0.85^{a}	4.31^{NS}	0.88 ± 0.13^{c}
N = 18		(1.52–2.13)	(1.30–1.80)	(0.58–1.23)	(3.54–5.24)	
IDDM	30–100	5.46^{c}	1.75^{c}	3.19^{a}	4.30^{NS}	0.38 ± 0.05^{a}
N = 22		(4.75–6.26)	(1.50–2.03)	(2.65–3.85)	(3.85–4.79)	
IDDM	101–300	32.14^{c}	6.75^{c}	13.58^{a}	5.96^{NS}	0.23 ± 0.03^{NS}
N = 16		(27.86–37.07)	(5.69–8.00)	(8.02–23.01)	(4.79–7.41)	
IDDM	>300	477	72.95	62.66	13.40	0.16 ± 0.02
N = 14		(330–693)	(48.75–109.14)	(41.11–95.50)	(8.34–21.53)	

SI: selectivity index (clearance of IgG/clearance of albumin), mean ± SEM.
× only 10, 10, 5, 5, and 10 cases were analyzed.
NS, a, b, and c are: non-significant ($2P > 0.05$), $2P < 0.05$, $2P < 0.02$, $2P < 0.01$, respectively, in comparison to the next group in the same column.

applied parametric statistics only after log transformations. Comparisons between groups were—after proper logarithm transformation—performed by *t*-tests after demonstrating significant differences between groups by ANOVA (Kruskall-Wallis).

Results

The groups of patients were fairly well matched according to sex, age, diabetes duration, diabetes regulation, and blood pressure with the exception of patients in group 4 (clinical nephropathy) who had higher HbA_{1c}, creatinine, and blood pressure compared with the other groups. The glomerular filtration rate (GFR) was significantly increased in most diabetic patients except in patients with clinical nephropathy ($U_{alb}V$ >300 mg/24 hr), who demonstrated a wide range of GFR (41 to 152 ml/min/1.73 m^2; Table 1).

In patients with normal $U_{alb}V$ (group 1) fractional IgG clearance was significantly increased, whereas the fractional albumin clearance and the fractional clearance of anionic IgG_4 were similar to that of controls (Table 2). Fractional IgG clearance was not correlated to age, diabetes duration, HbA_{1c}, or GFR. Since the fractional IgG clearance was increased and fractional albumin and IgG_4 clearances were unchanged, the relative IgG clearance (selectivity index SI = IgG clearance/albumin clearance) was significantly elevated ($2P = 0.011$) (Fig. 1). The increase in IgG/IgG_4 clearance did not reach statistical significance.

In patients with slightly elevated $U_{alb}V$ (30 to 100 mg/24 hr), (group 2) fractional IgG clearance was similar to that of diabetic patients with normal $U_{alb}V$. However, fractional IgG_4 clearance was now increased three- to fourfold in comparison with controls or patients demonstrating normal $U_{alb}V$. This increase was of the same magnitude as the increase in fractional albumin clearance. These changes resulted in a significant decrease in selectivity index, SI compared to diabetics with normal $U_{alb}V$. The IgG/IgG_4 clearance decreased too, but again without reaching statistical significance.

In patients with more marked microalbuminuria (100 to 300 mg/24 hr) (group 3) the fractional clearance of IgG was increased fourfold compared with diabetics of groups 1 and 2 and to similar degree as the fractional albumin clearance. Also, fractional IgG_4 clearance was further increased. A significant

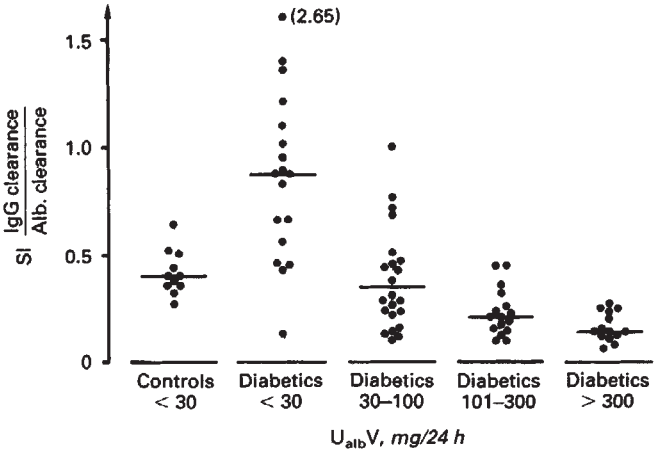

Fig. 1. *Selectivity index (clearance of IgG/clearance of albumin) in 11 controls and 70 patients with Type 1 (insulin-dependent) diabetes and different degrees of urinary albumin excretion ($U_{alb}V$).*

correlation between fractional albumin and fractional IgG clearances was seen in this group of patients alone and also when patients with clinical nephropathy (group 4) were included (r = 0.952, $2P < 0.01$, $N = 30$) (Fig. 2). A further decrease in SI was seen in groups 3 and 4 (Fig. 1), whereas no further change in IgG/IgG_4 clearances could be demonstrated.

The urinary excretion of β_2-microglobulin was higher in diabetic patients compared to controls, but the difference did not reach statistical significance and the fractional clearance of β_2-microglobulins was similar in all groups (Table 2). Only in patients with GFR below 80 ml/min/1.73 m^2 was the fractional β_2-microglobulin clearance clearly increased.

Discussion

All patients suffered from Type 1 diabetes. The serum samples were taken after the patient had relaxed for at least 30 minutes in the supine position to avoid hemoconcentration and increase of plasma protein in the upright position. Urine samples were kept at −20°C until analysis of the whole material, which was performed in random order within a few days.

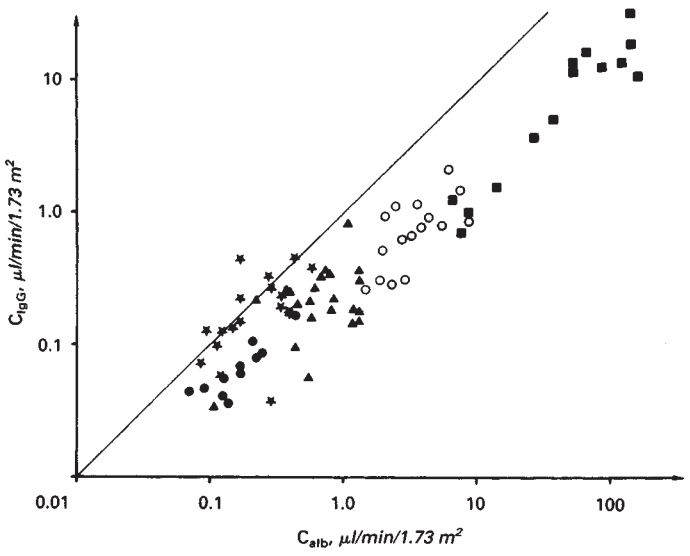

Fig. 2. *Albumin and IgG clearance in 11 control subjects and 70 patients with Type 1 (insulin-dependent) diabetes and different degrees of urinary albumin excretion.* (Group designation as in Fig. 1.) Log transformation was performed before the calculation of regression. Symbols are: (●) controls, $r = 0.864$, $y = -0.16 + 0.81x$, $2P < 0.01$; (*) IDDM, $U_{alb}V$ <30 mg/24 hr, $r = 0.510$, $y = 0.41 + 0.61x$, $2P < 0.05$; (▲) IDDM, $U_{alb}V$ 30 to 100 mg/24 hr, $r = 0.578$, $y = 0.14 + 0.66x$, $2P < 0.01$; (○) IDDM, $U_{alb}V$ 101 to 300 mg/24 hr, $r = 0.663$, $y = -0.13 + 0.78x$, $2P < 0.01$; (■) IDDM, $U_{alb}V$ >300 mg/24 hr, $r = 0.947$, $y = -0.97 + 1.04x$, $2P < 0.01$.

IgG_4 was only analyzed in 40 patients due to lack of sufficient specific antiserum. Ten controls and 10 + 5 + 5 + 10 diabetic patients from group 1 to group 4, respectively, were analyzed after random selection. Since the concentration of anionic IgG_4 in plasma and urine was only 3 to 4% of the total IgG concentration, total IgG was taken as representative of uncharged IgG. Glomerular filtration of plasma protein is restricted by glomerular basement membrane pore size and charge [23]. From experiments with dextrans [24], the mean glomerular pore radius is calculated to be of the same size as pores of fenestrated capillaries, which is about 55 Å [25]. A lognormal distribution of glomerular pore size has been demonstrated to fit well with experimental data [26]. The surface of the pores is, as in other endothelial pores, covered with highly anionic heparan sulphate [25], thus contributing to the charge selectivity of the GBM [27]. The regularly arranged anionic sites seen in the lamina rara of GBM from animals [28] and man [29, 30] might be equivalent to pores in the basement membrane. The fractional IgG clearance can be assumed to reflect either size non-selective pores (shunts) of about 100 Å Stokes radius or the very small fraction of relatively large pores (>55 Å) within the lognormal pore distribution [26]. Since our experimental findings seem to fit better to lognormal pore distribution, we will base our discussion on this model.

In diabetic patients with normal $U_{alb}V$ we unexpectedly found fractional IgG clearance to be significantly increased, indicating an increased large pore area or impaired tubular reabsorption. The latter assumption is supported by fractional dextran clearance studies demonstrating that pore size distribution in uncomplicated diabetic patients is identical with that of control subjects [5, 31, 32]. However, due to the very low fraction of large pores and the variance of such measurements, an increase of the large pore area can not be totally excluded from these experiments. The results of fractional β_2-microglobulin clearances in our patients also make impaired tubular reabsorption less likely (Table 2). Increased fractional IgG clearance has not been demonstrated earlier in normoalbuminuric diabetic patients. It might be correlated to increased intraglomerular pressure. The increase of fractional IgG clearance was not accompanied by a similar increase of fractional albumin clearance. Plasma albumin molecules have a significantly lower Stokes radius than IgG molecules (36 and 55 Å, respectively) and would therefore be able to pass much easier through pores of increased diameter than IgG if not retropulsive forces between the negative charge of the albumin molecule and the glomerular membrane inhibit the passage. The fact that the fractional albumin clearance was not increased in diabetic patients with normal $U_{alb}V$ thus indicates that the increased pore area in these patients is counterbalanced by increased anionic pore charge. This assumption is supported by the unchanged fractional clearance of anionic IgG_4 (Table 2). Since IgG_4 has the same molecular size as neutral IgG, the lack of a threefold increase of fractional IgG_4 can only be explained by an increase of retropulsive forces between the glomerular pore and IgG_4 molecules.

Increased restriction to the passage of anionic macromolecules has also been demonstrated in rats after three months of diabetes [33]. The decreased passage of anionic dextran macromolecules in this experiment was attributed to an increased negative charge density in the filtration barrier secondary to compositional alterations of the glomerular basement membrane [33]. The cause of increased anionic pore charge in diabetic patients with normal $U_{alb}V$ might be due to non-enzymatic glycation of GBM [34–36] or clogging of the GBM with anionic plasma proteins [37–41]. Our finding of increased fractional IgG clearance, but unchanged albumin and IgG_4 clearance in diabetic patients with normal $U_{alb}V$, can thus best be explained by a more negative pore charge in combination with either increased pore size or impaired tubular reabsorption.

In patients with slightly increased $U_{alb}V$ (30 to 100 mg/24 hr) other changes in pore charge seem to appear. In spite of unchanged fractional IgG clearance, the fractional albumin clearance was increased threefold. Two explanations seem to be possible: (1) formation of new electroneutral pores of less than 55 Å Stokes radius making it possible for albumin molecules to pass through, whereas IgG molecules cannot pass [5, 42]; or (2) loss of anionic charge within the existing pores of diabetic patients. Both possibilities would fit our recent finding of a decreased albumin/glycated albumin clearance ratio in these patients [36]. However, the highly significant increase in fractional IgG_4 clearance in these patients is not consistent with the formation of new small neutral pores. We therefore believe that patients with slightly increased $U_{alb}V$ are characterized by reduced anionic glomerular pore charge in comparison with diabetic patients with normal $U_{alb}V$. Since HbA_{1c} was similar as in diabetic patients with normal $U_{alb}V$, reduced non-enzymatic glycation of GBM as a cause of reduced anionic charge is unlikely. Also, loss of anionic plasma protein deposits in the GBM of these patients is unlikely, since the amount of anionic

plasma protein deposits within the GBM does not seem to be less in patients with diabetic nephropathy [34, 39]. We therefore believe that the decrease in anionic charge is due to loss of other anionic substances in the basement membrane such as acidic amino acid residues, sialic acid or heparan sulphate-proteoglycan. The concentration of acidic amino acids, however, has been found to be unchanged in human GBM from patients with diabetic nephropathy [10]. Only the content of sialic acid [10] and heparan sulphate [34, 43] has been shown to be reduced. Since sialic acid is present only on epithelial and endothelial cell surfaces, but not within the GBM [44], the decrease in anionic charge found in our studies is probably due to loss of heparan sulphate and/or decreased sulfation of heparan-sulfate-proteoglycan. It has been demonstrated earlier that loss of heparan sulphate in GBM results in albuminuria [45]. Furthermore, loss of anionic sites in diabetic patients with albuminuria has recently been demonstrated [30, 46]. It has also been shown several times that incorporation of SO_4^{--} into heparan sulphate of the GBM of diabetic animals is decreased [47–51] and that the concentration of heparan sulphate in GBM from diabetic patients might be decreased [34, 43]. Therefore it seems most likely that the initial increase of $U_{alb}V$ in insulin-dependent diabetic patients is caused by a reduction in the content or sulphation of heparan sulphate within the pores of the GBM and not by the formation of new pores.

In patients with more advanced microalbuminuria ($U_{alb}V$ 100 to 300 mg/24 hr, group 3), fractional IgG clearance was increased fourfold compared to patients of group 2. It is not possible from our studies to determine whether this increase in fractional IgG clearance was due to the formation of new large pores [26] or modification of original pores. We assume that loss of heparan sulphate-proteoglycan or decreased sulfation of heparan sulphate in basement membrane pores might well lead to microstructural disruption due to decreased crosslink formation to fibronection and collagen IV [45, 52–56]. The unchanged IgG/IgG_4 clearance in these patients compared to patients with less $U_{alb}V$ indicates that changes in pore size now dominate changes in charge.

Our findings in patients with $U_{alb}V$ greater than 300 mg/24 hr do not point to further qualitative changes in the GBM, but pore size or number are probably increasing further as indicated by the proportional increase of fractional IgG and albumin clearances (Fig. 2). The mean fractional clearance of β_2-microglobulin was slightly higher in diabetic patients compared to controls. However, the difference between these groups was not statistically significant. Only in patients with persistent proteinuria and GFR less than 80 ml/min/1.73 m^2 was plasma β_2-microglobulin elevated, indicating reduced kidney function. In these patients also the urinary β_2-microglobulin excretion was high and above the normal range.

Our findings in diabetic patients with increased $U_{alb}V$ are in close agreement with earlier findings by others [11, 32]. However, since diabetic patients with normal $U_{alb}V$ were not studied by these groups and clearance studies with IgG_4 to our knowledge have never been done before, these groups were unable to elucidate the difference in glomerular charge and size selectivity between controls and diabetic patients without and with slightly increased $U_{alb}V$.

Our study indicated changes in charge selectivity of the glomerular membrane in diabetic patients with normal $U_{alb}V$.

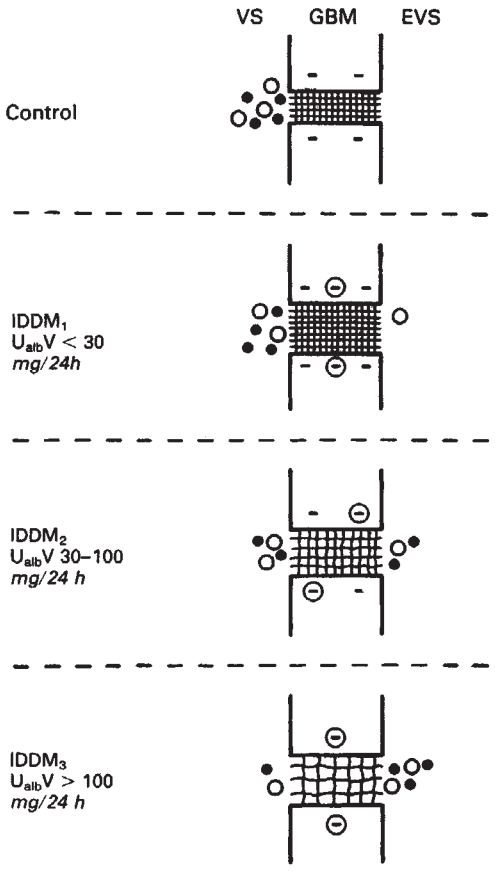

Fig. 3. *Diagram of a "pore" in the glomerular basement membrane (GBM).* Abbreviations are: VS, vascular space; EVS, extravascular space. Symbols are (●) negatively charged albumin; (○) neutral IgG; (÷) negative charges on the "pore" surface from i.a. heparan sulphate and sialic acid; (⊖) negative charges on the pore surface from non-enzymatic glycation of GBM proteins; (⊞) extracelluar matrix within the pores. Upper panel indicates the normal condition, the lower 3 panels the situation in IDDM: $IDDM_1$ $U_{alb}V$ <30 mg/24 hr; $IDDM_2$ $U_{alb}V$ 30-100 mg/24 hr; $IDDM_3$ $U_{alb}V$ >100 mg/24 hr. Note the gradual reduction of endogenous negative charges in patients with increased $U_{alb}V$. Note also the increased pore size in IDDM, and the gradual further enlargement of pores in patients with increased $U_{alb}V$.

Furthermore, we demonstrated that the increase in large pore area seen in patients with albuminuria is preceded by loss of charge selectivity (Fig. 3). We feel these alterations are likely due to loss of heparan sulphate in the GBM. The cause of the decrease in sulphation or loss of heparan sulphate is not fully understood but could be linked to genetic differences in the activity of N-deacetylase—the key enzyme in N-sulfation of heparan sulfate.

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