Diabetologia (1989) 32: 219–226

Diabetologia

Reviews

Albuminuria reflects widespread vascular damage*
The Steno hypothesis

T. Deckert, B. Feldt-Rasmussen, K. Borch-Johnsen, T. Jensen and A. Kofoed-Enevoldsen

Steno Memorial Hospital, Gentofte, Denmark

Summary. Albuminuria in Type 1 (insulin-dependent) diabetes is not only an indication of renal disease, but a new, independent risk-marker of proliferative retinopathy and macroangiopathy. The coincidence of generalised vascular dysfunction and albuminuria, advanced mesangial expansion, proliferative retinopathy, and severe macroangiopathy suggests a common cause of albuminuria and the severe renal and extrarenal complications associated with it. Enzymes involved in the metabolism of anionic components of the extracellular matrix (e.g. heparan sulphate proteoglycan) vulnerable to hyperglycaemia, seem to constitute the primary cause of albuminuria and the associated complications. Genetic polymorphism of such enzymes is possibly the main reason for variation in susceptibility.

Key words: Diabetes, albuminuria, extracellular matrix, heparan sulphate, vascular dysfunction.

Albuminuria indicates a poor prognosis in Type 1 (insulin-dependent) diabetic patients [1]. However, only about 35% of patients with Type 1 diabetes will ever develop Albustix, (Ames, Bucks, England) positive albuminuria [2], Why is albuminuria associated with such a poor prognosis, and why do only 35% of Type 1 diabetic patients develop Albustix positive albuminuria?

Onset of albuminuria

Albuminuria is not present at the onset of Type 1 diabetes. Shortly after the onset of Type 1 diabetes, the urinary albumin excretion rate (UalbV) is normal or sub-normal [3]. However, in the 35% of patients who later develop persistent proteinuria, UalbV increases in an exponential way at about 20% per year [4]. The rate of increase varies between patients and the intra individual variations of UalbV are quite large [5]. When UalbV is persistently more than 30 mg/24 h, it is clearly abnormal and we speak of incipient nephropathy. After a further 5–10 years, UalbV has increased to more than 300 mg/24 h, which is the macroalbuminuric range at which Albustix becomes positive. Thus, Albustix positive proteinuria is a late event in a long-lasting process which starts shortly after the onset of diabetes (Borch-Johnsen K, Skovgaard LT, Keiding N, Deckert T. To be published). But why only in 35% of patients, and why is prognosis so poor?

Albuminuria, more than an indicator of renal disease

Albuminuria is generally believed to reflect renal disease. This is also true in Type 1 diabetes as seen in Table 1 and Figure 1 which demonstrate changes in the glomerular filtration rate (GFR) in a trans-sectional study (Table 1) and during a 2-year prospective study in long-term Type 1 diabetic patients with different levels of UalbV (Fig. 1). A persistent decrease in GFR means loss of glomerular filtration surface due to advanced mesangial expansion [6, 7]. Research during recent years has, however, demonstrated that albuminuria in Type 1 diabetes is not only associated with renal alterations but also with proliferative retinopathy [8–11] and cardiovascular mortality [12]. An association with cardiovascular mortality is not only present in Type 1 diabetic patients but also in non-diabetic subjects [13–15] and Type 2 (non-insulin-dependent) diabetic patients [16–18]. The increased cardiovascular mortality in patients with albuminuria is only partly due to a higher prevalence of cardiovascular risk factors [19], such as smoking [20, 21], lipids [22, 23] high blood-pressure [8, 14, 16] and plasma fibrinogen [22, 24]. Thus, albuminuria is not only an indication of renal disease but also a new, strong and independent risk-marker of proliferative retinopathy and cardiovascular death.

* Given by T. Deckert as Claude Bernard lecture, Paris 1988

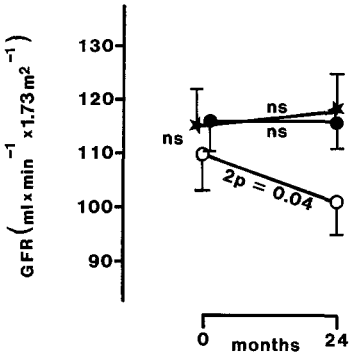

Fig. 1. Glomerular filtration rate (GFR) in 29 patients with conventional treated long-term Type 1 (insulin-dependent) diabetes, prospectively followed for 2 years (M ± SEM). * persistent normoalbuminuria (UalbV < 30 mg/24 h), $n=6$; ● UalbV at first observation 30–100 mg/24 h, $n=13$; ○ UalbV at first observation 101–300 mg/24 h, $n=10$. The difference of GFR after 24 months between group 2 and 3 was significant, $p=0.04$

This is an interesting observation. How does albuminuria indicate cardiovascular damage? Our hypothesis is that albuminuria reflects a more generalised vascular process which affects the glomeruli, the retina and intima of large vessels simultaneously. We therefore looked for indicators of generalised vascular dysfunction [25, 26] and studied the coincidence of some of the complications associated with albuminuria [8, 12, 27]. To assess alterations of the vascular system in general, measurement of the transcapillary escape rate (TER) of albumin and fibrinogen was undertaken and some markers of endothelial cell function were measured. As can be seen in Figure 2, long-term diabetic patients with normal UalbV had a normal albumin TER of about 5%. This means that 5% of the intravascular albumin mass will leave the vascular space per hour. But diabetic patients with elevated UalbV have a 50% increase in the albumin TER. Similar results were obtained with fibrinogen and a statistically significant ($p<0.01$) correlation was found between the albumin TER and that of fibrinogen ($r=0.72$) (Bent-Hansen L and Deckert T, to be published). Two observations were of interest. Firstly, an increase in albumin TER was observed in patients with the slightest increase of UalbV (Fig. 2). Secondly, the increase in TER was not due to increased vascular surface [28], longer diabetes duration, higher blood-pressure or poorer diabetic control [25] but only related to increased UalbV. If the increased albumin and fibrinogen TER reflects increased vascular permeability, these observations might indicate increased extravascular coagulation [29] which leads to an increased release of von Willebrand factor [30]. In fact, von Willebrand factor, a marker of endothelial dysfunction, was increased in albuminuric patients, but normal in long-term diabetic patients with normal UalbV [31, 32]. Similar results were seen with other markers of endothelial lesions such as angiotensin converting enzyme [33–35] and plasminogen activator [113]. Thus, albuminuria seems to indicate widespread vascular dysfunction.

The coincidence of albuminuria along with progressive mesangial expansion, proliferative retinopathy and/or macroangiopathy was striking. As can be seen in Figure 3 some mesangial expansion can be seen in nearly all long-term diabetic patients. But only in patients with increasing UalbV does mesangial expansion progress so seriously that the GFR begins to decline [6, 7]. Some cases of proliferative retinopathy can also be seen in patients with normal UalbV, but, as soon as UalbV increases the incidence of proliferative retinopathy increases enormously (Fig. 4) [8]. Also, some diabetic patients with normal UalbV will die from coronary heart disease (CHD), but the cumulative incidence of CHD increases remarkably in patients after the onset of albuminuria [27]. In the absence of albuminuria, mesangial expansion remains unimportant and never leads to renal insufficiency. Without albuminuria the incidence of proliferative retinopathy and cardiovascular events remains low. Thus, the process which leads to albuminuria would also seem to be a serious promotor of mesangial expansion, retinopathy and macroangiopathy.

Mechanism of the generalised process leading to albuminuria

What is the mechanism of this widespread process? It can hardly be exclusively related to hyperglycaemia or the associates of hyperglycaemia like the sorbitol pathway or non-enzymatic glycation. No doubt poor blood sugar

Table 1. Glomerular filtration rate (GFR) in 113 patients with Type 1 (insulin-dependent) diabetes and different levels of UalbV

	Sex ratio m/f	Age (years)	Diabetes duration (years)	HbA_{1C} (%)	GFR (ml/min × 1.73 m^2)
Control subjects $n=13$	13/10	33 ± 7		5.3 ± 0.4	104 ± 15[b, c, e]
Normoalbumin < 30 mg/24 h $n=38$, group 1	21/17	35 ± 7	15 ± 9	9.1 ± 1.2	116 ± 21[a, e]
Albuminuric 30–100 $n=32$, group 2	19/23	33 ± 9	18 ± 5	9.1 ± 1.2	117 ± 19[a, e]
100–300 $n=23$, group 3	13/10	32 ± 8	20 ± 8	8.9 ± 1.2	111 ± 20[e]
300 $n=20$, group 4	11/ 9	34 ± 9	19 ± 7	9.5 ± 0.9	85 ± 29[a, b, c, d]

Significant difference ($p<0.05$) compared with [a] control subjects, [b] group 1, [c] group 2, [d] group 3 and [e] group 4; Results are given as mean ± SD

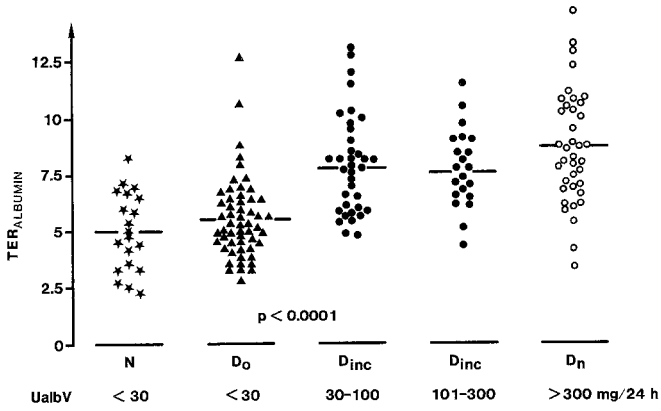

Fig. 2. Transcapillary escape rate of albumin (TER alb) in 151 patients with Type 1 (insulin-dependent) diabetes and different levels of UalbV (% per hour). N control subjects; D_o long-term diabetic patients with UalbV < 30 mg/24 h; D_{I1} UalbV 30-100 mg/24 h; D_{I2} UalbV 101-300 mg/24 h; D_n UalbV > 300 mg/24 h

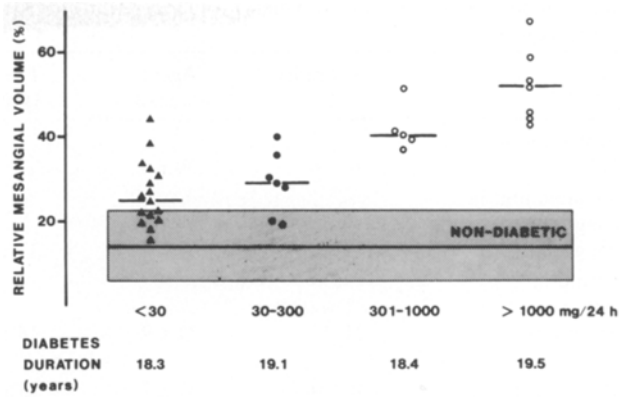

Fig. 3. Relative mesangial volume (%) in 35 patients with long-term Type 1 (insulin-dependent) diabetes (≥ 9 years) and different levels of UalbV (mg/24 h). Median duration of diabetes was similar in the different groups [6]. The dotted area represents the range in non-diabetic subjects (m ± SD) [112]

regulation plays an important role in the development of albuminuria [36-39]. However, the correlation between metabolic control and the development of persistent albuminuria is poor [38, 39]. Poor glycaemic control is a necessary, but not a sufficient condition to cause the development of albuminuria. This is also indicated in Figure 5 which demonstrates HbA_{1c} in Type 1 diabetic patients with albuminuria and long-term diabetic patients with Type 1 diabetes for more than 40 years but with normal UalbV. Thus, it would seem that some patients are susceptible to the deleterious effects of poor diabetes control, whereas others are resistant.

Elevation of blood-pressure can hardly in itself be the common cause of the generalised vascular dysfunction and organ-damage. It is true that elevated blood-pressure is seen early in diabetic patients with albuminuria as seen in Table 2, but the blood-pressure is not significantly elevated before UalbV is > 100 mg/24 h. Similar results were seen in a prospective study of 200 normoalbuminuric Type 1 diabetic patients. Among these patients 15 developed persistent microalbuminuria during a 5-year follow-up. These patients had similar blood-pressure readings to those who remained in the normoalbuminuric range for several years [40]. Thus, blood-pressure elevation does not appear simultaneously with, or before, but after the increase of UalbV, probably due to a disequilibrium between sodium reabsorption and glomerular filtration capacity, a hypothesis which is presently being tested. This does not mean that blood-pressure is without significance for the prognosis of diabetic patients with albuminuria. Increased blood-pressure might well be an independent promotor of mesangial expansion [41], retinopathy [8] and macroangiopathy [16] but it is not the cause of albuminuria, and the blood-pressure elevation per se can neither explain the high cardiovascular mortality [16] nor the deleterious mesangial expansion [42] and the high incidence of proliferative retinopathy [8]. Nor does increased intraglomerular pressure seem to be the cause of albuminuria [43-45].

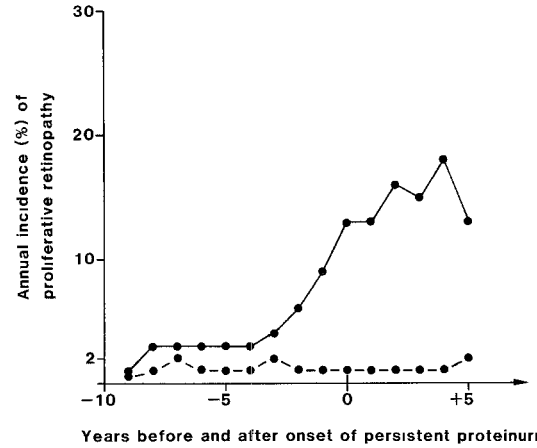

Fig. 4. The annual incidence (new cases per year) of proliferative retinopathy in 110 patients with Type 1 (insulin-dependent) diabetes who developed clinical nephropathy (UalbV > 300 mg/24 h) at 0. 110 age-, diabetes duration- and sex-matched patients who did not develop clinical nephropathy are shown as control subjects [9]

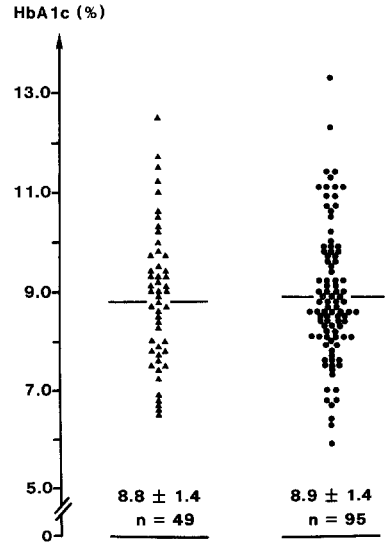

Fig. 5. HbA_{1c} in patients with long-term Type 1 (insulin-dependent) diabetes of juvenile onset. ▲ patients with UalbV 50-300 mg/24 h; ● patients who survived with diabetes for more than 40 years duration and normal UalbV

Table 2. Blood-pressure (BP) in 151 patients with long-term Type 1 (insulin-dependent) diabetes and different levels of UalbV. No antihypertensive therapy was used

	Sex ratio m/f	Age (years)	Diabetes duration (years)	HbA_{1c} (%)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
Control Subjects $n=20$	14/ 6	30±6		5.0±0.4	123± 9[d, e]	79± 7[c, d, e]
Normoalbuminuria <30 mg/24 h $n=55$, group 1	37/18	34±7	17±7	8.2±1.1	128±13[d, e]	82± 7[d, e]
Albuminuria 30-100 $n=38$, group 2	21/17	31±9	18±5	8.0±1.6	131±15[e]	84±10[a, e]
101-300 $n=20$, group 3	18/ 4	37±7	23±7	7.8±1.2	139±15[a, b, e]	87±10[a, b, e]
300 $n=38$, group 4	28/10	34±8	20±7	9.8±1.5	151±22[a, b, c, d]	95±10[a, b, c, d]

Significant difference ($p<0.05$) compared with [a] control subjects, [b] group 1, [c] group 2, [d] group 3 and [e] group 4; Results are given as mean±SD

Alteration of the composition of the extracellular matrix

We believe that the cause of this generalised process is a genetically determined alteration in the composition of the extracellular matrix. The evidence for the importance of genetic factors comes from several studies. 1) The incidence of albuminuria is significantly higher in males compared to females [1]. 2) Long-term Type 1 diabetic patients who have had diabetes for more than 40 years with normal UalbV have a distribution of tissue type antigens which is significantly different from Type 1 diabetic patients with nephropathy [46]. 3) Furthermore, albuminuria is more frequently observed in diabetic siblings of diabetic patients with albuminuria than in diabetic siblings of diabetic patients without albuminuria [47]. It has also been postulated that the Na^+/Li^+ counter transport activity in erythrocytes can be used as a genetic marker for patients who later develop albuminuria [48, 49]. However, this is not the case in Copenhagen. In ongoing studies we did not find that parents of diabetic patients with nephropathy have higher blood-pressure [50] than parents of patients without albuminuria.

The evidence for the involvement of the extracellular matrix comes from studies of the composition and charge of the glomerular basement membrane (GBM) and other filtration barriers in diabetic animals and human subjects [51-57]. The GBM is condensed extracellular matrix. It is negatively charged, partly due to its content of heparan sulphate [58-60]. It is, however, difficult to measure minor alterations in the complex composition of GBM. We therefore studied alterations in the glomerular charge selectivity which might reflect qualitative changes of the GBM. By measuring the renal clearance of pairs of plasma proteins which differ in charge but not in molecular size, alterations of the charge selectivity of the GBM can be identified. We have used nonglycated and glycated albumin [61, 62], glycated albumin being more negatively charged in comparison to nonglycated [62]. We have also used neutral IgG and the anionic charged IgG4 fraction [64, 65]. The ratio between the clearance of the less anionic and the more anionic plasma proteins is the selectivity index (SI). A decrease of SI indicates loss of the charge selectivity. With both pairs of plasma proteins we found a highly significant loss of charge selectivity in albuminuric patients with Type 1 diabetes (Fig. 6). These findings have been confirmed by others [55, 66, 67]. Interestingly, loss of charge selectivity could be seen already in patients with UalbV of 30-50 mg/24 h [65] i.e. at the very beginning of incipient nephropathy where size selectivity of the GBM seems to be normal [66, 68]. The combination of normal size selectivity and reduced charge selectivity strongly indicates that loss of charge selectivity is due to a reduction of negative charges of the GBM and not to increased pore size. These conclusions are strengthened by histochemical analyses of anionic sites within the GBM, which demonstrated a negative correlation between the density of negative charges and albuminuria in Type 1 diabetic patients [69, 70]. A reduction of fixed negative charge density induced by diabetes has been demonstrated in the GBM [71] as well as in Bruch's membrane [72], (the extracellular matrix between the chorioid and the pigment epithelium of the retina), on erythrocytes [73] and in the arterial intima [74]. These observations indicate that diabetes leads to a generalised reduction of negative charges of extracellular matrix and plasma membranes, reflecting qualitative changes in the composition of the membranes. In diabetic patients with albuminuria these alterations seem to be severe enough to induce changes in permeability.

Loss of heparan sulphate proteoglycan in diabetes

What does the loss of anionic charge mean in biochemical terms? There are several reasons to believe that the loss of anionic charge is due to a loss of normal heparan sulphate proteoglycan, the main glycosaminoglycan component of basement membranes of glomeruli [54, 75], aortic myomedial cells [76], mesangium [54, 77] and endothelial plasma membranes [78].

1) The loss of heparan sulphate in the GBM leads to loss of anionic sites and albuminuria [79-81].

2) Within the GBM a loss of heparan sulphate has been demonstrated in diabetic patients with nephropathy [53].

3) The multiplicity of the effects of heparan sulphate proteoglycan might well explain the strong association between vascular dysfunction and renal and extrarenal complications [82]. Heparan sulphate proteoglycan not only inhibits the glomerular filtration of albumin but also contributes to the integrity of the pore size of the GBM [83, 84]. Thus, loss of heparan sulphate proteoglycan has been demonstrated to lead to disruption of the microstructure of the GBM [84]. These alterations would explain the increase of fractional IgG clearance seen in albuminuric diabetic patients [55, 64] and the changes in dextran clearance in these patients [66, 68]. Heparan sulphate proteoglycan also strongly inhibits mesangial cell growth [85], and loss of heparan sulphate has been shown to be a strong promotor of mesangial expansion [85, 86]. It has also been shown that heparan sulphate proteoglycan in plasma membranes of endothelial cells have important antithrombogenic properties [87-89]. Thus, heparin-like components which accelerate thrombin-antithrombin complex formation have been identified in retinal microvascular endothelial cell preparations [90]. Loss of normal sulphated heparan sulphate in retinal capillaries might, therefore, contribute to the formation of microthrombi and/or platelet plugs followed by areas of non-perfusion, the forerunners of proliferative retinopathy in diabetes [91, 92]. In fact, increased platelet adhesion in vivo has been demonstrated in albuminuric diabetic patients [19]. The formation of platelet plugs will lead to increased local concentrations of platelet derived growth factor, potent mitogens of a number of cells including mesangial cells [93-95]. Besides antithrombogenic properties, heparan sulphate proteoglycan binds lipoprotein lipase [96], stimulates its activity [97] and inhibits smooth muscle cell proliferation in arteries [98]. Loss of heparan sulphate might, therefore, very well represent a serious promotor of atherosclerosis [82, 87, 99, 100].

4) Finally, diabetes affects heparan sulphate metabolism and leads to loss of normally sulphated heparan sulphate in extracellular matrix and plasma membranes [51, 54, 102]. Usually heparan sulphate is sulphated within the Golgi apparatus of many cells [103-105]. The key enzyme of sulphation is N-deacetylase [106]. After sulphation has taken place, heparan sulphate is incorporated into plasma and basement-membranes where it contributes to the anionic charge of the extracellular matrix [58-60] and the integrity of the collagen network [83, 84]. Loss of anionic charge in GBM and increased glomerular permeability due to decreased sulphation of glomerular heparan sulphate has been described in experimental membraneous nephropathy [80]. In diabetic animals inappropriate sulphation of heparan sulphate has been demonstrated [57, 107-110] probably due to impaired activity of the deacetylase enzyme [107].

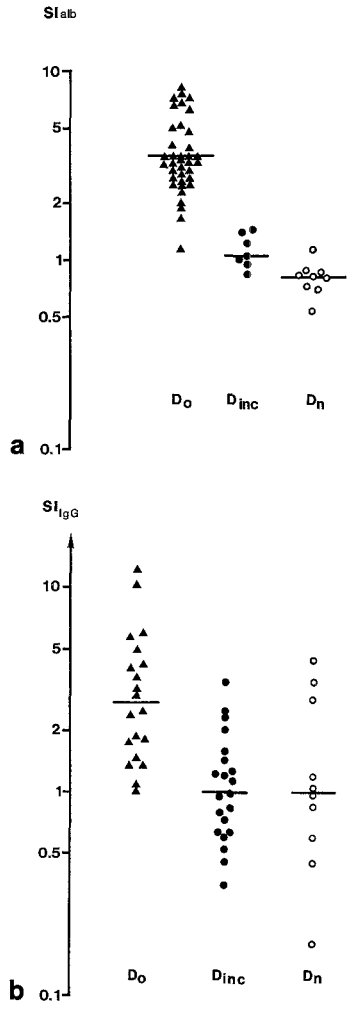

Fig. 6a and b. The selectivity index (SI) in patients with Type 1 (insulin-dependent) diabetes. SI alb = clearance of non-glycated albumin/clearance of glycated albumin (**a**). SI_{IgG} = clearance of IgG/clearance of IgG4 (**b**). D_o patients with normal UalbV; D_I patients with Ualbv 30-300 mg/24 h; D_n patients with UalbV > 300 mg/24 h

Therefore, we hypothesize that albuminuria and the associated complications are due to genetic polymorphism of enzymes involved in the metabolism of heparan sulphate proteoglycan e.g. N-deacetylase. Genetic polymorphism of diabetes-sensitive enzymes involved in the metabolism of glycosaminoglycans has been demonstrated in the Uppsala strain of Sprauge Dawley rats [111]. According to our hypothesis, patients who develop albuminuria are characterised by iso-enzymes which are extremely vulnerable to poor diabetes control (Fig. 7). In these patients a critical reduction of normal heparan sulphate would be expected, leading to albuminuria and progression of mesangial expansion, retinopathy and macroangiopathy, whereas persons equipped with iso-enzymes less vulnerable to hyperglycaemia would be protected. Thus, the polymorphism of enzymes involved in the metabolism of heparan sulphate proteoglycan might be the reason for the heterogeneous prognosis of poorly regulated diabetic patients and for the fact that only 35% of Type 1 diabetic patients develop albuminuria. Vulnerable to poor metabolic control, the presence

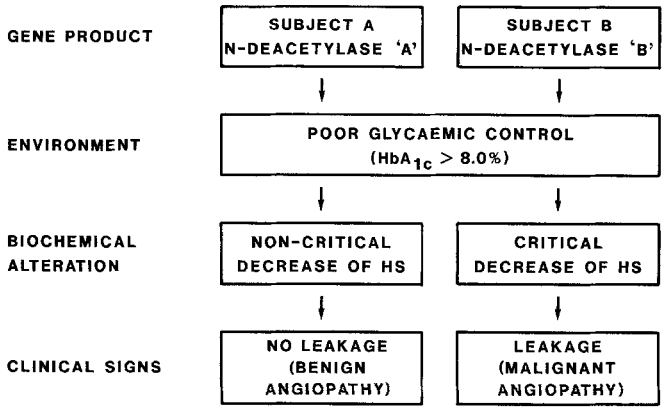

Fig. 7. Pathogenesis of albuminuria and its associated complications. HS = heparan sulphate proteoglycan

of this enzyme not only in epithelial and endothelial cells of the glomeruli but also in the mesangium, the retina and intima of large vessels, might explain why albuminuria in Type 1 diabetes reflects widespread vascular damage.

Acknowledgements. Besides the authors, A. Kverneland, L. Bent-Hansen, Elisabeth R. Mathiesen, Kirsten Nørgaard, Pablo Vidal, Benny S. Welinder, Marja Deckert and Hanne Foght have contributed to this work. Their enthusiasm and skilful assistance is highly appreciated.

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Dr. Torsten Deckert
Steno Memorial Hospital
Niels Steensensvej 2
DK-2820 Gentofte
Denmark