Diabetologia (1984) 26: 406–410

Diabetologia

Incipient nephropathy in Type 1 (insulin-dependent) diabetes

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Summary. Patients with Type 1 (insulin-dependent) diabetes without proteinuria were studied to define those patients who will later develop persistent proteinuria (more than 0.5 g protein/24 h). Two investigations were performed; 71 patients were studied longitudinally for 6 years and another 227 patients were studied cross-sectionally. All were less than 50 years of age and had developed diabetes before the age of 40 years. At entry into the study they had no proteinuria (Albustix method), had normal blood pressure and urinary albumin excretion rates <200 μg/min (normal $\leqslant 20$ μg/min). The best predictor of persistent proteinuria or an albumin excretion rate >200 μg/min was the initial urinary albumin excretion rate. During the longitudinal study, seven patients with an urinary albumin excretion rate of more than 70 μg/min at the start of the study developed persistent proteinuria or an albumin excretion rate >200 μg/min. In contrast, only three out of the remaining 64 patients with urinary albumin excretion rate $\leqslant 70$ μg/min developed urinary albumin excretion rate >200 μg/min. Patients with an urinary albumin excretion rate >70 μg/min are thus at risk of developing diabetic nephropathy. We designate this stage of renal involvement incipient nephropathy. Patients with incipient nephropathy were further characterized in the cross-sectional study. Compared with normoalbuminuric patients, patients with incipient nephropathy had increased systolic and diastolic blood pressure, but normal serum creatinine. The glomerular filtration rate was higher than normal in patients with incipient nephropathy though not different from that of normoalbuminuric patients.

Key words: Diabetic nephropathy, incipient nephropathy, proteinuria, urinary albumin excretion rate, glomerular filtration rate, blood pressure, Type 1 diabetes.

Diabetic nephropathy is the most serious complication of Type 1 (insulin-dependent) diabetes [1]. About 7 years after the onset of persistent proteinuria 50% of patients have died [2]. Anti-hypertensive treatment can slow, but not prevent, the deterioration in renal function in patients with diabetic nephropathy [3, 4]. Preventive measures at a very early stage of renal involvement, before the onset of persistent proteinuria, might prove more promising. It has previously been suggested that patients with no proteinuria, as estimated by the Albustix method but with increased urinary albumin excretion rate ($U_{alb}V$), are those who will later progress to diabetic nephropathy [5–7]. There is, however, still no agreement about the threshold level of $U_{alb}V$ that differentiates those at risk of developing diabetic nephropathy.

The aim of the present study was to define and describe those Type 1 diabetic patients who are at risk of developing persistent proteinuria.

Patients and methods

Longitudinal study

All patients with Type 1 diabetes attending the out-patient clinic during a 3-day period in 1974 and who had no proteinuria by the Albustix method were recorded. Seventy-one patients fulfilled the inclusion criteria of age below 50 years, diabetes onset before the age of 35 years, and duration of diabetes more than 2 years (Table 1). Sixteen of the patients had increased $U_{alb}V$ (>20 μg/min).

Cross-sectional study

Two-hundred and twenty-seven randomly selected patients with Type 1 diabetes and no proteinuria using the Albustix method were studied. Sixty-seven had elevated $U_{alb}V$. The patients were less than 50 years old, developed diabetes before the age of 40 years and had been diabetic for more than 5 years (Table 2).

The patients in the two groups were non-obese (body weight $<120\%$ of ideal body weight), had normal serum creatinine (<120 μmol/l) and diastolic blood pressures less than 95 mmHg. Patients with a positive urinary bacterial culture (Uricult, Orion, Helsinki, Finland) were excluded (seven out of 278 patients).

Table 1. Initial clinical data on patients in the longitudinal study (1974–1980)

Urinary albumin excretion (μg/min)	Sex M : F	Age (years)	Duration of diabetes (years)
⩽ 20 (n=55)	23:32	29 (13–50)	11 (2–36)
21– 70 (n= 9)	2: 7	28 (20–40)	15 (6–32)
71–200 (n= 7)	4: 3	31 (22–40)	19 (8–25)[a]

Results are expressed as median and range (n = number of patients).
[a] Significant difference from patients with $U_{alb}V \leqslant 20$ μg/min ($2p < 0.05$)

Table 2. Initial clinical data on patients in the cross-sectional study

Urinary albumin excretion (μg/min)	Sex M : F	Age (years)	Duration of diabetes (years)
⩽ 20 (n=160)	71:89	33 (18–50)	17 (5–29)
21– 70 (n= 45)	19:26	29 (19–45)[a]	16 (6–26)
71–200 (n= 22)	9:13	31 (21–44)	17 (6–30)

Results expressed as median and range (n = number of patients).
[a] Significant difference from patients with $U_{alb}V \leqslant 20$ μg/min ($2p < 0.01$)

Laboratory methods

The patients' urine samples were screened for proteinuria with Albustix (Boehringer Mannheim, Mannheim, FRG; sensitivity 200–300 mg/l) to exclude patients with Albustix-positive urine. The albumin concentration in the Albustix-negative urine samples was determined with the radial immunodiffusion method [9] (sensitivity 3 mg/l), and the protein concentration in the Albustix-positive urine samples was measured with both the biuret method (sensitivity 500 mg/l) [8] and the immunodiffusion method. The upper normal limit for $U_{alb}V$ in our laboratory is 20 μg/min (mean ±2 SD, n=19). The inter-assay coefficient of variation of the urinary albumin determination by immunodiffusion is 5.5%. The intra-individual coefficient of variation on five consecutive days of 22 in-patients was 47% ($U_{alb}V$ <20 μg/min). Intra-individual coefficient of variation of $U_{alb}V$ measured on two occasions with a 2–4 months interval (n=114) was 48% ($U_{alb}V \leqslant$ 20 μg/min), 58% ($U_{alb}V$ 21–70 μg/min) and 67% ($U_{alb}V$ > 70 μg/min).

Measurements of $U_{alb}V$ were based on 24-h urine samples collected at home or in hospital after careful instruction, except for 28 patients in the cross-sectional study, whose $U_{alb}V$ was based on measurements on 4-h urine samples.

β_2-microglobulin excretion was determined by radioimmunoassay (Phadebas, β_2 microtest, Pharmacia Diagnostics, Uppsala, Sweden) in the same 28 patients during 4-h collection periods (upper normal limit 2.1 g/l). The inter-assay coefficient of variation was 10%.

Glomerular filtration rate was measured after a single intravenous injection of Cr^{51}-EDTA (normal value: 103 ± 13 ml · min^{-1} · 1.73 m^{-2}; mean ±SD) [10].

Stable glycosylated haemoglobin (HbA_{1c}) was determined as described previously [11] (references range 4.1%–6.4%). Urinary glucose excretion was determined enzymatically. Serum creatinine was measured by a reaction rate method of Jaffe which eliminates pseudocreatinines (upper reference limit 120 μmol/l (men) and 100 μmol/l (women)). Inter-assay coefficient of variation was 2.5%. Blood pressure was recorded after 1 h rest in the sitting position in the out-patient clinic. Diastolic blood pressure was recorded as Korotkoff phase V.

Definitions

Persistent proteinuria was defined as a protein excretion of more than 0.5 g/24 h on at least four consecutive occasions at intervals of 2–4 months. $U_{alb}V$ of >200 μg/min corresponds to this level of proteinuria and patients with $U_{alb}V$ >200 μg/min are therefore grouped with those having persistent proteinuria.

Statistical analysis

Paired and non-paired t-tests were used for normally-distributed variables, and Wilcoxon tests (paired and non-paired) for non normally-distributed variables. Regression analyses of log $U_{alb}V$ values were used. χ^2 and Fisher's exact tests were used to compare degrees of retinopathy. Results are given as mean ±SD or median and range respectively.

Results

Longitudinal study

Of the 71 patients, five progressed to an $U_{alb}V$ > 200 μg/min and another five developed persistent proteinuria during the 6-year follow-up period. The most striking difference between those patients whose renal abnormality progressed and those who did not progress was the $U_{alb}V$ at start of the study (Table 3). $U_{alb}V$ in the two groups was 87 μg/min (range 6–111 μg/min) and 8 μg/min (range 2–64 μg/min), respectively ($2p <$ 0.001). At entry into the study, there were also smaller differences between the two groups with regard to duration of diabetes, systolic blood pressure and prevalence of retinopathy.

At entry into the study, seven patients had $U_{alb}V$ > 70 μg/min; all progressed to persistent proteinuria or $U_{alb}V$ >200 μg/min (Fig. 1). In contrast, only three out of the 64 remaining patients with $U_{alb}V \leqslant 70$ μg/min

Table 3. Initial clinical data on patients developing persistent proteinuria compared with patients not developing persistent proteinuria

Patients with	Age (years)	Duration of diabetes (years)	Initial urinary albumin excretion (μg/min)	Serum creatinine (μmol/l)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Retinopathy (%)
Persistent proteinuria or $U_{alb}V$ >200 μg/min (n=10)	31 ± 10	17 ± 6	87 (6–111)[b]	83 ± 12	130 ± 19[a]	83 ± 10	90[a]
$U_{alb}V \leqslant 200$ μg/min (n=61)	31 ± 10	13 ± 8	8 (2– 64)	79 ± 14	122 ± 9	80 ± 8	41

Figures given are median (range) or mean ±SD.
[a] $2p < 0.05$ Significant difference from the population who did develop persistent proteinuria or $U_{alb}V$ >200 μg/min;
[b] $2p < 0.001$ Significant difference from the population who did not develop persistent proteinuria or $U_{alb}V$ >200 μg/min.

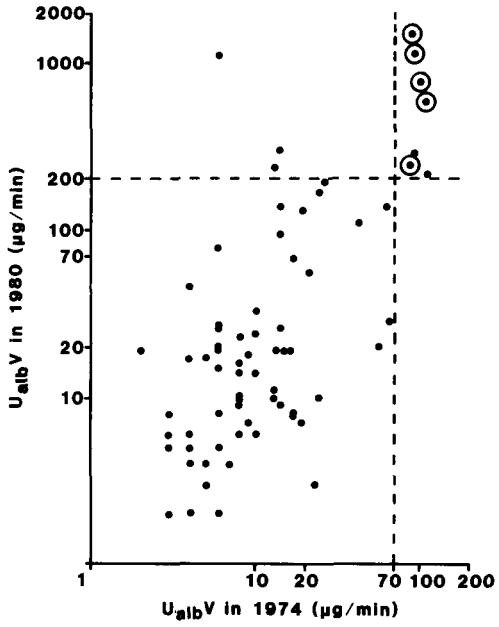

Fig. 1. Urinary albumin excretion rate ($U_{alb}V$) before (1974) and at follow-up (1980) in 71 patients with Type 1 diabetes. ⊙ = patients who developed persistent proteinuria

developed $U_{alb}V > 200$ µg/min and none developed persistent proteinuria. On the basis of this result, we classified the seven patients with $U_{alb}V$ of 71–200 µg/min as having 'incipient nephropathy'. At the start of the study, the group of patients with incipient nephropathy had longer duration of diabetes (Table 1) and higher systolic blood pressure (Table 4) than the normoalbuminuric group. All patients with incipient nephropathy had diabetic retinopathy.

Systolic blood pressure increased significantly in all three groups of patients (≤20, 21–70, 71–200 µg/min) during the study (Table 4). Diastolic blood pressure increased in patients with $U_{alb}V > 21$ µg/min, but most obviously in patients with incipient nephropathy. The initial blood pressure of patients who progressed to incipient nephropathy was not different from that of patients who did not progress (120/76 versus 121/81 mmHg). Serum creatinine increased in all three patient groups. Retinopathy was present in all the patients with incipient nephropathy at the beginning of the study, but in only 40% of the normoalbuminuric patients. The prevalence of retinopathy increased in both

Table 4. Urinary albumin excretion at the start of the longitudinal study and 6 years later in relation to serum creatinine, blood pressure, and retinopathy

Urinary albumin excretion (µg/min)	Urinary albumin excretion (µg/min)		Serum creatinine (µmol/l)		Blood pressure (mmHg)		Retinopathy (%)	
	Start of study	6 years later	Start of study	6 years later	Start of study	6 years later	Start of study	6 years later
≤20 (n=55)	8 (2– 19)	14 (2–1547)[c]	81±13	88±18[b]	122±11 / 81± 8	131±16[c] / 82± 9	40	67[c]
21–70 (n=9)	26 (21– 64)	56 (3– 188)	70±12[a]	86± 8[b]	115±11 / 70± 8[a]	134±11[b] / 77± 9	56	89[c]
71–200 (n=7)	91 (76–111)	570 (209–1451)[b]	82±13	108±30	134±13[a] / 87± 5	160±13[b] / 108± 8[c]	100[a]	100

Results expressed as median (range) or mean ± SD.
[a] 2 $p<0.05$ Significant difference from patients with $U_{alb}V \leq 20$ µg/min; [b] 2 $p<0.05$ Significant difference from start of study;
[c] 2 $p<0.01$ Significant difference from start of study

Table 5. Cross-sectional study: urinary albumin excretion in relation to HbA_{1c}, urinary glucose excretion, serum creatinine, blood pressure, glomerular filtration rate, urinary β_2-microglobulin excretion and retinopathy

Urinary albumin excretion (µg/min)	Median urinary albumin excretion (µg/min)	HbA_{1c} (%)	Urinary glucose (g/24 h)	Serum creatinine (µmol/l)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Glomerular filtration rate (ml·min^{-1}·1.73 m^{-2})	Urinary β_2-microglobulin excretion (µg/min)	No. of patients with retinopathy		
									None (%)	Background (%)	Proliferative (%)
≤ 20 (n=160)	6	8.9±1.4	13 (0–266)	83±11	124±14	79±8	122±15 (n=13)	80±39 (n=13)	45	47	8
21– 70 (n= 45)	32	9.3±1.7	22 (0–171)	81±11	123±12	79±10	126±22 (n=8)	113±68 (n=8)	44	47	9
71–200 (n= 22)	92	9.7±1.5 (n=13)	40 (0–140) (n=13)	82±11	131±11[a]	85±7[b]	125±26 (n=7)	93±29 (n=7)	23	55	23[a]

Values for urinary albumin excretion and urinary glucose excretion are not normally distributed and are given in median (range); the other values are mean ± SD (n = number of patients). [a] 2 $p<0.05$ significant difference from patients with $U_{alb}V \leq 20$ µg/min; [b] 2 $p<0.01$ significant different from patients with $U_{alb}V \leq 20$ µg/min

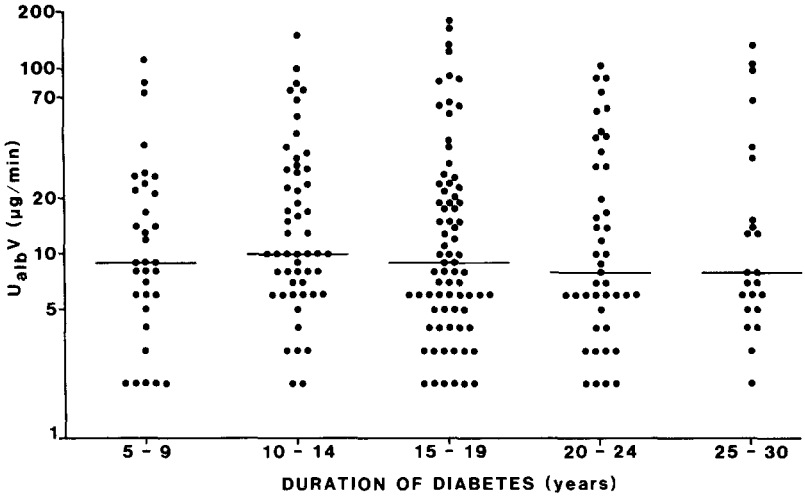

Fig. 2. Urinary albumin excretion ($U_{alb}V$) rate in 227 patients with Type 1 diabetes (cross-sectional study) and Albustix-negative urine as a function of duration of diabetes. The medians are indicated by horizontal lines

the normoalbuminuric group and in the group with $U_{alb}V$ 21–70 µg/min.

Cross-sectional study

Patients with incipient nephropathy were further characterized in the cross-sectional study (Table 2 and 5). The age of the patients with $U_{alb}V > 20$ µg/min was a little lower, but otherwise no significant difference was found between the normo- and the hyperalbuminuric groups (21–70 plus 71–200 µg/min) with respect to insulin requirement (not shown), duration of diabetes, serum creatinine (Table 5) or urinary glucose excretion. In the group of patients with incipient nephropathy, both systolic and diastolic blood pressures were significantly higher than in normoalbuminuric patients (Table 5). HbA_{1c} was significantly higher in the hyperalbuminuric group (> 20 µg/min) and a weak correlation was found between HbA_{1c} and log $U_{alb}V$ ($r=0.16$, $p<0.01$, $n=227$). $U_{alb}V$ was not correlated with degree of glycosuria. Twenty-three per cent of the patients with incipient nephropathy had proliferative retinopathy compared with 8% of the normoalbuminuric patients ($2p<0.05$). $U_{alb}V$ in the group with $U_{alb}V < 70$ µg/min was the same in patients with (9 µg/min, range 3–60 µg/min) as in patients without (9 µg/min, range 3–68 µg/min) proliferative retinopathy. $U_{alb}V$ was independent of duration of diabetes in those patients with $U_{alb}V$ 0–200 µg/min (Fig. 2).

Glomerular filtration rate was measured in 16 males and 12 females with a mean age of 32 years (range 21–44 years) and a mean duration of diabetes of 19 years (range 7–33 years). The mean glomerular filtration was 124 ± 17 ml · min^{-1} · 1.73 m^{-2} which is significantly higher than normal ($2p<0.001$). There was no difference in glomerular filtration between the normoalbuminuric and the hyperalbuminuric groups. Urinary β_2-microglobulin concentration was in the normal range, and there was no difference between the groups.

Discussion

The coefficient of variation of $U_{alb}V$ is considerable [12, 13]. We found a coefficient of variation of 47% in patients who collected 24-h urine samples in hospital after careful instruction. These patients were familiar with 24-h urine collection for determination of glucose excretion. The variation in albumin excretion expressed as a percentage of creatinine excretion was of the same order of magnitude as when expressed in µg/min. This suggests that the high coefficient of variation is not due to insufficient collection. We also checked that albumin was not lost due to adsorption to the container or due to freezing and storage (data not shown). The coefficient of variation in $U_{alb}V$ in young patients with Type 1 diabetes during standardized conditions with the patients recumbent for 1–2 h was 42%. The correlation between $U_{alb}V$ measured in 24-h urine samples and in urine samples collected during standardized conditions in the supine position for 1–2 h was high ($r=0.93$, $p<0.001$, $n=30$, $y=0.94x+1.67$; B. Feldt-Rasmussen, personal communication). This suggests that physical activity does not play a major role in the high coefficient of variation. It is therefore likely that the high variation in $U_{alb}V$ is real and not due to methodological factors.

Viberti et al. [14] found a high correlation between HbA_{1c} and $U_{alb}V$ in patients with Type 1 diabetes and $U_{alb}V$ between 4 and 80 µg/min. They included patients in very poor metabolic control. Our patients did not include such subjects and the variation in metabolic control seems to account for little of the variation in $U_{alb}V$ in the present study ($r^2=0.026$).

In the report by Viberti et al. [6] of 63 patients with Type 1 diabetes, seven out of eight patients with $U_{alb}V > 30$ µg/min developed persistent proteinuria during a 14-year follow-up period compared with two out of 55 patients with $U_{alb}V \leq 30$ µg/min. In the present study, all seven patients with $U_{alb}V < 70$ µg/min at entry into the longitudinal study progressed to persistent

proteinuria or $U_{alb}V > 200$ µg/min. In contrast, only three out of the 64 other patients increased the $U_{alb}V$ to > 200 µg/min. Mogensen et al. [7] found a mean annual increase in $U_{alb}V$ of 25 µg/min during a 6-year period in patients with initial $U_{alb}V$ between 15 and 300 µg/min. We found a mean annual increase of $U_{alb}V$ of 12 µg/min in the hyperalbuminuric (> 20 µg/min) and 0.7 µg/min in the normoalbuminuric (≤ 20 µg/min) patients.

In the cross-sectional study, $U_{alb}V$ was independent of duration of diabetes (Fig. 2) and independent of presence of proliferative retinopathy in patients with $U_{alb}V < 70$ µg/min. Thus prolonged duration of diabetes and presence of proliferative retinopathy are not automatically coupled to increased $U_{alb}V$.

Urinary albumin excretion is of glomerular origin since the excretion rate of β_2-microglobulin, an index of tubular function, was normal, as also shown previously [7, 14].

It is worthy of note that glomerular filtration rate and blood pressure were the same in the patients with an abnormal urinary albumin excretion rate of 20–70 µg/min as they were in the diabetic patients who were completely normoalbuminuric (Table 5). This observation suggests that neither a fall in glomerular filtration nor a rise in blood pressure are of major primary pathogenic importance for the rise in $U_{alb}V$ seen in the early stage of diabetic nephropathy.

Patients with incipient nephropathy had significantly higher systolic and diastolic blood pressures compared with patients with normal $U_{alb}V$. This confirms previous findings [2, 7]. However, at the start of the longitudinal study the blood pressure of patients who progressed to incipient nephropathy was not different from those who did not. Serum creatinine was normal and glomerular filtration higher than normal in incipient nephropathy confirming earlier results [15]. The decline in glomerular filtration in diabetic nephropathy is thus preceded by rises in $U_{alb}V$ and blood pressure.

In conclusion, patients with Type 1 diabetes who still have an Albustix-negative urine, but a $U_{alb}V >$ 70 µg/min, constitute a group with a high risk of developing persistent proteinuria or $U_{alb}V > 200$ µg/min. We therefore classified these patients as having incipient nephropathy. They are also characterized by having elevated systolic and diastolic blood pressures, but normal serum creatinine levels and increased glomerular filtration. Most also have retinopathy. Studies are needed to eludicate the pathogenesis of the leakage of albumin into the urine and of the elevated blood pressure in incipient nephropathy.

Acknowledgements. The skilful technical assistance of U. Søegaard is gratefully acknowledged. We also thank J. H. Olsen for secretarial assistance.

References

1. Knowles HC (1974) Magnitude of the renal problem in diabetes. Kidney Int 6: 2–7
2. Deckert T, Parving H-H, Andersen AR, Christiansen JS, Oxenbøll B, Svendsen P Aa, Telmer S, Christy M, Lauritzen T, Thomsen OF, Kreiner S, Andersen JR, Binder C, Nerup J (1982) Diabetic nephropathy. In: Eschwege E (ed) Advances in diabetes epidemiology. INSERM Symposium no. 22 Elsevier Biomedical Press, Amsterdam New York Oxford. pp 235–243
3. Mogensen CE (1982) Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. Br Med J 285: 685–688
4. Parving H-H, Andersen AR, Smidt UM, Svendsen P Aa (1983) Early and aggressive antihypertensive treatment reduces the rate of decline in kidney function in diabetic nephropathy. Lancet 1: 1175–1179
5. Parving H-H, Oxenbøll B, Svendsen P Aa, Sandahl-Christiansen J, Andersen AR (1982) Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin excretion. Acta Endocrinol (Copenh) 100: 550–555
6. Viberti GC, Jarrett RJ, Mahmud U, Hill RD, Argyropoulos A, Keen H (1982) Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet 1: 1430–1432
7. Mogensen CE, Christensen CK, Vittinghus E (1983) The stages in diabetic renal disease. Diabetes 32 (Suppl 2): 64–78
8. Richterich R (1965) Klinische Chemie. S. Kager, Basel New York, pp 212–213
9. Mancini G, Carbonara AO, Heremans JF (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2: 235–254
10. Granerus G, Aurell M (1981) Reference values for ^{51}Cr-EDTA clearance as measure of glomerular filtration rate. Scand J Clin Lab Invest 41: 611–616
11. Svendsen P Aa, Christiansen JS, Søegaard U, Welinder BS, Nerup J (1980) Rapid changes in chromatographically determined haemoglobin A_{1c} induced by short-term changes in glucose concentration. Diabetologia 19: 130–136
12. Mogensen CE (1971) Urinary albumin excretion in early and long-term juvenile diabetes. Scand J Clin Lab Invest 28: 183–193
13. Hemmingsen L, Skaarup P (1975) The 24-hour excretion of plasma proteins in the urine of apparently healthy subjects. Scand J Clin Lab Invest 35: 347–353
14. Viberti GC, Mackintosh D, Bilous RW, Pickup JC, Keen H (1982) Proteinuria in diabetes mellitus: role of spontaneous and experimental variation of glycemia. Kidney Int 21: 714–720
15. Deckert T, Poulsen JE (1981) Diabetic nephropathy: fault or destiny? Diabetologia 21: 178–183

Received: 25 May 1983
and in revised form: 15 March 1984

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