Histol Histopath (1989) 4: 129-135

Histology and Histopathology

Structural changes in glomeruli and proteinuria in streptozotocin diabetic rats

Farida M. Al-Awadi[1] and M.S. Al-Adnani[2]

Departments of [1]Biochemistry and [2]Pathology, Faculty of Medicine, Kuwait University, Kuwait

Summary. In streptozotocin induced diabetes in rats, excretion of urinary protein fractions were studied in relation to structural changes in the renal glomeruli, using light and transmission electron microscopy. After six weeks of induced diabetes only ß1 and ß2 plasma globulins were significantly elevated. The amount of excreted proteins and degree of glomerular changes were not proportional. In the initial stages (1-2 weeks) glomerular structural changes were very mild and were accompanied by significantly elevated proteinuria. This progressed (4-8 weeks) to moderate to prominent structural changes with intermittent proteinuria except for the fractions ß1 & ß2 which were elevated throughout the duration of the experiment. The amount of proteinuria was not proportional to changes in the plasma protein levels.

The following conclusions may be made:

1) The mild early glomerular abnormalities seem to be mainly due to acute metabolic disturbances.

2) An early indication of diabetic nephropathy is provided not only by albuminuria, but may also be an elevated excretion of betaglobulin fractions.

3) Decrease of albuminuria in the later stages of diabetes may be related to the deposition of albumin as a basement membrane - like material in the mesangium.

Key words: Diabetes mellitus, Proteinuria, Mesangial matrix, Basement membrane, Glomerulosclerosis

Introduction

Diabetic nephropathy is characterised by glomerulosclerosis especially in poorly controlled patients. Under normal conditions, the glomerular basement membrane is the major protein filtration barrier (Kirschbaum, 1981). This barrier is disturbed in diabetic nephropathy and results in the appearance of proteinuria (Belfiore, 1981; Brenner et al., 1981; Parving et al., 1982; Dahlquist et al., 1983). Attempts have been made to relate occurrence of selective proteinuria to the duration of diabetes mellitus in man, (Belfiore, 1981; Parving et al., 1982) and to determine whether the presence of compounds such as albumin in the urine (Parving et al., 1982) can indicate development of early nephropathy and be related to the duration of the disease and treatment with insulin.

The objective of this study is to determine the profile of urinary protein fractions in relation to the development of diabetes mellitus and to the structural changes in renal glomeruli of streptozotocin diabetic rats.

Materials and methods

Diabetes was induced in adult male, albino Wistar rats (200-250 g) by the intraperitoneal administration of streptozotocin (60 mg/kg body weight freshly dissolved in 0.01 mol/l citrate buffer, pH 4.5). The rats showing glycosuria subsequently received 2 IU protamine zinc insulin daily (subcutaneously) for the first week and then the same amount twice weekly for 2, 4, 6 and 8 weeks. The occurrence of diabetes mellitus was confirmed by demonstrating glucosuria (usually 24 hours after streptozotocin injection), with glucose indicator sticks (Rapignost, Hoechst, FRG) and hyperglycaemia as measured in 0.02 ml of whole blood with glucose oxidase kits (Bio Merieux, Maryl, Charbonnieres Lesbains, France) and UV 25 spectrophotometer (Beckman, Geneva, Switzerland). The animals were allowed free access to tap water and laboratory chow (E. Dixen and Son Ware Ltd., England). Only diabetic rats with an initial blood glucose of 12-15 mmol/l were included in the study, since from our experience, diabetes could be reversible with time in rats with blood glucose 7-10

Offprint requests to: Dr. Farida M. Al-Awadi, Department of Biochemistry, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat, 13110 Safat, Kuwait

mmol/l. Diabetes was again confirmed prior to sacrifice of animals for histopathological studies.

Blood and urine sampling

At the end of 1, 2, 4, 6 and 8 weeks of diabetes induction, normal and diabetic rats were placed individually in metabolic cages and fasted for 24 hours during which time urine samples were collected using toluene as preservative. Urine volumes were measured and aliquots were centrifuged for 5 min at 1500 g. These animals were then anaesthetized with phenobarbitone sodium (50 mg/kg body weight, intraperitoneally) and maintained at 37° C in a thermostatted restraining cage for blood sampling. Approximately 0.5 ml of blood was collected from rats' tails in heparinized tubes and then centrifuged at 1500 g for 10 min to collect plasma.

Measurement of proteins and creatinine

The total plasma protein was measured by the method of Lowry et al. (1951) and the urine protein by the method of Saifer and Gerstenfeld (1980). Creatinine in urine was measured by a kit method (Bio Merieux). For quantitation of protein fractions in plasma and urine, samples were desalted on 15.5 × 1 cm columns of Sephadex G100 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and eluates were concentrated in Minicon-A concentrators for macrosolutes (Scientific Systems Division Amicon Corporation, Lexington, Mass, U.S.A.) at 4° C. Proteins were separated by zone electrophoresis (Sartophor-Netzteil apparatus, Sartorius GmbH, Goettingen, Fed. Republic of Germany) on cellulose acetate strips (SM 11200, 70 × 145 BBN; Sartorius GmbH). Electrophoresis at 200 V for 75 min separated the albumin and α_1, α_2, β_1, β_2 & γ globulins. Staining was achieved with Ponceau S solution (BDH Chemicals Ltd., Poole, England) (Kohn, 1976). Electrophoretograms were scanned on an ACD-18 Automatic Computing Densitometer (Gleman Sciences, Inc. Ann Arbor, Michigan 48106, U.S.A.) and the area of each peak was expressed as a percentage of the total area. Concentrations of protein fractions were calculated by reference to measured total proteins and expressed as g/dl in plasma or g/g creatinine in urine. Values are presented as the mean ± standard error of the mean (SEM). Student's two tailed t-test was used to evaluate statistically significant differences, taking $P<0.05$ as statistically significant.

Histological, light and electronmicroscopic examination

Both kidneys of normal and diabetic rats were removed at the end of the assigned period (1, 2, 4, 6 and 8 weeks), divided longitudinally into two halves and immediately fixed in 10% neutral buffered formalin containing 5% sucrose, for 24-36 hours at 4° C. One half of each kidney was processed and embedded in paraffin for light microscopic examination (Leitz, Dialux 22Eb, West Germany). Consecutive 3-4 µm sections were stained individually with haematoxylin and eosin (H&E); periodic and Schiff's reagent (PAS); Gordon and Sweet reticulin; and Grocott's methanamine silver (GMS) stain. Part of the other half of each kidney was embedded in glycomethacrylate (Historesin, LKB), and sections 1-2 microns thick, were cut and stained with 1% toluidine blue, PAS and GMS. For electron microscopy 1-2 mm^3 pieces of cortical tissues were fixed in 3% glutaraldehyde for 2 hours and postfixed in 1% osmium tetraoxide and embedded in an araldite mixture. Thin sections were examined by a JEOL 1200 EX transmission electron microscope. The structural assessment was done independently from the biochemical evaluation. Also the histological findings were evaluated by an independent pathologist observer.

Results

Plasma proteins

Total and fractional plasma protein levels in normal and in diabetic rats at 1,6 and 8 weeks after the induction of diabetes are shown in Table 1. There was no significant change in total or fractional plasma protein levels one week after streptozotocin administration. However, there was an increase in β_1 ($P<0.05$) and β_2 globulins ($P<0.05$) at 6 weeks. Levels of albumin, α_1, α_2, and γ globulins showed no change throughout the test period.

Urinary proteins

Table 2 shows the total and fractional urinary proteins in normal and in diabetic rats at 1, 2, 4, 6 and 8 weeks after the induction of diabetes. A significant increase in the total urinary protein appeared from the first week and remained significantly high until the 8th week. Urinary albumin was significantly increased at one and two weeks but not thereafter. The α_1 and α_2 globulin excretion was intermittent. β_1 and β_2 globulins were abnormally high in quantity throughout the test period, while globulins were increased at 1,6 and 8 weeks. At the 8th week all urinary proteins were significantly increased except for albumin.

Histology, histochemistry and electron microscopy

Sections from multiple levels of each kidney were examined and tissues from the streptozotocin treated rats were compared with those from normal controls. One and two weeks after streptozotocin treatment, the kidneys showed mild changes consisting of focal and rather segmental mesangial hypercellularity with excess matrix but no basement membrane thickening of the capillary walls (Figs. 1, 2). At 6 to 8 weeks, however, diffuse sclerotic changes were evident with prominent mesangial and basement membrane thickening, and narrowing of capillary lumens (Figs. 3, 4). The tubules showed no remarkable changes. Some of the medium-sized blood vessels were also thickened. Light microscopic changes are summarized in Table 3.

The electron microscopy results confirmed the light microscopic changes especially for the 6 to 8 week

Table 1. Plasma protein concentrations after streptozotocin injection.

Rats	Total Protein	Albumin	α_1	α_2	β_1	β_2	γ
Normal n = 7	7.70 ± 0.13	1.9 ± 0.1	1.8 ± 0.05	0.8 ± 0.1	0.60 ± 0.07	1.70 ± 0.15	0.86 ± 0.14
Diabetic (1 week) (n = 5)	7.00 ± 0.16	1.7 ± 1.0	2.00 ± 0.08	0.80 ± 0.08	0.46 ± 0.04	1.40 ± 0.04	0.67 ± 0.05
P	ns	ns	ns	ns	ns	ns	ns
Diabetic (6 weeks) n = 5	8.9 ± 1.0	1.8 ± 0.2	1.6 ± 0.2	0.90 ± 0.15	0.9 ± 0.1	2.7 ± 0.4	1.0 ± 0.2
P	ns	ns	ns	ns	< 0.05	< 0.02	ns
Diabetic (8 weeks) (n = 5)	7.90 ± 0.05	1.70 ± 0.22	1.7 ± 0.2	1.00 ± 0.06	0.60 ± 0.03	1.8 ± 0.1	1.10 ± 0.02
P	ns	ns	ns	ns	ns	ns	ns

Results are expressed as g Protein/dl, mean ± SEM, "n" indicates number of animals.
P for concentrations at 6 and 8 weeks is calculated with reference to the calculations for the normal rats.
ns = non-significant.

Table 2. Urinary protein concentrations after streptozotocin injection.

Rats	Total Protein	Albumin	α_1	α_2	β_1	β_2	γ
Normal (n = 8)	0.70 ± 0.06	0.09 ± 0.01	0.090 ± 0.007	0.13 ± 0.01	0.17 ± 0.03	0.13 ± 0.02	0.07 ± 0.01
Diabetic (1 week) (n = 5)	1.90 ± 0.27	0.17 ± 0.01	0.18 ± 0.05	0.33 ± 0.08	0.66 ± 0.13	0.31 ± 0.05	0.2 ± 0.04
P	< 0.005	< 0.001	ns	< 0.05	< 0.005	< 0.01	< 0.02
Diabetic (2 weeks) (n = 5)	1.40 ± 0.12	0.23 ± 0.02	0.17 ± 0.03	0.26 ± 0.02	0.3 ± 0.05	0.27 ± 0.06	0.15 ± 0.04
P	< 0.001	< 0.001	< 0.025	< 0.001	< 0.05	< 0.05	ns
Diabetic (4 weeks) (n = 8)	1.00 ± 0.06	0.08 ± 0.01	0.13 ± 0.03	0.19 ± 0.04	0.31 ± 0.03	0.20 ± 0.02	0.11 ± 0.02
P	< 0.005	ns	ns	ns	< 0.01	< 0.05	ns
Diabetic 6 week (n = 10)	1.20 ± 0.04	0.11 ± 0.03	0.130 ± 0.017	0.190 ± 0.003	0.37 ± 0.05	0.190 ± 0.003	0.19 ± 0.02
P	< 0.001	ns	< 0.05	< 0.001	< 0.005	< 0.01	< 0.001
Diabetic (8 weeks) (n = 5)	1.50 ± 0.23	0.15 ± 0.07	0.30 ± 0.07	0.28 ± 0.02	0.30 ± 0.06	0.30 ± 0.07	0.14 ± 0.02
P	< 0.01	ns	< 0.02	< 0.001	< 0.025	< 0.05	< 0.01

Results are expressed as g protein/g creatinine, mean ± SEM, "n" indicates number of animals.
P for diabetic animals was calculated with reference to the calculations for the normal rats.
ns = non-significant

Table 3. Kidney structural changes after streptozotocin injection (*).

Duration of diabetes in weeks	Glomeruli			Other Vessels
	Mesangial Cells	Mesangial Matrix	Basement Membrane	
1-2	+(**)	+(**)	–	–
4	+ —▷ ++	+ —▷ ++	±	–
6	++	++	+	±
8	+	+++	++	+

(*) The scale used indicates as follows:
+: mild; ++: moderate; +++: very prominent;
and –: No remarkable changes.

(**) indicates focal changes

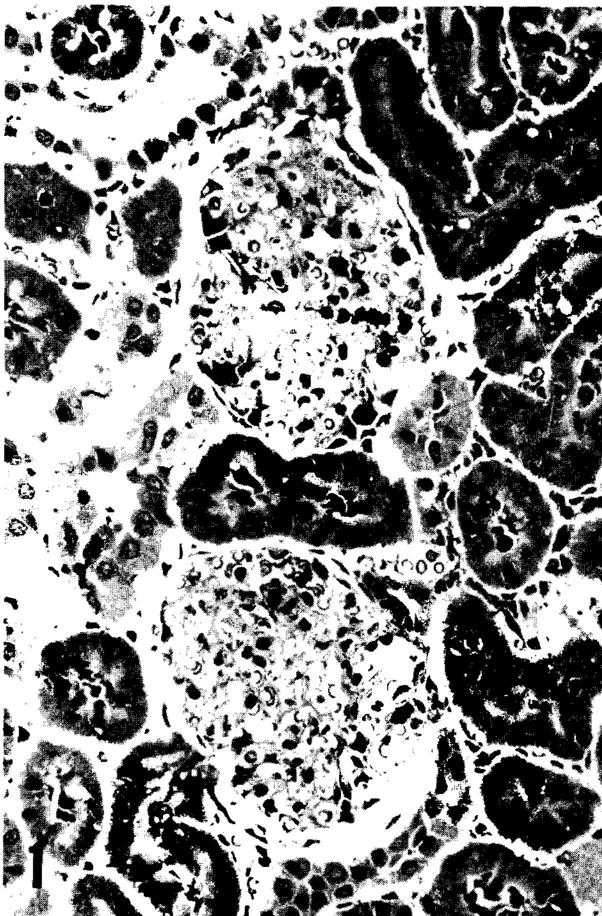

Fig. 1. Kidney from 2 weeks streptozotocin induced diabetes shows glomerular segmental mesangial hypercellularity H & E × 240

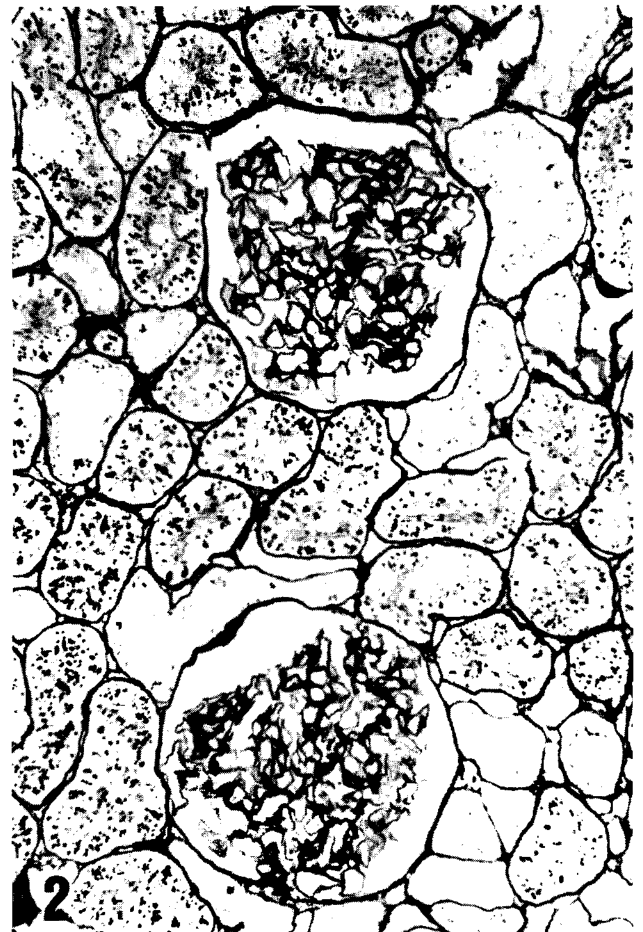

Fig. 2. Consecutive section to figure (1) shows prominent mesangial stalk and segmental mesangial matrix thickening. GMS × 240

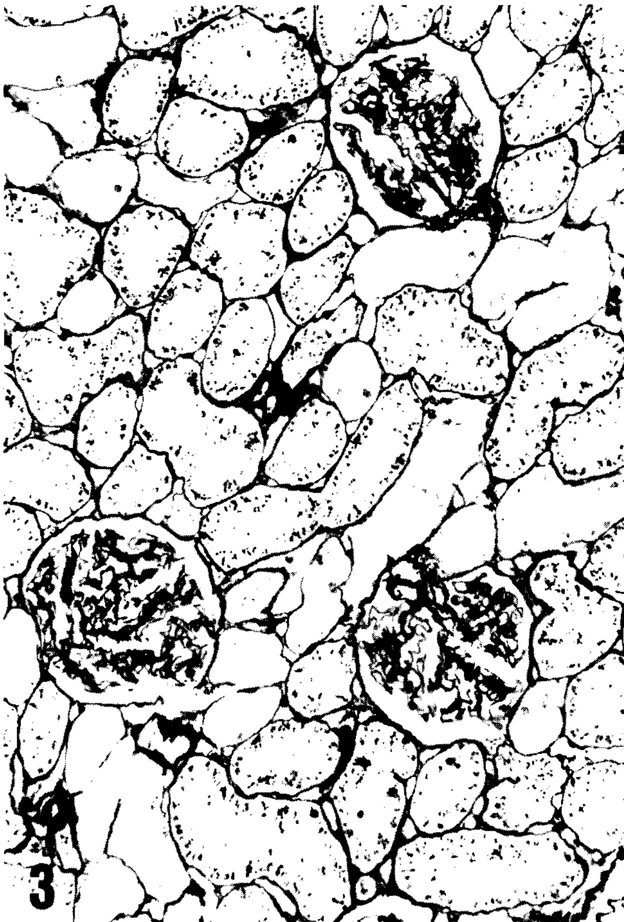

Fig. 3. Kidney from 6 weeks Streptozotocin induced diabetes showing thick mesangial matrix and basement membrane of most capillaries although many capillary lumina are still open. GMS × 200

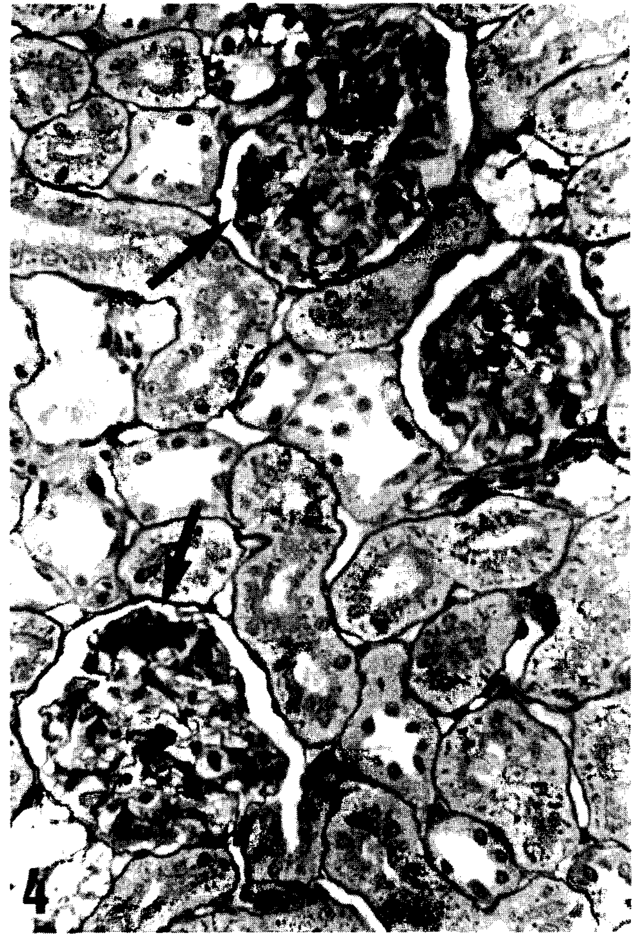

Fig. 4. Eight weeks Streptozotocin induced diabetic kidney shows remarkable matrix and basement membrane thickening with more exaggerated focal segment (Arrows) PAS × 200

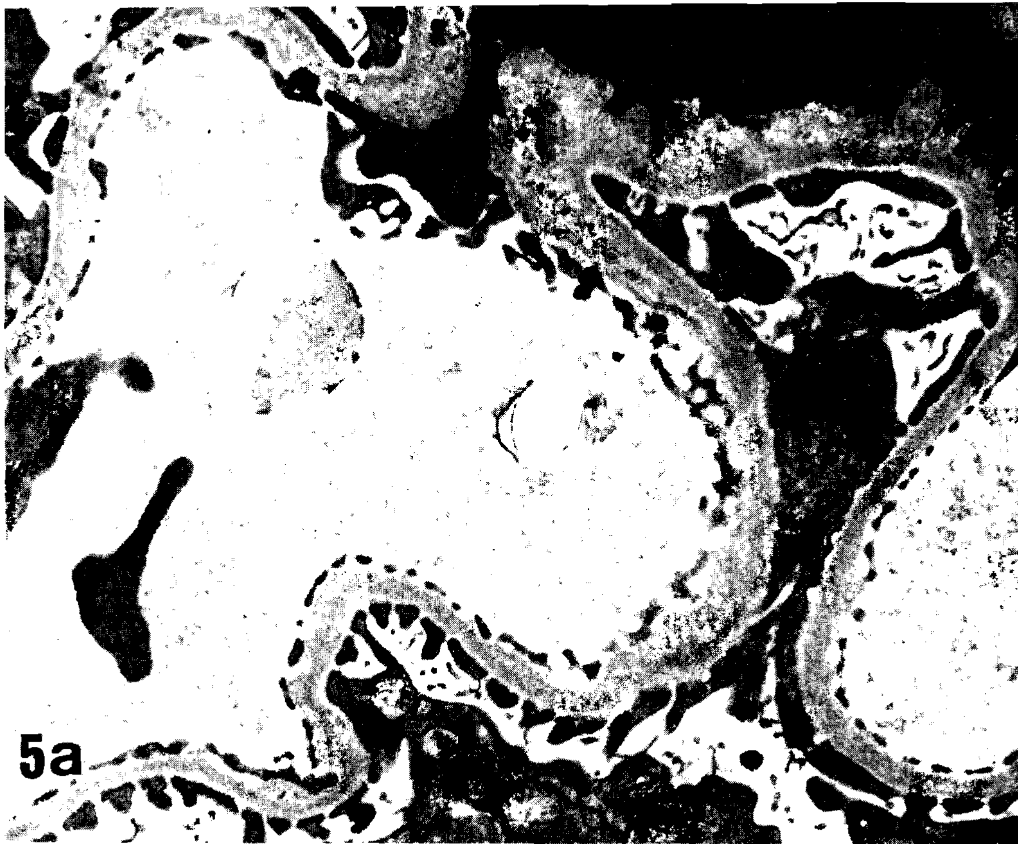

Fig. 5a. Electronmicrograph from kidney of 6 weeks streptozotocin treated rat showing uniform diffuse thickening of basement membrane of capillary wall. Uranyl acetate - Lead citrate × 8,000

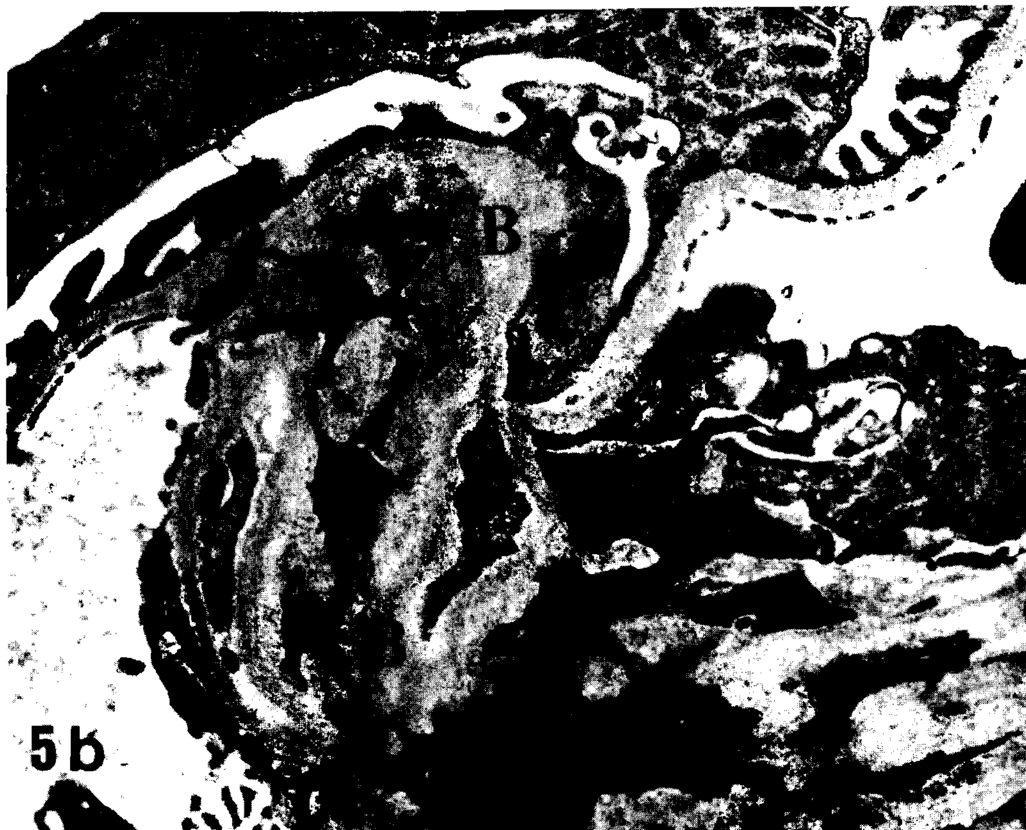

Fig. 5b. Electronmicrograph from kidney of 8 weeks streptozotocin treated rat. Noté the deposition of basement membrane-like material (B) in the mesengium. Uranyl acetate-lead citrate. × 8,000

specimens. These showed diffuse basement membrane thickening of almost all the capillaries and excess deposition of basement membrane-like material in the mesangium (Figs. 5a, b).

Discussion

It has been reported previously that 2-4 IU of insulin daily could cause a dramatic decrease in levels of proteinuria in streptozotocin diabetic rats (Pennel et al., 1981). In our study 2 IU of insulin were given intraperitoneally daily for the first week and twice weekly thereafter to avoid the toxicity of streptozotocin which might lead to death of some of the rats. Daily insulin treatment in the first week may explain the appearance of only minor structural changes in kidneys during the first and second weeks. The findings in this study suggest that proteinuria in the early stages may be due to metabolic changes in these animals since significant structural changes in the kidneys have not yet developed.

The total urinary protein excretion in the diabetic rats was not proportional to the degree of structural changes observed in the kidneys of different weeks of diabetes. This supports previous suggestions that diabetic nephropathy and associated hyperfiltration are attributable to the abnormal metabolism in diabetes such as hyperglycaemia (Brenner et al., 1981), elevation of some immunoglobulins (Westburg and Michael, 1972), and perhaps hyperlipoproteinaemia.

However, according to our results, the source of urinary protein may be mainly due to the mild structural changes observed during the first two weeks in the kidney since there was no significant change in the plasma protein levels during the test period.

It has been reported that ultrastructural changes in the kidney appear to be among the earliest that take place as detected in prediabetic youngsters (Camerini-Davalos et al., 1963) and later confirmed by Nash et al. (1975). However, these reports have not correlated such structural changes with urinary protein excretion.

Furthermore, during the first two weeks albuminuria was significantly high, and thereafter was non-significant. It has been reported that the non-enzymatic glycosylation proteins appear to be the common biochemical link between chronic hyperglycemia and a number of pathophysiological processes potentially involved in the development of long term diabetic complications (Brownlee et al., 1984). The report also stated that glycosylated proteins accumulate inside insulin-independent cells and on cell membrane proteins and that albumin is one of the proteins subjected to such an action. These findings may explain the disappearance of urinary albumin at later stages of induced diabetes concurrent with the increase in mesangial matrix as appeared in both light and electron microscopy. This of course does not rule out the possibility of deposition of other materials along with glycosylated albumin. Moreover, our results on albuminuria support the suggestion that early detection of albuminuria is a good parameter to indicate development of early diabetic

nephropathy (Parving et al., 1982). However, ß fractions could be another parameter since our results showed that they are significantly increased throughout the experiment.

The appearance of urinary globulins of large molecular weight (α, ß, γ), although intermittent, suggests that basement membrane thickening, although mild to moderate during the test period, could have affected permeability and hence contributed to protein leakage. This is in agreement with the report of Carrie and Myers (1980) who stated that selective increase in transglomerular passage of large molecules in the progressive loss of ability to discriminate between large molecules of different configuration, distinguish the glomerular capillary wall in diabetic nephropathy from that in minimal change nephropathy.

Finally, we may conclude that at early stages of diabetes proteinuria is mainly due to acute metabolic effects and early indication of renal damage may be associated not only with albuminuria but also excess excretion of ß globulin fractions. The later reduced albumin excretion may be partly due to deposition as basement membrane- like material in the mesangium which is observed to be of heterogeneous nature. Work is in progress to characterize the nature of the basement membrane-like material by immunohistochemical and ultrastructural methods.

Acknowledgements. We wish to thank Miss Ummukulthum Shamte and Miss. Mai Al-Agha for their skilled technical assisstance. This work was supported by Grant No. MG012 and MB011 from the University of Kuwait Research Council.

References

Belfiore F. (1981). Nephropathy in: Reviewed contents of Major Diabetes Congresses. Vol. 1. Belfiore F. (ed). Karger S. Basel. pp 106-107.

Brenner B.M., Hotettev T.H., Olson J.L., Rennke H.G. and Venkatachalam M.A. (1981). The role of glomerular hyperfiltration in the initiation and progression of diabetic nephropathy. Acta Endocrinol. 97 (Suppl. 242), 7-10.

Brownlee M., Vlassara H. and Cerami A. (1984). Non-enzymatic glycosylation and the pathogenesis of diabetic complications. Ann. Int. Med. 101, 527-537.

Camerini-Davalos R.A., Caulfield J.B., Rees S.B., Lozano-Castaneda O., Naldjian S. and Marble A. (1963). Preliminary observations on subjects with prediabetes. Diabetes 11, 308-317.

Carrie B.J. and Myers B.D. (1980). Proteinuria and functional characteristics of the glomerular barrier in diabetic nephropathy. Kidney Int. 17, 669-676.

Dahlquist G., Aperia A., Broberger O., Persson B. and Wilton P. (1983). Renal function in relation to metabolic control in children with diabetes of different duration. Acta Paediatr. Scand. 72, 903-909.

Kirschbaum B.B. (1981). Glomerular basement membrane and anti-GBM antibody disease (Review). Nephron 29, 205-208.

Kohn J. (1976). Cellulose acetate electrophoresis and immune-diffusion techniques. In: Chromatographic and Electrophoretic Techniques. Vol. II, 4th edition. Ivor Smith (ed). A William Heineman Medical Books Publication. Chicago. pp 103-104.

Lowry O.H., Rosebrough N.I., Farr A.L. and Randell R.I. (1951). Protein measurement with olin phenol reagent. J. Biol. Chem. 193, 265-275.

Nash D.A., Rogers P.W., Langlinasis P.C. and Bunn S.M. (1975). Diabetic glomerulosclerosis without glucose intolerance. Am. J. Med. 59, 191-199.

Parving H.H., Oxenboll B., Svendsen P.A., Christiansen J.S. and Anderson A.R. (1982). Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin exretion. Acta Endocrinol. (Copenh) 100, 550-555.

Pennel J.P., Millard M.M. and Ashby M.H. (1981). Proteinuria in the acutely diabetic rat and its response to insulin treatment. Diabetologia 21, 54-57.

Saifer A. and Gerstenfeld S. (1980). Determination of urinary proteins. In: Practical Clinical Chemistry. 5th edition. Varley H., Gowenlock A.H. and Bell M. (eds). William Heinemann Medical Books Ltd. London. pp 607.

Westburg N.G. and Michael A.F. (1972). Immunohistopathology of diabetic glomerulosclerosis. Diabetes 21, 163-174.

Accepted October 4, 1988