Diabetologia 13, 43–48 (1977)

Diabetologia

© by Springer-Verlag 1977

Glomerular Size and Structure in Diabetes Mellitus. II. Late Abnormalities

H. J. G. Gundersen and R. Østerby

Second University Clinic of Internal Medicine and the University Institute of Pathology, University of Aarhus, Åarhus, Denmark

Summary. A study of autopsy kidney material from six long-term diabetics and four controls was performed in order to elucidate the mechanism of the glomerular enlargement in long-term diabetics. The volume and the severity of the glomerular lesion were measured in each of a number of randomly selected, open glomeruli. The relative amount of solid material was taken as an expression of the severity of the glomerular lesion. In the long-term diabetics the volume of open glomeruli was almost doubled compared to that of controls and in the individual subject the enlargement was found to be inversely related to the relative amount of solid material in the glomeruli. This indicates that the enlargement of open glomeruli in long-term diabetics is due to a compensatory hypertrophy rather than to the excessive deposition of basement membrane material. The number of nuclei per open glomerulus was increased in long-term diabetics, but nuclear size was unchanged. Most of the long-term diabetics had a large number of occluded glomeruli, and the individual, relative number of such glomeruli correlated closely both with the duration of diabetes above 15 years and the concentration of creatinine in serum. It is concluded that the destruction of glomeruli due to diabetic microangiopathy is compensated for some years by hypertrophy of the least affected glomeruli. This compensatory hypertrophy of glomeruli might well account for the preservation of renal function in long-term diabetics for a number of years despite the progressive basement membrane lesions of diabetic microangiopathy.

Key words: Long-term diabetes, diabetic glomerulosclerosis, diabetic renal disease, glomerular size, compensatory glomerular hypertrophy, kidney function.

The development of diabetic glomerulopathy starts at the onset of diabetes. By 2 years there is a measurable thickening of the peripheral capillary basement membrane [9]. The diabetic microangiopathy progresses steadily over the years leading eventually to large accumulations of basement membrane material within the glomerular tuft, thereby reducing the capillary lumen and the capillary blood flow. On the other hand, studies of kidney function have shown that glomerular filtration rate (GFR) remains high and unchanged for many years of diabetes [5], until finally renal insufficiency sets in.

The solution of this paradox might lie in the fact that the glomeruli in long-term diabetics are larger than normal. An increase in glomerular size has previously been mentioned in several descriptions of the diabetic kidney and measurements have verified this observation [3, 10]. The glomerular enlargement might represent a compensatory hypertrophy of the functioning glomeruli, triggered by the loss of others, by analogy with that seen in other situations where a part of the kidney has ceased to function.

However, there is a quite different interpretation of the glomerular enlargement in long-term diabetes; the increase in size is thought to be due to an expansion of the tuft because of the accumulation of solid substances.

The aim of the present study was to elucidate the relationship between the persistently normal function and the progressive morphological changes of the glomeruli in long-term diabetes, and in particular to distinguish between the alternative hypotheses regarding the mechanism of the glomerular enlargement.

Material and Methods

Autopsy material was obtained from 4 non-diabetics and 6 long-term diabetics. Pertinent data for all subjects appear from Table 1. Renal cortical tissue was fixed in formalin, embedded in paraffin and cut into sections at 3 μ. The measurements were performed

Table 1. Clinical data and the relative number of occluded glomeruli in long-term diabetics and controls

Case no.	Age	Sex	Duration of diabetes years	Occluded glomeruli per cent	Causes of death
Long-term diabetics					
1	48	M	16	1	coronary occlusion
2	48	M	17	5	cardiac arrest
3	26	F	23	37	peritonitis
4	39	M	27	47	cerebral vascular disease
5	38	F	28	34	pneumonia
6	45	M	31	65	pneumonia
Controls					
7	51	F		< 1	subarachnoidal hemorrhage
8	31	M		< 1	subarachnoidal hemorrhage
9	27	M		< 1	cardiac arrest
10	35	F		< 1	hanging

after staining with haematoxylin and eosin. All glomerular cross-sections in which open capillary loops were visible, were termed "open", whereas the rest were termed "occluded".

The true glomerular volume was estimated in the following way: each glomerular cross-section was projected onto a drawing of a series of concentric circles, thereby permitting allocation of the class into which it fitted best. On the average 137 (82 to 197) cross-sections of glomerular tufts were classified in each specimen at a magnification of 580 ×. From the class-distribution of these cross-sectional areas the distribution of true volume of open and occluded glomeruli and its mean and variance were calculated according to the method of Saltikov [7]. The percentage of occluded glomeruli was calculated from the number of open and occluded glomeruli, which was estimated from the same distributions.

The composition and size of individual glomeruli were determined by a point counting procedure on serial sections. On the section in the middle of the series all open glomeruli within a pre-selected mid-cortical area (29 to 50 glomeruli) were identified and followed on every fourth section in both directions of the series until disappearance. The measurements were performed at a magnification of 900 × using a Leitz apochromatic, aplanatic 40/0.74 objective. The nuclei were counted and the number of points falling on nuclei, lumina and the solid part of the glomerular tuft, respectively, was estimated on the individual cross-sections of all selected glomeruli, employing a grid with a point densitiy of 16 points/10,000 μ^2. The sum of the three individual fractions – nuclei, lumina and solid – makes up the total glomerular tuft.

The total number of points falling on every fourth section of one tuft is proportional to the absolute volume of that tuft. The fractional number of points falling on the solid part of the glomerulus equals the fractional volume of solid, which is taken as an expression of the long-term diabetic lesion. The direction of the relationship within each subject between the size of individual glomeruli and their fraction of solid indicates which of the two before-mentioned mechanism is responsible for the glomerular enlargement. Counting of nuclei and point counting the area of nuclear cross-sections provides an estimate of the total number of nuclei per glomerular tuft and of their mean sectional area. Thereby it was possible to estimate whether cellular hyperplasia or hypertrophy was present.

The distribution of points between the glomerular components was evaluated in open glomeruli in 3 diabetics (Nos. 1, 2, 3) and 3 controls (Nos. 7, 8, 9) by one technician. In the remaining 3 diabetics and 1 control subject only nuclear cross-sections and points falling on nuclei were counted.

Detailed surveys of the morphometric methods and stereological principles are given in references numbers 2 and 8.

All absolute volumes pertaining to each individual were adjusted to a body surface of 1.73 m^2, based on a nomogram for height and weight [1]. Since distributions of volumes generally are log-normal the comparison of volumes between groups has been carried out on the logarithmically transformed values. The mean values calculated from these distributions equal the geometric means of the original values. Student's t-test and simple linear regression analysis were used in the statistical evaluation, employing a 5 per cent limit of significance.

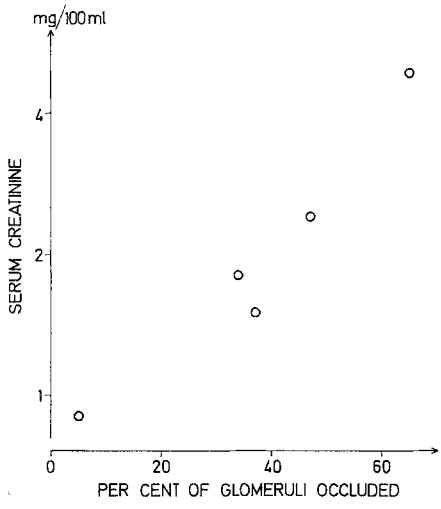

Fig. 1. The relationship between serum creatinine (mg/100 ml) and the relative number of occluded glomeruli (per cent) in five long-term diabetics. In the remaining diabetic a recent estimate of serum creatinine was not available. The coefficient of correlation is + 0.97; 2p = 0.0077. The ordinate is logarithmic

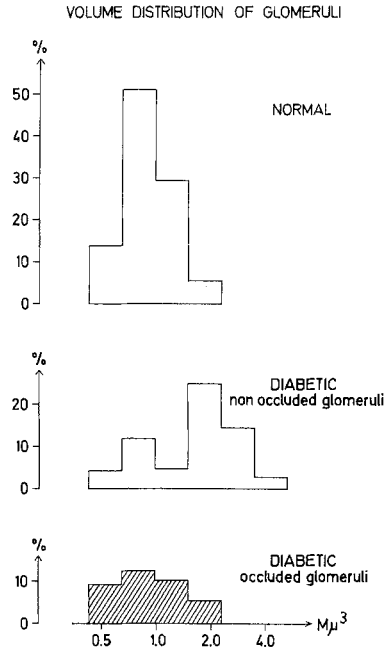

Fig. 2. The volume distribution of glomeruli in a normal subject (upper curve) and in a long-term diabetic (middle curve: open glomeruli, lower curve: occluded glomeruli). Glomerular cross-sections in which open capillary loops were visible, were termed "open"; the rest were termed "occluded". The abscissa is logarithmic

Results

The relative number of occluded glomeruli appears in Table 1. Most of the long-term diabetics show a considerably increased number of such glomeruli, and the increase is correlated both to the duration of diabetes (r = 0.95, 2p = 0.0039) and to the concentration of creatinine in serum (r = 0.97, 2p = 0.0077, see Fig. 1), the only parameter of renal function known in these patients.

Examples of the calculated distribution of true glomerular volume are shown in Figure 2. The individual mean glomerular volumes in controls and long-term diabetics are shown in Figure 3. The geometric mean volume of open glomeruli was 1.32 and 2.41 $M\mu^3$ in the two groups (2p = 0.010). The geometric mean volume of the occluded glomeruli was 1.71 $M\mu^3$ in the four diabetics, in whom a large proportion of such glomeruli was present. The mean size of the occluded glomeruli was smaller than that of the open glomeruli in all four cases. On average their volume was reduced by 23.1 ± 7.2 per cent (mean and SEM; 2p = 0.049).

The mean area of nuclear cross-sections was identical in the two groups, 17.4 ± 5.2 μ^2 v. 17.4 ± 1.9 μ^2 (mean and SD), whereas the number of nuclear cross

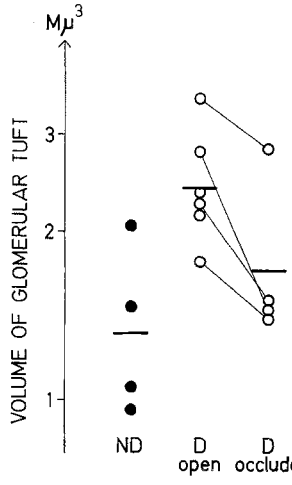

Fig. 3. The mean glomerular volume ($M\mu^3$) in four controls (ND) and six long-term diabetics (D) on a logarithmic scale. The glomeruli in long-term diabetics are divided into open and closed ones. Open circles connected with a line represent the mean volume of open and closed glomeruli, respectively, in the same subject. The horizontal bars indicate the group means

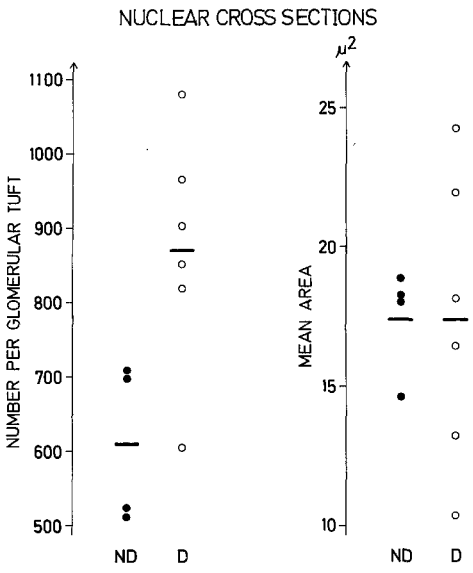

Fig. 4. The mean number of nuclear cross sections per open glomerulus in controls (ND) and in long-term diabetics (D) is given to the left. To the right is shown the mean sectional area (μ^2) of the nuclear cross sections in the same groups. The horizontal bars indicate the group means

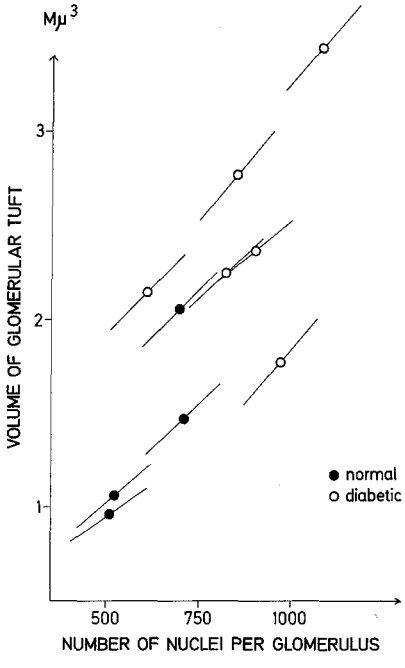

Fig. 5. Filled and open circles represent the individual mean number of nuclear cross sections per glomerulus and mean glomerular volume ($M\mu^3$) in normals and long-term diabetics, respectively. The line through each circle represents the individual relationship (the regression line) between the number of nuclei per glomerulus and the volume of the glomerulus (points), based on 29 to 50 glomeruli per individual. The slope of each line is proportional to the individual regression coefficient in the above relationship, whereas the length of the drawn line is arbitrarily fixed

sections per glomerulus was larger in the diabetics than in the controls, 870 ± 160 v. 610 ± 108; 2p = 0.022 (see Fig. 4). Since the mean nuclear cross sectional *area* was the same in the two groups the *number* of nuclear cross sections can be taken as an expression of the total number of nuclei per glomerulus in the two groups.

Figure 5 shows the relationship between the number of nuclei per glomerulus and the volume of glomeruli. Within each individual the relationship was statistically highly significant ($2p < 0.001$ in all cases), and the mean slope of the individual regression lines in diabetics, 0.279 ± 0.043 points/nuclear cross-section, was not different from that of normals, 0.233 ± 0.033 points/nuclear cross-section ($2p = 0.11$). The relationship between the number of nuclei per glomerulus and the volume of glomeruli among individuals was also statistically significant, $r = 0.80$, $2p = 0.0059$.

Figure 6 illustrates the relationship between glomerular size and fraction of solid within glomeruli from one diabetic subject. It appears that the relationship is inverse. Figure 7 shows the regression coefficients (the slopes of the linear regression lines) from the three diabetics and the three controls. The regression coefficients were statistically significantly different from zero in all diabetics but not in any of the controls. Figure 7 also shows that the *magnitude* of the slopes in diabetics tended to correlate to the proportion of occluded glomeruli, but the number of observations is too small to be considered for statistical analysis.

Discussion

The results of the present study have shown that the open, still functioning glomeruli in long-term diabetics are larger than normal.

The size of the nuclei is unchanged, but the number of nuclei (an expression of the number of cells) per glomerulus is increased in proportion to the increase in glomerular volume. This might be taken to indicate that the growth of the glomeruli in long-term diabetics is brougth about by cellular hyperplasia rather than hypertrophy.

The *inverse* relationship between individual glomerular size and the fraction of solid in glomeruli strongly supports the hypothesis that the enlargement of open glomeruli is compensatory to the loss of the function of the closed ones. If the glomerular enlargement were due mainly to deposition of basement membrane material the situation would have been the opposite one: a direct relationship between size and solid fraction would have been found.

Glomerular enlargement has previously been de-

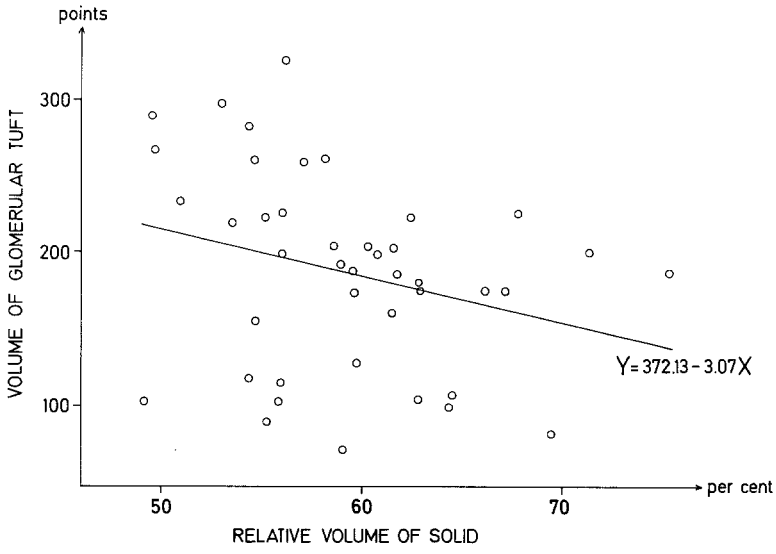

Fig. 6. The relationship between the relative volume of solid material (per cent) in individual glomeruli and the volume of the glomerulus (points). Each point represents one glomerulus followed by serial sectioning. All glomeruli are from that long-term diabetic who among the three shown in Fig. 7 had a median slope of the regression line

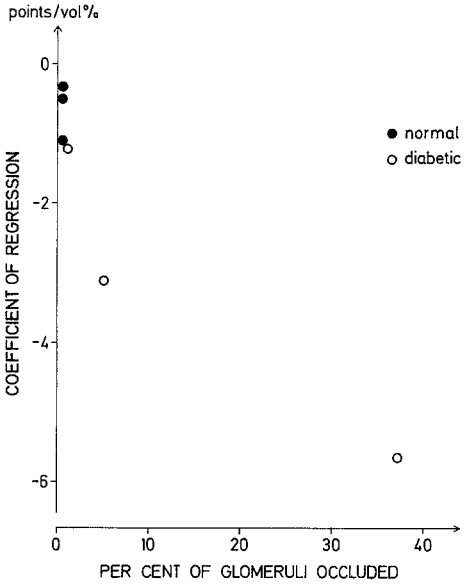

Fig. 7. The ordinate gives the coefficient of regression between the relative volume of solid material (per cent) in individual glomeruli and the volume of glomeruli (points) in three normal subjects and three long-term diabetics. Note that the figures on the ordinate are negative. The abscissa gives the relative number of occluded glomeruli (per cent) in each subject

scribed in newly diagnosed diabetics [11], in whom an increase of the luminal volume and the surface area of the capillaries is also found [4]. In a series of insulin-treated short-term diabetics (duration one to six years) the glomerular enlargement of 25 per cent was not statistically significant. However, the luminal volume was still increased in these patients [11]. In contradistinction to the present study, no indication of cellular hyperplasia was found in this early phase of the disease. The increase in GFR follows the same pattern as the enlargement of glomeruli, and it is known to remain elevated for many years of diabetes, decreasing only when albuminuria begins [5].

The estimates of absolute glomerular volumes in long-term diabetics in the present study cannot be compared directly to the figures in the above-mentioned study in early diabetes [11] since the latter was made on biopsy material. Such a comparison has been made in another biopsy study [10] revealing an increased glomerular size in long-term diabetics compared to short-term diabetics. Therefore, the glomerular enlargement in long-term diabetics is probably added to that present already from the start of the disease.

One rough morphological measure of this long-term diabetic kidney disease is the proportion of closed glomeruli. Closure of all capillaries in a glomerular cross section is only the terminal stage of a prolonged process during which the thickness of the peripheral basement membrane and the amount of basement membrane like material in the mesangium is steadily increasing. Therefore, the very close correlation between the duration of diabetes *above* approximately 15 years and the proportion of closed glomeruli does not mean that the long-term diabetic glomerulopathy starts at this time. It seems more reasonable to translate this correlation into the statement that it takes about 15 years for the microangiopathy to reach the terminal stage in single glomeruli. From this time the demand on the rest of the glomeruli to increase their function and size is likely to be more and more imperative. However, since the number of functioning glomeruli continues to decrease, the final outcome for the kidney as a

whole is cessation of function, unless means to delay the destructive process can be found. Lowering of the blood pressure is one way which is being studied at present [6].

From the results presented in this study we conclude that kidney function in long-term diabetics remains above critical limits due to a compensatory growth of some glomeruli which for a certain period balances the destruction of others.

Acknowledgments. We are very much indebted to Dr. M. Gregersen, Department of Forensic Medicine, for the normal kidney tissue. We also want to thank Mrs. Dorte Eilertsen, Mrs. Grethe Glerup, Mrs. Helen Hou Jørgensen and Mr. Erik Mouritzen for skilful technical assistance. The study was supported by grants from Statens lægevidenskabelige Forskningsråd, Nordisk Insulinfond, the University of Aarhus, P. Carl Petersens Fond, and Landsforeningen for Sukkersyge.

References

1. Diem, K., Lentner, C.: Scientific tables. Basel: Geigy 1970
2. Elias, H., Hennig, A., Schwartz, D. E.: Stereology: applications to biomedical research. Physiol. Rev. **51**, 158–200 (1971)
3. Klein, L., Butcher, D. L., Sudilovsky, O., Kikkawa, R., Miller, M.: Quantification of collagen in renal glomeruli isolated from human nondiabetic and diabetic kidneys. Diabetes **24**, 1057–1065 (1975)
4. Kroustrup, J. P., Gundersen, H. J. G., Østerby, R.: Glomerular size and structure in diabetes mellitus. III. Early enlargement of capillary surface. Diabetologia (in press)
5. Mogensen, C. E.: Kidney function and glomerular permeability to macromolecules in juvenile diabetes. Dan. med. Bull. **19** (Suppl. 3), 1–38 (1972)
6. Mogensen, C. E.: Renal function changes in diabetes. Diabetes **25**, 872–879 (1976)
7. Saltikov, S. A.: The determination of the size distribution of particles in an opaque material from a measurement of the size distribution of their sections. In: Stereology (ed. H. Elias), pp. 163–173. Berlin: Springer Verlag 1967
8. Weibel, E. R.: Stereological principles for morphometry in electron microscopic cytology. Int. Rev. Cytol. **26**, 235–302 (1969)
9. Østerby, R.: Early phases in the development of diabetic glomerulopathy. A quantitative electron microscopic study. Acta med. scand. Suppl. **574**, 1–82 (1975)
10. Østerby, R., Gundersen, H. J. G., Mogensen, C. E.: Increased glomerular size in short-term and long-term juvenile diabetics. Excerpta Medica, International Congress series no. 280, p. 191, Amsterdam 1973
11. Østerby, R., Gundersen, H. J. G.: Glomerular size and structure in diabetes mellitus. I. Early abnormalities. Diabetologia **11**, 225–229 (1975)

Received: August 20, 1976, and in revised form: October 15, 1976

Dr. R. Østerby
Department of Pathology
Kommunehospitalet
DK-8000 Århus C
Denmark