Fast Accumulation of Basement Membrane Material and the Rate of Morphological Changes in Acute Experimental Diabetic Glomerular Hypertrophy

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Summary. Renal glomerular structures have been studied during the initial phases of renal and glomerular hypertrophy in streptozotocin diabetic rats. – After four days of diabetes significant increases were found in the following absolute structural quantities: the tuft volume (25%), the surface area (42%) and volume (46%) of peripheral basement membrane, and capillary luminal volume (29%). No further changes in these quantities took place over the succeeding 47 days. The geometry of the capillaries, however, changed over this period: at 4 days the total capillary length had increased significantly (32%) with an unchanged average cross sectional area, whereas at 47 days the length had reverted to normal, but average cross sectional area had increased (30%). The increased surface of filtering area may be the morphological counterpart of increased glomerular filtration rate. The changes in glomerular volume were thus accompanied by changes in glomerular structural composition which after a few weeks returned to normal, in balance with the increased volume.

Key words: Experimental diabetes, basement membrane mass, kidney hypertrophy, glomerular hypertrophy, glomerular capillaries, filtration surface, capillary morphology, stereology.

In our light microscopic study of renal growth in early experimental diabetes [1] it was shown that glomerular enlargement is demonstrable after only 4 days of streptozotocin diabetes in the rat.

Earlier studies in acute onset, juvenile diabetes have shown a similar enlargement [2] and an increase in the glomerular capillary surface [3], as well as the

presence of increased amounts of glomerular capillary basement membrane (BM) material at the time of diagnosis or soon thereafter (Kroustrup, Østerby & Gundersen, unpublished observations). The results raised the question as to whether such structural changes were due to aberrant metabolism and developed in the few weeks from the onset of metabolic derangement prior to the diagnosis of diabetes.

The purpose of the present study was to examine glomerular ultrastructure during the early glomerular enlargement in the animal model of the metabolic disturbance and, specifically, to establish whether an increase in the amount of glomerular BM material was present in the very early stages.

Material and Methods

Animals

Adult female Wistar rats weighing 240 to 270 g at the start of the experiment were used. Diabetes was induced by injection of streptozotocin (55 mg/kg IP). Only animals with non-fasting blood glucose values above 300 mg/100 ml and a weight loss below 35 g at termination of the study were used. The left kidney was perfusion fixed in situ at 140 mmHg by use of 1% glutaraldehyde in modified Tyrode's buffer. The kidney was post-fixed in the perfusion medium for 90 minutes and stored in Tyrode's buffer for 1 week.

The animals used in the present study were all included in the material presented in the accompanying paper [1]. For the electron microscopic study 9 control rats (C), 9 rats with 4 days of streptozotocin diabetes (D4) and 8 rats with 47 days of streptozotocin diabetes (D47) were used.

Electron Microscopy

Tissue for electron microscopy was post-fixed in 1% OsO₄ for 1 h, dehydrated in acetone and embedded in Vestopal W. Thin (~50 nm) sections were stained with uranyl acetate and lead citrate. Three glomerular cross sections per animal were selected

Fig. 1. Cross section of glomerular tuft obtained at Low Magnification Electron Microscopy (LMEM) with superposed point- and line-lattice used in the stereological estimations. The polygon area is delineated on the micrograph with solid lines. The rectangles are the fields photographed at high magnification, see Fig. 2. The length of a single test line was 20 mm corresponding to 10 μ m

at random [4]. Each glomerular cross section was photographed in toto by Low Magnification Electron Microscopy (LMEM); final magnification was $\times 1900$. A systematic sample, comprising an average of 10 micrographs per cross section and covering $1/4$ of the total cross section, was photographed in a Jeol 100 C electron microscope by High Magnification Electron Microscopy (HMEM) to provide a final magnification of $\times 11180$. For measurements of the basement membrane thickness these exposures were further enlarged to give a final magnification of $\times 33180$, using the projection unit developed by Weibel [5].

Relative Structural Quantities

A comprehensive description of most of the stereological techniques employed in this study is given by Weibel [5]. On LMEM micrographs the minimal convex figure enclosing the epithelial side of the BM was drawn, a so-called string-polygon which is illustrated in Figure 1. On the LMEM micrographs all other compartment quantities were expressed in relation to this reference space, one of them being the naked tuft volume, namely the volume delineated by the bases of epithelial foot processes (i.e. the combined volume of mesangial regions, endothelium, peripheral BM, and capillary lumen). The naked tuft volume was in turn the reference volume employed in the quantitative analysis of the HMEM micrographs, see Figure 2. The individual tuft volume fraction was estimated by LMEM as,

$$V_V(\text{tuft/glomerulus}) = \frac{\Sigma P(\text{tuft})}{\Sigma P(\text{polygon})} \text{ mm}^3/\text{mm}^3$$

where $\Sigma P(X)$ is the total number of points hitting compartment X. The separate summation in numerator and denominator was carried out over the 3 glomeruli. The surface densities (S_V) of the peripheral BM and mesangial epithelial surfaces were estimated by for example:

Fig. 2. High Magnification Electron Microscopy (HMEM) micrograph with grid superposed, used for estimating fractional volumes of mesangial regions (one such is delineated by stippled lines), capillary luminal space, and endothelial and epithelial cells. For these structures the coarse points of the grid (intersections of lines) were used, whereas over the peripheral basement membrane and the basement membrane-like material in the mesangial regions the fine points were counted. The distance between coarse points was 30 mm which corresponds to 2.7 μ m

$$S_V(\text{peripheral BM/glomerulus}) = \frac{2\Sigma I(\text{peripheral BM})}{k \cdot \Sigma P(\text{polygon})} \text{ mm}^2/\text{mm}^3$$

where $\Sigma I(X)$ is the total number of intersections between the surface trace of compartment X and horizontal test lines (Fig. 1) and k is the real distance in mm between two adjacent test points. The length density (L_V) of glomerular capillaries was estimated by:

$$L_V(\text{capillaries/glomerulus}) = \frac{\Sigma Q(\text{lumina})}{k^2 \cdot \Sigma P(\text{polygon})} \text{ mm}/\text{mm}^3$$

where $\Sigma Q(\text{lumina})$ is the total number of luminal profiles, some of which were separated from each other only by an endothelial 'bridge'.

On the HMEM micrographs the fractional volumes of epithelium, endothelium, capillary lumen, mesangium, peripheral BM, and BM-like material in the mesangium were estimated by point counting with the tuft as reference volume.

Absolute Structural Quantities

BM thickness was determined by measuring orthogonal BM intercepts (Fig. 2). The empirical distribution of orthogonal intercept length, l_o , can then be transformed into the expected distribution of true BM thickness, t [6]. For the purposes reported here it suffices to notice two simple relations,

$$\bar{t}_h = \frac{8}{3\pi} \cdot \bar{l}_{o,h} \text{ and } \bar{t}_a = \frac{\pi}{4} \cdot \bar{l}_{o,a}$$

between harmonic (h) and arithmetic (a) mean thickness and the corresponding means of orthogonal intercept length.

The total volume of glomeruli per kidney, $V(\text{glom})$, in each animal was estimated by light microscopy in the companion study [1]. The delineation of glomerular profiles was identical at light microscopy and at LMEM (string-polygon in Figure 1). Thus, by multiplying the relative structural quantities defined above with the appropriate absolute reference volume absolute individual quantities are obtained, for example,

$$V(\text{lumen}) = V_V(\text{lumen/tuft}) \cdot V_V(\text{tuft/polygon}) \cdot V(\text{glom}) \text{ mm}^3$$

the three factors being estimated at descending magnification.

The total volume of peripheral capillary BM material was primarily estimated by,

$$V(\text{BM}) = S(\text{peripheral BM}) \cdot \bar{t}_a(\text{BM}) \text{ mm}^3$$

based on the relation $\bar{t}_a = \frac{V}{S}$ defined for membranes by Weibel & Knight [7]. S denotes surface.

Derived Structural Quantities

Given the fact that the glomerular volume is enlarged by over 30% in diabetic rats [1] it is of no particular interest to look for differences in the absolute glomerular structural quantities (and a valid null hypothesis would be difficult to specify). On the other hand, differences in glomerular composition are of considerable interest. However, the elements composing the glomerulus are only directly measurable in the case of volume fractions of dimension mm^3/mm^3 . Even for a constant shape, all other relative structural quantities with dimensions mm^3/mm^3 change when the glomerular volume changes, as illustrated for S_V in Figure 3. However, since the absolute quantities are known it is possible to construct estimators that have dimensions mm^3/mm^3 . In stereology such estimators are known as 'shape factors' and the particular members of this family employed here are independent of the absolute reference volume. For example, $S^{3/2}/V = 6 \cdot \sqrt{\pi} \sim 10.6$ for a sphere irrespective of its size and also therefore for an assembly of arbitrary spheres. By comparing such estimates from different groups we can test for *isomorphous* change of the structural quantity under consideration.

The average cross sectional area perpendicular to the capillary lumen was estimated by

$$\bar{A} = \frac{V_V(\text{lumen/polygon})}{L_V(\text{lumen/polygon})} \mu\text{m}^2 \text{ the estimator being described in the companion paper [1].}$$

Poiseuille's equation $\frac{p}{v} = 8\pi \cdot \eta \cdot \frac{l}{A^2}$ relates the geometry (length (l)) and cross sectional area (A)) of a capillary tube to function (volume flow (v)) and pressure drop due to resistance (p)), via a constant of the fluid (viscosity (η)). Topologically, the glomerular vascular bed is a mixture of parallel and serial capillaries, and apart from reconstruction via serial sections there exists

	A	B
$S_V = \frac{\pi \cdot d \cdot l}{l^3} = \frac{\pi}{2l} = 0.79l^{-1}$		$0.39l^{-1}$
$\frac{S^{3/2}}{V} = \frac{(\pi \cdot d \cdot l)^{3/2}}{l^3} = \left(\frac{\pi}{2}\right)^{3/2} = 1.97$		1.97

Fig. 3. Two isomorphous bodies where linear dimensions differ by a factor of 2. Calculation of the density of the cylindrical surface with the cube as reference volume shows that the surface density is decreased in B. The shape factor, $\frac{S^{3/2}}{V}$, on the other hand remains constant

no means for obtaining the correct average of the compound geometric variable $\left[\frac{l}{A^2}\right]$. We can only estimate $\frac{L}{(\bar{A})^2}$, total capillary length (L) divided by average cross sectional area squared, and it is related to $\left[\frac{l}{A^2}\right]$ through the unknown topology of the vascular bed. If one accepts that a change in glomerular topology, for example the relation between parallel and serial capillaries, is not altered by the acutely induced diabetic state, then changes in $\frac{L}{(\bar{A})^2}$ do reflect changes in $\frac{p}{v}$ due to changes in parameters of capillary geometry. Under the additional assumption of isomorphous growth of glomeruli relative changes in $\frac{L}{(\bar{A})^2}$ are proportional to relative changes in $\frac{p}{v}$.

Statistics

All experimental results within the different groups of animals were searched for extreme values or outliers, tests being based on attributes of the sample itself [8]. Extreme values were checked for errors in measurements and calculations and if none were found all results from that animal were excluded.

Estimates of population parameters are given in terms of means $\pm$ CV (Coefficient of Variation = $\frac{SD}{\text{mean}}$) enabling immediate recognition of parameters with low variation, i.e. $CV \leq 0.1$ [9], which implies the combined virtue of precise methods and low biological variation. Parameter differences are given in terms of mean difference $\pm$ CE (Coefficient of Error = $\frac{SE \text{ of mean difference}}{\text{mean difference}} = \frac{1}{\text{Student's } t}$) enabling immediate recognition of differences which are unlikely to be due to chance alone, i.e. $CE \leq 0.45$ implying statistical significance if the difference were the object of an a priori formulated null hypothesis. As stated in the introduction only one such was formulated in the present study: 'there is no increase in the amount of

Fig. 4. The total volume of glomerular capillary peripheral basement membrane (mm^3) per left kidney in individual rats. C, D4 and D47 denote control, 4 day, and 47 day diabetic rats, respectively

glomerular capillary BM material very early in experimental diabetes'. Most of the differences in this paper are therefore given for *descriptive* purposes, and the coefficients of error are given in order to indicate that judged by their own attributes the differences described are not likely to be just haphazardous.

Results

Figure 4 shows the volume of peripheral BM in the experimental groups calculated as the product of surface area and arithmetic mean thickness, both of which are shown in Table 1. The difference between mean volume in control rats ($1.2 \text{ mm}^3 \pm 0.13$, mean $\pm$ CV) and D4 rats ($1.8 \text{ mm}^3 \pm 0.20$) of $0.6 \text{ mm}^3 \pm 0.24$ (mean difference $\pm$ CE) is statistically highly significant ($2p = 0.00073$), judged by Student's t-test. No further change in peripheral BM volume is seen in D47 rats ($1.8 \text{ mm}^3 \pm 0.15$). This is, however, due to opposite changes in the two dimensions of membrane volume, surface area and thickness (see Table 1).

The link between the absolute glomerular volume determined by light microscopy [1] and the various relative morphometric parameters reported here at EM level is the tuft/polygon area fraction. This fraction is about 60% and it is very constant both within any of the experimental groups (CV from 1.8 to 6.5%) and between groups (CV under 5%).

The tuft volume was enlarged by 28% ($8 \text{ mm}^3 \pm 0.25$) in D47 rats compared to controls, but 83% of this enlargement had taken place already at 4 days of diabetes, and there is no difference between D4 and D47 rats.

The volume fractions of the tuft constituents (peripheral basement membrane, capillary luminal space, endothelium, and mesangium) and epithelium and the basement membrane-like material (BMLM) in the mesangium did not show any variation between the experimental groups. The volume fractions are ranked in Table 2 according to their inter-individual variation in the control group. The volume fraction of BMLM shows a 9.0-fold ± 0.33 larger inter-individual relative variance among control animals than that of peripheral BM.

The absolute volumes of the different glomerular compartments showed varying changes in the diabetic animals (Table 2). The total volume of peripheral BM, now calculated as $V_v(\text{peripheral BM}) \times V(\text{Tuft})$, was increased by 35% ($0.5 \text{ mm}^3 \pm 0.31$) in D4 rats compared to controls and showed no further increase in D47 rats ($0.1 \text{ mm}^3 \pm 2.9$). The less pronounced increase by 33% in BMLM was demonstrable in D47 rats only ($0.7 \text{ mm}^3 \pm 0.42$).

The capillary space was enlarged by 27% ($5 \text{ mm}^3 \pm 0.32$) in D4 rats and there was only an insignificant further increase in D47 rats ($1 \text{ mm}^3 \pm 1.7$). The endothelial volume only showed insignificant and inconsistent variations, whereas the capillary epithelial volume was enlarged by 30% ($3 \text{ mm}^3 \pm 0.26$) in D4 and showed no further change in D47 rats ($-1 \text{ mm}^3 \pm 1.2$). Mesangial volume showed no enlargement even after 47 days of diabetes ($1.7 \text{ mm}^3 \pm 0.53$).

The absolute surface of peripheral BM and of mesangium towards the urinary space was enlarged by 41 and 46% respectively in D4 rats (Table 1); accordingly the total filtration surface was enlarged by $51 \text{ cm}^2 \pm 0.16$. In D47 rats enlargements of 22 and 25% of the two surfaces remained (CE 0.32 and 0.38 respectively), but a decrease of the peripheral BM surface compared to D4 did show up ($-18 \text{ cm}^2 \pm 0.45$). Comparison of shape factors ($S^{3/2}/V$, Table 3) showed that at 4 days of diabetes both surfaces were enlarged considerably *above* that due to growth of the tuft (CE 0.16 and 0.31 respectively) whereas in D47 the absolute enlargements did *not* differ from those expected for a tuft of that volume (CE 1.5 for both).

Capillary luminal geometry changed markedly during the experimental period. In D4 rats the capillary space was elongated by 32% ($199 \text{ m} \pm 0.13$), with an unchanged average cross sectional area ($-1 \mu\text{m}^2 \pm 2.3$) whereas after 47 days of diabetes

Table 1. Glomerular structures, absolute quantities per left kidney. A_A (Tu/Pol) is the areal fraction of tuft per polygon. V (Tu) is total volume of the glomerular tuft. T_a is arithmetic mean thickness of the peripheral basement membrane. S (PBM) and S (Mes) are the surface areas of the peripheral basement membrane and mesangial regions, respectively. L (Cap) is total length of glomerular capillaries. $\bar{A}$ (Cap) is average capillary cross sectional area. For each group are given the means ($\bar{C}$, $\bar{D4}$ and $\bar{D47}$) and coefficients of variation (CV) and for comparison of groups the differences of means and their coefficients of error ($CE = \frac{1}{\text{Student's } t}$)

	A_A (Tu/Pol) mm ² /mm ²	V (Tu) mm ³	T_a nm	S (PBM) cm ²	S (Mes) cm ²	L (Cap) m	$\bar{A}$ (Cap) µm ²
Control rats	0.63	26	149	92	34	621	24
	0.60	25	163	78	32	556	28
	0.68	34	153	96	35	712	30
	0.65	29	136	93	31	630	22
	0.68	28	130	81	25	538	34
	0.71	30	134	92	27	638	29
	0.63	28	124	82	22	609	30
	0.67	29	130	105	31	613	28
	0.58	27	122	90	32	619	23
Mean ($\bar{C}$)	0.65	28	138	90	30	615	27
CV	0.065	0.10	0.10	0.095	0.15	0.081	0.14
4-day-diabetic rats	0.60	29	121	104	26	756	26
	0.59	35	122	134	40	896	24
	0.63	37	161	142	48	777	29
	0.62	36	137	133	48	876	23
	0.55	28	124	110	43	845	18
	0.63	30	146	110	37	744	25
	0.66	46	165	146	52	868	32
	0.63	37	158	122	51	752	30
	0.66	39	139	140	45	813	32
Mean ($\bar{D4}$)	0.62	35	141	127	43	814	27
CV	0.056	0.16	0.12	0.12	0.19	0.073	0.17
47-day-diabetic rats	0.61	32	171	94	34	564	33
	0.64	34	151	103	26	628	33
	0.61	35	152	116	35	728	32
	0.61	33	156	96	36	536	35
	0.62	31	188	88	34	571	36
	0.60	40	143	118	40	600	40
	0.63	46	167	122	50	796	36
	0.62	42	166	138	42	825	31
Mean ($\bar{D47}$)	0.62	37	162	109	37	656	35
CV	0.018	0.15	0.092	0.15	0.19	0.17	0.081
$\bar{D4}-\bar{C}$	-0.03	7	4	37	14	199	-1
CE	0.62	0.32	2.1	0.16	0.23	0.13	2.3
$\bar{D47}-\bar{C}$	-0.03	8	24	20	7	41	7
CE	0.45	0.25	0.29	0.32	0.38	1.0	0.23
$\bar{D47}-\bar{D4}$	-0.00	1	20	-18	-6	-158	8
CE	4.0	1.9	0.38	0.45	0.61	0.27	0.23

total capillary length was again normal ($\bar{D47}-\bar{C} = 41 \text{ m} \pm 1.0$). However, the average luminal cross sectional area was now enlarged by 26% ($7 \text{ } \mu\text{m}^2 \pm 0.23$, Table 1). Comparison of the shape factors L^3 (Capillary)/ V (Tuft) and $(\bar{A})^{3/2}$ (Capillary)/ V (Tuft) showed that at 4 days both structural quantities deviated in either direction from that expected from the enlarged

tuft volume (Table 3), but in $\bar{D47}$ rats they were within normal limits. The ratio $\frac{L}{(\bar{A})^2}$ (Capillary) of dimension length⁻³ was increased by 43% in $\bar{D4}$ rats, but after 47 days of diabetes its diminution by 34% below normal compares well with the concomitant increase of 28% in tuft volume of dimension length³.

Table 2. Glomerular volume fractions and absolute volumes per left kidney. The volume fractions (V_V) are given with the tuft as reference space for the peripheral basement membrane (PBM), capillary luminal space (Cap), endothelial (En) and epithelial cell (Ep), total mesangial region (Mes), and basement membrane-like material (BMLM). V denotes total volume. For each group are given the means ($\bar{C}$, $\bar{D4}$ and $\bar{D47}$) and coefficients of variation (CV) and for comparison of groups the differences of means and their coefficients of error ($CE = \frac{1}{\text{Student's } t}$)

	$V_V(\text{PBM}/\text{TU})$ mm^3/mm^3	$V_V(\text{Cap}/\text{TU})$ mm^3/mm^3	$V_V(\text{En}/\text{TU})$ mm^3/mm^3	$V_V(\text{Ep}/\text{TU})$ mm^3/mm^3	$V_V(\text{Mes}/\text{TU})$ mm^3/mm^3	$V_V(\text{BMLM}/\text{TU})$ mm^3/mm^3
Mean ($\bar{C}$)	0.049	0.60	0.12	0.35	0.24	0.074
CV	0.081	0.092	0.12	0.17	0.18	0.25
Mean ($\bar{D4}$)	0.054	0.61	0.11	0.38	0.22	0.074
CV	0.19	0.073	0.18	0.18	0.20	0.25
Mean ($\bar{D47}$)	0.054	0.62	0.10	0.34	0.23	0.078
CV	0.24	0.064	0.21	0.16	0.17	0.16
$\bar{D4}-\bar{C}$	0.005	0.02	-0.01	0.03	-0.01	0.000
CE	0.74	1.3	0.76	1.1	1.7	24
$\bar{D47}-\bar{C}$	0.005	0.02	-0.02	-0.01	-0.01	0.004
CE	0.95	1.0	0.48	2.1	2.5	2.1
$\bar{D47}-\bar{D4}$	0.000	0.00	-0.01	-0.04	0.01	0.003
CE	76	4.7	1.2	0.72	4.6	2.3
	V(PBM) mm^3	V(Cap) mm^3	V(En) mm^3	V(Ep) mm^3	V(Mes) mm^3	V(BMLM) mm^3
Mean ($\bar{C}$)	1.4	17	3.4	10	6.7	2.1
CV	0.091	0.13	0.16	0.18	0.21	0.31
Mean ($\bar{D4}$)	1.9	22	3.8	13	7.8	2.6
CV	0.23	0.17	0.21	0.11	0.27	0.30
Mean ($\bar{D47}$)	1.9	23	3.7	12	8.3	2.8
CV	0.19	0.15	0.25	0.18	0.26	0.19
$\bar{D4}-\bar{C}$	0.5	5	0.4	3	1.1	0.5
CE	0.31	0.32	0.80	0.26	0.75	0.68
$\bar{D47}-\bar{C}$	0.6	6	0.3	2	1.7	0.7
CE	0.26	0.25	1.3	0.44	0.53	0.42
$\bar{D47}-\bar{D4}$	0.1	1	-0.1	-1	0.5	0.2
CE	2.9	1.7	3.3	1.2	2.0	1.6

Table 3. Shape factors for surfaces and length, and the geometrical factor in Poiseuille's equation. V, S, and L denote total volume, surface, and length, respectively, whereas $\bar{A}$ is average cross sectional area. PBM and Mes are the interfaces between urinary space and peripheral basement membrane and mesangium, respectively. Cap denotes capillary luminal space. For each group are given the means ($\bar{C}$, $\bar{D4}$ and $\bar{D47}$) and coefficients of variation (CV) and for comparison of groups the differences of means and their coefficients of error ($CE = \frac{1}{\text{Student's } t}$)

	$\frac{S^{3/2}(\text{PBM})}{V(\text{TU})}$ $\text{mm}^3/\text{mm}^3 \times 10^{-3}$	$\frac{S^{3/2}(\text{Mes})}{V(\text{TU})}$ $\text{mm}^3/\text{mm}^3 \times 10^{-3}$	$\frac{L^3(\text{Cap})}{V(\text{TU})}$ $\text{mm}^3/\text{mm}^3 \times 10^{-15}$	$\frac{(\bar{A})^{3/2}(\text{Cap})}{V(\text{TU})}$ $\text{mm}^3/\text{mm}^3 \times 10^9$	$\frac{L}{(\bar{A})^2}(\text{Cap})$ $\mu\text{m}/\mu\text{m}^4 \times 10^{-6}$
Mean ($\bar{C}$)	30	5.8	8.3	5.1	0.83
CV	0.12	0.24	0.17	0.21	0.11
Mean ($\bar{D4}$)	41	8.2	15.8	3.9	1.18
CV	0.081	0.21	0.23	0.18	0.16
Mean ($\bar{D47}$)	31	6.2	8.1	5.7	0.54
CV	0.12	0.16	0.41	0.17	0.082
$\bar{D4}-\bar{C}$	11	2.4	7.5	-1.2	0.35
CE	0.16	0.31	0.17	0.36	0.48
$\bar{D47}-\bar{C}$	1	0.4	-0.2	0.6	-0.42
CE	1.5	1.5	5.7	0.56	0.35
$\bar{D47}-\bar{D4}$	-10	-2.0	-7.7	1.7	-0.77
CE	0.19	0.36	0.22	0.23	0.21

Discussion

Within 4 days of streptozotocin diabetes the glomeruli expand markedly and thereafter only small absolute volume changes are seen. The fast increases in many constituents are directionally non-uniform, giving rise to a pronounced anisomorphy at 4 days, but subsequent remodelling results in an isomorphous, enlarged glomerulus after 1½ months. These conclusions, based on comparisons between groups, are reached under the assumption that dimensional changes of the tissue during the preparative procedures are similar in the different groups. Under that assumption the magnitude of the shrinkage/swelling and its differential behaviour in different tissue components are of consequence only for the absolute value of the estimates – not for the direction of and the statistical confidence in the observed differences. Therefore, no effort was made to obtain measures of shrinkage/swelling in the present study.

The finding of significantly increased masses of peripheral BM and total glomerular BM in rats which have been hypoinsulinaemic and hyperglycaemic for less than 4 days indicates that a considerable acceleration of the basement membrane synthesis has taken place (assuming that the component which ultrastructurally and topologically is defined as BM is identical in the two situations). Even a total inhibition of basement membrane breakdown [10] would not lead to such changes. It is of considerable interest in this context that the amount of biochemically defined basement membrane protein per isolated glomerulus has been shown to be doubled in rats after 6 weeks of streptozotocin diabetes [11]. Furthermore, enzymes involved in the synthesis of basement membrane have been shown to be increased in experimental diabetes [12, 13].

A direct toxic effect of streptozotocin upon kidney morphology is ruled out by a number of facts. It is known that insulin treatment for 8 days to a very large extent prevents changes in both kidney weight and cell growth (protein/DNA ratio) [14] and that 6 months of insulin treatment prevents all morphological changes [15, 16, 17]. Moreover, in human diabetes all of the above morphological changes that have been looked for are present [2, 3]. Obviously, the changes observed in D4 rats can not be part of a normal morphological development and as regards the period between D4 and D47 it is known that kidney and glomerular size and the glomerular ultrastructure do not change within one or two months in adult rats ([14], Gøtzsche, Gundersen & Østerby, unpublished observations).

The rapidity with which these changes come about in the animal model does give support to the

idea that similar changes may also develop rapidly in diabetic patients. A growth of the glomerular capillary surface which commences with the metabolic derangement therefore seems to be a morphological change that may reasonably explain the increase in glomerular filtration rate (GFR) found already at the diagnosis of the disease [18]. Recently, a very close correlation has been obtained between simultaneous estimates of GFR and of glomerular filtration surface in short-term diabetic patients [19]. The increased glomerular function in diabetic patients is known to be reversible upon strict insulin treatment [20].

Preliminary results suggest that after metabolic normalization for 4 weeks by islet transplantation the increase in filtration surface persists [21]. This finding suggests that the glomerular structure-function relationship is a complex one. It is now well recognized that flow, viscosity, permeability, pressure gradients, and capillary geometry play their roles in determining GFR, but quantitative information regarding their actual contributions is scarce. An illustration of the geometrical and topological complexity involved is given in a recent paper by Shea [22]. The rapid and marked changes of capillary geometry (viz. $\frac{L}{(A)^2}$ in Table 3) indicate the magnitude by which one might err by not considering this fundamental structural quantity in models assuming, for example, filtration equilibrium in the diabetic kidney.

It remains to be clarified whether the early glomerular growth, which in the animal model settles with a normal glomerular structural composition at an enlarged volume, has any long-term implications. At present it is not known whether it is linked in any way to the diabetic microangiopathy, i.e. basement membrane *thickening*. The two morphologically completely different modes of basement membrane accumulation might well have a common causative metabolic factor.

The present results have shown that the accumulation of basement membrane material can be triggered fast by the metabolic aberration of diabetes. Other studies [21, 23] indicate that the accumulation of basement membrane material is not reversible after metabolic normalization.

We may now envisage the course of development of the irreversible diabetic microangiopathy. During periods of poor metabolic control excess capillary basement membrane material is laid down, and when good control is achieved it is not removed again. The main practical consequence would be that great efforts should be made to maintain the best possible control in diabetic patients.

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