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Effect of Islet Transplantation on the Glomerular Changes in Streptozotocin-Diabetic Rats

H. Wehner[1] *, W. Kösters[2], M. Strauch[2], and M. Staudenmeir[1]

[1] Institute of Pathology, General Hospital, D-7630 Lahr, Federal Republic of Germany
[2] Division of Clinical Nephrology, University of Heidelberg, D-6800 Mannheim Federal Republic of Germany

Summary. Glomerular changes develop in rats with streptozotocin diabetes. The structure of these lesions (nodular and diffuse glomerulosclerosis, mesangial cell proliferation, basement membrane thickening, glomerular aneurysms, fibrinoid caps, glomerular adhesions) is described in the present paper and the effect of normalization of metabolism by islet transplantation on the glomerular changes is studied with histological, immunohistological and morphometric methods.

Isogenous islets were transplanted into the portal vein of streptozotocin-diabetic rats after diabetes of 7 months' duration. The kidneys of normal, diabetic and transplanted animals of the same age were studied 2.5 months later. Studies of the kinetics of immunocomplexes in the mesangium were also performed.

The renal changes (glomerulosclerosis, mesangial cell proliferation) were largely reversible after islet transplantation and the blood glucose level and glucose tolerance were normalized. In the diabetic animals the delayed uptake and elimination of immunocomplexes in the mesangium was normalized after the transplantation.

It is possible, that the cause of the lesions is a functional disturbance of the mesangium induced by insulin deficiency and/or hyperglycaemia.

Key words: Streptozotocin diabetes – Islet transplantation – Diabetic glomerular lesions – Morphometry – Mesangium – Blood glucose – Immunocomplexes.

Rats with streptozotocin diabetes of long duration develop progressive glomerular changes. An increase in mesangial matrix has been demonstrated in light and electron microscopic studies. Immunohistological investigations have also

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Offprint requests to: Prof. Dr. med. H. Wehner, Institute of Pathology, General Hospital, Klostenstr. 19, D-7630 Lahr, Federal Republic of Germany

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shown changes in the mesangium, consisting of depositions of large quantities of immunoglobulins and complement (Mauer et al. 1975; Federlin et al. 1976).

Furthermore, thickening of the glomerular capillary wall has been demonstrated, and immunohistological studies described deposition of IgG, IgM and C_3 in the region of the glomerular capillary wall (Mauer et al. 1973, 1974, 1975, 1976). Here the glomerular changes are similar to alterations which occur in human diabetes mellitus of prolonged duration (Hägg 1974a and b; Mauer et al. 1976; Olsen 1969; Weil et al. 1976; Wehner 1977). Recent studies have shown that regression of the immunohistological and light microscopic changes is possible if normalization of glucose metabolism is achieved. This is evident from investigations with insulin treatment of diabetic animals (Wehner et al. 1978; Rasch 1979a and b) and from experiments with islet transplantation in experimentally induced diabetes in which farreaching normalization of the blood glucose level occured (Mauer et al. 1975; Federlin et al. 1976; Kösters et al. 1978; Bretzel et al. 1979; Wehner et al. 1979).

The purpose of the present study was to investigate to what extent hyperglycaemia leads to a morphological and functional alteration of the glomerular structures and whether regression of these changes can be demonstrated with histological, immunohistological and morphometric methods if the hyperglycaemia is normalized by islet transplantation.

Material and Methods

1. Experimental Animals and Healthy Controls

Inbred male Lewis rats (brother-sister mating over 67 generations) about 2.5 months old with an average weight of 310 g were used for the study. 83 animals remained untreated as controls over the 9.5 months of the experiments. Diabetes was induced in 208 animals (Table 1), although some animals died during the course of the experiments. The animals had free access to standard mixed food and water. Since there was a marked weight loss in the diabetic animals on this food, after four months all animals received a carbohydrate-low, protein-high diet (5% carbohydrate, 49.4% protein, 11.5% fat). The animals were weighed at regular intervals (controls every four weeks, diabetic and transplanted animals once weekly). After 9.5 months, i.e., at the age of approximately 12 months, the untreated nondiabetic controls were killed; histological and immunohistological studies were performed on the kidneys of 19 animals and additional morphometric studies in 3 animals (a total of 22 animals). 61 remained for studies of mesangial function (Table 1).

Table 1. Number of animals investigated in the various groups

	Controls	Diabetes	Transplantation
Histology Immunhistology Morphometry	19 +3 animals	35 +2 animals	19
Studies of mesangial function	61	28	25

2. Induction of Diabetes

Diabetes was induced in 208 animals by intravenous injection of streptozotocin in citrate buffer (pH 6) at a dose of 65 mg/kg body weight. Animals which exhibited blood glucose levels of more than 400 mg/100 ml 8 days after the injection were used for the further experiments; some of the animals died. Islet transplantation was carried out in 51 animals after 7 months duration of diabetes; islet transplantation was not performed in the other animals and they remained untreated over a further period of 2.5 months up to the end of the study. Histological and immunohistological studies were carried out on the kidneys of 35 diabetic animals and morphometric studies as well in a further 2 animals. Twenty eight animals remained for studies of mesangial function (Table 1).

3. Transplantation

A total of 51 animals which had been diabetic for more than seven months each recieved 600–800 islets under anaesthesia after opening the peritoneal cavity and exposing the portal vein. The islets were demonstrable histologically in the portal fields. Animals were sacrificed 2.5 months later. Histological, immunohistological and morphometric studies were performed on the kidneys of 19 animals, 25 remained fo studies of mesangial function. Seven animals died during the course of the experiments (Table 1).

4. Isolation of Pancreatic Islets

The pancreatic islets were isolated using a modification of the methods of Lacy and Kostianowsky (1967) and Shibata et al. (1976). The animals (inbred male Lewis rats) were deprived of food 12 h before death. Pancreatic tissue was fragmented mechanically, then incubated three times at 37° C with collagenase solution, first for 10 min and subsequently for four-minute periods, whereby the collagenase concentration was reduced each time by 50%. The material was then washed and suspended in Hank's solution at 4° C. The islets were collected under a stereomicroscope (magnification × 10) using a Pasteur pipette and preserved at 4° C. About 600 islets from three donor animals were isolated in this manner.

5. Blood Glucose Determination and Glucose Tolerance Test

The blood glucose level was measured (10^{00}–12^{00}) every four weeks in the normal control animals and at weekly intervals in the diabetic and transplanted animals. Blood (10µl) was obtained by puncture of the tail vein. Glucose was measured enzymatically by the hexokinase method in an autoanalyzer (LKB 8600).

The glucose tolerance test was performed after 24 h fasting at the end of the experiments in 15 controls, 10 diabetic animals and 15 transplanted animals. After drawing an initial blood sample the animals received 0.5 g glucose/kg body weight by i.v. injection. Tail vein blood (10 µl) was taken after 10, 20, 30, 40, 50, 60, and 120 min.

6. Preparation of IgG Complexes and Kinetic Studies

Lyophilized human IgG (Behring) was dissolved in 0.1 mol/l phosphate buffer (pH 6.8) and shaken in equal parts with 25% glutaraldehyde solution (dilution 1:500) for 48 h with cooling. Purification was carried out by dialysis against 0.5 mol/l ethanolamine solution and then again against PBS buffer (pH 7.2) in each case for 48 h. Control animals (61) 28 diabetic animals and 25 transplanted animals received 10 mg IgG complexes/kg body weight i.v. At intervals of 2, 4, 6, 8, 10, 16, 24, 36, and 48 h after injection groups of 5–10 animals were anaesthetized (Pentobarbital) and the kidneys removed with intact circulation. The quantity of IgG complexes deposited in the mesangium at time when the kidneys were removed was studied using direct immunofluorescence with FITC-labelled antisera against human IgG. Studies were performed on coded preparations

from each animal and 10 glomeruli were evaluated per animal, whereby the degree of fluorescence was classified as follows: 0 = negative, + = slightly positive, + + = positive, + + + = markedly positive. After the preparations had been evaluated they were decoded and allocated to the various experimental groups (blind test).

7. Histological Methods

Animals of all experimental groups were killed and the kidneys were removed; some were deep-frozen at once for immunohistological studies and the remainder were fixed in 4% buffered formalin and in Gendre solution for demonstration of glycogen. They were embedded in paraffin. HE and PAS stains were carried out and glycogen staining by the Best method was performed on the Gendre-fixed parts.

Renal tissue from cortical segments close to the surface were further fixed for two hours in buffered 2% osmic acid and embedded in plexiglass (methacrylate) for morphometric studies. Silver impregnation of sections 0.5–1.0 μ in thickness (ultra-microtome) was carried out according to Movat.

The light microscopic changes were studied in PAS preparations. The preparations were coded and were not allocated to the various experimental groups until after the evaluation. The individual histological changes were graduated semiquantitatively, whereby 0 signifies negative; further differentiation was carried out between slight, moderate and marked changes. Likewise thickening of the glomerular basement membrane was classified in this manner, i.e., it was not measured quantitatively but evaluated if light microscopic studies showed that it was widened.

8. Morphometric Methods

The morphometric studies were carried out on the silver impregnated sections 0.5–1.0 μ in thickness. The investigator was not aware from which experimental group the preparations originated on performing the evaluation. Allocation of the individual results to the experimental groups was not carried out until after the mathematical procedure (blind test). In this manner 10 different glomeruli per animal were evaluated. The determination was carried out with the help of the point counting technique (Weibel and Elias 1967; Wehner 1974).

The microscopic picture was projected on the ground-glass screen of a Glarex projection device (Zeiss) with an image scale of 1,000:1. A point-net was applied to the screen with a regular point distance corresponding to 5.0 μ. The number of points which fell on the glomerular area limited by Bowman's capsule corresponded to the total number of points. The number of points which lay over the mesangial structures corresponded to the mesangial point-number. The percentage mesangial fraction of the total glomerular area was calculated from same. Depending on the size between 300–500 points fell on one renal corpuscle, i.e., between 3,000 and 5,000 points were evaluated per animal.

9. Statistics

The results of the morphometric studies were analysed statistically with Student's t-test for different degrees of freedom. The limited value for the error probability was $2\,p < 0.01$.

10. Immunohistological Methods

The immunohistological studies were carried out on cryostat sections of the kidneys 3.0 to 6.0 μ in thickness which were deep-frozen immediately following removal. FITC-labelled antisera (Nordic) against rat IgG, β_1C and fibrinogen were used. The cryostat sections were incubated with these for 30 min and then washed with PBS buffer (pH 7.2). The specificity of the fluorescence was controlled by prior incubation with unlabelled antisera. The studies were carried out at the end of the experiments in 19 controls, 35 diabetic animals and 19 transplanted animals. The degree

of the fluorescence in the coded preparations was classified semiquantitatively in the following manner: Negative, slight, moderate and marked. After termination of the experiments the evaluated preparations were allocated to the corresponding experimental groups.

Results

1. Blood Glucose and Glucose Tolerance

Mean blood glucose levels in the untreated healthy control animals remained constant during the entire period of the experiments at 110 mg/100 ml. After streptozotocin blood glucose levels were 500 mg/100 ml. There was a slight but transient reduction (480–430 mg/100 ml) when the animals were transferred to the diet food. The blood sugar levels returned to normal in the transplanted animals within 72 h to values of 110 mg/100 ml; a reduction to mean values of 280 mg/100 ml was achieved in three animals.

The glucose tolerance test in the controls showed an increase in the blood glucose level to twofold the fasting level 10 min after the glucose injection. After 60 min the glucose concentration was already within the normal range again. In the diabetic animals a marked increase in the mean values from 350 mg/100 ml to 450 mg/100 ml was also observed after 10 min but the blood sugar concentration decreased far more slowly so that after 60 min the glucose concentration was still clearly above the basal value, i.e., the mean level was 420 mg/100 ml. In the transplanted animals the glucose tolerance test was consistent with that in the healthy controls.

2. Body Weight

At the start of the study the average weight of all animals was 310 g. The healthy control animals gained weight continuously; the mean weight of all animals was on average 130 g higher than the initial weight. In the first 8 weeks after induction of diabetes the body weight of the diabetic animals dec-

Table 2. Light microscopic renal changes in streptozotocindiabetic rats

1. Nodular glomerular changes	(37%)
2. Diffuse glomerulosclerosis	(100%)
3. Mesangial cell proliferation	(51%)
4. Basement membrane thickening	(14%)
5. Glomerular aneurysms	(23%)
6. Fibrinoid caps (exudative lesions)	(97%)
7. Glomerular adhesions	(11%)
8. Glycogen storage (not in the macula densa!)	(97%)
9. Interstitial nephritis (rare)	(~5%)
10. Tubular adenoma (in one animal)	

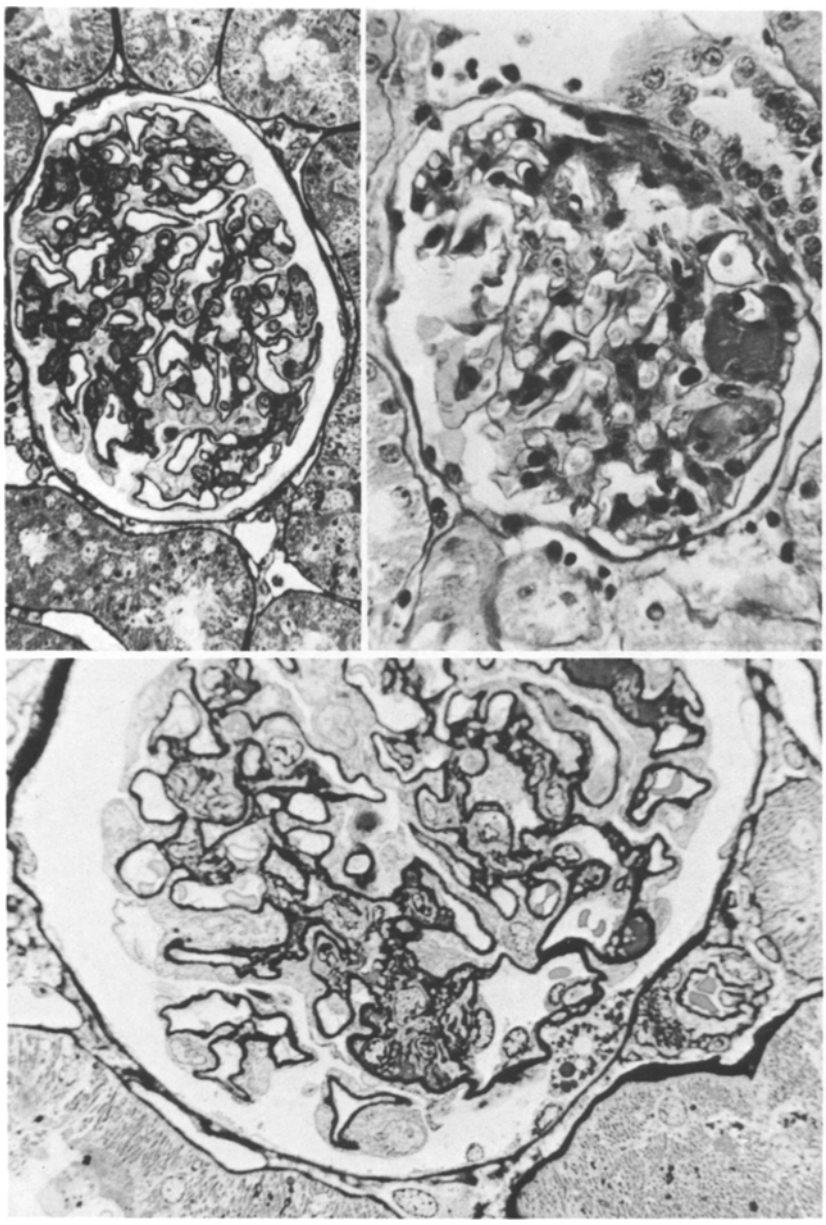

Fig. 1. Different forms and stages of nodular glomerular changes in streptozotocin diabetic rats (Top-left: Silver-impregnation after Movat, ×350. Top-right: PAS-reaction, ×350. Bottom: Movat's impregnation, ×825)

reased to a mean weight of 200 g. A slight increase in weight was apparent from the 9th to the 13th week of the study but there was then a further weight loss. After transfer to the diet food the animals showed an average weight gain of 260 g and the weight then remained constant up to the end of the experiments. After transplantation there was a marked increase in weight from on average 110 g to 290 g.

3. Light Microscopic Studies

Table 2 lists the various light microscopic findings in streptozotocin-diabetic rats.

Nodular Glomerular Changes. Nodular glomerulosclerosis is characterized by a nodular increase in mesangial matrix. The increase is more marked than the cell proliferation. A nodule results from an area whose central structure is predominantly cell-poor and homogenously hyaline, the margin exhibits in part a cell corona and in part ectatic capillaries. Frequently transitions from a diffuse form are found. Such nodules can be demonstrated even in the early stage (Fig. 1). Marked nodular changes were found at the end of the study in 4 of 35 diabetic animals, in 9 of 35 these nodules were only of moderate degree, e.g., in the early stages. In the transplanted animals nodular glomerulosclerosis was demonstrable in only one of 19 animals (Table 3).

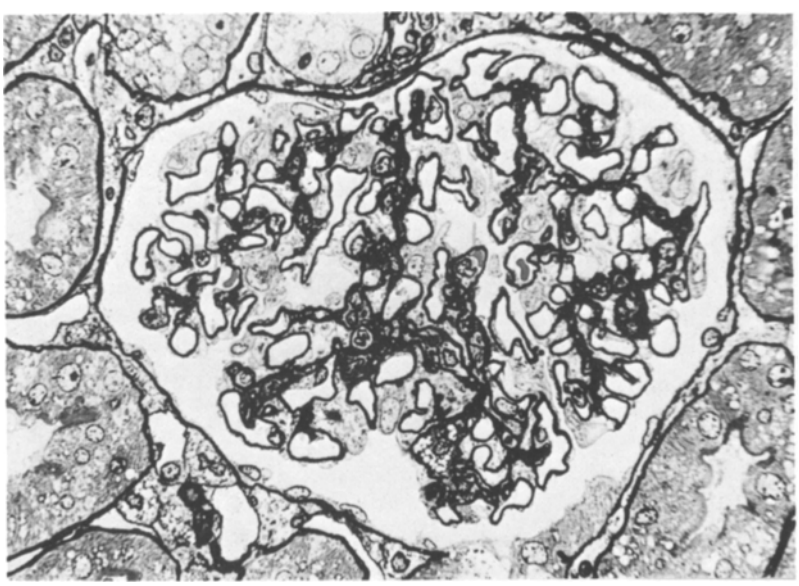

Fig. 2. Diffuse diabetic glomerulosclerosis with basement-membrane-like material in the mesangium (Silver-impregnation after Movat. × 512)

Table 3. Semiquantitative analysis of light microscopic glomerular changes in age-matched nondiabetic, diabetic and diabetic-transplanted rats

Glomerular lesions		Normal controls $n=19$	Diabetic rats	
			Untreated $n=35$	Transplanted $n=19$
Nodular Glomerulosclerosis	severe	∅	4/35	∅
	moderate	∅	9/35	1/19
Diffuse Glomerulosclerosis	severe	∅	15/35	∅
	moderate	6/19	20/35	1/19
	slight	5/19	∅	5/19
Exudative lesions		∅	34/35	13/19
Mesangial cell proliferation	severe	1/19	12/35	∅
	slight	2/19	6/35	3/19
Basement membrane thickening		∅	5/35	2/19
Aneurysms		2/19	8/35	2/19
Adhesions		1/19	4/35	2/19

Diffuse Glomerulosclerosis. Diffuse glomerulosclerosis is characterized by a diffuse increase of basement-membrane-like material in the mesangial matrix. This results in a diffuse increase in mesangial matrix (Fig. 2). A severe form of these lesions was not found in any of the control animals. Moderately severe diffuse glomerulosclerosis was demonstrable in 6 of 19 control rats and mild diffuse glomerulosclerosis in 5 of 19 control rats. Severe changes were found in 15 of 35 diabetic animals and moderate changes in more than 50%, i.e., in 20 of 35 animals. On transplantation severe diffuse glomerulosclerosis was no longer demonstrable, in 1 of 19 animals the alteration was moderately severe and in 5 of 19 animals it was mild (Table 3).

Exudative Changes. These were mainly so-called fibrinoid caps, i.e. plasma exudates between the basement membrane and endothelial cells (Fig. 3). Such alterations can also be observed in Bowman's capsule as so-called capsular drops. Exudative changes were not found in normal control rats but were present in 34 of 35 diabetic rats. Changes were still observed in transplanted rats in 13 of 19 animals (Table 3).

Cell Proliferation. Cell proliferation is an increase in local glomerular cells, particularly of mesangial cells, which were assessed on optical impressions (Fig. 4). A marked form of glomerula cell proliferation was observed in 1 of 19 normal control rats and a mild form in 2 of 19 animals. Cell proliferation was clearly apparent in 12 of 35 diabetic animals and a mild form was found in 6 of 35 animals. Only 3 of the 19 transplanted animals showed slight cell proliferation (Table 3).

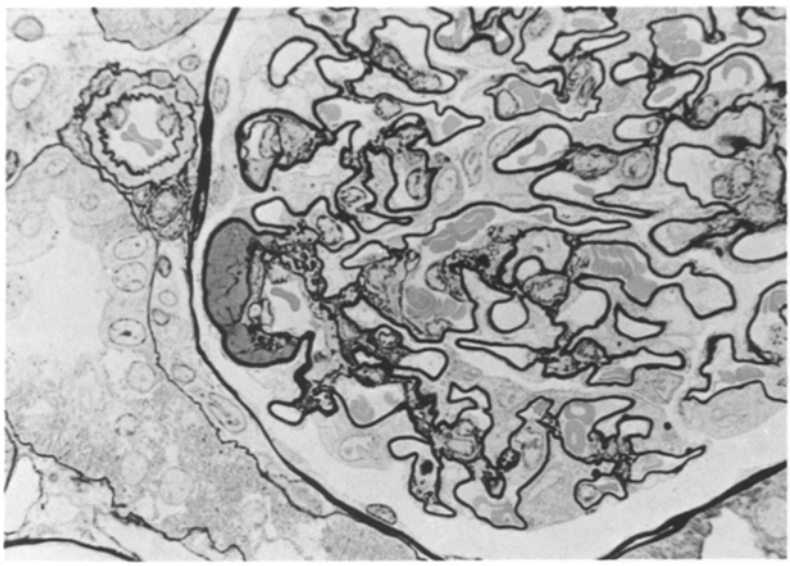

Fig. 3. Fibrinoid cap. Exudation between basement membrane and endothelial cells. (Silver-impregnation after Movat. × 750)

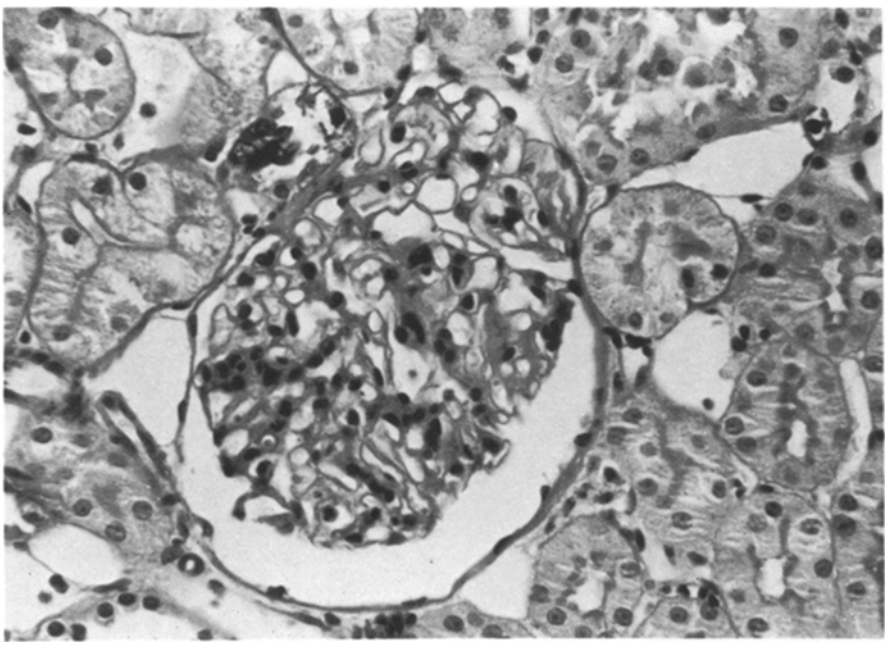

Fig. 4. Focal proliferation of mesangial cells. (PAS-reaction. × 350)

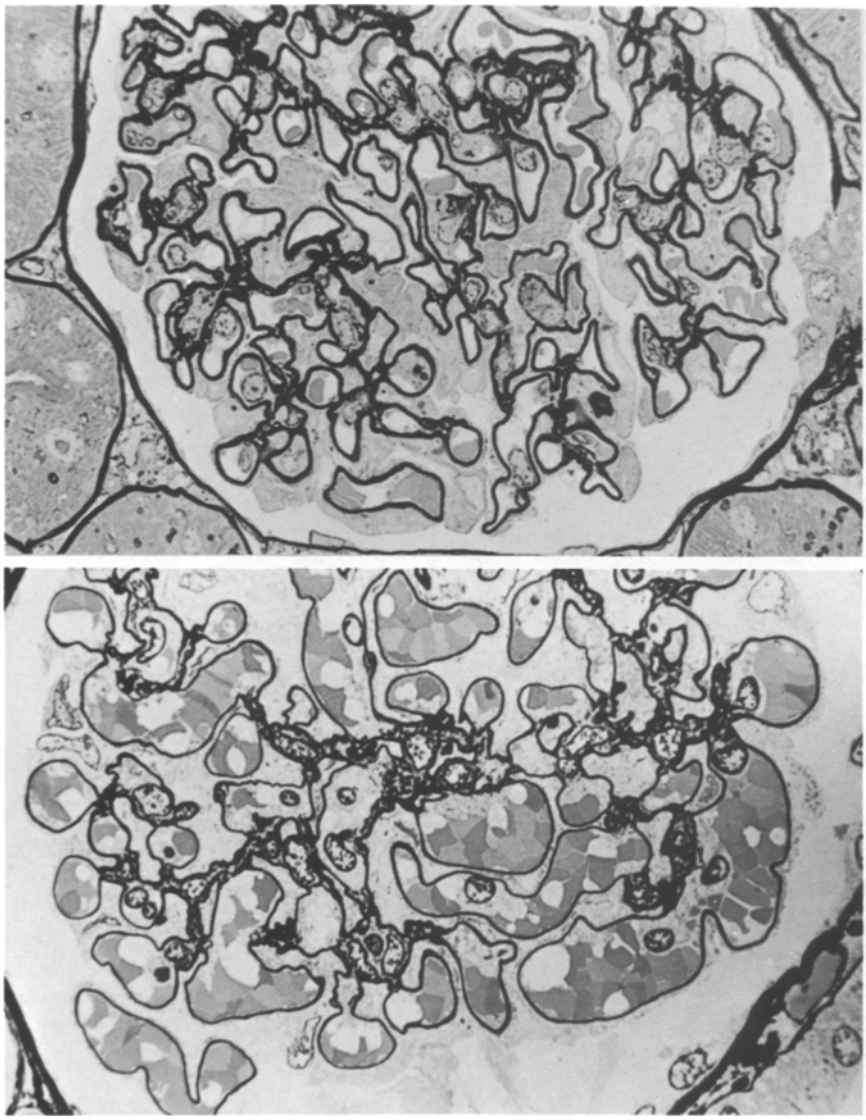

Fig. 5. Homogeneous thickening of the glomerular basement membrane (Bottom: For comparison a normal age correlated glomerulus), (Silver-impregnation after Movat. ×825)

Thickening of the Basement Membrane. These changes, which are only measurable in the early stage by electron microscopy, consist of a visible uniform homogeneous thickening of the basement membrane of the glomerular capillaries on light microscopic inspection (Fig. 5). We did not find this change in the normal controls but it was present in 5 of 35 diabetic animals. The kidneys of 2 of 19 transplanted animals showed this alteration (Table 3).

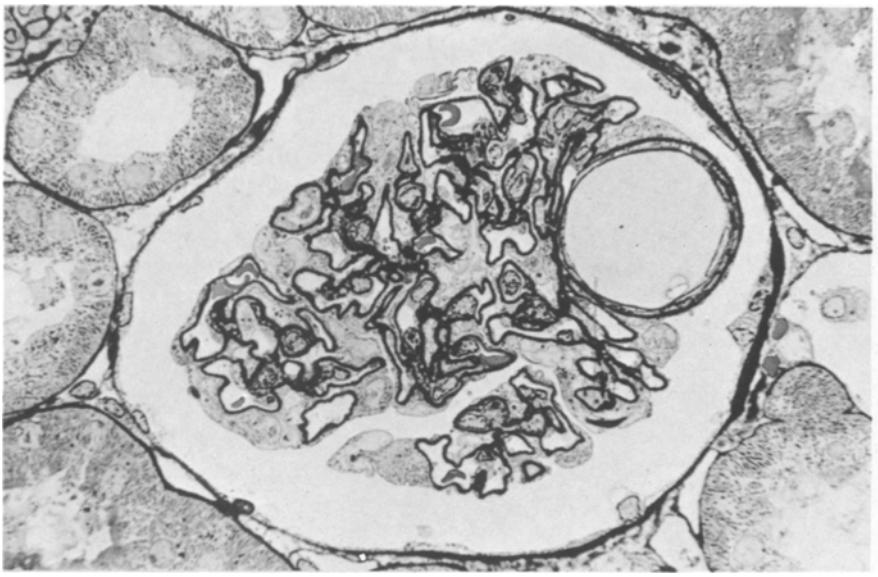

Fig. 6. Glomerular aneurysm. (Silver-impregnation after Movat. ×576)

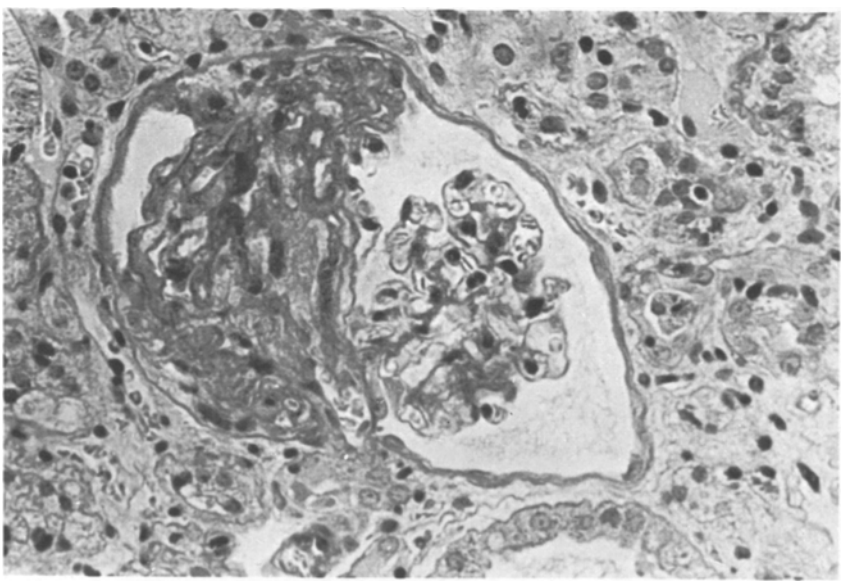

Fig. 7. Segmental sclerosis with a broad synechie (PAS-reaction. ×350)

Aneurysms. Aneurysmal dilatation of the glomerular capillaries particularly in the peripheral segments of the glomerular tuft (Fig. 6) were present in 2 of 19 control rats, in 8 of 35 diabetic animals, and in 2 of 19 transplanted animals (Table 3).

Adhesions. Adhesions of a part or of the whole capillary convolution with Bow-

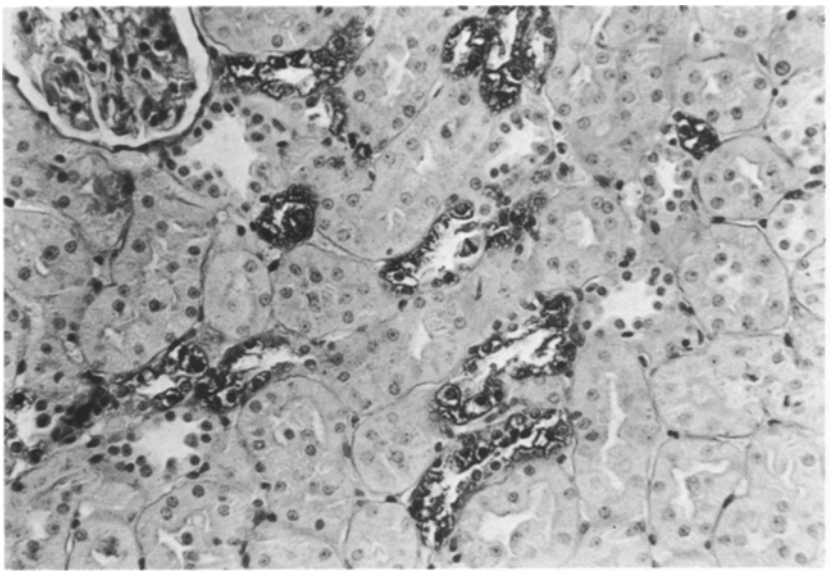

Fig. 8. Glycogen storage in the tubular epithelia (PAS-reaction. ×200)

man's capsule (Fig. 7) were found in one normal control animal, in 4 of 35 diabetic animals and in 2 of 19 transplanted animals (Table 3).

Glycogen Storage. No glycogen storage was found in the kidneys of normal animals but in contrast was detectable in 34 of 35 diabetic animals. Only 4 of 19 animals showed marked glycogen storage (Fig. 8).

4. Morphometry

The morphometric studies showed that in normal controls the mesangium accounted for 8.1% of the total glomerular area. This value increased to 13.4% in the diabetic animals; ($p < 0.01$) On transplantation the fraction of the mesangium decreases to almost normal values of 9.2% ($p < 0.01$) (Table 4).

Table 4. Morphometric analysis of the mesangial area (in per cent of the total glomerular area) in nondiabetic, untreated diabetic and transplanted-diabetic animals (p 0.01 = I vs II, I vs III, II vs III)

Normal controls $n=22$		Diabetic rats	
		Untreated $n=37$	Transplanted $n=19$
Mean	8.09	13.38	9.18
S D	0.57	0.92	0.81

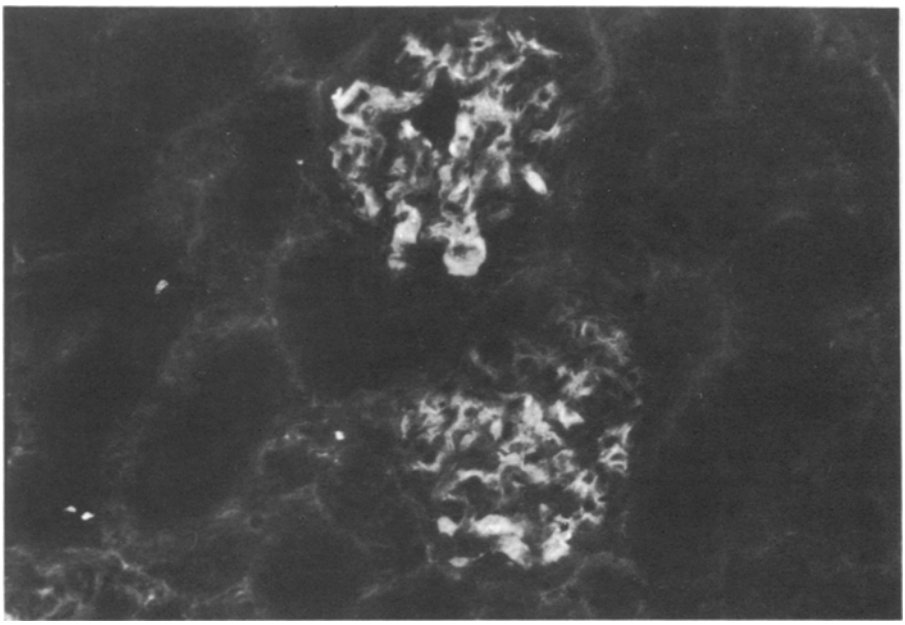

Fig. 9. Granular mesangial deposition of IgG in a diabetic glomerulus (× 250)

5. Immunohistology

Pronounced immunoglobulin depositions were not demonstrable in kidneys of normal rats; more than 70% were immunohistologically negative. Slight to moderate fluorescence for IgG was found however in 5 of 19 animals, in 4 of 19 for β_1C and slight fluorescence for fibrinogen in 3 of 19 animals. The fluorescence was in general granular and mainly demonstrable mesangially. Linear IgG deposition was also demonstrable along the glomerular capillary wall. In the diabetic animals a high percentage of immunoglobulins was found, partly also in linear form for IgG (Fig. 9). This was moderate to pronounced in 27 of 35 animals. There was slight to pronounced β_1C fluorescence in all animals, and moderately intense fluorescence for fibrinogen was found in 23 of 35 animals. On transplantation most animals were immunohistologically negative, 7 of 19 animals showed slight fluorescence for IgG, and 4 of 19 for β_1C and fibrinogen (Table 5).

6. Time Course of Mesangial Changes

In the untreated animals the quantity of immunocomplexes deposited in the mesangium was studied in 7 of 10 animals 2, 4, 6, 8, 10, 24, and 48 h after the injection. The deposition of immunocomplexes in the mesangium was most pronounced after 6 h and these were present in coarse lumpy granular form. Already after 2 h, i.e., 8 h after the injection, marked depositions were apparent in only 3 animals. 10, 24, and 48 h after the injection virtually no further

Table 5. Semiquantitative analysis of the immunohistological findings in age-matched nondiabetic, diabetic and transplanted animals (+++=marked, ++=moderate, +=slight, ∅=negative)

Degree of fluorescence		Normal controls $n=19$	Diabetic rats	
			Untreated $n=35$	Transplanted $n=19$
IgG	+++	∅	10/35	∅
	++	2/19	17/35	1/19
	+	3/19	7/35	6/19
	∅	14/19	1/35	12/19
β_1C	+++	∅	8/35	∅
	++	1/19	13/35	2/19
	+	3/19	14/35	2/19
	∅	15/19	∅	15/19
Fibrinogen	+++	∅	14/19	1/19
	++	∅	9/19	2/19
	+	3/19	12/19	2/19
	∅	16/19	∅	14/19

Table 6. Semiquantitative analysis of the mesangial fluorescence for immunocomplexes at various points of time after the injection in nondiabetic, diabetic and transplanted animals (a=number of animals, 10 glomeruli per animal were investigated) (∅=negative, +=slight, ++=moderate, +++=marked)

Group	Degree of fluorescence	Time after the injection							
		2	4	6	8	10	16	24	36 (h)
Control	+++			6					
	++		1	4	3				
	+	1[a]	2		4	1			
	∅	6	7		3	9		7	
Diabetic	+++						6		
	++					2			
	+					5	1		
	∅				7				7
Transplanted	+++			6	3				
	++			1	2				
	+	2			1				
	∅	5					5		

immunocomplexes were demonstrable. On the other hand no alterations were apparent in the diabetic animals after 8 h and the deposition of immunocomplexes was not present in full intensity until 16 h after the injection in 6 of 7 animals. After 36 h no further immunocomplexes were demonstrable. In the transplanted animals the immunocomplexes, as in the normal control animals, were markedly positive after 6 h in 6 of 7 animals; 16 h after the injection no immunohistological changes were demonstrable in any of the animals (Table 6).

Discussion

It would seem that streptozotocin diabetes, like alloxan diabetes, constitutes a reasonable model for studying diabetic glomerulopathy (Hägg 1974a and b; Mauer et al. 1975; Anjo and Couturier 1975; Weil et al. 1976; Bretzel et al. 1979). Streptozotocin-diabetic rats develop the histological criteria of diabetic glomerulopathy in a relatively short time. However age-matched control groups are necessary for such investigations since widening of the mesangium is observed with increasing age of the animals (Renold 1970; Camerini-Davalos et al. 1970; Olsen 1971). This is also shown by our investigations in which we observed diffuse widening of the mesangium to a high degree with increasing age in non-diabetic rats.

The number of glomerular nodules observed in the present study is far greater than that reported previously (Ditscherlein 1970). This is presumably due to the animals being severely diabetic (blood glucose 500 mg/100 ml), and also to the duration of diabetes. Furthermore, there is also the fact that demonstration of the early stages of nodular changes; particularly in the silver impregnated semithin sections, was included in the evaluation.

Our investigations also show that the transplantation of intact islets results in near-normalization of glucose metabolism and greatly improved control of the diabetes mellitus, as also shown by Slater et al. 1978. This near-normalization of metabolism leads to far-reaching regression of the glomerular changes. Thus the findings of other authors who also observed marked regression of the glomerular lesions after islet transplantation are confirmed. (Sutherland et al. 1975; Mauer et al. 1975, 1976; Federlin et al. 1976; Gray and Watkins 1976; Gray 1977; Slater et al. 1978). It is also evident from the renal transplantation studies of Lee et al. (1974) in which diabetic kidneys which were transplanted into normal recipients showed regression of the histological and immunohistological alterations of the glomeruli that the glomerular changes are reversible. So far this observation has been based on comparative morphological investigations, i.e. in perceptive findings. With the help of morphometric methods already used in the quantitative morphology of diabetic renal changes (Jidaka et al. 1968; Kawano et al. 1969; Wehner et al. 1972), we were able to confirm these findings and pub them on a quantitatively demonstrable basis. Similar results have been reported by Bretzel and Federlin (1977) and by Bretzel et al. (1979) although their absolute values were higher due to a different histological technique. Using morphometric methods the mesangial involvement in the initial changes of diabetes mellitus (Wehner and Majorek 1975) can be demonstrated as previously reported (Kimmelstiel 1968; Ditscherlein et al. 1970).

Not only is the morphology of the mesangium changed but its function is modified. The mesangium has a high capacity for phagocytosis of macromolecular material (Mauer et al. 1974). This property appears to be disturbed in diabetes. Thus in diabetic kidneys large quantities of immunoglobulins and complement are demonstrable in the mesangium (Hägg 1974a and b; Mauer et al. 1975; Weil et al. 1976; Slater et al. 1978). However it is still unclear as to whether the mesangium alone is responsible for the morphological renal

changes in diabetes mellitus and whether it is the key structure for understanding glomerular alterations. If one bears in mind the glomerular changes within the scope of diabetic microangiopathy then other factors which could play a role in this connection are evident. Recent investigation have shown genetic factors, the age at onset of the disease, and growth hormone level appear to play a significant role (Larkins et al. 1978). Likewise biochemical alterations of the basement membrane with changes of the permeability have been discussed (Westberg 1976). These disturbances of permeability are possibly the cause of the exudative lesions which we demonstrated and which hardly tend to regress. Genetic factors have also been discussed in this connection, i.e., the question as to whether the microangiopathy can occur independent of the metabolic disturbance. Here the findings are evidently dependent on the method and the type of tissue investigated (Gundersen et al. 1978; Siperstein et al. 1978). Immunocomplexes may also be important in the pathogenesis of diabetic microangiopathy. Thus Irvine et al. (1978) have demonstrated that the frequency of soluble immunocomplexes increased with the severity of the retinopathy in insulin-treated and oral hypoglycaemic-treated diabetics.

However our findings suggest that the functional defect of the mesangial cells constitutes an important factor in the pathogenesis of diabetic microangiopathy of the kidney, i.e. of diabetic glomerulosclerosis, whereby the circulating soluble immunocomplexes demonstrated by Irvine et al. (1978) possibly may be of relevance in that they are deposited in the mesangium and are not entirely eliminated. This can lead to a reaction in the mesangium in the form of increased formation of mesangial matrix and mesangial cell proliferation, as is known for the immunocomplex nephritides (McCluskey et al. 1962; Germuth and Rodriguez 1973; Mauer et al. 1973). In addition, close functional relations exist between basement membrane and mesangium (Hoyer et al. 1976). According to our investigations the disturbance of mesangial function can be provoked by the insulin deficiency and/or the hyperglycaemia.

The glomerular microaneurysms described by us have also been described in human glomerulosclerosis. Possible, by organisation of these aneurysms development of nodules may occur (Bloodworth 1978). In summary we are in agreement with Bloodworth (1978) that an interaction of many factors is responsible for the development of multiple vascular disease of diabetes.

References

Anjo A, Couturier E (1975) Mesangial changes of the renal glomerulus in long-term diabetic rats. Pathol Eur 10:21–27

Bloodworth JMB (1978) A re-evaluation of diabetic glomerulosclerosis 50 years after the discovery of insulin. Human Path 9:439–453

Bretzel RG, Federlin K (1977) Rückbildung diabetischer Nierenläsionen durch Inselzelltransplantation bei der Ratte. Quantitative Morphologie. Verh Dtsch Ges Inn Med 83

Bretzel RG, Breidenbach Ch, Hofmann J, Federlin K (1979) Islet transplantation in experimental diabetes of the rat. VI. Rate of regression in diabetic kidney lesions after isogeneic islet transplantation: Quantitative measurements. Horm Metab Res 11:200–207

Camerini-Davalos RA, Oppermann W, Mittl R, Ehrenreich T (1970) Studies of vascular and other lesions in KK-mice. Diabetologia 6:324–329

Ditscherlein G (1970) Zur Frage der Glomerulosklerose bei diabetischen Tieren. Z Ges Inn Med 25:281–293

Ditscherlein G, Kranz D, Marx I, Dena R (1970) Elektronenmikroskopische Untersuchungen an Rattennieren bei langdauerndem unbehandeltem Alloxan-Diabetes. Exp Pathol 4:222–239

Ditscherlein G, Guddat E (1971) Ergiebigkeit und Folgen der Nierenpunktion bei der Ratte. Z Urol 10:721

Federlin K, Bretzel RG, Schmidtchen K (1976) Islet transplantation in experimental diabetes of the rat V. Regression of glomerular lesions in diabetic rats after intraportal transplantation of isogeneic islets. Preliminary results. Horm Metab Res 8:404–406

Germuth FG, Rodriguez E (1973) Immunopathology of the renal glomerulus. Little Brown, Boston

Gray BN, Watkins E (1976) Prevention of vascular complications of diabetes by pancreatic islet transplantation. Arch Surg 111:254–257

Gray BN (1977) Transplantation of pancreatic islets in diabetes mellitus. Med J Australia 64:764–765

Gundersen HJG, Østerby R, Lundbaek K (1978) The basement membrane controversy. Diabetologia 15:361–363

Hägg E (1974a) Glomerular basement membrane thickening in rats with long-term alloxan diabetes. Acta Pathol Microbiol Scand Section A 82:211–219

Hägg E (1974b) Occurence of immunoglobulin and complement in the glomeruli of rats with long-term alloxan diabetes. Acta Pathol Microbiol Scand Section A 82:220–227

Hoyer JR, Elema JD, Vernier RL (1976) Unilateral renal disease in rat. II. Glomerular mesangial uptake of colloidal carbon in unilateral aminonucleoside nephrosis and nephrotoxic serum nephritis. Lab Invest 34:250–255

Jidaka K, McCoy J, Kimmelstiel P (1968) The glomerular mesangium: A quantitative analysis. Lab Invest 19:573–579

Irvine WJ, DiMario U, Guy K, Iavicoli M, Pozzilli P, Lumbroso B, Andreani D (1978) Immune complexes and diabetic microangiopathy. J Clin Lab Immunol 1:187–191

Kawano K, Arakawa M, McCoy J, Porch J, Kimmelstiel P(1969) Quantitative study of glomeruli. Focal glomerulonephritis and diabetic glomerulosclerosis. Lab Invest 21:269–275

Kimmelstiel P (1968) Diabetische Glomerulosklerose. Verh Dtsch Ges Inn Med 74:95–102

Kösters W, Wehner H, Strauch M (1978) Reversibility of glomerular lesions in long-term diabetic rats by islet transplantation. VIIth Internat Congr Nephrol Montreal

Lacy PE, Kostianovsky M (1967) Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16:35–39

Larkins RG, Marlin FJR, Heding LG, Tait BD (1978) Hormonal profile, blood sugar control and HLA patterns in long-term insulin dependent diabetes with and without vascular disease. Aust NZJ Med 8:465–471

Lee CS, Mauer SM, Brown DM, Sutherland DER, Michael AF, Najarian JS (1974) Renal transplantation in diabetes mellitus in rats. J Exp Med 139:793–800

Mauer SM, Sutherland DER, Howard RJ, Fish AJ, Najarian JS, Michael AF (1973) The glomerular mesangium III. Acute immune mesangial injury: A new model of glomerulonephritis. J Exp Med 137:553–570

Mauer SM, Fish AJ, Day NK, Michael AF (1974) The glomerular mesangium: II. Studies of macromolecular uptake in nephrotoxic nephritis in rats. J Clin Invest 53:431–439

Mauer SM, Steffes MW, Sutherland DER, Najarian JS, Michael AF, Brown DM (1975) Studies of the rate of regression of the glomerular lesions in diabetic rats treated with pancreatic islet transplantation. Diabetes 24:280–285

Mauer SM, Barbosa J, Vernier AL, Kjellstrand CM, Buselmeier TJ, Simmons RJ, Najarian JS, Goetz FC (1976) Development of diabetic vascular lesions in normal kidneys transplanted into patients with diabetes mellitus. New Engl J Med 295:916–920

Mauer SM, Steffes MW, Michael AF, Brown DM (1976) Studies of diabetic nephropathy in animals and man. Diabetes (Suppl 2) 25:850–857

McCluskey RT, Benacerraf B, Miller F (1962) Passive acute glomerulonephritis induced by antigen-antibody complexes solubilized in hapten excess. Proc Soc Exp Biol 111:764–768

Olsen TSt (1969) Diabetic glomerulosclerosis: A comparison between human and experimental lesions. Int Rev Exp Pathol 7:271–304

Olsen TSt (1971) Struktur und Ultrastruktur des Glomerulum beim humanen und experimentellen Diabetes. In: Bohle A, Schubert GE (eds), Fortschritte der Nephrologie FK Schattauer, Stuttgart-New York. pp 83–93

Rasch R (1979a) Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment. Glomerular basement membrane thickness. Diabetologia 16:319–324

Rasch R (1979b) Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment. The mesangial regions. Diabetologia 17:243–248

Renold AE (1970) Discussion to Renold AE, Burr J, Stauffacher W On the pathogenesis of diabetes mellitus: Possible usefulness of spontaneous hyperglycemic syndromes in animals In: Cerasi E, Luft R (eds) Nobel Symposium 13, Pathogenesis of diabetes mellitus. Almquist-Wiksell, Stockholm. p 232

Shibata A, Ludvigsen CW, Naber StP, McDaniel ML, Lacy PE (1976) Standardization of a digestion-filtration method for isolation of pancreatic islets. Diabetes 25:667

Siperstein MD, Feingold KR, Bennett PH (1978) Hyperglycaemia and diabetic microangiopathy. Diabetologia 15:365–367

Slater DN, Mangnall Y, Fox M (1978) Transplantation of pancreatic islet tissue and the control of diabetes mellitus. Invest Cell Pathol 1:65–97

Sutherland DER, Steffes MW, Mauer SM, Brown DM, Najarian JS (1975) Reversal of the secondary lesions of diabetes by islet transplantation in the rat. Transplant Proc 7:747–749

Wehner H (1974) Quantitative pathomorphology of the glomeruli in the human kidney. Veröffentl Pathol 95:1–67

Wehner H (1977) Morphologie der diabetischen Nephropathie. Münch Med Wschr 119:489–492

Wehner H, Höhn D, Faix-Schade U, Huber H, Walzer P (1972) Glomerular changes in mice with spontaneous hereditary diabetes. Lab Invest 27:331–340

Wehner H, Majorek B (1975) Early glomerular changes in streptozotocin diabetes of the guinea pig. Virchows Archiv Pathol Anat 368:179–189

Wehner H, Wagner H, Podmaniczky A, Heidbrink V, Kiessling B (1978) Influence of short-term insulin therapy on the mesangial structure in young KK mice. Nephron 22:460–472

Wehner H, Kösters W, Strauch M (1979) Die Wirkung der Inseltransplantation auf die glomerulären Veränderungen bei Streptozotocin-diabetischen Ratten. Verh Dtsch Ges Pathol 63:503

Weibel ER, Elias H (1967) Quantitative methods in morphology. Springer Berlin-Heidelberg-New York

Weil R, Nozawa M, Koss M, Weber C, Reemtsma K, McIntosh R (1976) The kidney in streptozotocin diabetic rats. Morphologic, ultrastructural, and function studies. Arch Pathol Lab Med 100:37–49

Westberg NG (1976) Biochemical alterations of the human glomerular basement membrane in diabetes. Diabetes (Suppl 2), 25:920–924

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