Diabetologia (1989) 32: 845–857

Diabetologia

Effects of very mild versus overt diabetes on vascular haemodynamics and barrier function in rats

G. Pugliese[1, 4], R. G. Tilton[1], A. Speedy[1], K. Chang[1], E. Santarelli[1, 4], M. A. Province[3], D. Eades[1], W. R. Sherman[2] and J. R. Williamson[1]

[1] Departments of Pathology and [2] Psychiatry, and [3] Division of Biostatistics, Washington University School of Medicine, St. Louis, Missouri, USA, and [4] Endocrinologia, Clinica Medica 2, "La Sapienza" University, Rome, Italy

Summary. Rats injected i.p. with a single dose of nicotinamide (250 mg/kg) 15 min prior to i.v. injection of streptozotocin (65 mg/kg) develop a very mild form of diabetes characterized by slight elevations of plasma glucose, increased levels of HbA_1, and reduced insulin secretion in response to an i.v. glucose tolerance test. These rats gain weight normally and they are not hyperphagic, glycosuric, or polyuric. The effects of this very mild form of diabetes vs overt streptozotocin diabetes of three months duration on regional vascular ^{131}I-albumin clearance, blood flow (assessed by 15 μm ^{85}Sr-microspheres), and renal filtration function were examined in male Sprague-Dawley rats. Plasma glucose levels of rats with mild diabetes were 7.4±0.9 (mean±SD) (mmol/l) vs 6.5±0.6 for control rats and 31.3±6.0 for overtly diabetic rats. HbA_1 levels were increased 1.4 fold in mildly diabetic and 2.3 fold in overtly diabetic rats. Vascular clearance of ^{131}I-albumin was markedly increased in ocular tissues (anterior uvea, retina, and choroid), sciatic nerve, aorta, new (subcutaneous) granulation tissue, and kidney of both diabetic groups, although increases in overtly diabetic rats exceeded those in the mildly diabetic group (2.2–4.6 times control animals vs 1.6–3.3 times, respectively). Likewise, both overt and very mild diabetes markedly increased glomerular filtration rate (~1.8 times and 1.2 times control animals, respectively), urinary excretion of endogenous albumin (~9 times and 4 times) and IgG (~15 times and 4 times), as well as regional blood flow in the anterior uvea, choroid, and sciatic nerve. Increases in tissue sorbitol levels were much larger in overtly diabetic rats (generally 10–20 times control animals) than in mildly diabetic rats (1.5–3 times controls). myo-Inositol levels were significantly decreased only in lens and sciatic nerve of overtly diabetic rats. These observations indicate that even very mild diabetes is associated with vascular functional changes which develop more slowly than in overtly diabetic rats, but are disproportionately large (in view of the minimal increases in glycaemia and tissue polyol levels) compared to those in overtly diabetic rats.

Key words: Nicotinamide, streptozotocin, albumin permeation, glomerular filtration rate, blood flow, urinary protein excretion.

Despite the apparent wide variability in the relationship between the degree of metabolic control and the onset and progression of diabetic complications, the link between hyperglycaemia and complications is no longer questioned by most investigators. Although not all studies are in agreement, a growing number of reports indicate that the duration and/or severity of diabetes are positively correlated with the cumulative incidence and/or severity of all of the late complications of diabetes including retinopathy [1–16], nephropathy [4, 16–24], neuropathy [4, 8, 16, 25–27], and large vessel disease [4, 8]. On the other hand, very little is known about the degree of glycaemic control required to avoid vascular complications of diabetes. Indeed, characteristic diabetic neuropathy, retinopathy, and/or nephropathy are occasionally observed in individuals with minimal or no evidence of carbohydrate intolerance as well as in newly diagnosed diabetic patients [28–36].

Recent studies in animal models of diabetes indicate that many of the early structural and functional vascular changes associated with poorly controlled diabetes are linked to increased metabolism of glucose to sorbitol [37]. These changes include retinal microaneurysms in dogs [38], thickening of basement membranes in retinal and glomerular capillaries in dogs [38, 39] and in retinal capillaries of rats [40–42], and increased vascular albumin permeation and blood flow in ocular tissues, sciatic nerve, and kidney of rats [43–46]. In rats with overt (severe) streptozotocin diabetes of three weeks duration, vascular albumin permeation is markedly in-

creased in ocular tissues, sciatic nerve, aorta, and kidney, as well as in new granulation tissue formed in the diabetic milieu [45]. Inhibitors of aldose reductase completely prevent these increases in vascular albumin permeation.

In contrast, rats with very mild diabetes of three weeks' duration manifest increased albumin permeation in new granulation tissue and kidney (although not as marked as in overtly diabetic rats) but not in the other tissues enumerated above [45]. These mildly diabetic rats are neither hyperphagic nor polyuric and they gain weight normally. Twenty-four hour urine glucose excretion is increased less than twice that of control values (vs ~150-fold increases in overtly diabetic rats). Plasma glucose levels are elevated only ~1.15 times controls (vs 4.8-fold for overtly diabetic rats) and increases in HbA_1 levels are approximately one third of those observed in overtly diabetic rats. This mild form of diabetes was produced by injecting nicotinamide immediately prior to injection of streptozotocin, a technique which has been widely used to prevent induction of diabetes by streptozotocin [47–52]. In our hands, however, the protective effect of nicotinamide administered in this fashion is incomplete and virtually all of the rats develop a very mild form of diabetes as described.

The present study was undertaken to: 1) assess and compare the effects of very mild vs overt diabetes of three months duration on regional blood flow and vascular filtration function, 2) examine the relationship of these changes to glycaemia and tissue sorbitol and myo-inositol levels, and 3) further characterize the structural and functional abnormalities of pancreatic B cells in this model of mild diabetes.

Materials and methods

Experimental design

Male Sprague-Dawley rats, initially weighing ~300 g, were divided in four groups: (1) non-diabetic control rats ($n=21$), (2) non-diabetic rats given a single injection of nicotinamide (250 mg/kg body weight in 154 mmol NaCl/l) i.p. at the start of the study ($n=19$), 3) overtly diabetic rats ($n=14$) in which diabetes was induced by i.v. injection of 65 mg/kg body weight streptozotocin (Sigma Chemicals, St. Louis, Mo, USA), and 4) rats with very mild diabetes ($n=24$) produced by injecting nicotinamide (250 mg/kg body weight) i.p. 15 min prior to injecting streptozotocin (65 mg/kg body weight). At the time of induction of diabetes, a piece of sterile polyester fabric was implanted s.c. in all rats to induce formation of new granulation tissue as in previous experiments [45, 53]. Rats were fed a diet of pelleted food (Ralston Purina Rodent Lab Chow 5001, St. Louis, Mo, USA) and allowed water ad libitum.

Body weights were measured weekly, plasma glucose levels (non-fasting) were measured biweekly using the hexokinase method [54], and nonenzymatic glycosylation of Hb was determined by the thiobarbituric acid method [55] from blood taken immediately prior to killing the animals. Twenty-four hour urine volumes ($ml \cdot kidney^{-1} \cdot 24\,h^{-1}$) and food consumption ($g \cdot 100\,g$ body $weight^{-1} \cdot 24\,h^{-1}$) were measured at 6, 9, and 12 weeks. Three months after induction of diabetes, the animals were randomly assigned to three different experimental protocols: (1) assessment of vascular permeation by ^{131}I-albumin (BSA) and glomerular filtration rate (GFR) (six to nine rats per group), (2) assessment of regional blood flow and tissue polyol levels (six rats per group), and (3) i.v. glucose tolerance tests (eight rats per group; overtly diabetic rats were not subjected to glucose challenge).

Protocol for assessment of regional vascular albumin permeation, GFR, and urinary albumin and IgG excretion in the same animal

In order to investigate relationships between GFR, vascular albumin permeation, and urinary albumin excretion in diabetic rats we have utilized an experimental protocol which permits the assessment of GFR (estimated by renal plasma clearance of ^{57}Co-EDTA) and regional and renal vascular permeation by ^{131}I-BSA concurrently in the same animal following collection of 24 h urine samples for quantification of urinary excretion of endogenous albumin and IgG. Because conventional methods for assessment of GFR by inulin clearance involve cannulation of the bladder followed by continuous infusion of inulin and collection of urine samples over an extended time period, we were concerned that these interventions and the very long period of anaesthesia required might impact on the assessment of vascular albumin permeation. Therefore, we have utilized an alternative approach for the assessment of GFR based on plasma clearance of ^{57}Co-EDTA during a 2-min tracer circulation time [56] as described below.

Preparation of radiolabelled tracers

Purified monomer BSA was iodinated with 1 mCi of either ^{125}I or ^{131}I (NEN Research Products, Boston, Mass., USA) using the lactoperoxidase method [57] as previously described [58]. ^{57}Co-EDTA was prepared by the method of Bridge et al. [59]. Immediately prior to use an aliquot was passed through a 4 cm column of Dowex 50 (Sigma Chemicals, St. Louis, Mo, USA), to remove trace amounts of free $^{57}Co^{++}$.

Animal preparation

Rats were anaesthetised with inactin (Byk Gulden, Konstanz, FRG) (~100 mg/kg body weight injected i.p.), the left femoral vein and both iliac arteries were cannulated with polyethylene tubing (0.58 mm internal diameter) filled with heparinized saline (400 U heparin per ml of 154 mmol NaCl/l), and the trachea was cannulated and connected to a small rodent respirator for continuous ventilatory support. The femoral vein cannula was used for tracer injection, the left iliac artery cannula was connected to a withdrawal pump for GFR (and for blood flow, see below) measurements, and the right iliac artery was connected to a pressure transducer for continuous monitoring of blood pressure. The right iliac artery cannula was also used for withdrawing arterial blood samples during the experiment.

At time 0, ^{131}I-BSA (in 0.2 ml of 154 mmol NaCl/l) was injected i.v.; 0.3 ml arterial blood samples were obtained 1 and 5 min later. Immediately following each arterial withdrawal an equal volume of 154 mmol NaCl/l was injected i.v.. Eight min after time 0, 0.1 ml of ^{57}Co-EDTA and 0.1 ml of ^{125}I-BSA (the vascular reference marker), were injected simultaneously and the withdrawal pump was started at 0.34 ml/min. Two min later an arterial blood sample was obtained, the pump was stopped, the heart was excised to stop all blood flow, and various tissues including one kidney were sampled and total bladder urine was recovered for quantitation of each tracer by gamma spectrometry.

Each tissue sample was thoroughly rinsed in 154 mmol NaCl/l to remove all contaminating blood. Each eye was rinsed in 154 mmol NaCl/l to remove blood contaminating the surface, then quickly frozen in liquid nitrogen. While still frozen, eyes were cut with a razor blade into anterior and posterior segments just posterior to the ora ser-

rata and the lens was removed from the anterior segment. The posterior segment was allowed to thaw partially, the vitreous was removed and discarded, and the entire retina was dissected free from the choroid-sclera portion. Corresponding tissues from both eyes were pooled prior to quantification of their tracer content. Tissue and urine samples as well as arterial plasma samples were weighed, counted in a gamma spectrometer interfaced with a Hewlett-Packard 1000 A computer in which the data were corrected for background and spillover, then stored for subsequent analysis.

Calculation of ^{131}I-BSA vascular clearance and GFR

Vascular clearance of ^{131}I-BSA in tissues was calculated as described previously [60] and expressed as μg plasma·g tissue wet weight^{-1}·min^{-1}. Very briefly, ^{131}I-BSA activity in each tissue was corrected for tracer contained within vessels which was determined by multiplying ^{125}I-BSA activity in the tissue by the ratio of ^{131}I-BSA/^{125}I-BSA activities in the arterial plasma sample obtained at the end of each experiment. The vascular-corrected ^{131}I-BSA tissue activity was then divided by a time-averaged ^{131}I-BSA plasma activity (obtained from plasma samples taken at 1, 5, and 10 min after tracer injection) and by the tracer circulation time (10 min) and normalized per g tissue wet weight.

Renal clearance of ^{131}I-BSA was obtained similarly except that half of the bladder urine ^{131}I-BSA activity was added to the vascular-corrected ^{131}I-BSA kidney activity (assuming that both kidneys contributed equally to urine production). A quantitative estimate of GFR based on plasma clearance of ^{57}Co-EDTA was also calculated in the same manner as for ^{131}I-BSA clearance using the time-averaged ^{57}Co-EDTA plasma activity obtained from the withdrawal pump blood sample [56].

Since the renal content of ^{131}I-BSA and ^{57}Co-EDTA includes tracer that has permeated postglomerular peritubular capillaries into the interstitium as well as tracer filtered by glomerular capillaries into the urinary space, it is important to note that the interstitial space constitutes ~13% of the wet weight of the kidney [61-63]. Thus, even if the renal interstitial space is fully equilibrated with plasma ^{57}Co-EDTA after 2 min of tracer circulation, the error resulting from interstitial accumulation of tracer is less than 10% in control rats and is reduced to less than 5% in diabetic rats (because of the increase in GFR). On the other hand, plasma clearance of ^{131}I-BSA during the 10 min tracer circulation time is a relatively small fraction of the interstitial space of the kidney and we do not know how much of the tracer clearance represents glomerular filtration vs permeation of peritubular capillaries into the interstitium. Therefore, the increase in renal ^{131}I-BSA clearance in diabetic rats must be viewed as evidence of an increase in overall renal filtration of radiolabelled tracer.

Assessment of urinary excretion of endogenous albumin and IgG

Six, nine, and twelve weeks after initiating the study rats were placed in individual metabolic cages with free access to food and water, and 24 h urine samples were collected in jars containing sodium azide (1 mg), NaEDTA (7.4 mg), and protease inhibitors (0.25 mg N-ethylmaleimide, 0.17 mg of phenylmethylsulfonyl fluoride, and 1 μg of Pepstatin). Albumin and IgG concentrations were quantified from an aliquot of urine by radial immunodiffusion assay [56] and excretion rates were expressed as mg·kidney^{-1}·24 h^{-1}.

Protocol for assessment of regional blood flow

Regional blood flows (expressed as ml blood·g tissue wet weight^{-1}·min^{-1}) were measured by using ^{85}Sr-labelled, 15 μm diameter microspheres (NEN Research Products, Boston, Mass, USA) as described previously [46, 56, 60]. Animals were prepared as described above for assessment of albumin permeation and GFR except that the femoral vein was not cannulated and the chest wall was opened to expose the heart for subsequent microsphere injection. When the blood pressure had stabilized (~10 min), the withdrawal pump was started at 0.34 ml/min and the microspheres were injected slowly over a 10–15 s interval via the left ventricular chamber. The withdrawal pump was stopped 90 s later, the heart was excised, and tissues were sampled and processed for gamma spectrometry as described above after extraction of sorbitol and myo-inositol as described below.

Quantification of tissue polyol levels

Tissue polyol levels were quantified as their butylboronate derivatives by gas chromatography-mass spectrometry by a method recently reported by Eades et al. [64] which permits separation and quantification of sorbitol, galactitol, mannitol, and myo-inositol. Very briefly, approximately 50 mg of tissue was extracted with 1 ml of water at 100° C for 15 min in the presence of the internal standard d6 - myo-inositol (30 ng/μl). The extract was deproteinized by the addition of 100 μl each of 0.3 normal zinc sulfate and 0.3 normal barium hydroxide and then centrifuged. The pellet was utilized for quantification of ^{85}Sr by gamma spectrometry as described above. The supernatant was lyophilized and derivatized with n-butylboronic acid (100 mg/ml extract) for analysis of polyols as their triboronate derivatives. The gas chromatographic-mass spectrometric analysis was performed using electron ionization at 70 electron volts with helium carrier gas at 1 ml/min with a 10:1 split ratio in a 15 meter DB-17 capillary column at 208° C run isothermally. Elution times were 1.7 min for mannitol, 2.07 min for sorbitol, 2.37 min for myo-inositol, and 2.56 min for galactitol.

Protocol for i. v. glucose tolerance test

Overnight fasted rats were anaesthetized with inactin (100 mg/kg body weight injected i.p.), and a femoral vein and carotid artery were cannulated for glucose injection and blood sampling, respectively. At time 0, an arterial blood sample was withdrawn into a syringe containing EDTA, 0.5 g/kg body weight glucose was injected i.v. [65], and arterial blood was sampled 1, 2, 4, 10, and 20 min later. These plasma samples were used for the assessment of glucose and insulin concentrations by the hexokinase method [54] and radioimmunoassay [66], respectively, using rat insulin as the standard. The inter and intra insulin assay coefficients of variation were 10.2 and 9.8%, respectively.

Morphology of pancreatic islets

A portion of the pancreas was removed at the time of killing and placed in 10% formalin containing 10% calcium acetate for examination of the islets of Langerhans by light microscopy. The tissue was rinsed thoroughly in buffer prior to embedding in paraffin using standard techniques. Five μm sections were stained with hematoxylin and eosin or aldehyde fuchsin, and were examined without knowledge of the group designation.

Statistical analysis

Except for urine volumes and protein excretion data, all data are expressed as means ± SD. Because of the large variance in urine volume and protein excretion data, these parameters are expressed as the antilogs of the mean (mean ± 1 SD in parentheses) of *ln* transformed data.

Since all of the parameters assessed in control rats and in non-diabetic control rats injected with nicotinamide were identical, data from both control groups were combined in the tables and for all statistical analyses. Relationships between regional ^{131}I-BSA clearance and blood flow, parameters of renal function and glycaemia were as-

sessed by Spearman's rank correlation coefficient [67]. Effects of diabetes on parameters of renal function which were measured repeatedly (at six, nine, and twelve weeks) were assessed by a repeated measures analysis of variance [68].

An analysis of variance of all parameters was performed using the SAS general linear models procedure [69]. In order to reduce potential type I errors related to multiple comparisons, overall differences among groups for each parameter were assessed by the Van der Waerden test [70], a global test which makes no assumptions regarding data distribution. If the Van der Waerden test indicated that differences among groups were statistically significant at $p < 0.01$ for a given parameter, pair-wise comparisons were assessed by least square means. Because of the large variance in tissue polyol levels and in some parameters of renal function, i.e., urinary protein excretion, a nonparametric (rank order) Blom transformation [71] of all of the data was performed prior to assessment of differences between groups.

Although a relatively large number of comparisons can be made for the data shown in some of the tables, only a small number of them address the hypotheses tested in this investigation. The remaining comparisons either confirm previous observations or are descriptive in nature and not central to the hypotheses investigated. For this reason we have reported uncorrected p values (based on two-tailed tests of significance) for the relevant comparisons shown in the tables. p values corrected for multiple comparisons (by the Bonferroni method, [72]) have been provided for the data shown in each of the tables. The number of comparisons used for Bonferroni corrections in each table was determined by multiplying the three paired comparisons between groups by the number of parameters in the table for which the Van Der Waerden test was significant at $p < 0.01$.

Results

Metabolic parameters

Differences in body weight, plasma glucose, HbA_1, and food consumption shown in Table 1 all attest to the mildness of the diabetic milieu in the "mild" diabetic group. Basal arterial plasma levels of insulin and glucose did not differ between controls and mildly diabetic rats (Fig. 1 and 2). While plasma glucose levels of the mildly diabetic group did not differ significantly from those of either control group at any time during the IVGTT, the total glucose area under the curve was increased by ~25% (14.0 ± 1.8 mmol/l · min and 11.5 ± 1.2, respectively; $t = 4.1$, $p < 0.001$). In contrast, 1 min after glucose injection plasma insulin levels in the mildly diabetic group were 50% lower than controls and remained ~50% of the control values at every time interval thereafter (total insulin areas under the curve were 1.0 ± 0.6 $\mu g \cdot ml^{-1} \cdot min$ and 2.2 ± 0.6, respectively; $t = 4.7$, $p < 0.001$).

Table 1. Effects of overt and very mild diabetes on metabolic parameters

	Control rats	Overt diabetic rats	Mild diabetic rats
(n)	(14-24)	(9-21)	(12-19)
Body weight			
Initial (g)	306 ± 15[a]	292 ± 14	296 ± 14
Final (g)	460 ± 46	250 ± 43[d]	472 ± 23[e]
Plasma glucose mmol/l	6.4 ± 0.6	31.3 ± 6.0[d]	7.4 ± 0.9[d, e]
HbA_1[b]	1.12 ± 0.16	2.53 ± 0.33[d]	1.56 ± 0.15[d, e]
Food consumption[c]			
6 weeks	6.2 ± 1.1	12.0 ± 2.5[d]	5.8 ± 1.1[e]
9 weeks	5.9 ± 1.0	12.6 ± 2.4[d]	5.9 ± 0.7[e]
12 weeks	5.7 ± 0.7	19.0 ± 2.7[d]	5.7 ± 0.4[e]
Blood pressure mm Hg	128 ± 17	107 ± 9	121 ± 11

[a] Mean ± SD; number of animals in parentheses *(n)*. [b] nmol of hydroxymethylfurfural per mg protein. [c] $g \cdot 100\,g$ body $weight^{-1} \cdot 24\,h^{-1}$. Significantly different from controls: [d] $p < 0.0001$. Significantly different from overt diabetics: [e] $p < 0.0001$. After Bonferroni adjustment (see text for details) for multiple [18] comparisons, p values < 0.0001 remain significant at < 0.002

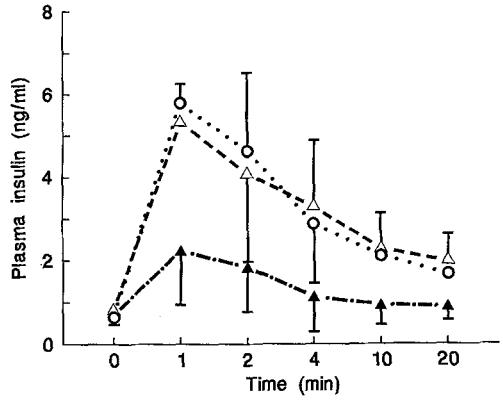

Fig. 1. Plasma glucose levels (x ± SD) during an i.v. glucose tolerance test (0.5 g glucose injected/kg body weight). Controls (○), nicotinamide-injected controls (△), and mildly diabetic rats (▲)

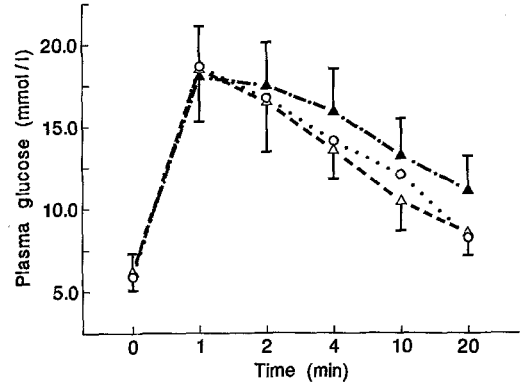

Fig. 2. Plasma insulin levels (x ± SD) during an i.v. glucose tolerance test (0.5 g glucose injected/kg body weight). Groups as described in legend of Figure 1

Histologic changes in the islets of Langerhans

There were no obvious differences in the appearance of the islets of Langerhans in any of the four groups of rats in hemotoxylin-eosin stained sections. Aldehyde fuchsin stained sections (Fig. 3) revealed moderate degranulation of B cells of the mildly diabetic group, marked or moderate degranulation of B cells in the overtly diabetic group, and no difference in B cell granulation of the group given nicotinamide alone vs the control group.

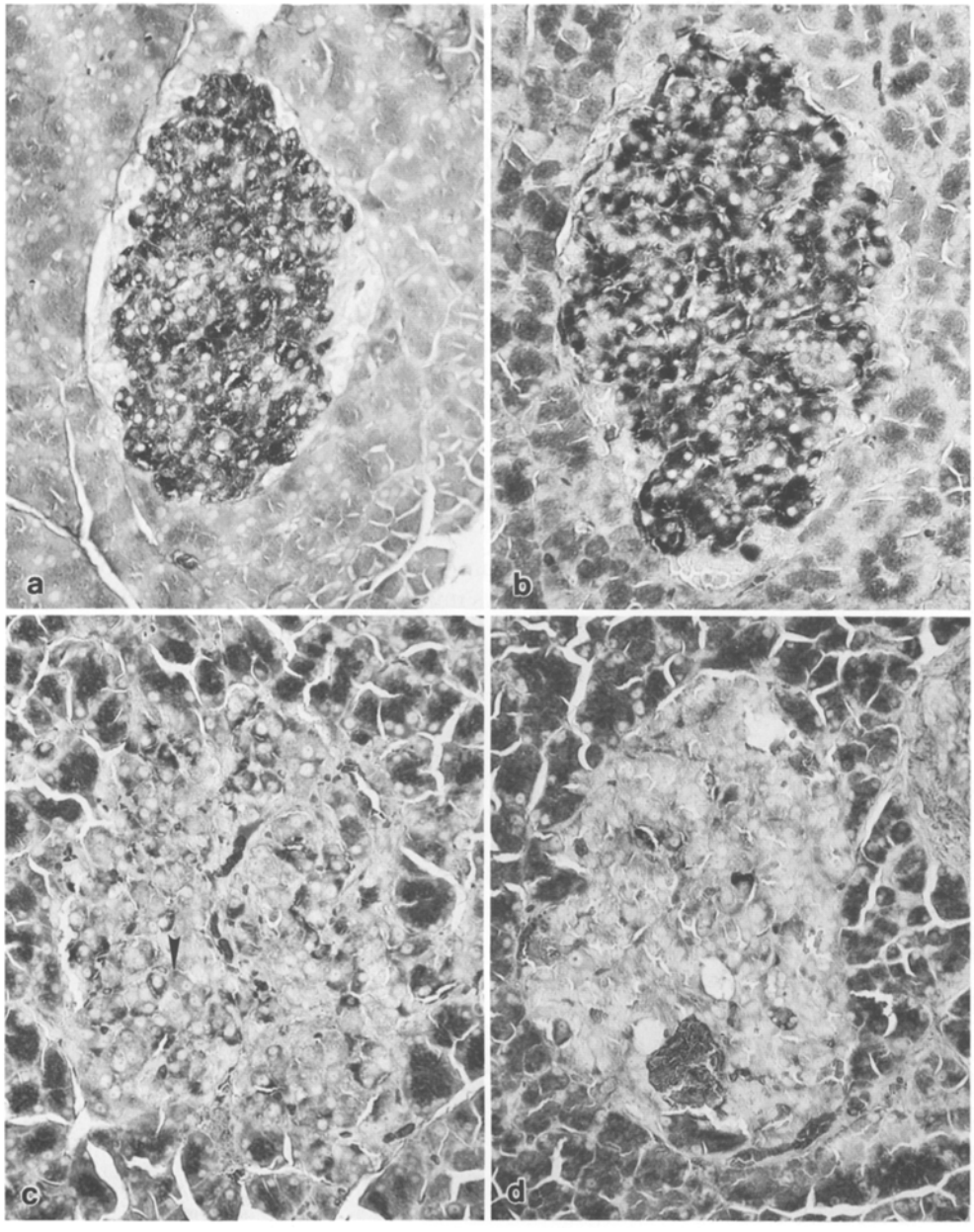

Fig. 3. (a–d) Light photomicrographs of aldehyde fuchsin-stained sections of islets of Langerhans. 280×. **a** Control rat – numerous well-granulated B cells. **b** Nicotinamide controls – numerous well-granulated B cells identical to control rats. **c** Mild diabetes – numerous B cells many of which are moderately degranulated arrowhead. **d** Overt diabetes – marked degranulation of B cells

Kidney filtration function

Van der Waerden tests indicated that group differences were significant at $p < 0.0001$ for renal clearance of ^{131}I-BSA and GFR at twelve weeks and at $p < 0.005$ to < 0.0001 for urine volume and 24 h urinary excretion of albumin and IgG at six, nine, and twelve weeks. Group differences were not significant for kidney weight ($p < 0.41$) or renal blood flow per kidney ($p < 0.37$). Although absolute kidney weights (Table 2) did not differ among the three groups of rats, if expressed per 100 g body weight they were increased 1.9 fold in the overtly diabetic group but still did not differ from controls in the mildly diabetic group. Likewise, while renal blood flow per kidney did not differ among the groups, if expressed per g kidney renal blood flow was increased in the overtly diabetic group ($p < 0.001$) vs all other groups (Table 2). Renal ^{131}I-BSA clearance and GFR were significantly increased approximately twice and 1.4 fold in the overtly and in the mildly diabetic groups, respectively (Table 2).

Urine volumes in mildly diabetic rats were not significantly increased in contrast to the approximately 19 fold increase in urine volume in the overtly diabetic group (Table 2). Urinary albumin and IgG excretion (Table 2 and Fig. 4) were significantly increased ($p < 0.0001$ by repeated measures analysis of variance) in mildly and overtly diabetic rats vs control rats and in overtly vs mildly diabetic rats. Excretion of both proteins also increased significantly over time for all three groups together ($p < 0.0001$ by repeated measures analysis of variance). This was accounted for by ~2.4 fold increases

Table 2. Parameters of renal function after 12 weeks of very mild vs overt diabetes

	Control rats	Overt diabetic rats	Mild diabetic rats
Kidney weight			
g	1.55 ± 0.15 (24)[a]	1.64 ± 0.21 (11)	1.60 ± 0.17 (13)
g/100 g body weight	0.33 ± 0.03	0.63 ± 0.14[c]	0.36 ± 0.03[f]
Renal blood flow			
ml·kidney^{-1}·min^{-1}	6.5 ± 0.8 (9)	7.1 ± 1.2 (4)	6.4 ± 1.0 (4)
ml·g kidney^{-1}·min^{-1}	4.1 ± 0.1	4.6 ± 0.2[e]	4.1 ± 0.1[i]
^{57}Co-EDTA clearance			
g plasma·kidney^{-1}·min^{-1}	0.84 ± 0.09 (14)	1.64 ± 0.14 (7)[c]	1.04 ± 0.02 (8)[c, g]
g plasma·g kidney^{-1}·min^{-1}	0.55 ± 0.06	0.98 ± 0.12[c]	0.65 ± 0.05[d, g]
^{131}I-BSA clearance			
mg plasma·kidney^{-1}·min^{-1}	3.36 ± 0.60 (14)	7.82 ± 1.67 (7)[c]	5.78 ± 0.29 (8)[c, h]
mg plasma·g kidney^{-1}·min^{-1}	2.21 ± 0.34	4.65 ± 0.84[c]	3.63 ± 0.31[c, h]
(n)	(24)	(12)	(18)
Urine volume			
ml·kidney^{-1}·24 h^{-1}	4.9 (2.4–10.1)[b]	93.0 (75.3–114.8)[c]	7.4 (4.9–11.2)[f]
Urine protein			
µg·kidney^{-1}·24 h^{-1}			
Albumin	286 (109–750)	3241 (1426–7363)[c]	1220 (197–7576)[e, j]
IgG	134 (56–318)	2010 (1018–3970)[c]	562 (136–2328)[c, h]

[a] Mean ± SD; number of animals in parentheses *(n)*. [b] Antilog of mean (mean ± lSD) of $\log_e$ transformed data. Significantly different from control rats: [c] $p < 0.0001$; [d] $p < 0.0005$; [e] $p < 0.001$. Significantly different from overt diabetic rats: [f] $p < 0.0001$; [g] $p < 0.001$; [h] $p < 0.005$; [i] $p < 0.01$. After Bonferroni adjustment for multiple [27] comparisons, p values < 0.0001, < 0.0005, and < 0.001 remain significant at $p < 0.003$, < 0.015, and < 0.03, respectively. ^{131}I-BSA = albumin

in albumin and IgG excretion in overt diabetics ($p < 0.007$) and by approximately twofold increases in excretion of both proteins ($p < 0.001$) in mild diabetic animals (Fig. 4). Corresponding ~1.2-fold increases in excretion of both proteins in control rats were not statistically significant. Thus, urinary albumin and IgG excretion at twelve weeks were increased four times the control rats in the mildly diabetic rats without an increase in urine volume in contrast to the 11- and 15-fold increases in albumin and IgG excretion associated with a ~19-fold increase in urine volume in overtly diabetic rats.

Group differences for urinary albumin concentration were statistically significant at $p < 0.01$ only at twelve weeks (Fig. 4), but were significant for urinary IgG concentration at nine and twelve weeks ($p < 0.0004$ and $p < 0.005$, respectively). The urine concentration of albumin and IgG in mildly diabetic rats was approximately four times higher ($p < 0.0001$) than in control rats and in overtly diabetic rats at six, nine, and twelve weeks. The concentration of albumin and IgG did not increase significantly with time in any of the groups (Fig. 4).

Regional blood flows

Van der Waerden scores indicated that significant group differences in blood flow were present in anterior uvea ($p < 0.0008$), choroid-sclera ($p < 0.002$), and sciatic nerve ($p < 0.003$), but not in the retina ($p < 0.63$), new granulation tissue ($p < 0.08$), or forelimb muscle ($p < 0.024$). Blood flow was significantly increased 1.2–1.5 times control values in the anterior uvea, posterior uvea, and sciatic nerve (but not in forelimb muscle, retina, or new granulation tissue) in both mild and overtly diabetic animals (Table 3). The failure of the 50% increase in forelimb muscle blood flow in overt diabetics to achieve statistical significance may reflect an overly conservative criterion for determination of statistically significant group differences (i.e., $p < 0.01$), since the p value for the Van der Waerden test was significant at $p < 0.024$ and p values for paired comparisons were significant for overtly diabetic vs control rats at $p < 0.03$ and vs mildly diabetic rats at $p < 0.01$.

Regional ^{131}I-BSA clearance

Group differences in ^{131}I-BSA clearance were significant at $p < 0.0001$ for anterior uvea, choroid-sclera, retina, sciatic nerve, aorta, and new granulation tissue; they were not significant for heart, skin, forelimb muscle, or testis ($p < 0.45$ to 0.98). In both overtly and mildly diabetic rats ^{131}I-BSA clearance was significantly in-

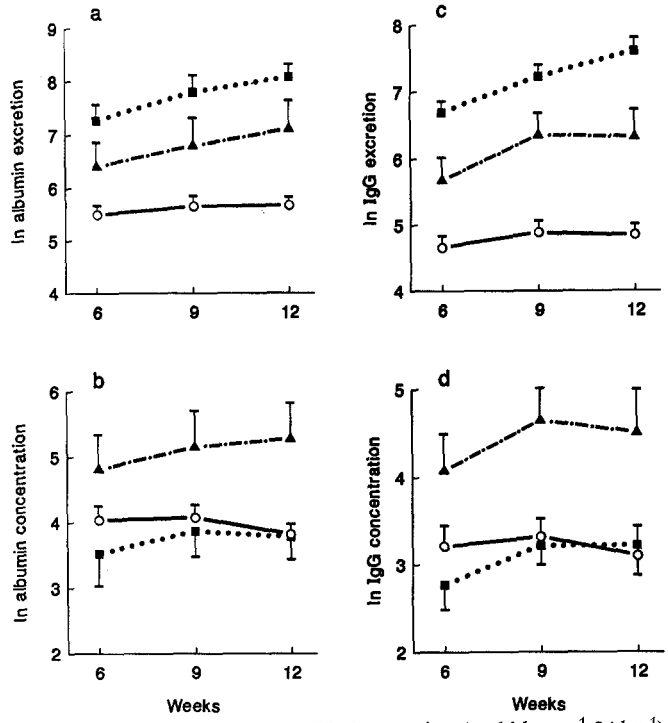

Fig. 4a–d. Urinary albumin and IgG excretion (µg kidney^{-1} 24 h^{-1}) and concentration (µg/ml urine) as ln mean ± SEM. Control rats (○—○), mild diabetic rats (▲-·-▲), and overt diabetic rats (■----■)

Table 3. Effects of overt and very mild diabetes on regional blood flows

	Control rats	Overt diabetic rats	Mild diabetic rats
(n)	(9)	(4)	(5)
Eye			
Anterior uvea	0.77 ± 0.04[a]	1.36 ± 0.16[b]	1.04 ± 0.09[b, e]
Choroid-sclera	1.42 ± 0.09	2.09 ± 0.23[b]	1.86 ± 0.19[c]
Retina	0.44 ± 0.02	0.45 ± 0.04	0.46 ± 0.04
Sciatic nerve	0.13 ± 0.01	0.16 ± 0.02[d]	0.20 ± 0.02[b, f]
Granulation tissue	0.15 ± 0.02	0.17 ± 0.01	0.18 ± 0.02
Forelimb muscle	0.08 ± 0.01	0.12 ± 0.02	0.08 ± 0.01

[a] Mean ± SD in ml·g wet weight^{-1}·min^{-1}; number of animals in parentheses *(n)*. Significantly different from controls: [b] $p < 0.0001$; [c] $p < 0.001$; [d] $p < 0.03$. Significantly different from overt diabetics: [e] $p < 0.0001$; [f] $p < 0.03$. After Bonferroni adjustment for multiple [9] comparisons, p values < 0.0001, < 0.001, and < 0.005 remain significant at $p < 0.001$, < 0.01, and < 0.05, respectively

Table 4. Effects of overt and very mild diabetes on ^{131}I-BSA tissue clearance[a]

	Control rats	Overt diabetic rats	Mild diabetic rats
(n)	(14)	(9)	(8)
Eye			
Anterior uvea	181 ± 49[b]	405 ± 84[c]	294 ± 17[c, e]
Choroid-sclera	197 ± 38	558 ± 125[c]	424 ± 96[c, g]
Retina	23 ± 9	75 ± 15[c]	43 ± 15[d, e]
Sciatic nerve	33 ± 14	154 ± 28[c]	108 ± 38[c, h]
Aorta	142 ± 35	534 ± 161[c]	277 ± 91[c, f]
Granulation tissue	52 ± 16	157 ± 24[c]	131 ± 34[c]
Heart	238 ± 50	251 ± 81	208 ± 81
Skin	59 ± 23	59 ± 21	64 ± 18
Forelimb muscle	17 ± 7	17 ± 3	18 ± 8
Testis	402 ± 138	407 ± 105	403 ± 75

[a] μg plasma·g tissue wet weight^{-1}·min^{-1}. [b] Mean ± SD; number of animals in parentheses *(n)*. Significantly different from control rats: [c] $p < 0.0001$; [d] $p < 0.001$. Significantly different from overt diabetic rats: [e] $p < 0.00001$; [f] $p < 0.0025$; [g] $p < 0.04$, [h] $p < 0.02$. After Bonferroni adjustment for multiple [18] comparisons, $p < 0.0001$ and < 0.001 remain significant at $p < 0.002$ and < 0.02, respectively. ^{131}I-BSA = albumin

creased (Table 4) in anterior uvea (2.2 and 1.6-fold, respectively, vs control rats), choroid-sclera (2.8 and 2.2-fold), retina (3.3 and 1.9-fold), sciatic nerve (4.7 and 3.3-fold), aorta (3.8 and 2.0-fold), and new granulation tissue (3.0 and 2.5-fold).

Relationship between changes in vascular filtration function and glycaemia

The incremental increase in vascular clearance (filtration) of ^{131}I-BSA in various tissues (as well as renal clearance of ^{57}Co-EDTA and excretion of endogenous albumin and IgG) per unit increase in HbA_1 in mild diabetic vs the control rats is shown in Figure 5 as a ratio of the corresponding increase in vascular ^{131}I-BSA clearance per unit increase in HbA_1 for overt vs mild diabetic rats. For example, in the sciatic nerve of mildly diabetic rats plasma clearance of ^{131}I-BSA was increased by 75 μg plasma·g nerve^{-1}·min^{-1} (from data in Table 4) with a corresponding increase of 0.44 nmol hydroxymethylfurfural (HMF)/mg Hb (from data in Table 1). Thus, the increase in plasma clearance of ^{131}I-BSA per nmol increase in HMF/mg Hb in mildly diabetic vs control rats was 170 μg plasma/Δ nmol HMF; in contrast, the increase in ^{131}I-BSA clearance in overtly vs mildly diabetic rats was only 47 μg plasma/Δ nmol HMF. The ratio of these incremental increases in ^{131}I-BSA clearance per unit increase in HbA_1, i.e., 170÷47, is 3.6 as shown in Figure 5.

Similar 3-4.5 fold increases in ^{131}I-BSA clearance per nmol increase in HMF/mg Hb in mildly vs overtly diabetic rats were observed in the anterior uvea, posterior uvea, and kidney. An even higher ratio of 6.7 was observed in new granulation tissue vessels. Thus, in these tissues the incremental increase in vascular ^{131}I-BSA clearance per unit increase in glycaemia was much larger in rats with mild diabetes than in those with severe diabetes. In contrast, the ratios were only 1.2 in aorta, 1.4 in retina, 0.7 for GFR and 24 h urinary excretion of IgG, and 1.0 for urinary excretion of albumin. These lower ratios indicate that vascular albumin permeation (and glomerular filtration of ^{57}Co-EDTA and endogenous proteins) increased relatively linearly in these tissues with increasing glycaemia throughout the range of blood glucose levels observed in these experiments. Since the method used to assess ^{131}I-BSA clearance in the kidney in these experiments includes (and may reflect primarily) ^{131}I-BSA filtered across peritubular capillaries as well as that filtered across glomerular capillaries (as discussed in the Methods section),

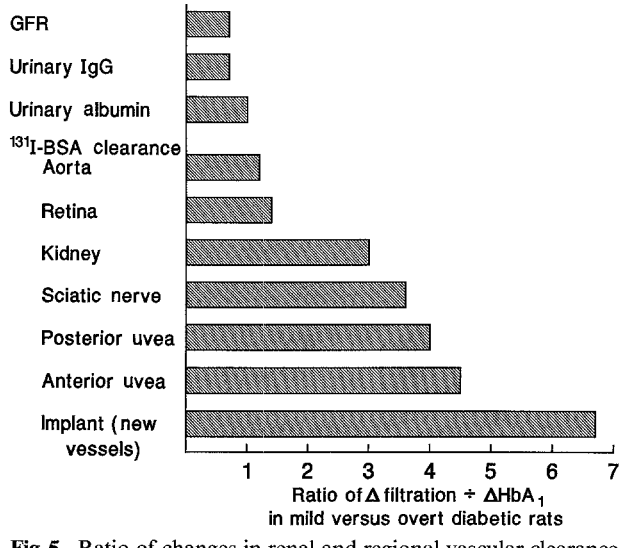

Fig. 5. Ratio of changes in renal and regional vascular clearance (filtration) of ^{131}I-BSA, ^{57}Co-EDTA, and endogenous albumin and IgG to changes in glycaemia (HbA_1) in mildly and overtly diabetic rats.

* Δ filtration (mild diabetics − controls) ÷ Δ HbA_1 (mild diabetics − controls)

Δ filtration (overt − mild diabetics) ÷ Δ HbA_1 (overt − mild diabetics)

Table 5. Effects of overt and very mild diabetes on tissue sorbitol and myo-inositol levels

	Control rats	Overt diabetic rats	Mild diabetic rats
(n)	(11)	(6)	(6)
Eye			
Anterior uvea			
Sorbitol	29±11[a]	448±291[b]	61±25[d, h]
myo-inositol	537±92	547±131	575±70
Choroid-sclera			
Sorbitol	18±8	312±104[b]	48±15[d, h]
myo-inositol	115±209	1026±118	1158±77
Retina			
Sorbitol	36±27	543±126[b]	79±22[f, i]
myo-inositol	1259±531	1697±384	1936±943
Optic nerve			
Sorbitol	0.01±0.00	168±123[b]	4±0[g]
myo-inositol	10946±2014	13822±3237	11878±2486
Lens			
Sorbitol	576±341	6325±2254[b]	1272±364[e, h]
myo-inositol	1033±265	96±22[b]	808±268[i]
Sciatic nerve			
Sorbitol	106±24	1182±191[b]	157±32[e, h]
myo-inositol	3214±378	2233±354[c]	3089±416[i]
Aorta			
Sorbitol	4±5	23±14[d]	12±9
myo-inositol	1517±370	1932±839	1479±417
Granulation tissue			
Sorbitol	1.4±0.4	26±4[b]	7±3[e, h]
myo-inositol	507±75	596±67	513±92
Erythrocyte			
Sorbitol	10±4	53±37	13±3
myo-inositol	36±13	49±27	36±6

[a] Mean ± SD in nmol/g tissue; number of animals in parentheses (*n*). Significantly different from control rats: [b] $p<0.0001$; [c] $p<0.0005$; [d] $p<0.001$; [e] $p0.005$; [f] $p<0.01$. Significantly different from overt diabetic rats: [g] $p<0.0001$; [h] $p<0.005$; [i] $p<0.01$. After Bonferroni adjustment for multiple [30] comparisons, $p<0.0001$, <0.0005, and <0.001 remain significant at $p<0.003$, <0.015, and <0.03, respectively

changes in GFR and urinary protein excretion are undoubtedly better indices of glomerular capillary filtration function than changes in ^{131}I-BSA clearance per unit change in glycaemia shown in Figure 5.

Tissue polyol data

Group differences for tissue sorbitol levels were significant for every tissue shown in Table 5 at $p<0.01$ to 0.0001 except for erythrocytes ($p<0.025$). In most tissues sorbitol levels were increased 1.5–2.5 times the control values in mildly diabetic rats vs 10–15 fold increases in overtly diabetic rats. Group differences in myo-inositol levels were significant by the Van der Waerden test only for lens ($p<0.0015$) and sciatic nerve ($p<0.005$). These group differences were attributable to marked decreases in myo-inositol levels in the lens and sciatic nerve of overtly diabetic rats (Table 5).

Correlations

Correlations between regional tissue ^{131}I-BSA clearance values, mean plasma glucose levels, HbA_1, urinary albumin and IgG excretion, and GFR are shown in Table 6. Both mean plasma glucose and HbA_1 values correlated strongly with ^{131}I-BSA clearance (in those tissues in which ^{131}I-BSA clearance was increased by diabetes) as well as with GFR and urinary excretion of endogenous albumin and IgG; in some tissues *r* values were higher for HbA_1 than for mean plasma glucose values and in others the converse was observed. Both GFR and renal ^{131}I-BSA clearance were strongly correlated with ^{131}I-BSA clearance in all ocular tissues as well as in the sciatic nerve, aorta, and new granulation tissue; while urinary excretion of endogenous albumin and IgG also were strongly correlated with ^{131}I-BSA clearance in these same tissues, the correlations were not quite as strong as those for GFR and ^{131}I-BSA clearance. ^{131}I-BSA clearances in ocular tissues, kidney, nerve, aorta, and new granulation tissue were all significantly correlated with each other ($p<0.001$); however, the correlations tended to be slightly stronger between ocular tissues, sciatic nerve, kidney, and aorta ($r=0.72$–0.88) than for retina and new granulation tissue ($r=0.64$–0.77).

Blood flow in the anterior uvea was strongly correlated with that in the choroid ($r=0.83$, $p<0.0001$) and sciatic nerve ($r=0.55$, $p=0.018$) but not with that in the retina ($r=0.19$, $p=$ NS); blood flow in the choroid was significantly correlated with retinal blood flow ($r=0.46$, $p=0.05$) as well as blood flow in the sciatic nerve ($r=0.54$, $p=0.02$). In none of the ocular tissues or in sciatic nerve was blood flow significantly correlated with renal blood flow. Granulation tissue blood flow was strongly correlated with that in the anterior uvea ($r=0.64$, $p=0.01$) but not with blood flow in any of the other tissues examined.

Discussion

These observations confirm and extend our previous findings that blood flow and vascular albumin permeation are markedly increased in overtly diabetic rats in tissues which correspond to sites of clinically significant vascular disease in human diabetic patients [43–45]. The demonstration that very mild diabetes of three months duration increased vascular albumin clearance in every tissue in which albumin clearance is increased in overtly diabetic rats is of particular interest in view of the absence of any detectable increase in vascular albumin permeation (except in the kidney and granulation tissue) after mild diabetes of only three weeks' duration [45]. These observations suggest that: (1) functional consequences of mild glycaemia develop earlier and are more marked in the kidney and in new vessels formed in the diabetic milieu than in ocular tis-

Table 6. Correlations* between regional tissue ^{131}I-BSA clearance, plasma glucose levels, HbA_1, urinary albumin and IgG excretion, and glomerular filtration rate

	^{131}I-BSA Clearance											
	1	2	3	4	5	6	7	8	9	10	11	12
Plasma glucose		0.73	0.82	0.54	0.62	0.75	0.80	0.66	0.83	0.65	0.79	0.64
HbA_1			0.74[b]	0.57	0.62	0.62[d]	0.64[e]	0.67[d]	0.64[e]	0.82	0.80	0.74[b]
Glomerular filtration rate				0.74	0.79	0.85	0.87	0.81	0.83	0.76	0.90	0.77
Urinary albumin excretion					0.89	0.66[a]	0.65[a]	0.57[c]	0.63[a]	0.62[b]	0.72	0.62[e]
Urinary IgG excretion						0.80	0.71	0.78	0.64[a]	0.66[a]	0.77	0.66[a]
^{131}I-BSA clearance												
Kidney							0.83	0.79	0.73	0.72	0.78	0.76
Anterior uvea								0.88	0.77	0.84	0.81	0.75
Posterior uvea									0.64	0.88	0.83	0.70
Retina										0.69	0.77	0.72
Sciatic nerve											0.83	0.70
Aorta												0.75
Granulation tissue												

* Spearman correlation coefficients: $n = 24$–31 except for correlations involving urinary albumin excretion, urinary IgG excretion, and plasma glucose ($n = 50$–55) and correlations involving HbA_1 ($n = 16$ for correlations involving HbA_1 and clearance data, and $n = 40$ for correlations of HbA_1 with urinary albumin excretion, urinary IgG excretion, and plasma glucose). All correlations (r values) are significant at $p < 0.0001$ except those indicated: [a] $p < 0.0005$, [b] $p < 0.001$, [c] $p < 0.002$, [d] $p < 0.005$, [e] $p < 0.01$. After Bonferroni adjustment for multiple [66] comparisons, p values < 0.0001, < 0.0005, and < 0.001 remain significant at $p < 0.007$, < 0.03, and < 0.07, respectively. ^{131}I-BSA = albumin

sues, sciatic nerve, and aorta, but that (2) very mild diabetes of sufficient duration leads to increased vascular albumin clearance in the latter tissues, albeit less severe than in overtly diabetic rats.

In contrast to the relatively long duration of mild diabetes required to develop significant increases in vascular albumin clearance in ocular tissues, sciatic nerve, and aorta, albumin clearance is increased in all of these tissues three to seven days after the onset of overt diabetes and reaches essentially maximal values in three to four weeks. On the other hand, in keeping with observations of other investigators [73, 74], urinary excretion of endogenous albumin and IgG continued to increase with increasing duration (between 6 and 12 weeks) of mild as well as overt diabetes; after 12 weeks, urinary albumin excretion in mildly diabetic rats was similar to that in rats with overt diabetes of six weeks' duration.

The finding that the incremental increase in vascular albumin clearance per unit increase in glycaemia in mild vs overt diabetic rats varies considerably in different tissues (Fig. 5) has several interesting implications for the role of hyperglycaemia per se in the onset and progression of vascular filtration changes associated with late complications. First, there may be no distinct glycaemic threshold below which vascular filtration changes associated with diabetes can be predictably avoided in any tissue. Second, in some tissues such as the anterior and posterior uvea, sciatic nerve, and new vessels formed in the diabetic milieu, there may be a relatively moderate level of glycaemia above which relatively little additional vascular injury is incurred. Third, in other tissues such as the retina, aorta, and kidney there may be no threshold of glycaemia above which further vascular injury is not incurred. Fourth, improvement of glycaemic control in diabetic subjects with mild to moderately elevated plasma glucose levels may be more efficacious than reducing marked elevations in glucose to moderate levels in preventing or retarding the onset and progression of vascular injury.

The demonstration that tissue sorbitol levels were significantly elevated in most tissues of mildly diabetic rats in which albumin clearance was increased is consistent with the likelihood that increased metabolism of glucose via the sorbitol pathway is important in mediating vascular functional changes in subjects with very mild, as well as in those with very poorly controlled, diabetes [37]. It can be readily appreciated from the data in Tables 3 and 5 that increases in vascular albumin clearance per unit increase in sorbitol level were much greater in mildly diabetic rats than in overtly diabetic rats (in keeping with the relationship between vascular filtration changes and glycaemia as discussed above). On the other hand, since tissue sorbitol levels represent the difference between rates of sorbitol synthesis and oxidation to fructose, differing sorbitol levels in different tissues do not necessarily imply differences in the rate of sorbitol metabolism or in the importance of sorbitol metabolism in the pathogenesis of associated vascular functional changes which are markedly reduced or completely prevented in all tissues by inhibitors of aldose reductase [37, 75].

The finding that an increase in vascular albumin clearance in one tissue was generally strongly correlated with corresponding increases in other tissues is consistent with the observations of Feldt-Rasmussen [76] that the whole body transcapillary escape rate of albumin is increased in patients with (but not in those without) microalbuminuria. This observation also has interesting and potentially important implications regarding the relationships between clinically significant vascular complications in human diabetic patients. First, the strong

correlations between increased ^{131}I-BSA clearance in the aorta, ocular tissues, nerve, and kidney suggest the possibility that increased vascular permeation by plasma macromolecules (including atherogenic lipoproteins) may play an important role in the pathogenesis of accelerated macrovascular as well as microvascular complications of diabetes and is consistent with the concurrence of clinically significant complications in these same tissues [3, 4, 8, 12, 18, 20, 77–82]. Since permeation of the anterior uveal vasculature by tracers can be readily assessed (by their accumulation in the anterior chamber aqueous humour) by fluorometric techniques in humans [83], the strong correlations between ^{131}I-BSA clearance in the anterior uvea, retina, nerve, and kidney (as well as GFR) suggest the feasibility of monitoring changes in anterior uveal vascular tracer permeation as an index of vascular filtration changes in other tissues.

In view of the small number of animals available for assessment of blood flow, the absence of statistically significant differences between groups in which mean blood flow differed appreciably from each other may simply reflect the small sample size. This explanation, however, would not appear to account for the absence of significant differences in blood flow in the retina and new granulation tissue in which the mean and SD of blood flow were very similar for overtly diabetic and control rats. The absence of significant differences in blood flow in these tissues is in contrast to previous studies in which blood flow was appreciably increased in these same tissues in rats with overt diabetes of 6 weeks duration [43, 44]. The apparent decreases in blood flow (in retina and granulation tissue) with longer duration of diabetes are consistent with evidence that GFR is increased in humans early after the onset of poorly controlled diabetes but falls to below normal levels with progression of renal vascular disease [84–89]. The greater increase in sciatic nerve blood flow in the mildly diabetic rather than in overtly diabetic rats may correspond to an earlier stage in the evolution of haemodynamic changes corresponding to the increased blood flow observed in galactose-fed rats and in rats with overt diabetes of shorter duration in previous studies [37, 46]. The discordance between the marked increases in ^{131}I-BSA clearance but not of blood flow in retinai and granulation tissue is consistent with similar observations in other studies [43, 46] and supports the hypothesis that increased vascular albumin permeation induced by diabetes is largely due to impaired endothelial junctional integrity (rather than a consequence of haemodynamic changes) while changes in blood flow reflect impaired contractile function of smooth muscle cells in resistance arterioles.

Since blood flow was not increased in all tissues of diabetic rats and since increases in blood flow were not associated with a change in mean arterial blood pressure, vascular resistance must be selectively decreased in the affected tissues. This would permit transmission of arterial blood pressure further downstream beyond the resistance arterioles resulting in microvascular hypertension in the absence of any increase in (arterial) blood pressure. The only vessels in which an increase in transcapillary hydraulic pressure has been reported in diabetic animals are glomerular capillaries [74, 90, 91]. The finding that diabetes-induced increases in GFR (and in blood flow per g kidney in overtly diabetic rats) were not accompanied by an increase in mean arterial pressure is consistent with observations of numerous other investigators in diabetic rats [74, 90–93]. Thus, the present observations support the hypothesis that microvascular hypertension develops in selected tissues early after the onset of diabetes [85, 89]. On the other hand, they do not clarify the disputed nature of the role of microvascular hypertension in the pathogenesis of diabetic microvascular disease [94]. The decreased resistance of the microvasculature in these tissues may also make them more susceptible to the impact of an elevation in systemic blood pressure. This could explain, at least in part, the association between arterial blood pressure and the development and/or progression of diabetic retinopathy and nephropathy [95–98]. Indeed, in diabetic rats with hypertension glomerular lesions are more severe than in normotensive diabetic rats [99], vascular albumin permeation is increased in the eyes, kidneys, and aorta [100], and albuminuria is dramatically increased to ~15 times that of normotensive diabetic rat values.

The fact that increases in GFR and in ^{131}I-BSA clearance (but not blood flow) as well as increased urinary excretion of endogenous albumin and IgG in mild as well as in overtly diabetic rats were highly significant regardless of how the data were expressed (i.e., per kidney, per g kidney, or per 100 g body weight (data not shown)) attests to marked alterations in glomerular filtration function which are independent of renal hypertrophy and differences in body weight and/or composition. Although renal hypertrophy and increases in GFR are often associated phenomena in diabetic animals [94] the present observations (and conclusions) are consistent with those of Christiansen et al. [101] and of Wiseman et al. [102] that improved glycaemic control in human diabetes resulted in reduction of GFR without any change in renal size.

The apparent absence of renal hypertrophy (absolute kidney weight) in the diabetic rats in this study contrasts with the increased kidney weight observed in rats with diabetes of shorter duration [74, 90–92, 103–106, and unpublished studies]; this discrepancy may simply reflect the relatively larger kidney size in the control rats (attained because of the longer duration of this study). While the possibility that the absence of renal hypertrophy may also be related to the severity of diabetes (as reported by Hostetter et al. [90]) cannot be excluded, it is of interest that GFR was markedly increased in overtly diabetic rats (twice that of the control rats) in contrast to the decrease in GFR to 0.7 times that of control rats ob-

served by Hostetter et al. The finding that kidney size in overtly diabetic rats was increased twice that of controls, if expressed per 100 g body weight, may reflect, in part, marked differences in body composition (as well as body weight) of diabetic and control rats related to loss of adipose tissue in diabetic rats.

While the GFR in control rats is at the lower end of the range of values reported by other investigators, it is in good agreement with values reported by Ortola et al. [92] and Craven et al. [105]. Since GFR values in overtly diabetic rats agree closely with values reported by many other investigators [90–92, 103, 104, 106], we think it is unlikely that the relatively lower GFR values in the control rats reflect basic methodological differences in assessing GFR. In any case, under the conditions of these experiments, the method we have used to assess GFR clearly distinguishes between the control, overtly diabetic, and mildly diabetic rats.

The demonstration of significant haemodynamic and vascular albumin permeation changes in ocular tissues, aorta, sciatic nerve, and kidney of rats with very mild diabetes is of interest because of its implications regarding the pathogenesis of diabetic complications as well as for current investigations addressing the feasibility of preventing late complications of diabetes by normalisation of blood glucose levels. The HbA_1 values suggest that time-averaged blood glucose levels were considerably higher than the plasma glucose levels shown in Table 1 which are minimally elevated relative to the control rats. On the other hand, the minimal increase in plasma glucose levels during an i.v. glucose tolerance test, the normal food consumption and body weight gain, and the minimal increase in urine volume (together with minimal glycosuria) are all consistent with the likelihood that plasma glucose levels rarely exceeded the renal threshold, which in the rat appears to be in the range of 11–14 mmol/l [107]. This would correspond to plasma glucose levels commonly observed in human diabetic patients.

The lower plasma insulin levels in mildly diabetic rats vs control rats at all times during the i.v. glucose tolerance test indicate that these rats are slightly insulinopoenic. This impairment of insulin secretion in response to a glucose load is consistent with the moderate degranulation of B cells observed in aldehyde fuchsin stained sections (Fig. 3). Thus, the human counterparts of this animal model of mild diabetes would include: (1) non-insulin-requiring diabetes associated with mild insulin deficiency rather than insulin resistance, and (2) the early stage of Type 1 (insulin-dependent) diabetes preceding the onset of severe hyperglycaemia and ketosis.

Acknowledgements. The excellent secretarial assistance of Ms. A. Stone is gratefully appreciated. This research was supported by grants from the National Institutes of Health (EY 06600 and HL 30678) and by the Kilo Diabetes and Vascular Research Foundation.

References

1. Caird FI, Pirie A, Ramsel TG (1968) Diabetes and the eye. Blackwell, Oxford
2. Jarrett RJ, Keen H (1976) Hyperglycaemia and diabetes mellitus. Lancet II: 1009–1012
3. Pirart K (1978) Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Diabetes Care 1: 168–188, 252–263
4. West KM (1978) Epidemiology of diabetes and its vascular lesions. Elsevier, New York, pp 402–439
5. Palmberg P, Smith M, Waltman S, Krupin T, Singer P, Burgess D, Wendtlant T, Achtenberg J, Cryer P, Santiago J, White N, Kilo C, Daughaday W (1981) The natural history of retinopathy in insulin-dependent juvenile-onset diabetes. Ophthalmology 88: 613–618
6. Frank RN, Hoffman WH, Podgor MJ, Joondeph HC, Lewis RA, Margherio RR, Nachazel DP Jr, Weiss H, Christopherson KW, Cronin MA (1982) Retinopathy in juvenile-onset Type 1 diabetes of short duration. Diabetes 31: 874–882
7. Ballard DJ, Melton LJ III, Dwyer MS, Trautmann JC, Chu C-P, O'Fallon WM, Palumbo PJ (1986) Risk factors for diabetic retinopathy: a population-based study in Rochester, Minnesota. Diabetes Care 9: 334–342
8. Knuiman MW, Welborn TA, McCann VJ, Stanton KG, Constable IJ (1986) Prevalence of diabetic complications in relation to risk factors. Diabetes 35: 1332–1339
9. Krolewski AS, Warram JH, Rand LI, Christlieb AR, Busick EJ, Kahn CR (1986) Risk of proliferative diabetic retinopathy in juvenile-onset Type 1 diabetes: a 40-year follow-up study. Diabetes Care 9: 443–452
10. Nathan DM, Singer DE, Godine JE, Harrington CH, Perlmuter LC (1986) Retinopathy in older Type 2 diabetics: association with glucose control. Diabetes 35: 797–801
11. Monson JP, Koios G, Toms GC, Kopelman PG, Boucher BJ, Evans SJW, Alexander WL (1986) Relationship between retinopathy and glycaemic control in insulin-dependent and non-insulin-dependent diabetes. J Royal Soc Med 79: 274–276
12. Sterky G, Wall S (1986) Determinants of microangiopathy in growth-onset diabetes. Acta Paed Scand [Suppl] 327: 5–45
13. Weber B, Burger W, Hartmann R, Hovener G, Malchus R, Oberdisse U (1986) Risk factors for the development of retinopathy in children and adolescents with Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 29: 23–29
14. Klein BE, Moss SE, Klein R (1987) Longitudinal measure of glycemic control and diabetic retinopathy. Diabetes Care 10: 273–277
15. Davis MD (1988) Diabetic retinopathy: a clinical overview. Diabetes Metab Rev 4: 291–322
16. Hanssen KF, Dahl-Jorgensen K, Lauritzen T, Feldt-Rasmussen B, Brinchmann-Hansen O, Deckert T (1986) Diabetic control and microvascular complications: the near-normoglycaemic experience. Diabetologia 29: 677–684
17. Kamenetzky SA, Bennett PH, Dippe SE, Miller M, LeCompte PM (1974) A clinical and histological study of diabetic nephropathy in the Pima Indians. Diabetes 23: 61–68
18. Fabre J, Balant LP, Dayer PG, Fox HM, Vernet AT (1982) The kidney in maturity onset diabetes mellitus: a clinical study of 510 patients. Kidney Int 21: 730–738
19. Viberti GC, Mackintosh D, Bilous RW, Pickup JC, Keen H (1982) Proteinuria in diabetes mellitus: role of spontaneous and experimental variation of glycemia. Kidney Int 21: 714–720
20. Holman RR, Dornan TL, Mayon-White V, Howard-Williams J, Orde-Peckar C, Jenkins L, Steemson J, Rolfe R, Smith B, Barbour D, McPherson K, Poon P, Rizza C, Mann JI, Knight AH, Bron AJ, Turner RC (1983) Prevention of deterioration of renal and sensory-nerve function by more intensive management of insulin-dependent diabetic patients. A two-year randomised prospective study. Lancet I: 204–208

21. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR (1985) The changing natural history of nephropathy in Type 1 diabetes. Am J Med 78: 785–794
22. Feldt-Rasmussen B, Mathiesen ER, Deckert T (1986) Effect of two years of strict metabolic control on progression of incipient nephropathy in insulin-dependent diabetes. Lancet II: 1300–1304
23. Kofoed-Enevoldsen A, Borch-Johnsen K, Kreiner S, Nerup J, Deckert T (1987) Declining incidence of persistent proteinuria in Type 1 (insulin-dependent) diabetic patients in Denmark. Diabetes 36: 205–209
24. Viberti G (1988) Etiology and prognostic significance of albuminuria in diabetes. Diabetes Care 11: 840–845
25. Graf RJ, Halter JB, Halar E, Porte D (1979) Nerve conduction abnormalities in untreated maturity-onset diabetes: relation to levels of fasting plasma glucose and glycosylated hemoglobin. Ann Int Med 90: 298–303
26. Thomas PK, Eliasson SG (1984) Diabetic Neuropathy. In: Dyck PJ, Thomas PK, Lambert EH, Bunge R (eds) Peripheral neuropathy, Vol II. Saunders, Philadelphia, pp 1773–1810
27. Vinik A, Mitchell B (1988) Clinical aspects of diabetic neuropathies. Diab Metab Rev 4: 223–253
28. Freedman LR (1957) Inapparent diabetes mellitus as a cause of renal insufficiency due to Kimmelstiel-Wilson lesions. Bull Johns Hopkins Hosp 100: 132–138
29. Collens WS, Silverstein JN, Dobkin GB (1959) Case of a diabetic with a Kimmelstiel-Wilson syndrome and a normal glucose tolerance. Ann Int Med 50: 1282–1288
30. Ellenberg M (1963) Diabetic complications without manifest diabetes. JAMA 183: 926–930
31. Harrington AR, Hare HG, Chambers WN, Valtin H (1966) Nodular glomerulosclerosis suspected during life in a patient without demonstrable diabetes mellitus. N Engl J Med 275: 206–208
32. Strauss FG, Argy WP, Schreiner GE (1971) Diabetic glomerulosclerosis in the absence of glucose intolerance. Ann Int Med 75: 239–242
33. Harrington JT, Garella S, Stilmant MM, Chazan JA (1973) Renal failure as the initial manifestation of diabetes mellitus. Arch Intern Med 132: 249–251
34. Nash DA, Rogers PW, Langlinais PC, Bunn SM Jr (1975) Diabetic glomerulosclerosis without glucose intolerance. Am J Med 59: 191–199
35. Winaver J, Teredesai P, Feldman HA, Stachura I, Cavallo T, Puschett JB (1979) Diabetic nephropathy as the mode of presentation of diabetes mellitus. Metabolism 28: 1023–1030
36. Chan JYC, Cole E, Hanna AK (1985) Diabetic nephropathy and proliferative retinopathy with normal glucose tolerance. Diabetes Care 8: 385–390
37. Williamson JR, Tilton RG, Chang K, Kilo C (1988) Basement membrane abnormalities in diabetes mellitus: relationship to clinical microangiopathy. Diabetes Metab Rev 4: 339–370
38. Engerman RL, Kern TS (1984) Experimental galactosemia produces diabetic-like retinopathy. Diabetes 33: 97–100
39. Kern TS, Engerman RL (1987) Kidney morphology in experimental hyperglycemia. Diabetes 36: 244–249
40. Frank RN, Keirn RJ, Kennedy A, Frank KW (1983) Galactose-induced retinal capillary basement membrane thickening: prevention by sorbinil. Invest Ophthalmol Vis Sci 24: 1519–1524
41. Chandler ML, Shannon WA, DeSantis L (1984) Prevention of retinal capillary basement membrane thickening in diabetic rats by aldose reductase inhibitors. Invest Ophthalmol Vis Sci 25 [Suppl]: 159 (Abstract)
42. Robison WG Jr, Kador PF, Akagi Y, Kinoshita JH, Gonzalez R, Dvornik D (1986) Prevention of basement membrane thickening in retinal capillaries by a novel inhibitor of aldose reductase, Tolrestat. Diabetes 35: 295–299
43. Tilton RG, Chang K, Pugliese G, Eades DM, Province MA, Sherman WR, Kilo C, Williamson JR (1989) Prevention of hemodynamic and vascular albumin filtration changes in diabetic rats by aldose reductase inhibitors. Diabetes 38: 1258–1270
44. Williamson JR, Chang K, Tilton RG, Allison W, Ashton T, Kilo C (1987) Increased ocular blood flow and ^{125}I-albumin permeation in diabetic rats are aldose reductase-linked phenomena. Invest Ophthalmol Vis Sci 28: 69 (Abstract)
45. Williamson JR, Chang K, Tilton RG, Prater C, Jeffrey JR, Weigel C, Sherman WR, Eades DM, Kilo C (1987) Increased vascular permeability in spontaneously diabetic BB/W rats and in rats with mild versus severe streptozotocin-induced diabetes: prevention by aldose reductase inhibitors and castration. Diabetes 36: 813–821
46. Tilton RG, Chang K, Weigel C, Eades D, Sherman WR, Kilo C, Williamson JR (1988) Increased ocular blood flow and ^{125}I-albumin permeation in galactose-fed rats: inhibition by Sorbinil. Invest Ophthalmol Vis Sci 29: 861–868
47. Lazarow A, Liambies J, Tausch AJ (1950) Protection against diabetes with nicotinamide. J Lab Clin Med 36: 249–258
48. Schein PS, Cooney DA, Vernon ML (1967) The use of nicotinamide to modify the toxicity of streptozotocin diabetes without loss of antitumor activity. Cancer Res 27: 2324–2332
49. Dulin WE, Wyse BM (1969) Reversal of streptozotocin diabetes with nicotinamide. Proc Soc Exp Biol Med 130: 992–994
50. Stauffacher W, Burr I, Futzeit A, Beaven D, Veleminsky J, Renold AE (1970) Streptozotocin diabetes: time course of irreversible β-cell damage; further observations on prevention by nicotinamide. Proc Soc Exp Biol Med 133: 194–200
51. Lazarus SS, Shapiro SH (1973) Influence of nicotinamide and pyridine nucleotides on streptozotocin and alloxan-induced pancreatic β cell cytotoxicity. Diabetes 22: 499–501
52. Wahlberg G, Carlson LA, Wasserman J, Ljungqvuist A (1985) Protective effect of nicotinamide against nephropathy in diabetic rats. Diabetes Res 2: 307–312
53. Kilzer P, Chang K, Marvel J, Rowold E, Jaudes P, Ullensvang S, Kilo C, Williamson JR (1985) Albumin permeation of new vessels is increased in diabetic rats. Diabetes 34: 333–336
54. Lowry OH, Passonneau JV (1972) A flexible system of enzymatic analysis. Academic Press, Orlando, p 174
55. McFarland KF, Catalano EW, Day JR, Thorpe SR, Baynes JW (1979) Nonenzymatic glucosylation of serum proteins in diabetes mellitus. Diabetes 28: 1011–1014
56. Tilton RG, Pugliese G, Chang K, Kilo C, Williamson JR (1989) Effects of hypothyroidism on vascular ^{125}I-albumin permeation and blood flow in rats. Metabolism 38: 471–478
57. Krohn KA, Welch MJ (1974) Studies of radioiodinated fibrinogen. I. Lactoperoxidase iodination of fibrinogen and model compounds. Int J Appl Radiot Isot 25: 315–323
58. Kilzer P, Chang K, Marvel J, Kilo C, Williamson JR (1985) Tissue differences in vascular permeability changes induced by histamine. Microvasc Res 30: 270–285
59. Bridge JHE, Bersohn MM, Gonzalez F, Bassingthwaighte JB (1982) Synthesis and use of radiocobaltic EDTA as an extracellular marker in rabbit heart. Am J Physiol 242: 671–676
60. Williamson JR, Holmberg SW, Chang K, Marvel J, Sutera SP, Needleman P (1989) Mechanisms underlying atriopeptin-induced increases in hematocrit and vascular permeation in rats. Circ Res 64: 890–899
61. Bell DR, Pinter GG, Wilson PD (1978) Albumin permeability of the peritubular capillaries in rat renal cortex. J Physiol 279: 621–640
62. Wolgast M, Larson M, Nygren K (1981) Functional characteristics of the renal interstitium. Am J Physiol 241: F105–F111
63. Larson M, Sjoquist M, Wolgast M (1984) Renal interstitial volume of the rat kidney. Acta Physiol Scand 120: 297–304
64. Eades DM, Williamson JR, Sherman WR (1989) Rapid analysis of sorbitol, galactitol, mannitol and myo-inositol mixtures from biological sources. J Chromatography 490: 1–8
65. Tancrede G, Rousseau-Migneron S, Nadea A (1982) Beneficial effects of physical training in rats with a mild streptozotocin-induced diabetes mellitus. Diabetes 31: 406–409
66. Morgan CR, Lazarow A (1963) Immunoassay of insulin: two antibody system: plasma insulin levels of normal, subdiabetic, and diabetic rats. Diabetes 12: 115–126

67. Hollander M, Wolfe D (1973) Nonparametric Statistical Methods. Wiley & Sons, New York
68. Cole IWL, Grizzle IE (1966) Applications of multivariate analysis of variance to repeated measures experiments. Biometrics 22: 810–828
69. Anderson TW (1958) An introduction to multivariate statistical analysis. Wiley & Sons, New York
70. Lehman EL (1975) Nonparametrics: statistical methods based on ranks. Holden-Day, San Francisco
71. Blom G (1958) Statistical estimates and transformed beta variables. Wiley & Sons, New York
72. Miller RG Jr (1981) Simultaneous statistical inference. Springer, Berlin Heidelberg New York
73. Mauer SM, Brown DM, Matas AJ, Steffes MW (1978) Effects of pancreatic islet transplantation on the increased urinary albumin excretion rates in intact and uninephrectomized rats with diabetes mellitus. Diabetes 27: 959–964
74. Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM (1986) Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. J Clin Invest 77: 1925–1930
75. Goldfarb S, Simmons DA, Kern EFO (1986) Amelioration of glomerular hyperfiltration in acute experimental diabetes mellitus by dietary myo-inositol supplementation and aldose reductase inhibition. Transact Assoc Am Phys 99: 67–72
76. Feldt-Rasmussen B (1986) Increased transcapillary escape rate of albumin in Type 1 (insulin-dependent) diabetic patients with microalbuminuria. Diabetologia 29: 282–286
77. Mogensen CE (1984) Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med 310: 356–360
78. Jarrett RJ, Viberti GC, Argyropoulos A, Hill RD, Mahmud U, Murrells TJ (1984) Microalbuminuria predicts mortality in non-insulin-dependent diabetes. Diabetic Med 1: 17–19
79. Borch-Johnsen K, Andersen PK, Deckert T (1985) The effect of proteinuria on relative mortality in Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 28: 590–596
80. Krolewski AS, Kosinski EJ, Warram JH, Leland OS, Busick EJ, Asmal AC, Rand LI, Christlieb AR, Bradley RF, Kahn CR (1987) Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. Am J Cardiol 59: 750–755
81. Jacobson SH, Fryd D, Sutherland DER, Kjellstrand CM (1988) Treatment of the diabetic patient with end-stage renal failure. Diabetes Metab Rev 4: 191–200
82. Mogensen CE, Schmitz A, Christensen CK (1988) Comparative renal pathophysiology relevant to IDDM and NIDDM patients. Diabetes Metab Rev 4: 453–483
83. Krupin T, Waltman SR (1985) Fluorophotometry in juvenile-onset diabetes: long-term follow-up. Jpn J Ophthalmol 29: 139–145
84. Mogensen CE (1976) Progression of nephropathy in long-term diabetics with proteinuria and effect of initial antihypertensive treatment. Scand J Clin Lab Invest 36: 383–388
85. Parving H-H, Viberti GC, Keen H, Christiansen JS, Lassen NA (1983) Hemodynamic factors in the genesis of diabetic microangiopathy. Metabolism 32: 943–949
86. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC (1984) Structural-functional relationships in diabetic nephropathy. J Clin Invest 74: 1143–1155
87. Michels LD, O'Donnell MP, Keane WF (1984) Glomerular hemodynamic and structural correlations in long-term experimental diabetic rats. J Lab Clin Med 103: 840–847
88. Mogensen CE (1986) Early glomerular hyperfiltration in insulin-dependent diabetics and late nephropathy. Scand J Clin Lab Invest 46: 201–206
89. Zatz R, Brenner BM (1986) Pathogenesis of diabetic microangiopathy: the hemodynamic view. Am J Med 80: 443–453
90. Hostetter TH, Troy JL, Brenner BM (1981) Glomerular hemodynamics in experimental diabetes mellitus. Kidney Int 19: 410–415
91. Jensen PK, Christiansen JS, Steven K, Parving H-H (1981) Renal function in streptozotocin-diabetic rats. Diabetologia 21: 409–414
92. Ortola FV, Ballermann BJ, Anderson S, Mendez RE, Brenner BM (1987) Elevated plasma arterial natriuretic peptide levels in diabetic rats. J Clin Invest 80: 670–674
93. Carbonell LF, Salom MG, Garcia-Estan J, Salazar FJ, Ubeda M, Quesada T (1987) Hemodynamic alterations in chronically conscious unrestrained diabetic rats. Am J Physiol 252: H900–H905
94. O'Donnell MP, Kasiske BL, Keane WF (1988) Glomerular hemodynamic and structural alterations in experimental diabetes mellitus. FASEB J 2: 2339–2347
95. Rosenstock J, Raskin P (1986) Early diabetic nephropathy: assessment and potential therapeutic interventions. Diabetes Care 9: 529–545
96. Kohner EM, Sharp PS (1987) Diabetic retinopathy. In: Alberti KGMM, Krall LP (eds) The diabetes annual/3. Elsevier Science, Amsterdam, pp 252–288
97. Mogensen CE (1987) Early diabetic renal involvement and nephropathy: can treatment modalities be predicted from identification of risk factors? In: Alberti KGMM, Krall LP (eds) The diabetes annual/3. Elsevier, Amsterdam, pp 306–324
98. Viberti G (1988) Recent advances in understanding mechanisms and natural history of diabetic renal disease. Diabetes Care 11 [Suppl 1]: 3–9
99. Mauer SM, Steffes MW, Azar S, Kupcho-Sandberg S, Brown DM (1978) The effect of Goldblatt hypertension on the development of glomerular lesions of diabetes mellitus in the rat. Diabetes 27: 738–744
100. Tilton RG, LaRose L, Prater C (1986) Additive effects of hypertension- and diabetes-induced increased albumin permeation in eyes, aorta, and kidney of rats. Diabetes 33: 164
101. Christiansen JS, Gammelgaard T, Tronier B, Svendsen PA, Parving H-H (1982) Kidney function and size in diabetics before and during initial insulin treatment. Kidney Int 21: 683–688
102. Wiseman MJ, Saunders AJ, Keen H, Viberti GC (1985) Effect of blood glucose control on increased glomerular filtration rate and kidney size in insulin-dependent diabetes. N Engl J Med 312: 617–621
103. Michels LD, Davidman M, Keane WF (1981) Determinants of glomerular filtration and plasma flow in experimental diabetic rats. J Lab Clin Sci 98: 869–885
104. Jensen PK, Steven K, Blaehr H, Christiansen JS, Parving H-H (1986) Effects of indomethacin on glomerular hemodynamics in experimental diabetes. Kidney Int 29: 490–495
105. Craven PA, Caines MA, DeRubertis FR (1987) Sequential alterations in glomerular prostaglandin and thromboxane synthesis in diabetic rats: relationship to the hyperfiltration of early diabetes. Metabolism 36: 95–103
106. Jensen PK, Christiansen JS, Steven K, Parving H-H (1987) Strict metabolic control and renal function in the streptozotocin diabetic rat. Kidney Int 31: 47–51
107. Bowman RH, Maack T (1972) Glucose transport by the isolated perfused rat kidney. Am J Physiol 222: 1499–1504

Received: 2 March 1989
and in revised form: 21 August 1989

Dr. R. G. Tilton
Department of Pathology
Box 8118
Washington University School of Medicine
660 South Euclid Avenue
St. Louis, MO 63110
USA