Renal

THE KIDNEY IN DIABETES MELLITUS

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Medical Grand Rounds

August 5, 1982

The kidney is a major target organ for the adverse consequences of diabetes mellitus. Diabetic nephropathy is a term that includes the morphologic findings of diabetic glomerulosclerosis and arteriolonephrosclerosis, as well as the functional abnormalities of proteinuria and renal failure. Table 1 lists the major causes of death in patients with diabetes. In patients less than 20 years of age, renal disease

Table 1. Causes of diabetic patient mortality

Causes of death	Patients		Patients diagnosed at age, *N*	
	N	%	< 20 (%)	20+ (%)
Renal	615	9	229 (48)	386 (6)
Cardiovascular	4,613	67	132 (28)	4,481 (71)
Other	1,572	23	111 (24)	1,461 (23)
Total	6,800	99	472 (100)	6,328 (100)

accounts for 48% of the mortality. For patients older than 20 years at the time the disease began, renal disease accounts for only 6% of the mortality while cardiovascular causes account for 71% of the mortality. This is in keeping with the known predilection of diabetes to affect small vessels in the younger patients, and larger vessels in the older patients. Figure 1 depicts the cumulative risk for development of nephropathy in juvenile diabetic patients. It is unknown

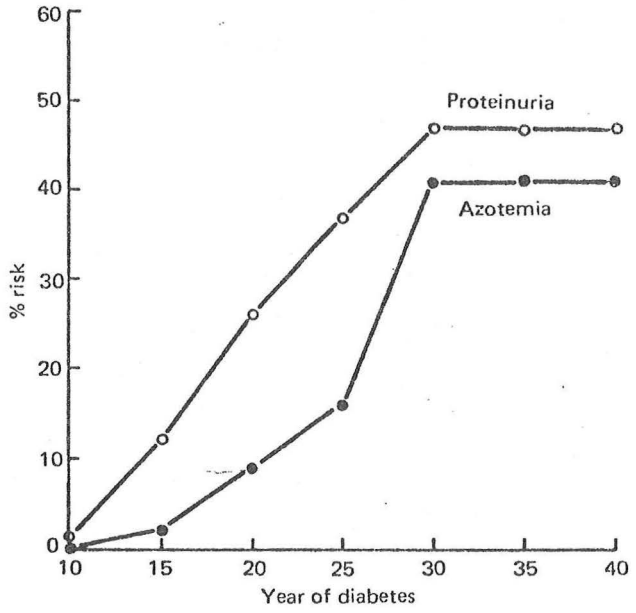


Fig. 1. Cumulative risk in years of nephropathy developing in juvenile diabetic patients on an unmeasured diet.

whether diabetes developing in the older patient will have the same cumulative risk for development of renal disease as that seen in the

juvenile patient. The reason for this is the complicating presence of atherosclerosis in the older age group. Figure 2 is from the Joslin Clinic experience and shows the time of onset of complications for a group of 112 patients. As renal failure progressed there was a

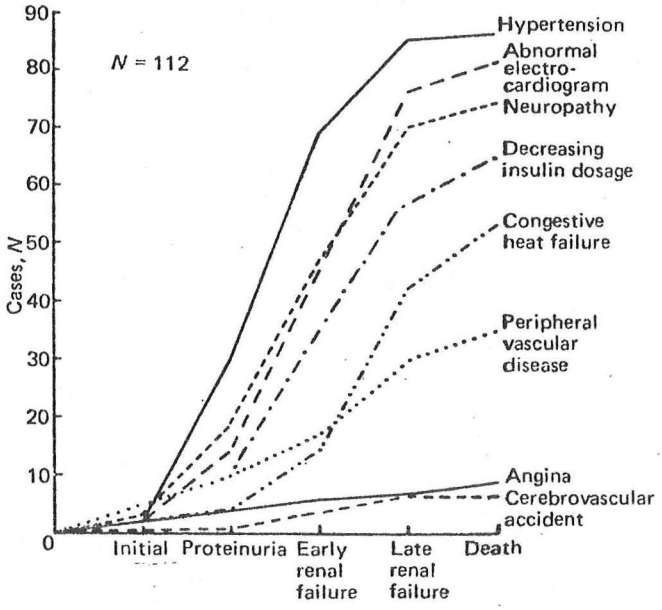


Fig. 2. Development of diabetic complications with progression of renal disease in 112 diabetic patients.

significant increase in the number of patients who developed the complications listed. All patients had some degree of retinopathy at the time of the onset of proteinuria and the retinopathy grew progressively worse as renal function deteriorated.

Even with the use of hemodialysis and renal transplantation, renal disease continues to represent a formidable obstacle in the care of the diabetic patient. How this renal disease develops in the diabetic and whether its progression can be slowed or arrested is a matter of continued debate. The most prominent morphologic change of the diabetic kidney is basement membrane thickening. Two major theories have arisen to explain this change. One theory suggests that the metabolic defect of diabetes leads to basement membrane thickening. The other theory suggests that an independent, but genetically determined process accounts for the thickening of the capillary basement membrane in the kidney and elsewhere throughout the body. Although these theories are not mutually exclusive, historically physicians have leaned on one or the other viewpoint to justify their approach to blood sugar control in the diabetic patient, i.e. strict versus loose. I will not try to reconcile these opposing viewpoints today except to point out that our current understanding of the expression of diabetes and its complications in any individual stresses an interplay between genetic and environmental factors. However, for this discussion I am limiting my presentation to a review of clinical and experimental work that has appeared largely in the last 10 years that emphasizes the important role that the abnormal metabolic environment of diabetes has on kidney function and structure.

Diabetic nephropathy virtually never develops in the absence of overt, longstanding hyperglycemia. Isolated cases of diabetic nephropathy occurring in the absence of hyperglycemia are reported, but the cases are not without alternative explanations. Once established, diabetic glomerulosclerosis leads inexorably to end stage kidney disease. No form of therapy seems to be able to reverse this disorder and to survive, patients require either hemodialysis or renal transplantation. Neither of these forms of therapy is as successful in the diabetic as it is in patients with most other forms of renal disease. I will not discuss the therapy of the end stage diabetic kidney today. Rather, I will review with you primarily those supraphysiologic changes in kidney function characteristic of early diabetes. This phenomenon has been appreciated for many years, but only recently has attention shifted to its potential importance and contribution to understanding the late renal deterioration so characteristic of diabetic nephropathy.

The following pages will review the pathophysiology of the diabetic kidney from three perspectives: 1) gross and microscopic anatomical changes, 2) changes in the glomerular basement membrane (GBM), both biochemically and as they apply to the development of proteinuria, and 3) the pathophysiology of the hemodynamic changes. Each of these sections will attempt to relate these findings to understanding the clinical counterparts. While each area will be discussed separately, it will become apparent that they are closely interrelated. The final sections will discuss a plausible hypothesis to explain these data and how they might apply to the prevention and/or reversal of diabetic nephropathy.

Much of the information presented here is derived from studies of streptozotocin diabetes in the rat. With a few exceptions this and the other experimental models are remarkably similar in morphologic and functional manifestations to human diabetic nephropathy. The major differences between rat and man are the absence in the rodent of 1) nodular glomerulosclerosis, and 2) the lack of glomerular arteriolar hyalinosis.

MORPHOLOGY

Short term insulin dependent diabetes mellitus is associated with increased kidney size, Figure 3, whether measured by intravenous pyelogram or ultrasound (1,2). In such patients a 24% increase in kidney volume

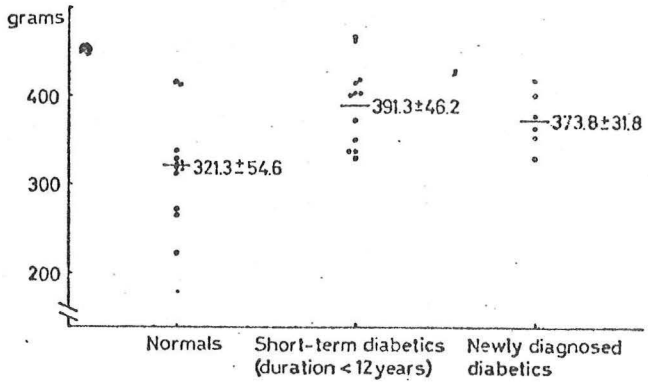


Fig. 3. Kidney weight in normal and diabetic subjects (1).

can be demonstrated when compared with non-diabetic controls (2). Increased volume of the kidney is also found in experimental streptozotocin diabetes in the rat (3). In the rat, renal growth is due primarily to hypertrophy and is characterized by an increase in the RNA/DNA ratio (3). The mechanism involved in diabetic renal hypertrophy is unclear, but the abnormal milieu of the diabetic patient or animal seems certainly to be involved, since renal hypertrophy only occurs in animals with uncontrolled hyperglycemia. If plasma glucose levels are normalized using insulin begun shortly after the induction of experimental diabetes in the rat, renal hypertophy does not occur (4). Similarly, juvenile diabetics treated with insulin have a reduction in kidney size (5).

By light microscopy both the glomeruli and tubules are enlarged (6). In experimental diabetes in the rat, there is nonuniformity of renal growth when the structures of the nephron are examined. Initially, the glomerulus in the diabetic animal (D) grows faster than the rest of the kidney, Figure 4, (7). This pattern of accelerated glomerular

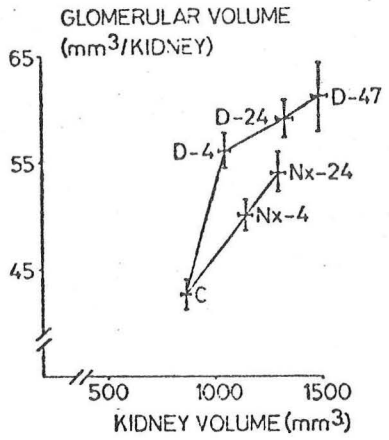


Fig. 4. Glomerular and kidney growth in rats with experimental diabetes (D) and with contralateral nephrectomy (Nx),(7).

growth may be contrasted to the proportionate growth of glomerulus and kidney volume occurring after contralateral nephrectomy in normal animals (Nx).

In experimental diabetes, renal hypertrophy results in enlargement of the surface area of the glomerular capillaries (8,9). Transplantation of pancreatic islet cells into highly inbred Louis rats reverses the hyperglycemia and much of the increase in kidney weight, Figure 5, but over a 4 week period of normoglycemia does not normalize the total amount of glomerular basement membrane material, Figure 6, (9). While this

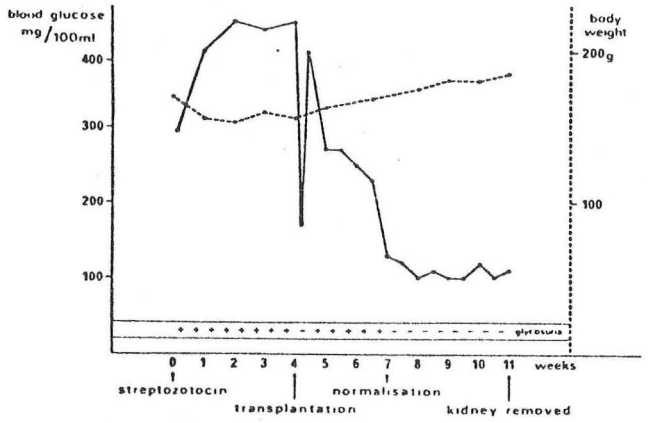


Fig. 5. Effect of transplantation. Nonfasting blood glucose, body weight, and glycosuria in one of the transplanted animals(9).

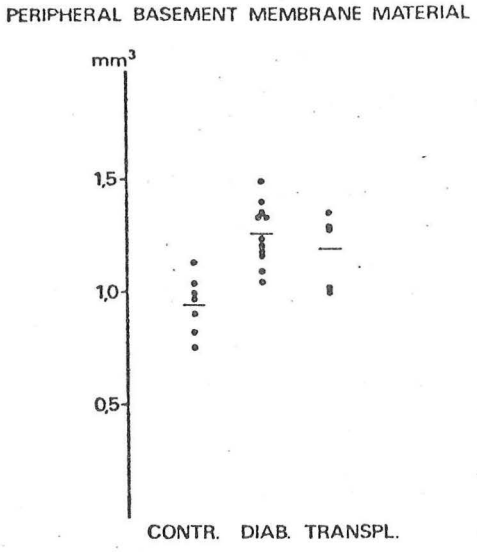


Fig. 6. Amount of peripheral basement membrane material in control, diabetic, and 4 weeks post transplantation (9).

study does not exclude the possibility that prolonged periods of normoglycemia could normalize the change in the basement membrane material, it does suggest that reversal of diabetic glomerular lesions may be a very slow process following normalization of hyperglycemia. The increased synthesis of glomerular basement membrane material has been demonstrated very soon after the onset of experimental diabetes, but there is at present no evidence to indicate that the glomerular basement membrane material produced is abnormal (10).

In short term diabetic animals, the amount but not the thickness of the peripheral basement membrane is increased. However, after six months of diabetes there is significant thickening of the basement membrane that is preventable by strict insulin treatment (4). This thickening of the GBM may be irreversible. If experimental diabetes in the rat is allowed

to exist untreated for 7 months and the animals then receive a pancreatic islet cell transplant and are followed for another 6 months, there is no tendency to reverse the thickening of the basement membrane (11). These observations may have clinical significance when approaching the question of prevention or reversal of glomerular basement membrane alterations in human diabetes.

The major light microscopic abnormalities in human diabetic nephropathy are : 1) thickening of the glomerular basement membrane, 2) an increase in mesangial matrix material which is the forerunner of the most striking glomerular lesion, diffuse glomerulosclerosis, and 3) subintimal hyaline thickening of both afferent and efferent arterioles. The early changes in the basement membranes and mesangium described in the experimental models of diabetes also occur in the kidneys of humans with diabetes. Osterby (6) showed that glomerular basement membrane thickening and expansion of the glomerular mesangial area are not present at the onset of Type I diabetes in young people. When these patients were studied again between 1 1/2 and 2 years after the onset of diabetes, there were definite changes observable in the glomeruli, Figure 7.

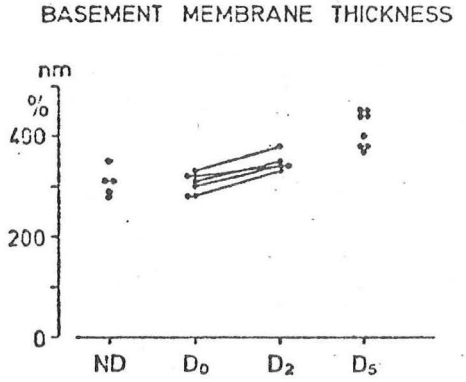


Fig. 7. Harmonic mean thickness of GBM in normal controls (ND), juvenile diabetics at the time of diagnosis (D_0), and after 2 (D_2) and 5 years (D_5) of the disease. The lines connect values from individuals rebiopsied after 1.5 and 2.5 years (6).

After five years of diabetes the changes were clear-cut. Further incriminating the importance of the diabetic milieu in the morphologic changes is the observation that normal kidneys transplanted into diabetic patients develop characteristic arterial lesions and mesangial thickening, including nodular glomerulosclerosis within four years (12). Lastly, there is a brief account of a patient by Mauer et al (13) who received a successful simultaneous pancreas and kidney transplant from the same donor and had no evidence of diabetic nephropathy in the graft four years after graft transplantation.

In addition to these changes in gross kidney size and the alterations in glomerular basement membrane area and thickness, clinical and experimental diabetes is associated with a parallel increase in mesangial matrix material (6). The mesangium can be considered part of the reticuloendothelial system and has the capacity to take up and process macromolecules from the circulation (14). Mauer (15) has demonstrated that mesangial processing of macromolecules in diabetic rats is impaired and the abnormality is most apparent in those areas where the

the mesangial thickening is the greatest. Mauer (13) has suggested that these abnormalities may be related to the GBM changes in several ways. Since GBM protein is one macromolecule apparently processed and cleared by the mesangium, delayed turnover of GBM material in the face of increased GBM deposition would result in thickening of the GBM. Secondly, and possibly more important in leading to end stage kidney, mesangial expansion might lead to encroachment on the sub-endothelial space and eventually compromise and occlude the capillary lumen, reduce blood flow, and ultimately lead to glomerulosclerosis and obsolescence.

In summary, the morphologic changes of early diabetes include an increase in gross kidney weight, nephron hypertrophy with a preferential increase in the size of glomeruli compared to tubules, an early increase in glomerular basement membrane area, a late increase in glomerular basement membrane thickness, and an increase in mesangial area. How all of these changes come about and how they interrelate is not completely known, but the biochemical changes described next are probably involved.

GLOMERULAR BASEMENT MEMBRANE

1. Biochemical changes. Brown et al. (16) recently reviewed the biochemistry of the diabetic basement membrane. Glomerular basement membrane is a complex, porous matrix that is negatively charged and plays an important role in regulating the filtration of proteins from serum into the urinary space. The GBM with its polyanionic covering is schematically depicted in Figure 8. The negative charge is due

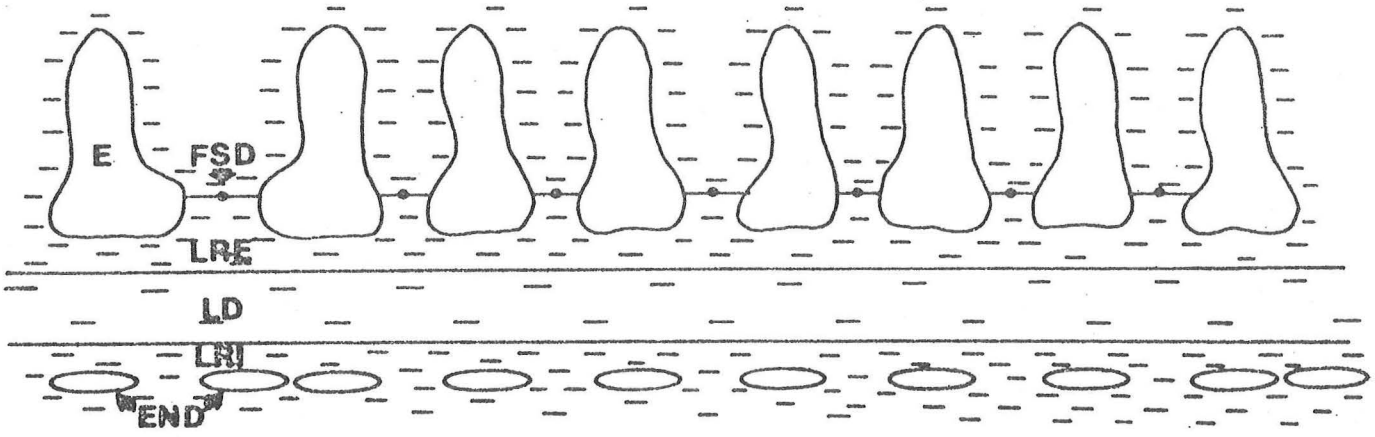


Figure 8. Schematic representation of the GBM showing the anionic covering (-) in the glomerular extracellular matrix. END=endothelium; LRI=lamina rara interna; LD=lamina densa; LRI=lamina rara externa; FSD=filtration slit diaphragm; E=ephthelial foot process.

primarily to sialic acid residues and glycosoaminoglycans incorporated into the GBM. GBM in diabetes is chemically different from normal GBM in several ways. Kefalides (17) demonstrated that human diabetic glomerular basement membrane has a reduced content of sialic acid. The GBM also has increased quantities of glycine, hydroxylysine, hydroxyproline, and disaccharide, all of which are present in the collagen portion of the membrane (18,19). Thus, the GBM in diabetic nephropathy is characterized by an increase in collagen related components and a fall in components that contribute to its negative charge. The collagen-like components of glomerular basement membrane turn over very slowly, at a rate approaching that of fibrillar collagen (20). In contrast, incorporation and turnover studies of amino acids in the glomerular basement membranes demonstrated that the specific activity of hydroxyproline, an amino acid in high quantity in collagen, was twice normal in diabetic GBM (10). These findings suggest that the accumulation of excess basement membrane in diabetes is the result of increased synthesis rather than delayed breakdown.

Several mechanisms have been offered to explain these morphological and biochemical changes in the GBM in diabetes. The changes that occur in the glomerulus of the diabetic kidney are very similar to those occurring in renal hypertrophy. The glomerulus in diabetes enlarges in volume without an increase in the number of glomerular cells, the surface area of the glomerular basement membrane increases, and RNA content and synthesis rate of the renal cortex are increased (6,21). Cortes et al. (22) studied renal RNA incorporation, immunoreactive insulin, and immunoreactive glucagon in streptozotocin diabetic rats with different degrees of diabetic control using continuous intravenous insulin. In animals with diabetes for 48 hours, insulin administration had the predictable effects of lowering plasma glucose and glucagon, Figure 9. Untreated diabetes lead

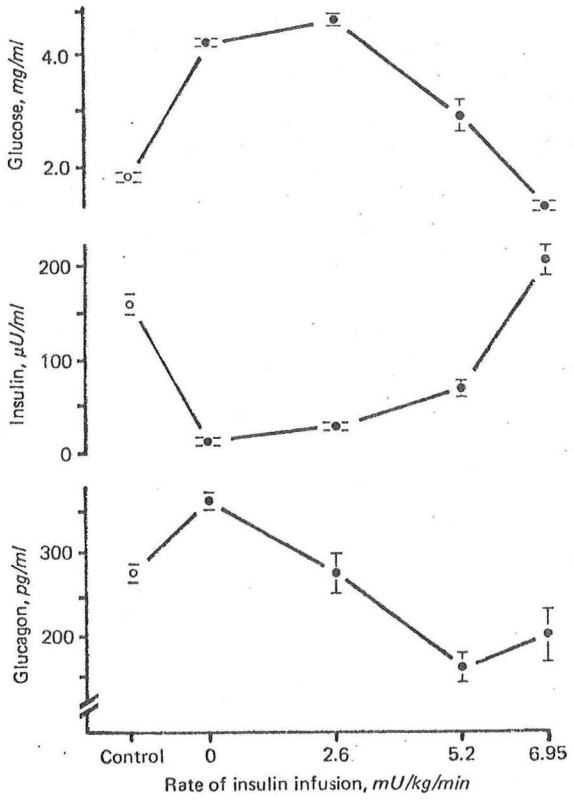
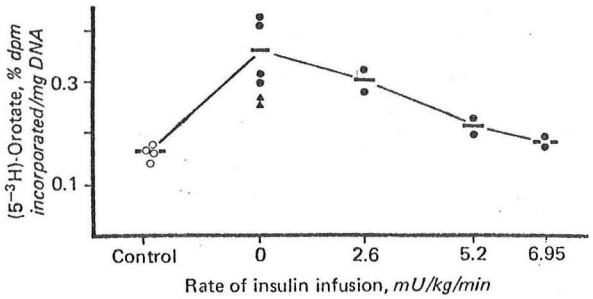


Figure 9. Plasma glucose, immunoreactive insulin, and immunoreactive glucagon in 48 hour diabetic rats. Effect of insulin infusion.

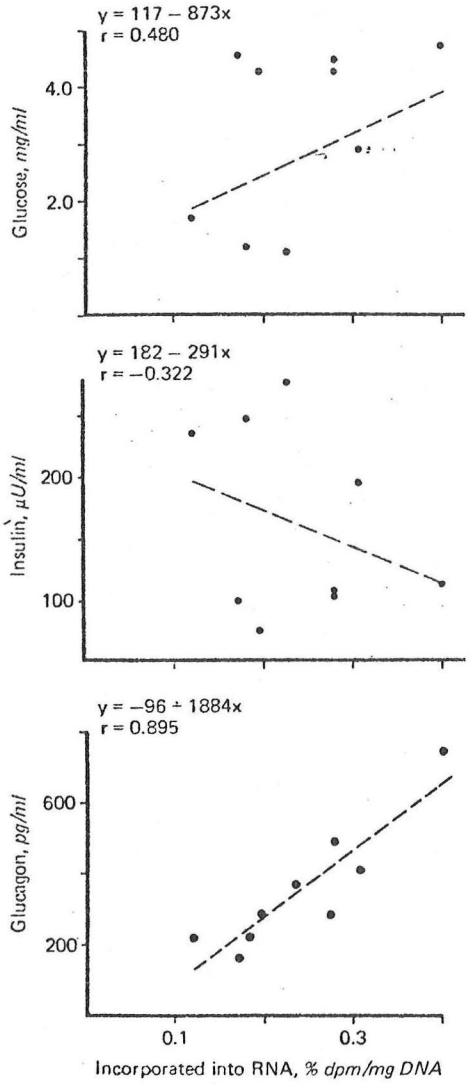
to increased renal RNA synthesis [(5-^{3}H)-Orotate incorporation], but this could be normalized by continuous insulin infusion, Figure 10.

Fig. 10. Incorporation of radiolabeled orotate into glomerular RNA in 48 hr diabetic rats. Effect of insulin infusion (22).



Analysis of the data indicated that RNA incorporation correlated with changes in the glucagon level (r=.895, p .01), but not with changes in plasma glucose (r=.480, NS) or plasma insulin (r=-.322, NS), Figure 11.

Fig. 11. Relationship between incorporation of radiolabeled orotate into glomerular RNA and the plasma concentration of glucose, immunoreactive insulin, and immunoreactive glucagon in 48 hour diabetic rats (22).



Hyperglucagonemia of some degree is present in most forms of diabetes, and complete reversal to normal values is probably only possible with continuous insulin infusion (23). Thus, the changes in RNA incorporation after suppression of hyperglucagonemia with insulin suggest that in early diabetes elevated glucagon levels may be an important factor in the hypertrophy and biochemical changes in the glomerulus.

Nonenzymatic glycosylation of glomerular proteins may be another factor in the early and late glomerular changes in diabetes. Nonenzymatic glycosylation of many proteins throughout the body is enhanced in the diabetic state and has been proposed as the common link between hyperglycemia and the functional and structural abnormalities of diabetes. It is possible that glycosylation may alter the physical and chemical properties of certain proteins and may well be the pathogenic link to the chronic complications of diabetes (24). For example, glycosylation of lens crystallin appears to be involved in cataract formation (25).

A number of proteins have been demonstrated to undergo glycosylation in human and experimental diabetes including hemoglobin, albumin, erythrocyte membranes, proteins, lens crystallins, and aortic collagen (26-30). In the glycosylation reaction of hemoglobin A, glucose attaches via the free amino group at the N-terminus of the beta chain forming a labile Schiff base which rearranges to form a stable ketoamine derivative, Figure 12. Nonenzymatic glycosylation of other body proteins involves a similar reaction. Rahbar (31) discovered that a minor component of

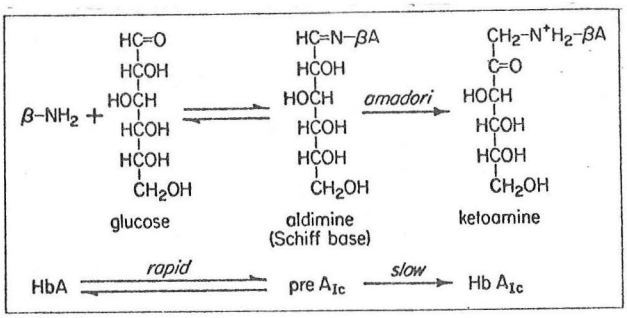


Fig. 12. Reaction pathway for the synthesis of Hb A_{1c}.

hemoglobin A, hemoglobin A_{1c}, was increased 2-3 times normal in the red cells of patients with diabetes, Figure 13. Hemoglobin A_{1c} and several

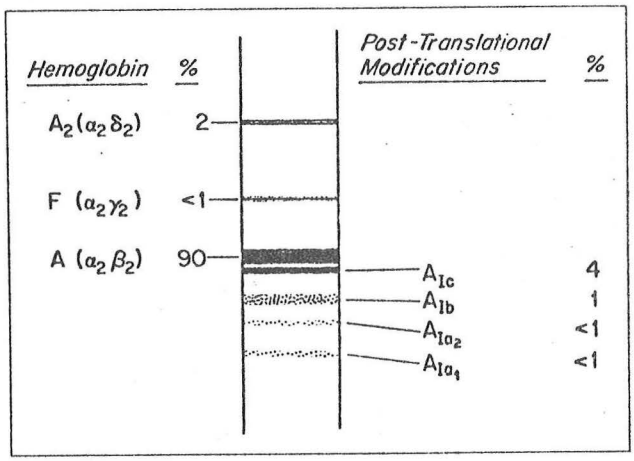


Fig. 13. Hemoglobin components of normal red blood cells separated by gel electrofocusing.

others in low concentrations represent post-translational modifications of hemoglobin A. Hemoglobin A_{1c} is formed at a rate dependent on the glucose concentration to which the erthyrocyte is exposed, Figure 14.

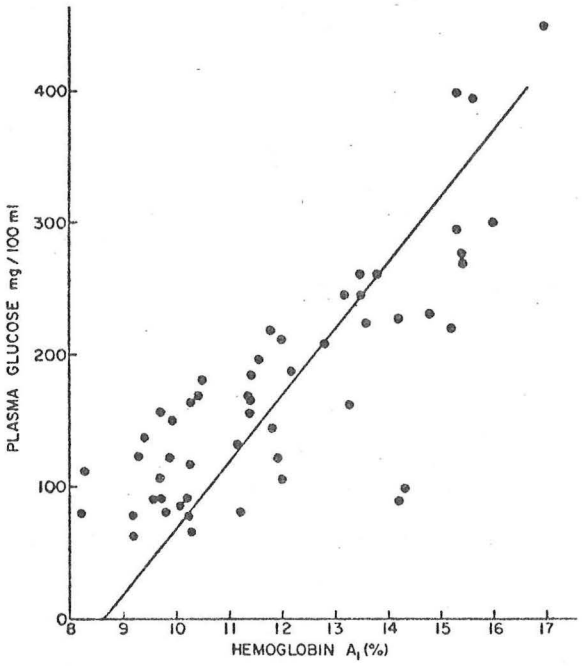


Fig. 14. Relationship between plasma glucose and Hb A_{1c} in a group of diabetic patients.

Although these non-enzymatically glycosylated hemoglobins have a greater affinity for oxygen than normal hemoglobin, they are present in relatively low concentrations and oxygenation of the intact red cells is probably not significantly lowered. This research has lead to the discovery that other proteins in the body are glycosylated during uncontrolled hyperglycemia and that these changes may affect their function. Cohen (32) has shown in vitro that glomerular basement membrane undergoes non-enzymatic glycosylation and that this effect is directly proportionate to the glucose concentration in the medium, Figure 15.

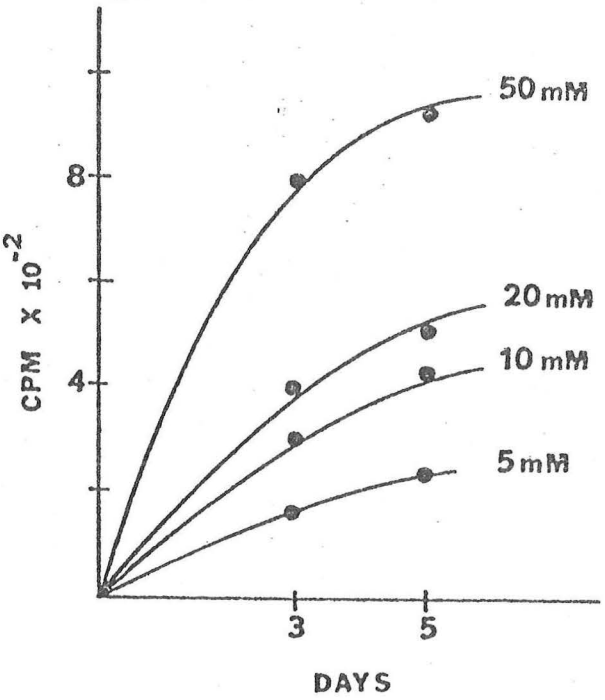


Fig. 15. Time and concentration dependent incorporation of radiolabeled glucose into rat GBM. Each curve represents a different medium glucose concentration.

The specific glomerular basement membrane fraction that contains the collagen components is the primary site of nonenzymatic glycosylation, with the lysine and hydroxylysine residues as the most likely site. How nonenzymatic glycosylation could affect glomerular basement membrane structure or function is not yet clear, but Cohen et al (32) has hypothesized that glycosylation of lysine and hydroxylysine residues would decrease the availability of the amino acids for collagen cross linking, while keto amino linkage of free amino groups could alter electrochemical properties of the involved proteins. Such changes might lead to permeability changes in the glomerular basement membrane. Furthermore, continued glycosylation of a basement membrane that has a very slow turnover rate would lead to progressive accumulation of the abnormal collagen and probably interfere with normal anabolic and catabolic functions of the glomerular basement membranes.

Glycosylation of proteins may injure the GBM in a more indirect way. Circulating concentrations of glycosylated albumin are increased in the plasma of diabetic patients (28). Albumin concentration in glomerular and tubular basement membranes of diabetic individuals is increased (33). It is possible that these circulating glycosylated albumin molecules might deposit and damage the GBM. This possibility is supported by a study in mice in which injection of glycosylated proteins lead to thickening of the GBM (34).

Thus, if a link can be established between nonenzymatic glycosylation of body proteins, whether it be hemoglobin, lens crystallin, or basement membrane collagen, and the longterm complications of diabetes, then a strong case could be made for meticulous diabetic control.

2. Proteinuria. One of the primary functions of the GBM is to prevent movement of large molecular weight proteins and red blood cells into the urine. The presence of persistent, clinically detectable proteinuria in diabetes usually signifies an abnormality at the level of the GBM and is almost invariably accompanied by diabetic glomerulosclerosis and some degree of retinopathy. Development of clinically detectable proteinuria is a very slow process and represents the first clinical sign of diabetic nephropathy in most patients. It is a predictable forerunner of renal failure. In one large study in which the initial evidence for diabetic nephropathy was proteinuria, the average duration of diabetes at the time proteinuria began was 17 years (35). Although the duration of diabetes preceeding the onset of proteinuria was variable, once renal deterioration appeared, there was little variation in the subsequent course. Early renal failure, as demonstrated by a rise in the BUN and the serum creatinine, occurred 19.4 years after the onset of diabetes. Late renal failure (serum creatinine greater than 5 mg/100 ml or symptoms of uremia) developed 21.6 years after the onset of diabetes. In contrast, diabetic patients who do not develop proteinuria rarely develop renal failure (36). When the level of proteinuria exceeds 3 grams per day such patients will usually lose all renal function within six years (36). While

proteinuria in the nephrotic range (greater than 3 grams per 24 hours) is a characteristic of late diabetic nephropathy, in the first few years of diabetes, proteinuria is absent by the usual clinical tests as well as by a sensitive radioimmunoassay technique, Figure 16, (1).

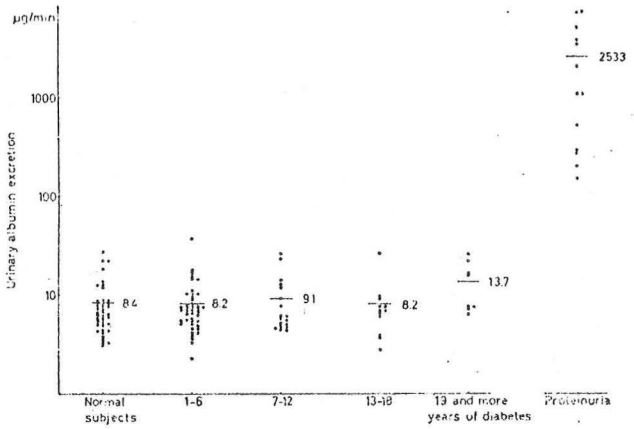


Fig. 16. Urinary albumin excretion in 97 young male diabetics with and without proteinuria and in 41 normals.

After several years of diabetes, patients with baseline normal or slightly elevated albumin excretion show a selective increase in albumin excretion following exercise, Figure 17, (37). The mechanism for this exercise induced proteinuria is unknown, but the hemodynamic changes at the level of the kidney resulting from the exercise may be involved. This will be discussed in a later section. Early in diabetes the level of metabolic control has an important effect on the degree of albuminuria and suggests that the proteinuria is not due to a structural abnormality of the kidney. Pennell (38) studied protein excretion and urinary glucose excretion in streptozotocin diabetic rats before, during, and after a continuous subcutaneous insulin infusion and demonstrated a prompt reduction in

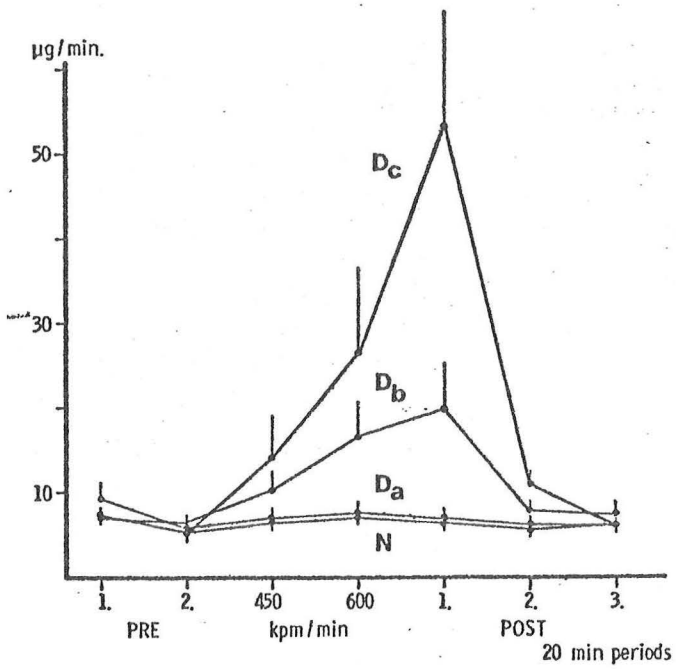


Fig. 17. Urinary albumin excretion during exercise in 11 normals (N), 6 diabetics with 0-1 years of diabetes (D_a), 18 diabetics with 2-11 years of diabetes (D_b), and 7 diabetics with 16-20 years of diabetes (D_c).

proteinuria, Figure 18. Viberti et al (39) studied the effect of improved control of blood glucose on markers of renal glomerular and tubular function.

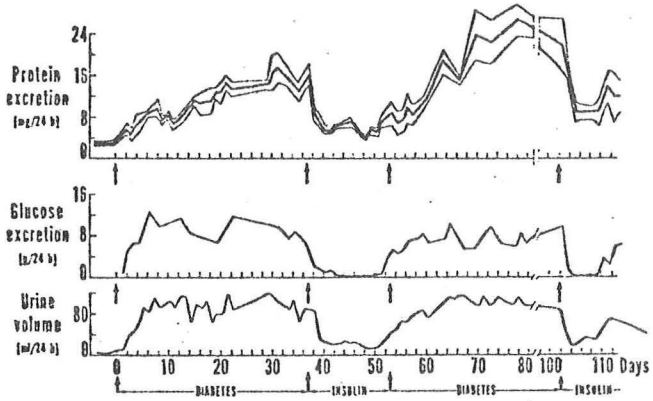


Fig. 18. Excretions of urine, glucose, and protein in 6 rats given continuous insulin for 2 periods after induction of streptozotocin diabetes.

Using a radioimmunoassay, they studied seven patients with elevated albumin excretion before and during a continuous, subcutaneous insulin infusion for a period of 1-3 days. Urinary albumin excretion was significantly reduced with insulin therapy, Figure 19. With the nephrotic syndrome of late diabetes, there is heavy, non-selective proteinuria both of albumin and of immunoglobulins (40). Unlike most other glomerular diseases in which protein excretion tends to fall in parallel with reduction in the glomerular filtration rate, in

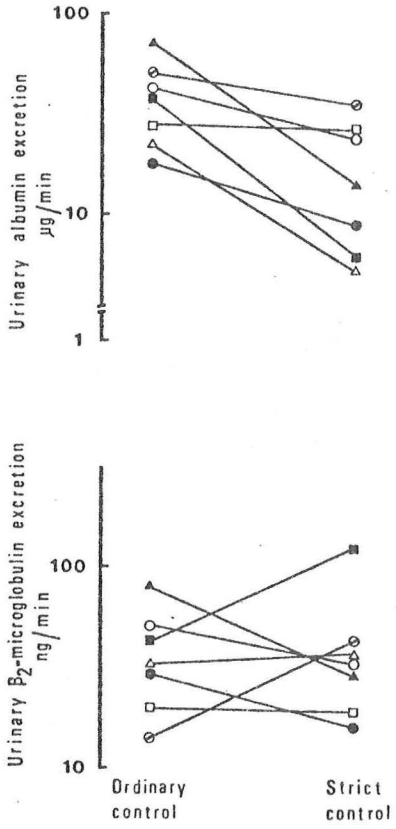


Fig. 19. Urinary excretion (log scale) of Albumin and beta 2 microglobulin during ordinary and strict glycemic control in 7 long term diabetics undergoing continuous subcutaneous insulin infusion (39).

advancing diabetic nephropathy, protein excretion may persist at massive levels despite a severely reduced glomerular filtration rate.

The mechanism of protein loss in diabetic nephropathy is unclear. There does not seem to be an increase in "pore size" of the glomerular basement membrane, but there does appear to be a selective increase in transglomerular passage of large molecular weight substances (40,41). However, in man the renal clearance of dextran molecules of different sizes is reduced within a few weeks when diabetes is controlled with insulin (42). Concomitant with insulin treatment was a reduction in glomerular filtration rate. These authors concluded that, since enlargement of glomerular size and surface area are known to be unchanged by as much as one month of insulin treatment, the alteration in dextran clearance must have been due to hemodynamic factors. There is evidence that the early proteinuria in the diabetic kidney and possibly other forms of renal disease may result from increases in glomerular hydrostatic pressure and blood flow (43,44). Along with these hemodynamic effects, there also appears to be a metabolic effect at the level of the glomerular basement membrane that independently accounts for the proteinuria. However, this metabolic effect may be a late consequence of diabetes. Pennell et al (41) have shown that chronic streptozotocin diabetic rats have proteinuria in the absence of an alteration in GFR. A similar process seems to occur in man. Within the first 2-5 years of diabetes when proteinuria is usually absent by standard tests, a thickening of the glomerular basement membrane can be demonstrated. The thickening progresses over many years and is associated with an increase in proteinuria. Thus, two changes are occurring in the basement membrane in diabetes. First, very early in diabetes, concomitant with the increase in glomerular filtration rate, there is an increase in the glomerular filtration surface area. Secondly, the thickness of glomerular basement material increases with advancement of the diabetes. It is this latter factor that is thought to be related to the late proteinuria, while the former basement changes and the hemodynamic changes leading to the increase in the glomerular filtration rate are thought to account for the early proteinuria. Thus, the early, subclinical selective proteinuria of juvenile diabetes is probably due to hemodynamic factors and may not be a specific effect of diabetes. The heavy, nonselective proteinuria of late diabetes is a more specific effect of diabetes and is probably related to abnormalities of glomerular basement membrane. It is likely that the heavy proteinuria of late human diabetes represents a combination of hemodynamic and basement membrane derangements. Mauer et al (45) demonstrated enhanced albuminuria after unilateral nephrectomy performed in diabetic animals suggesting that both hemodynamic and metabolic glomerular basement membrane alterations combined to increase protein excretion.

In summary, evidence from clinical and experimental diabetes indicates that the proteinuria of diabetes is probably multifactorial. The early subclinical and highly selective proteinuria of early diabetes appears to be the result of altered hemodynamic factors at the level of

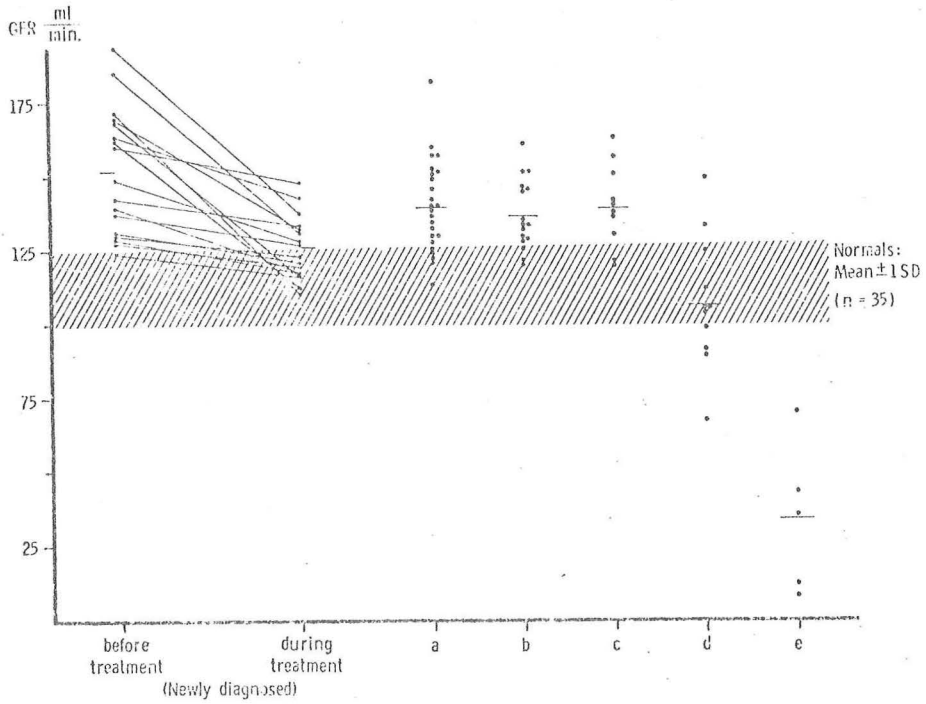
the glomerulus. The late, nonselective, heavy proteinuria appears to be due to an alteration in the glomerular basement membrane. It is this late, heavy proteinuria that appears to be involved in the production of diabetic glomerulosclerosis. Since the albuminuria of streptozotocin diabetes can be prevented by islet cell transplantation and diabetic glomerulosclerosis halted by transplantation of diabetic kidneys to normal animals, the most logical interpretation of the results is that it is the diabetic milieu that leads to the proteinuria. Several studies demonstrating that diabetic glomerulosclerosis can be prevented by strict insulin treatment are compatible with this view (46,47).

PHYSIOLOGY OF DIABETIC NEPHROPATHY

One of the paradoxes of end stage diabetic kidney disease is that the earliest changes in kidney physiology brought about by the diabetic state is an increase in glomerular filtration rate and renal plasma flow (2,48), Figure 20. During early diabetes, the glomerular filtration rate is above normal and only begins to fall with development of overt,

Fig. 20

GFR in newly diagnosed diabetics, before treatment and during treatment, and in diabetics with a) duration 1 to 12 years, b) no proteinuria and duration > 15 years, c) intermittent proteinuria, d) constant and pronounced proteinuria but normal serum-creatinine, e) severe retinopathy and increased serum-creatinine



clinically detectable proteinuria.

Hostetter and his associates (49) using micropuncture techniques have investigated the hemodynamic changes that occurred in early streptozotocin diabetes in the rat. This study examined the 4 factors controlling glomerular filtration rate: 1) glomerular plasma flow, 2) systemic oncotic pressure, 3) glomerular transcapillary hydraulic pressure difference, and 4) glomerular capillary ultrafiltration coefficient (K_f). K_f represents the product of the glomerular capillary hydraulic permeability and the total surface area available for filtration. Munich-Wister rats were made diabetic by the injection of intravenous streptozotocin and non-ketotic hyperglycemia was maintained by daily low dose insulin administration. The animals were studied after 1-2 months of diabetes when no glomerular lesions were apparent by light microscopy. The

micropuncture studies demonstrated two important hemodynamic changes. First, renal vasodilatation of the afferent and efferent arteriole of the glomerulus lead to increased renal plasma flow and 2) the transcapillary hydraulic pressure gradient across the glomerular basement membrane was increased. There was no change in systemic oncotic pressure and no change in K_f. They concluded that the hyperperfusion and hyperfiltration of early diabetes could be explained on the basis of alterations in vascular resistance. Moreover, such changes would explain the presence of protein in the urine of diabetic patients, since changes in glomerular pressure and flow influence the driving forces for macromolecules (50).

It is appropriate at this point to ask how these early hemodynamic changes in the diabetic kidney might lead to the late consequences of diabetes, i.e. reduction of the glomerular filtration rate and heavy proteinuria. The answer to this question appears to be that hyperfiltration per se leads to glomerular damage. It is known that reduction of the nephron mass leads to compensatory hypertrophy of the remaining nephrons. This change is accompanied by increased glomerular plasma flow and increased GFR. At least in the rat, this phenomenon has been associated with a progressive glomerulopathy characterized by mesangial enlargement and eventual glomerulosclerosis (51,52). The pathophysiology of the renal ablation model has been characterized by Hostetter et al (53) using techniques very similar to those used in the diabetic rat. They demonstrated in normal rats with 1 5/6 nephrectomy that hyperfiltration associated with renal ablation lead to a four fold increase in protein excretion by the remnant kidney. The mechanism responsible for the proteinuria appeared to be both a change in pore size as well as an alteration of the charge on the glomerular basement membrane. Associated with the changes in protein excretion were an increase in the mesangial deposition of plasma proteins. They postulated that excessive mesangial deposition of circulating macromolecules might serve to increase the number of mesangial cells, promote mesangial matrix deposition, and be the forerunner of diabetic glomerulosclerosis. To test this hypothesis, they prevented hyperfiltration from developing in the remnant kidney by reducing the dietary protein, a known stimulus to renal hypertrophy. This was accomplished and the glomerular structural changes were prevented as well as the hemodynamic alterations.

These results from diabetic rats and rats undergoing renal hypertrophy may apply to the mechanism of progressive renal failure that accompanies many kidney diseases irrespective of the inciting event. Since progression of renal failure seems to occur even after the inciting event has been removed, these studies raise the interesting possibility that alterations of renal hemodynamics through the mechanism described above may be the final common pathway for progressive renal failure. In the case of diabetes, support for this hypothesis comes primarily from studies in experimental animals. Steffes et al (45) demonstrated in streptozotocin diabetic rats that mesangial abnormalities were far worse in diabetic animals subjected to unilateral nephrectomy. They hypothesized

that altered microcirculatory dynamics influenced the rate of development of complications. A logical extension of these findings is that protection of the diabetic kidney from hyperperfusion might protect the animal from accelerated glomerulosclerosis. Mauer (54) studied this question both in diabetic and in normal animals using the one clip renal artery hypertension model. He found that diabetic rats with a unilateral renal artery clip had a distinct asymmetry in the glomerular lesion. The unclipped kidney which was exposed to an elevated arterial pressure showed more extensive glomerular injury than the contralateral clipped kidney that appeared to be protected. The most prominent lesion was a PAS positive mesangial matrix thickening. Since both kidneys were exposed to the same diabetic environment, it was apparent that the pathologic lesion resulted from the altered intrarenal hemodynamics. An interesting experiment of nature suggests that a similar phenomenon may be operative in human diabetes. Berkman and Rifkin (55) reported a 63 year old patient with longstanding diabetes and unilateral renal artery stenosis. At autopsy there was a marked asymmetry in the glomerular lesion with the unstenosed kidney showing nodular glomerulosclerosis, the classic Kimmelstiel-Wilson lesion, while the stenosed kidney had only mild to moderate degrees of mesangial thickening.

A number of factors resulting from the diabetic state might be responsible for the hemodynamic alterations. Acute hyperglycemia increases the glomerular filtration rate in man and in animals, Figure 21, (56,57). Glucagon and growh hormone are high in uncontrolled diabetes and both are capable of causing modest increases in glomerular filtration rate (58,59). In normal man a short term infusion of glucagon sufficient to cause a four fold rise in plasma glucagon over control values caused a 9% increase in glomerular filtration rate (60). Christensen (61) studied renal hemodynamics in seven well-controlled insulin dependent diabetics before and after the administration of growth hormone for one week. During this time glomerular filtration rate increased from 122 to 131 ml/min and renal plasma flow increased from 535 to 569 ml/min. Similar elevations in glomerular filtration rate have been demonstrated in normal men given growth hormone over

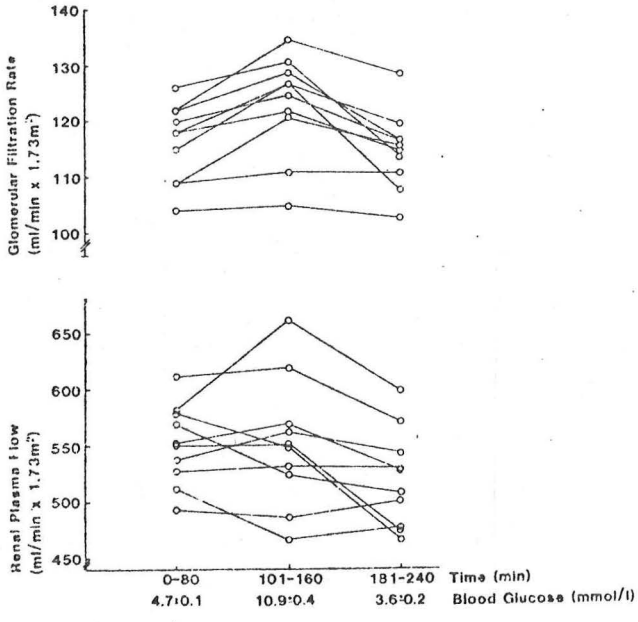


Fig. 21. Glomerular filtration rate and renal plasma flow in ten normal subjects before, during and after glucose infusion (57).

several days (59).

Alterations in vasoactive mediators have been suggested as possible explanations for the hyperperfusion of early diabetes (43). However, it is difficult at present to get a consensus from the literature about the importance of the changes that have been reported in circulating vasoactive substances (62-64). In vitro studies have demonstrated diminished contractile response in aortas from diabetic rabbits exposed to norepinephrine (65a). While this hyporesponsiveness to circulating vasoconstrictors might suggest that diabetic blood vessels are under enhanced vasodilating influences, there is no evidence to suggest that one important group of circulating vasodilators, namely the prostaglandins, is increased in diabetes. In fact, experimental diabetes in the rat is associated with a reduction in vascular prostacyclin content, the latter being an important circulating vasodilator (64). In humans with early diabetes vascular prostacyclins appear to be reduced (65b,66). Thus, there is not a good candidate to take on the role of a renal vasodilator to explain the renal hyperperfusion and hyperfiltration of early diabetes.

The late renal hemodynamic effects of diabetes are a reversal of the early defects. Renal blood flow and glomerular filtration rate begin to fall at the time detectable proteinuria occurs. Hypertension is invariably present, presumably the result of sodium retention. Exchangeable body sodium in diabetes is increased, even in the absence of kidney or heart failure (71). In addition, there is enhanced pressor sensitivity to angiotensin II or norepinephrine in diabetics with retinopathy or mild hypertension (72,73). Such reports suggest that exaggerated pressor sensitivity to normal plasma levels of norepinephrine might play a role in the hypertension of diabetes. A recent report by Beretta-Piccoli (74) demonstrated that pressor responsiveness to norepinephrine in normotensive nonazotemic diabetic patients was increased. Since cardiovascular hyperresponsiveness appears to occur in the normotensive stage of diabetes mellitus, this alteration in conjunction with the excess total body sodium might predispose to the development of hypertension.

A change in rheologic or flow characteristics of the blood in diabetes also may participate in the hemodynamic changes (67). Poorly controlled diabetes is associated with increased plasma viscosity (68,69), reduced red cell deformability (70), and increased red cell agglutination (70). Simpson (67) has suggested that resistance to flow by viscous blood in the efferent arteriole would lead to high intraglomerular pressures, hyperfiltration, and albuminuria.

Figure 22 from a review by Hostetter et al (43) incorporates much of the data cited above and provides a useful schema for future investigation regarding the mechanisms involved in the renal disease of diabetes. In this formulation, hyperperfusion and hyperfiltration represents the net effect of several contributing systems, including extracellular fluid volume expansion, renal hypertrophy with its

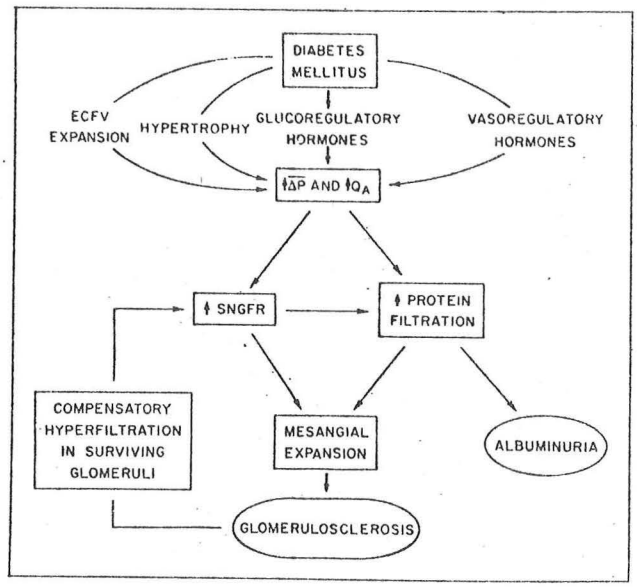


Figure 22 Hypothetical role of glomerular hyperfiltration in the initiation and progression of diabetic nephropathy. Hyperfiltration is stimulated by some feature(s) of the diabetic state such as extracellular fluid volume (ECFV) expansion due to hyperglycemia, renal hypertrophy, increased growth hormone and glucagon secretion, and/or altered levels of, or vascular responsiveness to, vasoactive hormones. Increases of the glomerular transcapillary hydraulic pressure gradient ($\overline{\Delta P}$) and glomerular plasma flow rate (Q_A) are responsible for the hyperfiltration which increases transglomerular protein filtration leading to albuminuria and mesangial deposition of circulating proteins. The latter effect eventuates in mesangial expansion and ultimately glomerulosclerosis. This initial loss of functioning nephrons exerts a positive feedback stimulus to compensatory hyperfiltration in less affected surviving glomeruli, in turn contributing to their eventual destruction. SNGFR = single-nephron glomerular filtration rate.

resulting effect on the filtration surface area, glucoregulatory hormones, and the vasoregulatory hormones. With time, hyperfiltration leads to increased protein filtration and albuminuria. Hyperfiltration of albumin and other macromolecules leads to increased reabsorption of these substances and hypertrophy of the mesangial area. Mesangial expansion combined with basement membrane thickening may then lead to encroachment on the capillary lumens and ischemia-induced glomerulosclerosis. This in turn leads to compensatory hypertrophy and hyperfiltration of the remaining nephrons with repetition of the cycle until renal failure ensues.

PREVENTION AND REVERSAL OF DIABETIC NEPHROPATHY

The studies reviewed here pose many challenges for the future and perhaps give some reason to be optimistic that diabetic renal complications some day may be preventable. Two questions seem appropriate at this point. Is it possible to prevent diabetic nephropathy with standard modes of therapy? The answer is probably, no. However, renal injury in diabetic animals can apparently be prevented by avoidance of hyperglycemia through the use of continuous intravenous insulin or with pancreatic islet cell transplantation. These studies also indicate that good control must be started early and must be continuous, since diabetic GBM changes are very slow to reverse. Continuous insulin perfusion pumps have not been used long enough to determine whether they will have a preventive effect on the development of renal disease of diabetes. However, clinical and experimental animal data suggest that reversal of the diabetic milieu and prevention of hyperglycemia reverses hyperfiltration, hyperperfusion, and renal hypertrophy.

The second question is this: Is it possible to retard to progression of diabetic nephropathy? The answer is, maybe. Mogensen (75) has demonstrated in a small group of patients with renal insufficiency secondary to diabetes that antihypertensive treatment was capable of altering the progressive decline in glomerular filtration rate occurring in the untreated state, Figure 23. Six male insulin dependent, proteinuric

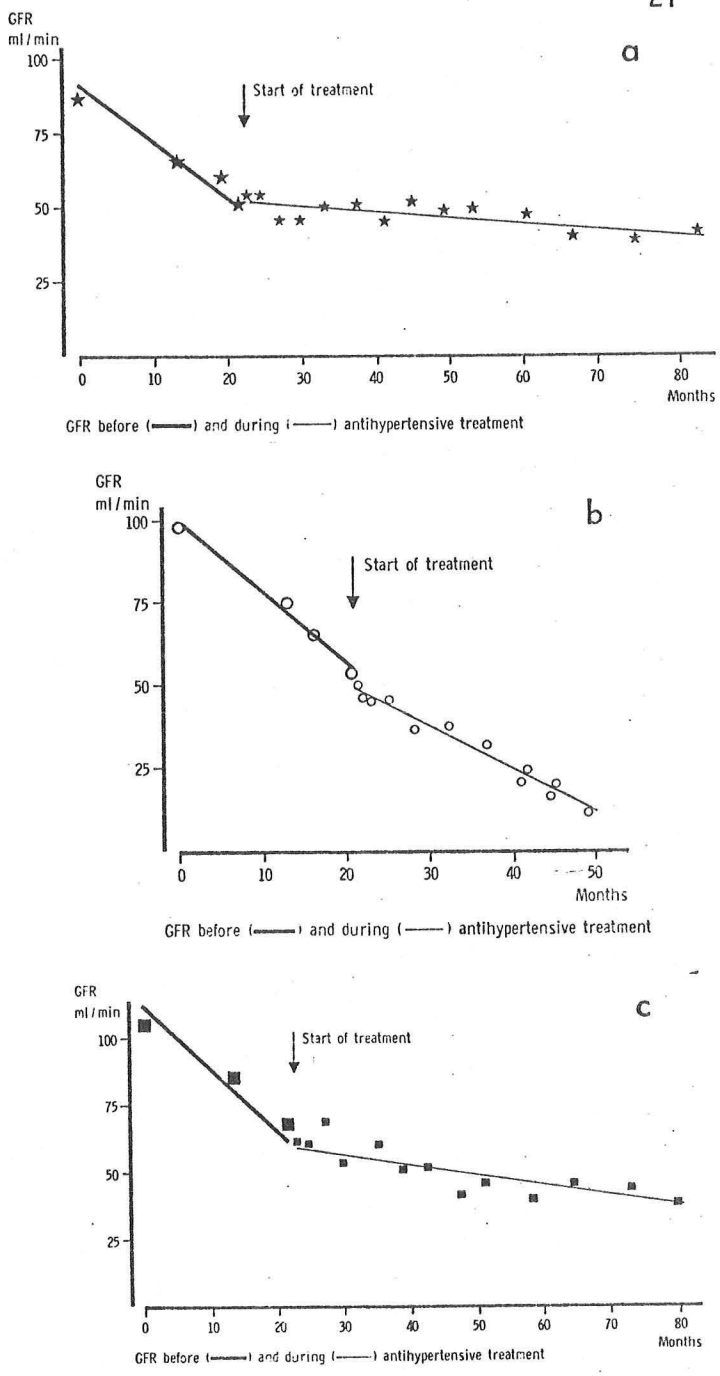


Fig. 23. Effect of antihypertensive treatment on the progression of renal disease in three proteinuric diabetic patients (75).

patients with long-term diabetes were followed for two years with moderate hypertension and for a subsequent four years during antihypertensive therapy with a beta blocker, a vasodilator, and furosemide. With reduction of blood pressure, there was a concomitant reduction in albumin clearance when expressed as a fraction of the glomerular filtration rate, Figure 24.

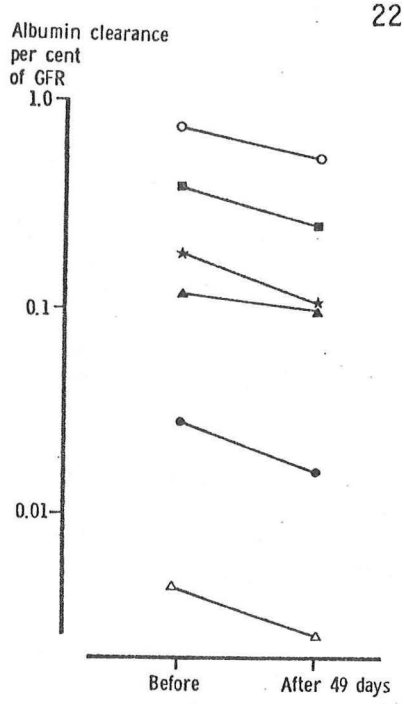


Fig. 24 Percentage albumin clearance before and during initial antihypertensive treatment (75).

These changes occurred in the absence of a change in fasting plasma glucose or insulin requirement. Takazakura et al (76) using serial renal biopsies in 23 diabetic patients reported that lack of progression of the glomerular lesion correlated with good control of the blood sugar. However, there were other equally significant correlations of progression of glomerular lesions with the type of diabetes (juvenile progressed faster than adult), degree of obesity (lean persons progressed faster than obese), and type of treatment (insulin treated patients progressed faster than those treated with oral agents).

What does the future hold for the control of kidney disease in the diabetic patient? Servo mechanical blood glucose regulators which are now being used in selected patients offer the hope that blood glucose can be normalized on a 24 hour basis. If animals studies are correct and hyperglycemia is as important as it seems in the initiation and progression of renal hemodynamic abnormalities leading to diabetic nephropathy, then careful control of hyperglycemia might prevent diabetic nephropathy. It is too early to decide whether pancreatic islet cell transplantation in humans will be feasible, but animal studies have been encouraging.

REFERENCES

1. Mogensen, C.E: Renal function in diabetes. Diabetes 25:872-79, 1976.

2. Sandahl Christiansen, J., Gammelgaard, J., Frandsen, M., and Parving H.-H: Increased kidney size, glomerular filtration rate and renal plasma flow in short-term insulin-dependent diabetics. Diabetologia 20:451-456, 1981.

3. Seyer-Hansen, K: Renal hypertrophy in streptozotocin-diabetic rats. Clin Sci Mol Med 51:551-555, 1976.

4. Rasch, R: Functional and structural abnormalities in the kidneys of IDDM-studies on the prevention of glomerulopathy in diabetic rats. Acta Endocrinol 97(Suppl 242): 43-44, 1981.

5. Mogensen, C.E., Andersen, J.J.F: Increased kidney size and glomerular filtration rate in untreated juvenile diabetics: Normalization by insulin-treatment. Diabetologia 11:221-224, 1975.

6. Osterby, R., Gundersen, H.J.G: Glomerular size and structure in in diabetes mellitus. Early abnormalities. Diabetologia 11: 225-229, 1975.

7. Seyer-Hansen, K., Hansen, J., Gundersen, H.J.G: Renal hypertrophy in experimental diabetes. Diabetologia 18:501-505, 1980.

8. Kroustrup, J.P., Gundersen, H.J.G., Osterby, R.: Glomerular size and structure in diabetes mellitus. III. Early enlargement of the capillary surface. Diabetologia 13:207-210, 1977.

9. Gotzsche, O., Gundersen, H.J.G., and Osterby, R: Irreversibility of glomerular basement membrane accumulation despite reversibity of renal hypertrophy with islet transplantation in early experimental diabetes. Diabetes 30, 481-485, 1981.

10. Brownlee, M., Sprio, R.G.: Glomerular basement membrane metabolism in the diabetic rat. Diabetes 28, 121-125, 1979.

11. Steffes, M.W., Brown, D.M., Basgen, J.M., Matas, A.J., and Mauer, S.M: Glomerular basement membrane thickness following islet transplantation in the diabetic rat. J. Lab. Invest. 41:116-118, 1979.

12. Mauer, S.M., Barbosa, J., Vernier, R.L., Kjellstrand, C.M., Buselmeier, T.J., Simmons, R.L., Najarian, J.S., and Goetz, F.C: Development of diabetic vascular lesions in normal kidneys transplanted into patients with diabetes mellitus. N. Engl. J. Med. 295:916-920, 1976.

13. Mauer, S.M.,Steffes, M.W., and Brown, D.M: The kidney in diabetes. Am. J. Med. 70:603-612, 1981.

14. Mauer, S.M., Fish, A.J., Day, N.K., Michael, A.F.: The glomerular mesangium. II. Quantitative studies of mesangial function in nephrotoxic nephritis in rats. J. Clin. Invest. 53:431, 1974.

15. Mauer, S.M., Steffes, M.W., Chern, M., Brown, D.M: Mesangial uptake and processing of macromolecules in rats with diabetes mellitus. Lab. Invest. 41:401, 1979.

16. Brown, D.M., Andres, G.A., Hostetter, T.H., Mauer, S.M., Price, R., and Venkatachalam, M.A: Kidney Complications. Diabetes, 31 (Suppl. 1) 71-81, 1982.

17. Kefalides, N: Biochemical properties of human glomerular basement membranes in normal and diabetic kidneys. J. Clin. Invest. 53:403-407, 1974.

18. Canivet, J., Cruz, A., and Moreau-Lalande, H: Biochemical abnormalities of the human diabetic glomerular basement membrane. Metabolism 28:1205-1210, 1979.

19. Beisswenger, P.J., and Sprio, R.G: Studies on the human glomerular basement membrane: composition, nature of the carbohydrate units and chemical changes in diabetes. Diabetes 22: 180-193, 1973.

20. Price, R.G., and Sprio, R.G: Studies on the metabolism of the renal glomerular basement membrane: turnover measurements in the rat with the use of radiolabelled aminoacids. J. Biol. Chem. 252:8597-8602, 1977.

21. Cortes, P., Levin, N.W., Martin, P.R: Ribonucleic acid synthesis in the renal cortex at the initiation of compensatory growth. Biochem. J. 158:457-470, 1976.

22. Cortes, P., Dumler, F., Venkatachalam, K.K., Goldman, J., Sury Sastry, K.S., Venkatachalam, H., Bernstein, J., and Levin, N.W.: Alterations in glomerular RNA in diabetic rats: roles of glucagon and insulin. Kid. Internat. 20:491-499, 1981.

23. Raskin, P., Pietri, A., Unger, R.H: Changes in glucagon levels after four to five weeks of glucoregulation by portable insulin infusion pumps. Diabetes 28:1033-1035, 1979.

24. Koeing, R.J., and Cerami, A: Hemoglobin A_{1c} and diabetes mellitus. Annu. Rev. Med. 31:29-34, 1980.

25. Stevens, V.J., Rouzer, C.A., Monnier, V. M., and Cerami, A: Diabetic cataract formation: potential role of glycosylation of lens crystallins. Proc. Natl. Acad. Sci. USA 75:2918-2922, 1978.

26. Koenig, R.J., Peterson, C.M., Jones, R.L., Saudek, C., Lehrman, M. and Cerami, A: The correlation of glucose regulation and hemoglobin A_{1c} in diabetes mellitus. N. Engl. J. Med. 295:417-420, 1976.

27. Yue, D.K., Morris, K., McLennan, S., and Turtle, J.R: Glycosylation of plasma protein and its relation to glycosylated hemoglobin in diabetes. Diabetes 29:296-300, 1980.

28. Guthrow, C.E., Morris, M.A., Day, J.F., Thorpe, S.R., and Baynes, J.R: Enhanced non-enzymatic glucosylation of human serum albumin in diabetes mellitus. Proc. Natl. Acad. Sci. USA 76:4258-4261, 1979.

29. Bailey, A.J., Robins, S.P., and Tanner, M.J.A: Reducible components in the proteins of human erythrocyte membrane. Biochem. Biophys. Acta 434:51-57, 1976.

30. Rosenberg, H., Modrak, J.B., Hassing, J.M., Al-Turk, W.A., and Stohs, S.J: Glycosylated collagen. Biochem. Biophys. Res. Commun. 91:498-501, 1979.

31. Rahbar, S: An abnormal hemoglobin in red cells of diabetics. Clin. Chim. Acta. 22:296-298, 1968.

32. Cohen, M.P., Urdanivia, E., Surma, M., and Ciborowski, C.J.: Nonenzymatic glycosylation of basement membranes. In vitro studies. Diabetes 30:367-371, 1981.

33. Michael, A.F., Brown, D.M.: Increased concentration of albumin in kidney basement membranes in diabetes mellitus. Diabetes 30:843-846, 1981.

34. McVerry, B.A., Fisher, C., Hopp, A., and Huehns, E.R: Production of pseudodiabetic renal glomerular changes in mice after repeated injections of glycosylated proteins. Lancet 1:738-740, 1980.

35. Goldstein, H.H: Discussion: The problem of end-stage diabetic nephropathy. Kidney Int. 6 (Suppl. 1):521, 1974.

36. Watkins, P.J., Blainey, J.D., Brewer, D.B., Fitzgerald, M.G., Malins, J.M., O'Sullivan, D.J., and Pinto, J.A: The natural history of diabetic renal disease. Quart. J. Med. 41:437, 1972.

37. Mogensen, C.E., Osterby, R., and Gundersen, H.J.G: Early functional and morphologic vascular renal consequences of the diabetic state. Diabetologia 17:71-76, 1979.

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

39. Viberti, G.C., Pickup, J.C., Jarrett, R.J., Keen, H: Effect of control of blood glucose on urinary excretion of albumin and β_2 microglobulin in insulin-dependent diabetics. N. Engl. J. Med. 300:638-641.

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

41. Pennell, J.P., Yanagawa, N., Hwang, K.H., Millard, M.M., Bourgoignie, J.J: Glomerular selective permeability to macromolecular neutral dextrans in experimental diabetes. Diabetologia 20: 223-227, 1981.

42. Parving, H.-H., Rutili, F., Granath, K., Noer, I., Deckert, T., Lyngsoe, J., and Lassen, N.A.: Effect of metabolic regulation on renal leakiness to dextran molecules in short-term insulin-dependent diabetics. Diabetologia 17:157-160, 1979.

43. Hostetter, T.H., Helmut, H.G., and Brenner, B.M: The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. Amer. J. of Med. 72:375-379, 1982.

44. Olson, J.L., Hostetter, T.H., Rennke, H.G., Brenner, B.M., Venkatachalam, M.A: Altered charge and size selective properties of the glomerular wall:a response to reduced renal mass. Kid. Internat. 16:857, 1979.

45. Steffes, M.W., Brown, D.M., and Mauer, S.M: Diabetic glomerulopathy following unilateral nephrectomy in the rat. Diabetes 27: 35-41, 1978.

46. Mauer, S.M., Steffes, M.W., Sutherland, D.E.R., Najarian, J.S., Michael, A.F., and Brown, D.M: Studies of the rate of regression of the glomerular lesions in diabetic rats treated with pancreatic islet transplantation. Diabetes 24:280-285, 1975.

47. Lee, C.S., Mauer, S.M., Brown, D.M., Sutherland, D.E.R., Michael, A.F., and Najarian, J.S: Renal transplantation in diabetes mellitus in rats. J. Exp. Med. 139:793-800, 1974.

48. Mogensen, C.E: Glomerular filtration rate and renal plasma flow in short-term and long-term juvenile diabetes mellitus. Scand. J. Clin. Lab. Invest. 28:91-100, 1971.

49. Hostetter, T.H., Troy, J.L., Brenner, B.M: Glomerular hemodynamics in experimental diabetes. Kidney Int. 19:410-415, 1981.

50. Deen, W.M., Bohrer, M.P., Brenner,B.M: Macromolecule transport across glomerular capillaries:application of pore theory. Kidney Internat. 16:353-365, 1979.

51. Purkerson, M.L., Hoffsten, P.E., and Klahr, S: Pathogenesis of the glomerulopathy associated with renal infarction in rats. Kidney Internat 9:407-417, 1976.

52. Shimamura, T., Morrison, A.B.: A progressive glomeruloscerosis occurring in partial five-sixths nephrectomy. Am. J. Pathol. 79:95-101, 1975.

53. Hostetter, T.H., Olson, J.L., Rennke, H.G., Venkatachalam, M.A., Brenner, B.M: Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. Am. J. Physiol. 241:F85-F93, 1981.

54. Mauer, S.M., Steffes, M.W., Azar, S., Kupcho-Sandberg, S., and Brown, D.M: The effect of Goldblatt hypertension on the development of glomerular lesions of diabetes mellitus in the rat. Diabetes 27:738-744, 1978.

55. Berkman, J., and Rifkin, H: Unilateral nodular diabetic glomerulosclerosis. (Kimmelstiel-Wilson): report of a case. Metabolism 22:715-722, 1973.

56. Mogensen, C.E: Glomerular filtration rate and renal plasma flow in normal and diabetic man during elevation of blood sugar levels. Scand. J. Clin. Lab. Invest. 28:177-182, 1971.

57. Christiansen, J.S., Frandsen, M., Parving, H-H: Effect of intravenous glucose infusion on renal function in normal man and in insulin dependent diabetes. Diabetologia 21:368-373, 1981.

58. Parving, H-H., Christiansen, J.S., Noer, I., Tronier, B., Mogensen, C.E: The effect of glucagon infusion on kidney function in short-term insulin-dependent juvenile diabetes. Diabetologia 19:350-354, 1980.

59. Corvilain, J., Abramow, J: Some effects of human growth hormone on renal hemodynamics and on tubular phosphate transport in man. J. Clin. Invest. 41:1230-1235, 1962.

60. Parving, H.H., Noer, J., Kehlet, H., Mogensen, C.E., Svendsen, PA_A, Heding, L: Effect of short-term glucagon infusion on kidney function in normal man. Diabetologia 13:323-325, 1977.

61. Sandahl Christiansen, J., Gammelgaard, J., Frandsen, M., Orskov, H., and Parving, H.-H: Kidney function and size in type I (insulin dependent) diabetic patients before and during growth hormone administration for one week. Diabetologia 22:333-337, 1982.

62. Christlieb, A.R: Renin, angiotensin, and norepinephrine in alloxan diabetes. Diabetes 23:962-970, 1974.

63. Christensen, N.J: Plasma norepinephrine and epinephrine in untreated diabetics, during fasting and after insulin administration. Diabetes 23:1-8, 1974.

64. Harrison, H.E., Reece, A.H., and Johnson, M: Decreased vascular prostacyclin in experimental diabetes. Life Sci. 23:351-356, 1978.

65a. Sullivan, S., and Sparks, H.V: Diminished contractile response of aortas from diabetic rabbits. Am. J. Physiol:Heart Circ. Physiol. 236(2):H301-H306, 1979.

65b. Silberbauer, K., Schernthaner, G., Sinzinger, H., Piza-Katzer, H., and Winter, M: Decreased vascular prostacyclin in juvenile onset diabetes. N. Engl. J. Med. 300:366-367, 1979.

66. Johnson, M., Reece, A.H., and Harrison, H.E: An imbalance in arachidonic acid metabolism in diabetes. Adv. Prostaglandin Thromboxane Res. 8:1283-1286, 1980.

67. Simpson, L.O: Further views on the basement membrane controversy. Diabetologia 21:517-519, 1981.

68. Lowe, G.D.O., Lowe, J.M., Drummond, M.M., Reith, S., Belch, J.J.F., Kesson, C.M., Wylie, A., Foulds, W.S., Forbes, C.D., MacCuish,A.C., Manderson, W.G: Blood viscosity in young male diabetics with and without retinopathy. Diabetologia 18:359-363, 1980.

69. Skovberg, F., Nielsen, AaV., Schlictkrull, K., Ditzel, J: Blood viscosity in diabetic patients. Lancet 1:129-131, 1966.

70. Schmid-Schonbein H., Volger, E: Red cell aggregation and red cell deformability in diabetes. Diabetes 25:897-902, 1976.

71. DeChatel, R., Weidmann, P., Flammer, F., Ziegler, W.H., Beretta-Piccoli, C., Vetter, W., Reubi, F.C.: Sodium, renin, aldosterone, catecholamines, and blood pressure in diabetes mellitus. Kidney Internat 12:412-421, 1977.

72. Christlieb, A.R., Janka, H.U., Kraus, B., Gleason, R.E., Icasas-Cabral, E.A., Aiello, L.M., Cabral, B.V.,Solano, A: Vascular reactivity to angiotensin II and to norepinephrine in diabetic subjects. Diabetes 25:268-274, 1976.

73. Weidmann, P., Beretta-Piccoli, C., Keusch, G., Gluck, Z., Mujagic, M., Grimm, M., Meier, A., Ziegler, W.H: Sodium-volume factor, cardiovascular reactivity and hypotensive mechanism of diuretic therapy in mild hypertension associated with diabetes mellitus. Am. J. Med. 67:779-784, 1979.

74. Beretta-Piccoli, C., Weidmann, P: Exaggerated pressor responsiveness to norepinephrine in nonazotemic diabetes mellitus. Am. J. Med. 71:829-835, 1981.

75. Mogensen, C.E: Progression of nephropathy in long-term diabetics with proteinuria and effect of initial anti-hypertensive treatment. Scand. J. Clin. Lab. Invest. 36:383-388, 1976.

76. Takazakura, E., Nakamoto, Y., Hayakawa, H., et al: Onset and progession of diabetic glomerulopathy. Diabetes 24:1, 1975.