Medical Staff Conference

The Pathogenesis and Prevention of Diabetic Nephropathy

These discussions are selected from the weekly staff conferences in the Department of Medicine, University of California, San Francisco. Taken from transcriptions, they are prepared by Drs Homer A. Boushey, Associate Professor of Medicine, and David G. Warnock, Associate Professor of Medicine, under the direction of Dr Lloyd H. Smith, Jr, Professor of Medicine and Associate Dean in the School of Medicine. Requests for reprints should be sent to the Department of Medicine, University of California, San Francisco, School of Medicine, San Francisco, CA 94143.

LLOYD H. SMITH, JR, MD:* *When insulin was discovered, more than 60 years ago, it was at first assumed that diabetes mellitus would no longer be a lethal disease. The treatment would be simply by hormone replacement and would be as successful as the treatment of myxedema by thyroid extract. This, of course, has not turned out to be the case. Diabetic patients, although rarely dying of ketoacidosis, are heir to severe complications in the vascular system, often with devastating results in the heart, retina, nerves and kidneys. In this conference Dr Rodney Omachi will review for us the current concepts of how diabetes injures the kidney and what can be done to prevent, or at least to slow, this process.*

RODNEY OMACHI, MD:† Diabetic nephropathy is now, or soon will be, the most frequent cause of end-stage renal disease in the United States. It accounted for about 25% of all new cases of end-stage renal disease as of 1980, when its incidence was increasing and approaching that of all types of glomerulonephritis combined.[1] Patients with insulin-dependent diabetes have a 40% risk for diabetic nephropathy, which most frequently develops after 10 and before 20 years of diabetes. The yearly incidence declines after 20 years, suggesting that susceptibility to this complication is predominantly in this subset of about 40% of the total population.[2] In patients with maturity-onset diabetes, the prevalence of a urine protein level of over 500 mg a day reaches 35% after 20 years. Although these older patients often die of diabetic complications other than uremia,[3] the highest incidence of end-stage diabetic nephropathy is in the 45- to 64-year age group, and the incidence in the over-65-year age group is twice as high as in the 14- to 44-year age group.[1] Our experience at the University of California, San Francisco, confirms this high incidence and this age distribution. Of 22 consecutive cases of diabetic nephropathy presenting with end-stage renal disease, the onset of our patients' diabetes was before age 30 in a third, between ages 30 and 40 in a third and over age 40 in a third. The median age at initiation of dialysis was 58. Life-table analysis showed a 50% mortality after three years on dialysis, which is disappointing but better than the results generally reported. In short, diabetic nephropathy is a frequent cause of renal failure and strikes patients of all ages. Fortunately, in the past five years there have been major advances in our understanding of the pathogenesis of diabetic nephropathy and the development of a potential both to prevent it and to arrest its progression.

The Stages of Diabetic Kidney Disease

Figure 1 is a map of the two routes that patients with diabetes currently follow, with an increasing duration of diabetes from left to right and increasing severity of renal involvement from top to bottom.[4,5] Microalbuminuria is defined as albuminuria that is below the level of routine chemical detection—about 150 mg per day—but abnormal and detectable by more sensitive immunoassay. Renal function is abnormal at the onset of diabetes but is supranormal rather than impaired. Increased glomerular capillary pressure and glomerular filtration rate accompanied by microalbuminuria occur during the severe hyperglycemia of uncontrolled diabetes and partially abate with routine control of the hyperglycemia. By the time diabetes has been diagnosed, the kidneys are enlarged but the glomeruli are histologically normal. After only three to five years of diabetes, mild diabetic histologic changes have occurred in virtually all persons with diabetes. Glomerular capillary basement membranes are abnormally thick, and the glomerular mesangium is increased in area. These changes are milder but similar in kind to the changes of overt diabetic nephropathy shown in Figure 2. Microalbuminuria may be precipitated by exercise or severe hyperglycemia but is not present at rest. Most patients with insulin-dependent diabetes continue with these mild changes without increasing severity, but in a substantial minority the disorder progresses to a stage called incipient diabetic nephropathy. These patients have no measurable histologic progression on renal biopsy, but certain renal functional features

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ABBREVIATIONS USED IN TEXT

Ig = immunoglobulin
PG = prostaglandin

indicate their high risk of progressing to overt diabetic nephropathy and also appear important in the pathogenesis of overt nephropathy. These functional features are a more extremely elevated glomerular filtration rate, microalbuminuria at rest and a tendency to arterial hypertension. Intensive insulin therapy in different studies does or does not result in reversal of the hyperfiltration and microalbuminuria, but the natural history with routine diabetic care is progression within ten years to overt diabetic nephropathy, defined operationally as persistent clinical proteinuria in the absence of evidence of other renal disease. Overt nephropathy is characterized by expansion of the mesangium to occupy more than 37% of the total glomerular area, illustrated in Figure 2, and by inexorable loss of renal function, with half of the patients reaching end stage within ten years. The rate of this progression is not slowed by intensive control of diabetes but is slowed by control of secondary hypertension, which becomes overt at this stage. The evidence for these stages in diabetic kidney disease has been developed in insulin-dependent diabetes mellitus, but I will mention its applicability to non-insulin-dependent diabetes. In each stage there is an interplay between the metabolic abnormalities of diabetes and the resultant hemodynamic abnormalities in the kidneys that, at the critical stage of incipient nephropathy, can result in self-perpetuating renal damage.

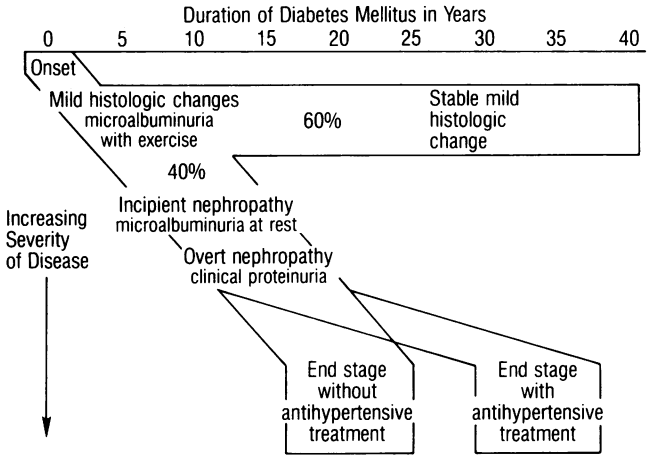

Figure 1.—A map of the renal courses which insulin-dependent diabetics currently follow.

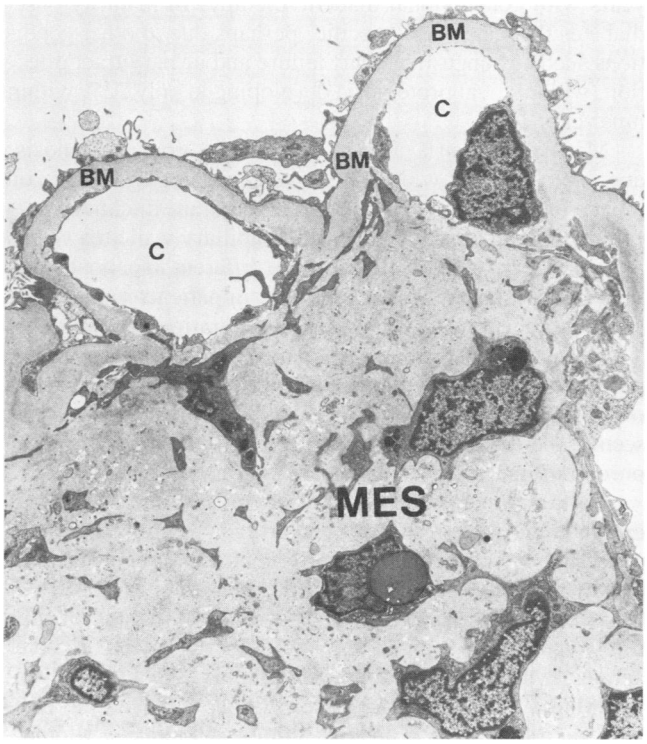

Figure 2.—Overt diabetic nephropathy. An electron micrograph (× 5,000) of a typical glomerulus showing a greatly expanded mesangial area **(MES)** encroaching on the capillary lumina **(C)**, and threefold thickening of the peripheral capillary basement membrane **(BM)**. The capillary lumen is separated from the mesangium only by fenestrated endothelium and not by basement membrane.

The mechanism of proteinuria differs at each stage in this map.[6] Microalbuminuria occurs at onset (associated with extreme hyperglycemia), with mild histologic changes (precipitated by exercise) and with incipient nephropathy at rest. This microalbuminuria is initially accompanied by a proportional increase in renal clearance of immunoglobulin (Ig)G. This conforms with microalbuminuria being due to increased glomerular pressures and fluxes rather than intrinsic changes in the glomerular membrane. With increasing microalbuminuria at rest, proteinuria then becomes selective for negatively charged albumin, consistent with a loss of the negative ionic charge on the glomerular filtration barrier. In human and experimental diabetes, glomeruli show decreased anionic sialic acid and heparan sulfate incorporation into glycoproteins and proteoglycans. Therefore, in the stage of incipient nephropathy the hemodynamically induced microalbuminuria may become compounded by this biochemical membrane defect. Finally, when renal failure develops, increased leakage of IgG and other large molecules into the urine occurs, consistent with the development of isolated membrane defects. At least in part, this accounts for the appearance of massive and nonselective proteinuria as a terminal event.[7]

The Kidney at Onset of Diabetes

When diabetes mellitus is first diagnosed, the glomerular filtration rate is already elevated 30% to 40% above normal.[8,9] This is not due to any increase in extracellular fluid volume.[8] Plasma glucagon and growth hormone levels are often elevated, and infusion of these hormones in normal persons results in increased glomerular filtration, but these hormones are not the major mediators of this effect of hyperglycemia. About half of this increase in the glomerular filtration rate reverses with only eight days of near-normal metabolic control, despite no change in plasma glucagon or growth hormone levels.[9] The remaining hyperfiltration may be a result of a 30% increase in kidney size, which does not reverse with short-term metabolic control, although in diabetic rats the hypertrophy is prevented by tight control.[10]

Glomerular prostaglandins appear to be major mediators of the hyperfiltration of diabetes. Isolated perfused rat kidneys respond to severe increases in perfusate glucose levels with immediate and sustained vasodilatation and increased glomerular filtration. This response is prevented by blockade

of prostaglandin synthesis.[11] Glomeruli and cultured mesangial cells isolated from diabetic rats produce increased amounts of prostaglandin (PG) E_2, and urinary excretion of PGE_2 is increased in diabetic rats. In patients with short-term diabetes, inhibition of prostaglandin synthesis by salicylate reverses the increased glomerular filtration.[12]

Microalbuminuria, as a consequence of hyperfiltration, is present before insulin treatment and abates after only a few days of insulin therapy.[13] Exercise increases the microalbuminuria, but even this increase is reversed by insulin treatment for two weeks.[14]

Mild Diabetic Changes

In human diabetes, peripheral capillary basement membrane thickening and expansion of mesangial matrix are absent at the onset of diabetes but are discernible after only two years. After five years of diabetes, basement membrane thickness is increased by 30%.[15] Nondiabetic identical twin offspring of patients with diabetic nephropathy have glomerular basement membranes of normal thickness, whereas the thickness of quadriceps muscle capillary basement membranes in nondiabetic and diabetic twins exceeds normal in about a third of both groups. Glomerular basement membranes are thickest in those persons with diabetes with thickened muscle capillary basement membranes.[16] Whether overt diabetic nephropathy is more likely to develop in these patients is not known. Thickening of glomerular basement membranes and mesangial matrix also occurs in most patients with diabetes due to pancreatic disease[17] and in rats, dogs and monkeys with experimentally induced diabetes. Moreover, thickening of glomerular basement membranes and mesangial matrix as well as hyalinosis of both afferent and efferent arterioles, specific for diabetes, will develop in nondiabetic kidneys transplanted into diabetic recipients.[18] Studies so far have reported an absence of these changes in renal transplants done along with successful pancreatic transplantation.[19] Therefore, the diabetic state is necessary and sufficient for the development of these mild histologic changes, but genetic factors may modulate the degree of change.

The mechanism of these histologic changes has been elucidated in rats with streptozocin-induced diabetes. The renal pool size of uridine triphosphate and diphosphate glycosides is moderately increased.[20] These are substrates used in the synthesis of glycoproteins, which are the major components of glomerular capillary basement membrane and mesangial matrix. A variety of enzymes involved in glomerular protein glycosylation have been shown to be present in increased activity,[21] and glycosylation of glomerular basement membrane and mesangial protein has been shown to be increased.[22] After six to eight months of experimental diabetes, glomerular basement membrane thickness increases by 50% and the volume of mesangial matrix is increased. In part, the mesangial thickening and increase in mesangial cells may also be a reflection of mesangial injury resulting from changes in glomerular hemodynamics. As shown in Figure 2, the mesangium is separated from the glomerular capillary lumen only by the endothelium and not by glomerular basement membrane, and plasmic flow carries circulating macromolecules through mesangial channels.[23] IgG, IgM and C3 enter the mesangium in this way in experimentally induced diabetes. After pancreatic islet cell transplantation, their deposition is rapidly reduced, and subsequently mesangial thickness returns toward normal.[24]

In cases of diabetes, kidneys with mild histologic changes are characterized functionally by progression of the hyperfiltration and hypertrophy present at diagnosis of diabetes. Poor metabolic control is positively correlated with higher glomerular filtration,[25] which is positively correlated with greater renal size.[26] Intensive metabolic control for one year substantially reverses the hyperfiltration but not the renal hypertrophy, so that a return to conventional insulin therapy immediately results in the return of hyperfiltration.[27] In contrast to the situation at the onset of diabetes, conventional insulin therapy at the stage of mild histologic changes does not prevent microalbuminuria with levels of exercise that do not cause microalbuminuria in normal controls.[14] Microalbuminuria apparently does not develop in children with exercise, in contrast to the case with adults, after a comparable duration of diabetes.[28] Perhaps this is related to the observation that, in juvenile-onset diabetes, the prevalence of diabetic nephropathy correlates better with attained age than with the duration of diabetes.[2] This suggests that some process resulting in microalbuminuria with exercise and necessary for the development of overt nephropathy usually begins with puberty.

Incipient Diabetic Nephropathy

Microalbuminuria at rest of a level greater than 15 to 30 μg per minute, unlike exercise-induced microalbuminuria, develops in only a minority of persons with insulin-dependent diabetes and indicates, with high sensitivity and specificity, that overt diabetic nephropathy will develop within 10 to 14[4,5] years with conventional diabetic therapy. In maturity-onset diabetes the test is less specific, perhaps due to other conditions such as congestive heart failure and urinary tract infection, with clinical proteinuria developing in only 22% within nine years.[3]

Microalbuminuria at rest, despite its grave prognosis, does not correlate with more severe histologic changes on renal biopsy. Glomerular basement membrane thickness, percent mesangial area and peripheral capillary wall area versus mesangial capillary wall area are no different in patients with microalbuminuria at rest from those in patients without microalbuminuria matched for age and duration of diabetes.[29] This absence of correlation is particularly striking because percent mesangial area correlates well with the presence of overt diabetic nephropathy. The still mild histologic changes seem to be only a necessary background on which overt nephropathy develops.

Certain functional characteristics associated with microalbuminuria at rest appear to trigger the progression to overt diabetic nephropathy. Early in the development of microalbuminuria, at levels below 70 μg per minute, glomerular filtration is more extremely elevated as compared with the mild hyperfiltration in patients of similar duration of diabetes and age who do not have microalbuminuria and do not progress to have clinical proteinuria.[5] In patients with more severe microalbuminuria and then clinical proteinuria, glomerular filtration falls back to normal and then subnormal. Arterial pressure is also significant—though not in the overtly hypertensive range—in the group with microalbuminuria.[5,30]

Several observations suggest that metabolic control of diabetes is a major factor in whether incipient and then overt

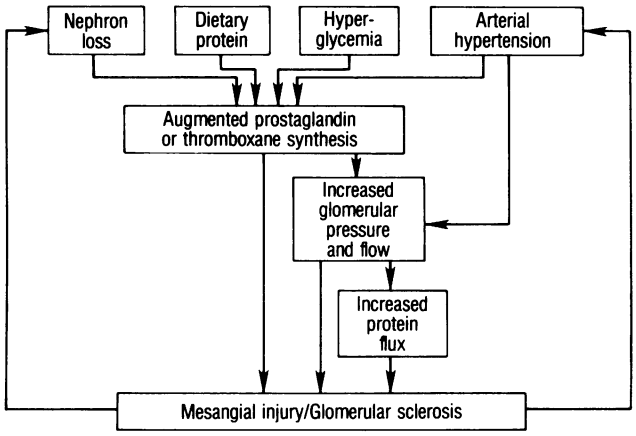

Figure 3.—Proposed mechanism resulting in glomerular sclerosis.

diabetic nephropathy will develop. As mentioned above, experimental hyperglycemia in isolated kidneys and in vivo can produce the hemodynamic renal changes, which can then produce microalbuminuria. Patients with microalbuminuria on average have higher glycosylated hemoglobin and mean plasma glucose levels on 24-hour profiles than patients without microalbuminuria.[6,30] Moreover, the risk of developing clinical proteinuria is considerably greater[2] and the duration of diabetes until the development of proteinuria is shorter[31] in patients with more poorly controlled diabetes compared with patients with better controlled diabetes of similar age and duration of disease. Clinical proteinuria has also been reported to reverse with pancreatic islet transplantation in diabetic rats with unilateral nephrectomy[32] and in two diabetic human kidneys transplanted into nondiabetic recipients.[33] In some controlled studies, intensive insulin therapy has resulted in rapid reversal of microalbuminuria,[6] and this improvement is sustained at least eight months.[34] Another controlled study, however, has shown no improvement in microalbuminuria with similar strict metabolic control in similar patients followed for one year. This latter study also did not find the reduced glomerular filtration during strict metabolic control reported in other studies, and the patients had lower initial glomerular filtration rates than might be expected.[35] It might be speculated that the patients in the latter study had already experienced a decline in their glomerular filtration rate and that the metabolically influenced mechanism of glomerular hyperfiltration and microalbuminuria had been lost. This leads us now to consider the nonmetabolic factors in diabetes that may result in progression of nephropathy.

Role of Hemodynamic Factors

Brenner and colleagues[36] first presented many of the concepts in Figure 3 to explain why chronic renal failure tends to progress inexorably, even in models of surgical ablation of renal mass in which the remaining nephrons are normal. A loss of nephron mass leads to compensatory hyperfunction in the remaining nephrons, which in the short run tends to maintain renal function. This sustained single-nephron hyperfiltration, however, or some determinant thereof, appears to be ultimately detrimental to the glomerulus. Glomerular capillary pressure is determined by intra-arterial pressure and by the ratio of efferent (postglomerular) arteriolar resistance to afferent (preglomerular) resistance. Hyperglycemia, dietary protein intake and arterial hypertension, as well as reduced renal mass, result in an increase in single-nephron glomerular filtration and glomerular capillary pressures and flows. This augments the glomerular transcapillary convective flux of plasma proteins and is reflected in the development of microalbuminuria in incipient diabetic nephropathy. The plasma proteins thus leaked accumulate in the mesangium by plasmic flow, mentioned above, stimulating proliferation of mesangial cells and matrix and leading to glomerular sclerosis. Glomerular hypertension may also cause glomerular injury directly. The resulting glomerular sclerosis causes progressive arterial hypertension and compensatory hyperfiltration of the remaining glomeruli, thereby closing a positive feedback loop favoring progressive glomerular injury. The theory fits well with the features of incipient diabetic nephropathy mentioned above: extreme elevation in the glomerular filtration rate, elevation in filtration fraction (reflecting increased glomerular capillary pressure), sustained microalbuminuria and often significant arterial hypertension. The theory also leads to certain predictions that have now been tested experimentally in rats made diabetic with streptozocin.

The first prediction is that changes in dietary protein will affect the development of diabetic nephropathy. If diabetic rats are given a high-protein diet, their kidney weight, glomerular capillary pressure and glomerular filtration rate are higher than in nondiabetic rats on high-protein diets or in diabetic rats on low-protein diets. The most striking abnormality is increased glomerular capillary pressure due to disproportionate afferent arteriolar dilatation, which is absent in the other groups. As predicted, notable progressive albuminuria, mesangial expansion and glomerular sclerosis develop in only the diabetic rats on a high-protein diet.[37] Like hyperglycemia, dietary protein loading increases glomerular filtration in association with augmented production of glomerular prostaglandins and thromboxanes.[38]

Another prediction of the theory is that a reduced renal mass would predispose to the development of diabetic nephropathy. Unilateral nephrectomy plus induction of diabetes in rats results in the development of mesangial matrix thickening within one year, whereas neither maneuver alone does so.[39] Moreover, successful pancreatic transplantation does not result in reversal of the mesangial thickening in rats with one kidney removed, whereas it does reverse the mesangial thickening that develops after a year in diabetic rats with intact renal mass.[40] Renal prostaglandin synthesis is increased in cases of chronic renal failure and with dietary protein loading and hyperglycemia.[41]

The final prediction of the theory is that pharmacologic normalization of glomerular hemodynamics would prevent the development of diabetic nephropathy. The angiotensin I-converting enzyme inhibitor enalapril reduces systemic blood pressure and normalizes the glomerular capillary pressure in diabetic rats on normal protein diets. It also prevents the progressive mild albuminuria and the mild glomerular sclerosis that develops at 14 months.[42]

An alternative interpretation of the above data on the effects of dietary protein, enalapril and a reduced renal mass on diabetic nephropathy is that they may have effects mediated directly by prostaglandin or thromboxane synthesis (or both) rather than through hemodynamic changes. Aspirin prevents both the increase in PGE_2 synthesis and the progressive de-

cline in glomerular filtration rate in long-term diabetes in rats.[43] In some nondiabetic models, recent preliminary reports, so far in abstract only, have dissociated a single-nephron glomerular filtration rate from the development of glomerular sclerosis. In experimental glomerulonephritis in rats, high protein intake does not increase the glomerular filtration rate but does result in increased glomerular prostaglandin and thromboxane synthesis and increased proteinuria. In this model, enalapril reduces glomerular prostaglandin and thromboxane synthesis[38] and reduces proteinuria but does not reduce the glomerular filtration rate.[44] Accelerated glomerular sclerosis develops in spontaneously hypertensive rats on a high-protein diet. Enalapril reduces the glomerular sclerosis without reducing the glomerular capillary pressure and with an increase in the glomerular filtration rate and blood flow. This beneficial effect of enalapril is not simply due to reduced arterial pressure. Hydralazine also reduces arterial pressure and increases the glomerular capillary pressure, glomerular filtration rate and blood flow but increases glomerular sclerosis in this model.[45]

Hyperglycemia, dietary protein, arterial hypertension and loss of renal mass thus all appear to predispose to the development of progressive glomerular sclerosis experimentally through changes either in glomerular hemodynamics or in prostaglandin or thromboxane synthesis. The above findings have now been found to have clinical relevance in overt diabetic nephropathy, a stage in which manipulation of factors other than metabolic control appears promising.

Overt Diabetic Nephropathy

Once persistent proteinuria develops due to diabetic nephropathy, conventional treatment cannot prevent a subsequent inexorable loss of renal function.[2] The structural feature best correlating with overt diabetic nephropathy is mesangial enlargement. In one series of patients with insulin-dependent diabetes studied by renal biopsy, when mesangium accounted for more than 37% of total glomerular volume, persistent proteinuria and hypertension were invariably present. Moreover, the absolute peripheral capillary filtering surface increased with mild mesangial expansion, was greatest when mesangium accounted for 27% to 37% of the total area and then fell off considerably when the mesangium was more than 37%.[29] Thus, at this stage hypertension and a reduction in surface area for glomerular filtration predispose to inexorable progression.

Prospectively, intensive metabolic control has been shown effective in preventing deterioration in renal function in only one study, in patients with background retinopathy with a normal creatinine clearance not characterized as to proteinuria.[46] In one retrospective study, the duration of persistent proteinuria until the development of elevated creatinine concentrations was correlated with a median postprandial blood glucose concentration, but once the serum creatinine level was elevated, a decay in renal function was not correlated with the blood glucose.[31]

There is good evidence that controlling hypertension effectively preserves renal function in patients with diabetic nephropathy. In retrospective studies, the rate of decay in renal function varies greatly among patients, is constant with time in any one patient and is faster in patients with persistent hypertension[31] and in those whose hypertension is less well controlled.[47] Statistically significant increases in blood pressure are present even in incipient diabetic nephropathy, and overt hypertension is present in most insulin-dependent patients with overt nephropathy with a normal serum creatinine level.[48] Aggressive antihypertensive treatment results in a decrease in proteinuria and slowing of the average rate of decline in glomerular filtration rate from 0.84 to 1.23 ml per minute per month without treatment to 0.39 to 0.49 ml per minute per month with treatment both in historically controlled[49,50] and prospective randomly controlled[51] studies. At these rates, the time required for deterioration of the glomerular filtration rate from 110 ml per minute to 10 ml per minute would be 7 to 10 years without treatment and 17 to 23 years with aggressive treatment of hypertension.

It is not established at present which antihypertensive agents will best preserve renal function in diabetic nephropathy. In the studies cited above, metoprolol, hydralazine and a diuretic were used. Thiazide diuretics should probably have a major role in that the hypertension of early diabetic nephropathy is associated with sodium retention and increased pressor responsiveness to infused angiotensin and norepinephrine, and all of these are reversed by thiazides.[52] Clonidine decreases plasma norepinephrine levels and, at least in rats with three fourths of their kidney mass removed, is effective in retarding the progression of renal failure and proteinuria.[53] Clonidine also is effective in relieving some cases of diabetic diarrhea.[54] Hydralazine should perhaps be replaced by enalapril if the above findings in hypertensive rats on high-protein diets[45] can be applied to diabetic nephropathy. As mentioned above, the use of enalapril has resulted in better preservation of renal function in experimental diabetes.[42] This has been attributed perhaps to inhibition of angiotensin II-mediated uptake of macromolecules and disruption of their clearance from the mesangium[55] or perhaps to inhibited prostaglandin or thromboxane synthesis.[42] Captopril, another inhibitor of angiotensin-converting enzyme, has been reported in low doses to decrease proteinuria in some cases of azotemic diabetic nephropathy.[56] High-dose captopril should probably be avoided due to a slight risk of its inducing membranous glomerulonephritis superimposed on diabetic nephropathy.

One small study suggests that dietary protein restriction may be efficacious in human diabetic nephropathy and in experimental diabetes. Ten patients with chronic renal insufficiency from diabetes were studied on 1 to 1.5 grams per kg per day protein intake and then on 0.6 grams per kg per day. The urine protein level decreased by 3% to 77% at one month and by 22% to 83% at three months, the serum albumin level remained normal or increased to normal and the serum creatinine level and creatinine clearance remained stable for a mean of 6.7 months follow-up.[57]

Conclusions

In its early stages, diabetic nephropathy is characterized by mild histologic changes and striking functional changes that are caused by the metabolic abnormalities of diabetes and can be ameliorated by strict metabolic control. Within about ten years, microalbuminuria at rest appears in a substantial minority of persons with diabetes and, with conventional insulin therapy, is followed within ten years by overt diabetic nephropathy. Intensive insulin therapy may or may not reverse the microalbuminuria and the associated glomerular

hyperfiltration. Overt diabetic nephropathy initiates a vicious cycle, and at this stage metabolic control is less influential than treatment of arterial hypertension in determining the subsequent decline in renal function. Experimental evidence has accumulated supporting the mediating role of glomerular hemodynamics or prostaglandin or thromboxane metabolism in the progression of diabetic nephropathy. Results of clinical trials on the effects of long-term intensive insulin therapy in patients with microalbuminuria and on the effects of inhibitors of angiotensin-converting enzyme, dietary protein restriction and perhaps prostaglandin or thromboxane inhibitors are likely to revolutionize the prevention and treatment of diabetic nephropathy.

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