Acta Medica et Biologica
Vol. 38, (Suppl.), 81-91, 1990

Onset and Progression of Diabetic Glomerulosclerosis. A Pathogenetic Role of Segmentally Turbulent Glomerular Circulation.

Yasushi NAKAMOTO, Shigeki INOMATA*, Eisuke TAKAZAKURA, Tadashi YASUDA and Neil L. R. TATE*****

*First and Third Departments of Internal Medicine, Akita University School of Medicine, **Division of Medicine, Kurobe Municipal Hospital, Japan. ***English editor

Received October 12, 1989

Summary. **A total of 390 kidney specimens (361 biopsies and 29 autopsies) from 353 patients with diabetes mellitus were reviewed. There were 48 cases with IDDM (58 kidney specimens) and 305 cases with NIDDM (332 specimens). The diffuse and nodular lesions were graded by Gellman's criteria, and arteriolar hyalinosis by our previous criteria. We analyzed sequential features in the development of the diabetic renal injuries and compared them with IDDM and NIDDM. The diffuse lesion, which began to appear as segmental thickening of the mesangial area, was characterized by the deposition of strongly PAS-positive materials, most probably glycosylated serum proteins. As the diffuse lesion progressed to grades II and III, the small nodule was formed with the locally accentuated deposition of the glycosylated proteins. The blood flow of surrounding capillaries of the nodule frequently began to stagnate, and as long as the stagnation persisted, the capillary lumina were gradually enlarged and bulged with the eventual development of capillary microaneurysm filled with red blood cells. This sequence of events indicates that a segmentally turbulent intraglomerular circulation has occurred. The widened perinodular or perimesangial spaces with or without the capillary microaneurysm were slowly occupied again by the glycosylated proteins in a stratified pattern, ultimately leading to larger nodules. A few cases showed mesangial disintegration or mesangiolysis, but this did not result in the larger nodule, only to be recanalized. Fibrin cap was structurally identical to the capillary aneurysm, but lacked red blood cells in it. This difference seemed to be well explained by blood skimming processes; the former with accumulation of already skimmed plasma, and the latter with that of skimmed red blood cells. Thus, the segmental turbulence of glomerular circulation played an important role in the progression of diabetic glomerulosclerosis. This intraglomerular hemodynamic perturbation appeared to be enhanced by intraglomerular hypertension, especially in IDDM, and simultaneous hyalinosis of the afferent and efferent arterioles.**

INTRODUCTION

The kidney can develop a wide variety of functional and morphologic derangements under the environment of diabetes mellitus, especially of the insulin-dependent type.

Functionally, the glomerular filtration rate (GFR) often increases to supernormal levels and renal plasma flow (RPF) remains generally unchanged or with less of an increase, thereby resulting in an elevated filtration fraction (FF). This hyperfiltration has been attributed to the elevation of the transcapillary pressure gradient rather than to the increment of the glomerular filtration surface area as noted by Parving et al.[1] This has also been confirmed in moderately hyperglycemic diabetic rats with micropuncture techniques[2]. The increased transcapillary pressure enhances the permeability of the capillary walls which in turn induces diabetic microalbuminuria[3].

Morphologically, following the description of the nodular lesion by Kimmelstiel and Wilson[4], additional diabetic lesions in the kidney, i.e., diffuse, exudative, arteriolar lesions, and thickening of the glomerular and tubular basement membrane have been delineated. Moreover, glomerulocapillary and arteriolar microaneurysms in the diabetic kidneys

have recently been recognized[5,6].

However, it remains unclear how the functional alterations contribute to the pathomorphogenesis of various diabetic lesions, and, furthermore, whether or not there are any interrelations among these morphologic changes. In this report, we analyzed sequential features in the development of the diabetic renal injuries and compared them with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). We concluded that a segmental turbulence of glomerular circulation plays an important role in the progression of diabetic glomerulosclerosis.

MATERIALS AND METHODS

A total of 390 kidney specimens (361 biopsies and 29 autopsies) taken from 353 patients with diabetes mellitus were reviewed. Among them, 265 specimens (240 biopsies and 25 autopsies) from 230 patients were examined in Kanazawa University Hospital by two of the authors (E. T. and Y. N.) between 1962 and 1978, and their details have been described elsewhere[6-8]. The remaining 125 specimens were obtained from 123 patients in Akita University Hospital between August, 1979 and June, 1989. In those patients who underwent serial histological examinations, each specimen was separated by more than two-year intervals. The type of diabetes mellitus was determined by diabetologists (E.T. and S. I.), and there were 48 patients with IDDM (58 kidney specimens) and 305 patients with NIDDM (332 specimens).

All the kidney tissues were routinely processed, i.e., fixed in 10% phosphate-buffered formalin, embedded in paraffin, cut at a thickness of 2 μm and stained with hematoxylin and eosin (H & E), periodic acid Schiff (PAS), Heidenhain's azocarmine aniline blue (Azan), and Jone's periodic acid methenamine silver (PAM). Since each stained slide mounted five or more sections, in all, 20 or more sections were semi-serially evaluated. The degree of the diffuse and nodular lesions was graded on a scale of O through IV according to the criteria of Gellman et al.[9], and the extent of arteriolar hyalinosis was classified according to our criteria as previously described[8]. The exudative lesions were divided into the so-called capsular drop and fibrin cap. The capillary microaneurysm in the glomeruli was defined as a cystic dilation of the glomerular tuft with size of more than half the lobule, usually filled with red blood cells.

GFR and RPF were measured with the method of one-shot intravenous infusion (taking 10 to 15 min) of sodium thiosulfate and sodium paraaminohippurate, and FF was calculated. The results of this renal function test were adopted only when examined within one or two weeks before or after the renal biopsy.

Statistical analysis was performed using student t test or Fisher's exact test, and the data were expressed as mean±SEM. A p-value less than 5% was regarded as significant.

RESULTS

1. Sequential features in the development of glomerular pathology

The diffuse lesion, which began to apper as segmental thickening of mesangial area (Fig. 1A), was characterized by the deposition of strongly PAS-positive materials. At an advanced stage, it could induce a conspicuous mesangial expansion, often simulating small nodules (Fig. 1B). However, before progressing to such an extent, a majority of glomeruli managed

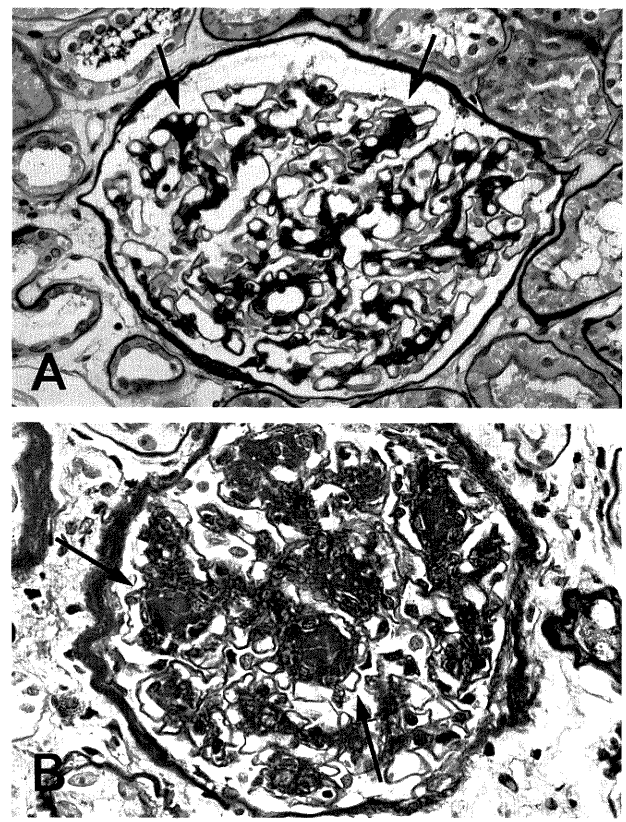


Fig. 1. The diffuse lesion. **A.** Grade I lesion. There is segmental thickening of mesangial area (arrows). PAS, ×400. **B.** Mesangial area extremely expanded by the deposition strongly PAS-positive substances, often simulating small nodules (arrows). PAS, ×400

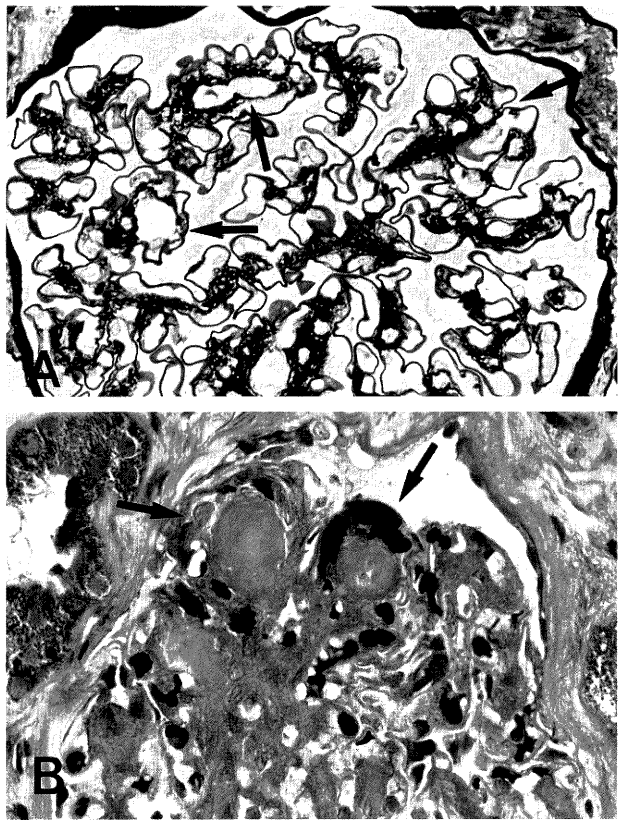


Fig. 2. Intraglomerular circulation at an early stage. **A.** Cracking or splitting of the mesangium (arrows), early adaptatory processes of intraglomerular circulation. PAM, ×400. **B.** There are two small nodules (arrows), and the surrounding capillaries are congested with rouleau-formed red blood cells in one of them (the right one). Azan, ×400

to maintain the intraglomerular circulation by cracking or splitting the thickened mesangium, which secured new routes for the circulation (Fig. 2A). Once the small nodule was formed with the locally accentuated deposition of the PAS-positive proteins, the blood flow of surrounding capillaries frequently stagnated, as judged from the presence of rouleau-formed red blood cells (Fig. 2B). As long as the stagnation of capillary circulation persisted, the capillary lumina were gradually enlarged and bulged (Fig. 3A), sometimes leading to large glomerular or capillary microaneurysms filled with red blood cells (Fig. 3B), although, in several cases, the widened lumina were occupied by foamy materials. In such situations, the nodularly expanded mesangium, from which the capillary microaneurysm was derived, was often concavely compressed (Fig. 3C), clearly indicating that the pressure within the microaneurysm was considerably elevated. Meantime, every effort was being made to sustain the intraglomerular segmental blood flow by utilizing all cracks or clefts even within the nodule (Fig. 4A). However, this effort was not rewarded on some occasions, since these newly opened but distorted canals were easily occluded, resulting in the retention of serum protein components (Fig. 4B), or foamy or fatty substances (Fig. 4C).

As the diffuse lesion progressed to grade II or III, the occurence of mesangial disintegration or mesangiolysis was occasionally observed. Fig. 5A shows an example of such a mesangiolytic focus with loosened mesangial matrices. Through a semi-serial observation, a majority of mesangiolytic changes were traced to be part of the perinodularly widened space of the nodule (Fig. 5B and C). Only in four instances did they not belong to the nodular lesion. This *de novo* mesangiolytic area was usually recanalized subsequently, and its development toward the nodular lesion was not confirmed. Then, the widened capillary spaces surrounding the nodule or the advanced diffuse lesion as a means of adaptation to maintain the disturbed intraglomerular circulation, were gradually filled up with stratified substances, thus leading to the larger or, in some, gigantic nodules (Fig. 6A and B). This newly formed part of the nodule was generally less PAS-positive than the original nodule or diffuse lesion. The capillary walls of the larger nodule expanded by massive contents were often ruptured, thereby spitting out the contents into Bowman's space (Fig. 6C). In this particular picture, the fibrillarly laminated structure indicates several directions of deposition and leakage.

Fibrin cap, which is one of the exudative lesions, was characterized by the retention of hyaline substances, probably of serum proteins, within the widened capillary lumina (Fig. 7A). Seen in PAM-stained section, this structure was consistent with the capillary microaneurysms (Fig. 7B). However, fibrin cap was different from the latter in that it contained little, if any, red blood cells.

2. Comparison between IDDM and NIDDM

Filtration fraction (FF). Fig. 8 shows a correlation of grades of the diffuse lesion with FF from patients in whom it was measured. Although the difference between IDDM and NIDDM patients was not statistically substantiated, FF of IDDM tended to be higher than that of NIDDM throughout all grades of the diffuse lesion. In addition, it is noteworthy that FF of both types of DM was more elevated than normal in patients with the grades 0 through II.

Glomerular hypertrophy. Glomerular size in IDDM

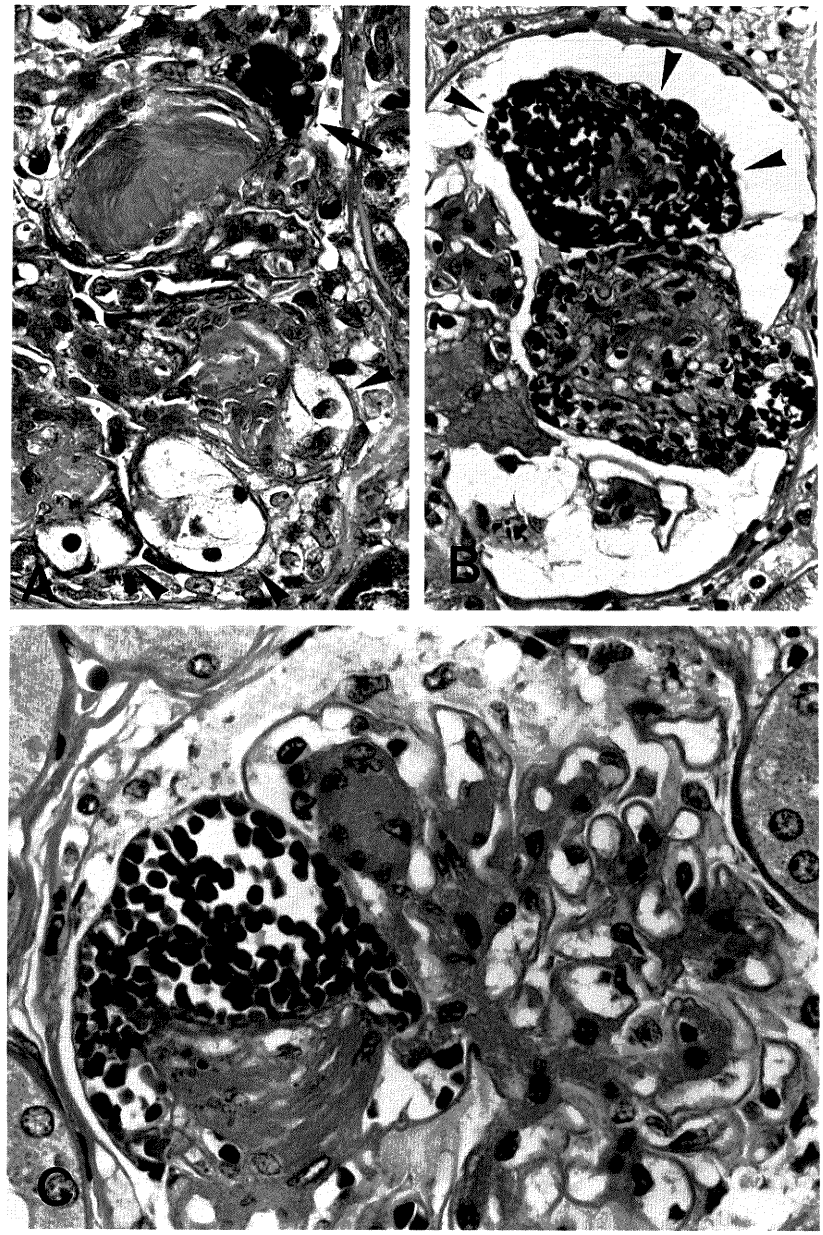


Fig. 3. Capillary microaneurysms. **A.** A small capillary aneurysm filled with red blood cells, adjacent to a nodule of moderate size (arrow). In the lower part, there are three widened lumina devoid of red blood cells but instead filled with foamy materials (arrow heads). Azan, ×400. **B.** A larger microaneurysm (arrow heads). H & E, ×400. **C.** A well-developed microaneurysm. The nodularly expanded mesangium, somewhat loosely composed, is concavely compressed. PAS, ×400

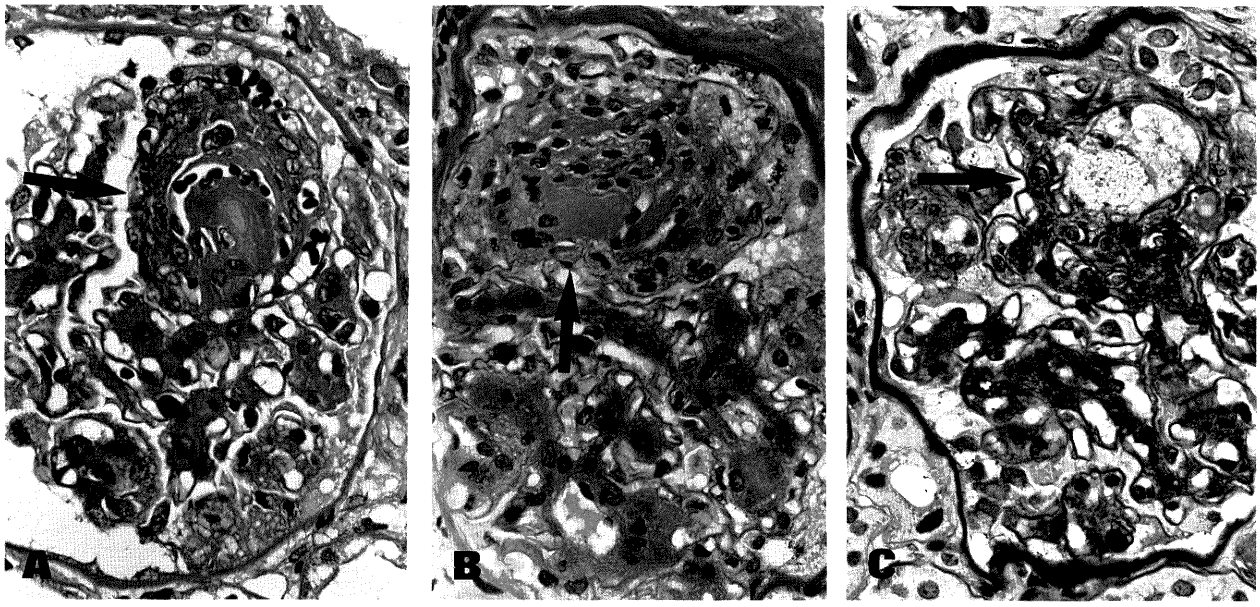


Fig. 4. Intraglomerular circulation at a later stage. **A.** Blood flow utilizing cracks or clefts within a nodule (arrow). H & E, ×400. **B.** The cracks were occluded by the retention of serum protein components (arrow). PAS, ×400. **C.** A crack was filled with foamy or fatty substances (arrows). This spherial expansion indicates an elevated intraluminal pressure. PAS, ×400

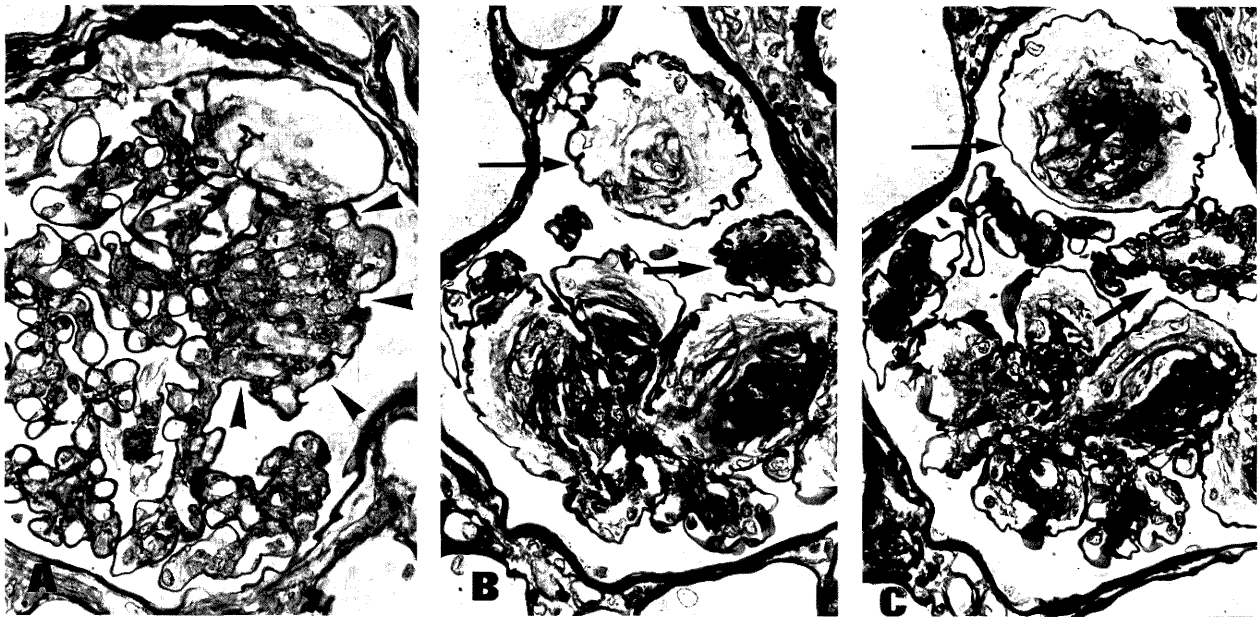


Fig. 5. Mesangiolysis. **A.** Mesangial disintegration or mesangiolysis (arrow heads). PAM, ×400. **B.** Another mesangiolytic focus (thin arrow) and an area of expanded mesangium (thick arrow). PAM, ×400. **C.** Subsequent section disclosed that the mesangiolytic site seen in B, was part of a nodule (thin arrow). This kind of perinodular disintegration (it can occur also around an extremely expanded mesangium) perhaps can best be termed peri-mesangiolysis. The previous mesangial expansion (thick arrows) was subsequently recanalized. PAM, ×400

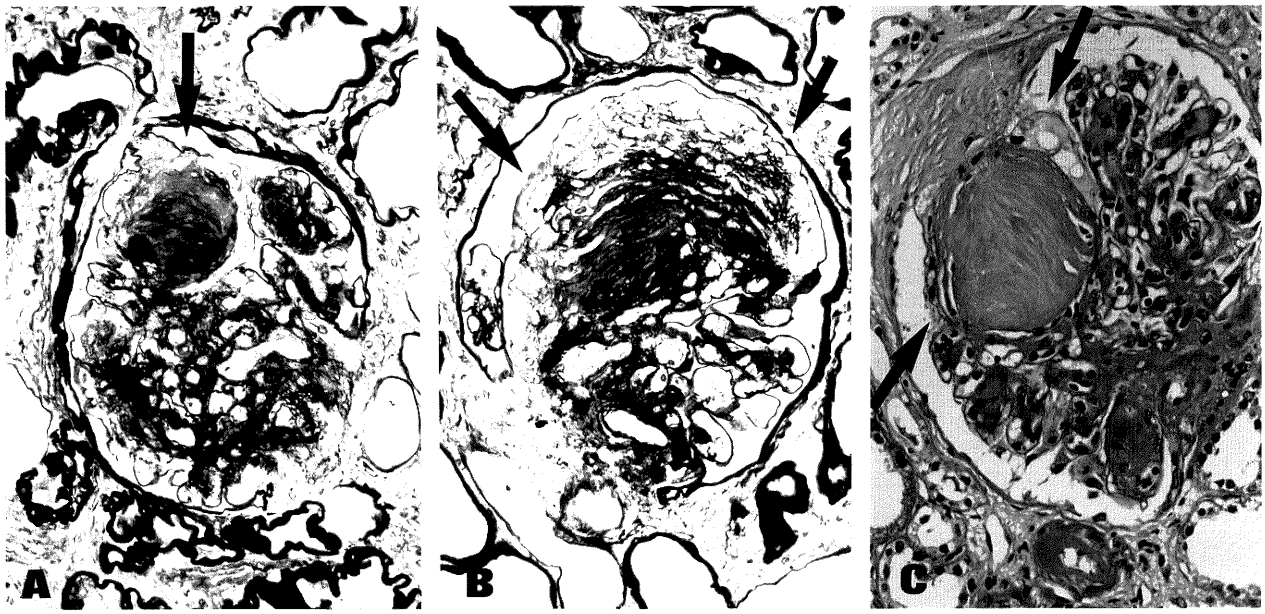


Fig. 6. The established nodular lesions. **A.** Accumulated materials (less darkly stained) in a layered fashion upon the original small nodule or expanded mesangium (more darkly stained) (arrow). PAM, ×320. **B.** A gigantic nodule is being formed (arrows), PAM, ×400. **C.** Another larger nodule (arrows). Note the direction of laminated structure within the nodule which indicates accumulation and leakage. H & E, ×400

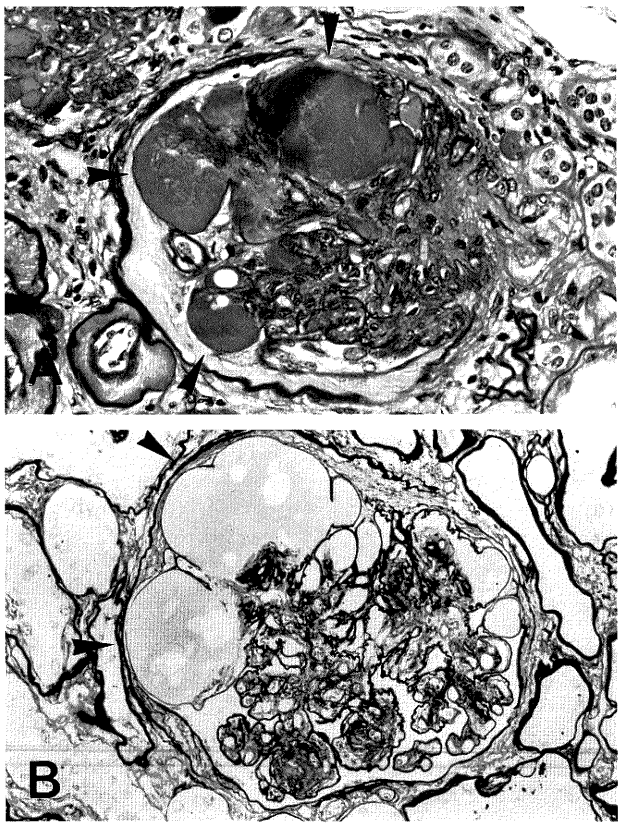


Fig. 7. Fibrin cap. **A.** Large fibrin caps composed of serum protein components with various densities and fat droplets (arrows). PAS, ×320. **B.** This structure was essentially identical to the capillary microaneurysm, but devoid of red blood cells. PAM, ×320

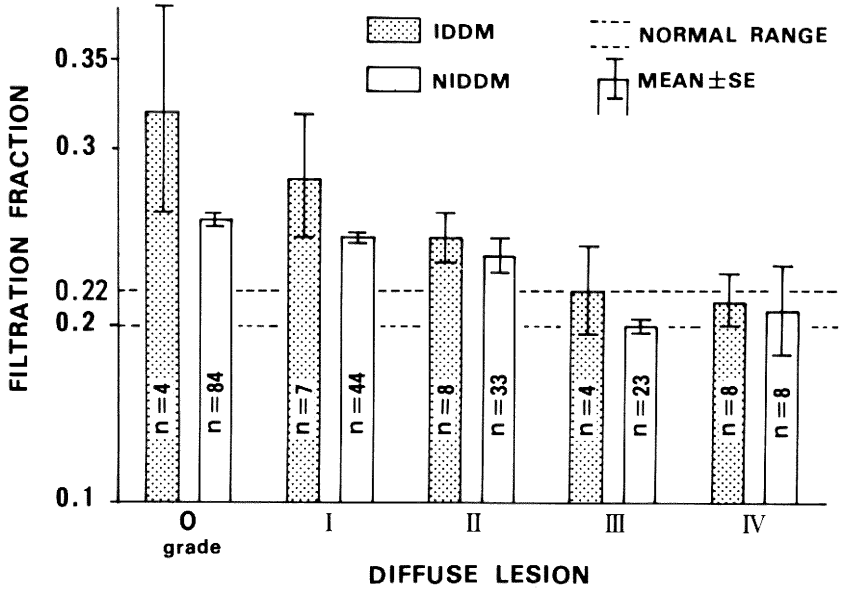


Fig. 8. A correlation of filtration fraction (FF) with the degree of the diffuse lesion.

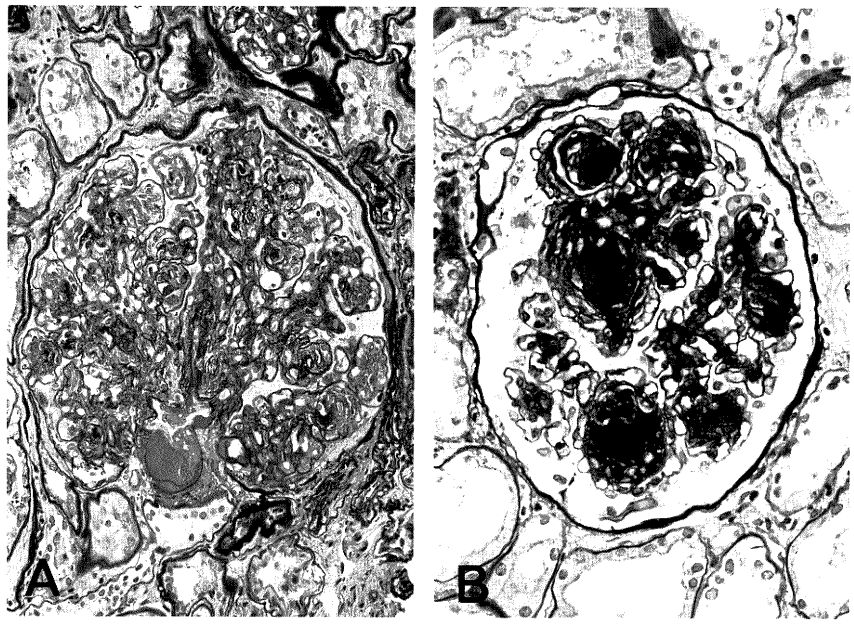


Fig. 9. Glomerular hypertrophy. **A.** Extreme glomerular hypertrophy seen in a case of IDDM. PAS, ×200. **B.** Ordinary glomerular size in a NIDDM patient. PAS, ×400

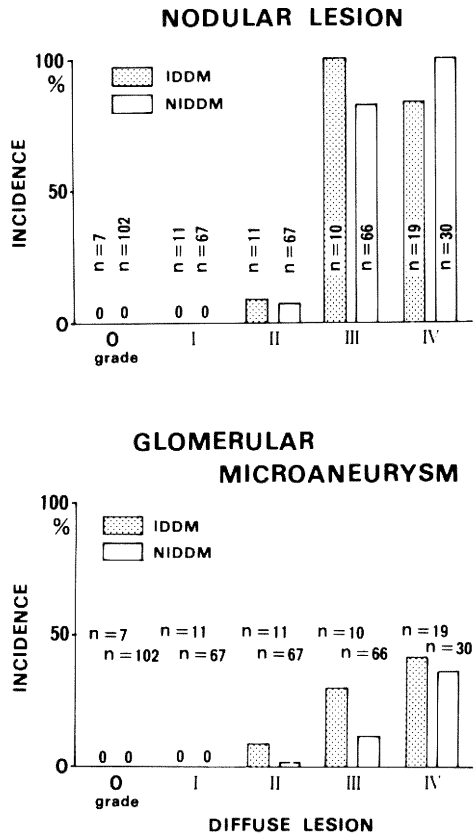


Fig. 10. The incidences of the nodular lesion and the glomerular capillary microaneurysm in relation to the degree of the diffuse lesion.

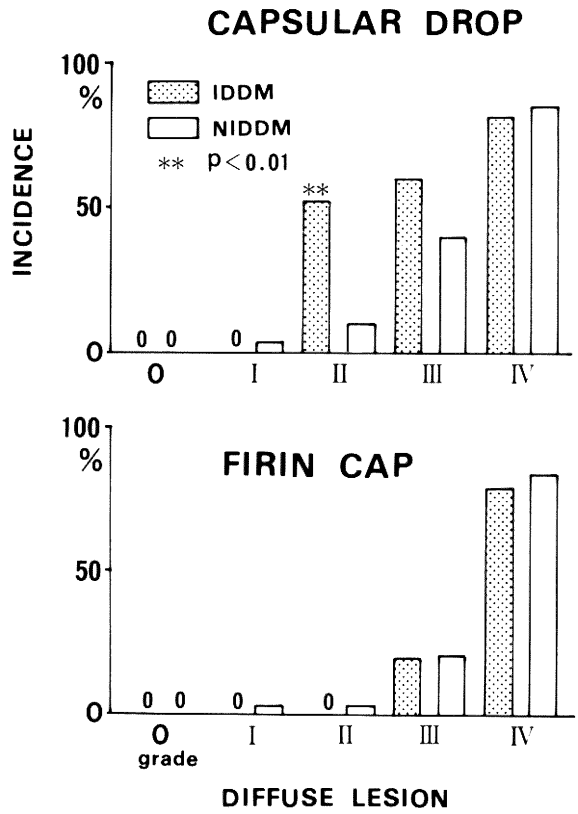


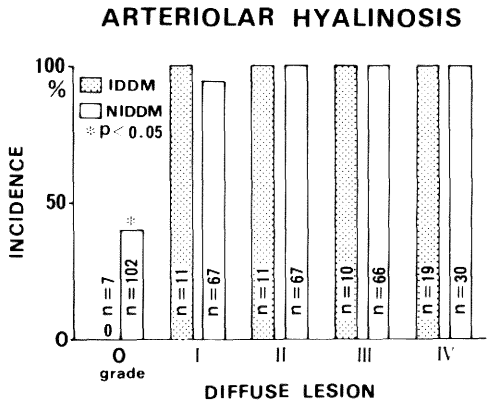
Fig. 11. The incidences of capsular drop and fibrin cap. The case number in each category is the same as in Fig. 10.

cases was widely scattered, and was not necessarily larger than that of NIDDM. Nevertheless, extreme glomerular hypertrophy (Fig. 9A) was exclusively observed in IDDM cases, as compared with ordinary glomerular size in NIDDM[10] (Fig. 9B).

The nodular lesion and glomerular microaneurysm. As seen in the upper column of Fig. 10, the nodular lesion began to emerge from grade II of the diffuse lesion and its incidence was precipitously increased in grades III and IV in both types of DM. However, a few patients progressed to the end-stage diffuse lesion without any nodular formation. The glomerular microaneurysm also started to be formed at the grade II and thereafter increased gradually in contrast to the nodular lesion, and the frequency of the microaneurysm remained less than 50% even at grade VI of the diffuse lesion. When compared with the two types of DM, IDDM subjects tended to show the microaneurysm more often than NIDDM, but the difference was not significant (Fig. 10, lower).

The exudative lesions. Capsular drop, one of the exudative lesions, was seen more frequently in IDDM, especially at grade II of the diffuse lesion, which was significant ($p<0.01$), although, thereafter, the difference was not statistically supported (Fig. 11, upper). Fibrin cap, another exudative lesion, developed in both types of DM in a similar fashion. Although fibrin cap iteself is a nonspecific marker of the end-stage kidney disease, its universality at grade IV of the diffuse lesion was rather characteristic of diabetic kidney disease (Fig. 11, lower).

Arteriolar hyalinosis. This was the constant accompanist of the diffuse lesion after its grade I in both types of DM. However, arteriolar hyalinosis preceded the diffuse lesion in about 40% of cases with NIDDM. This was significant ($p<0.05$) against patients with IDDM, probably reflecting a higher onset age of those with NIDDM (Fig. 12, upper). Simultaneous hyalinosis of the afferent and efferent arterioles, a characteristic finding of diabetic nephropathy, again commenced at grade II of the diffuse lesion regardless of diabetic type, and thereafter became more



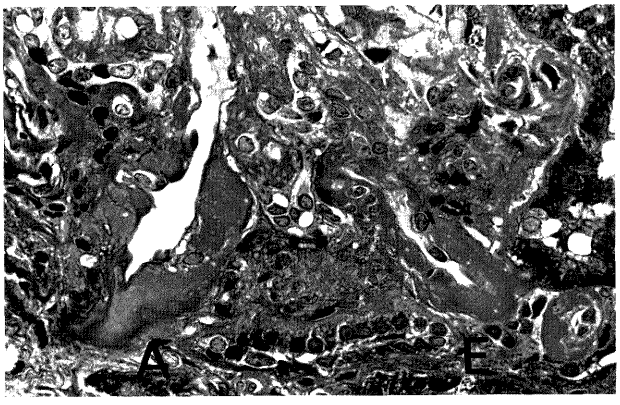


Fig. 12. Arteriolar hyalinosis. The upper part. The incidence of arteriolar hyalinosis in relation to the diffuse lesion. The lower part. Simultaneous hyalinosis of the afferent (A) and efferent (E) arterioles. Azan, ×400

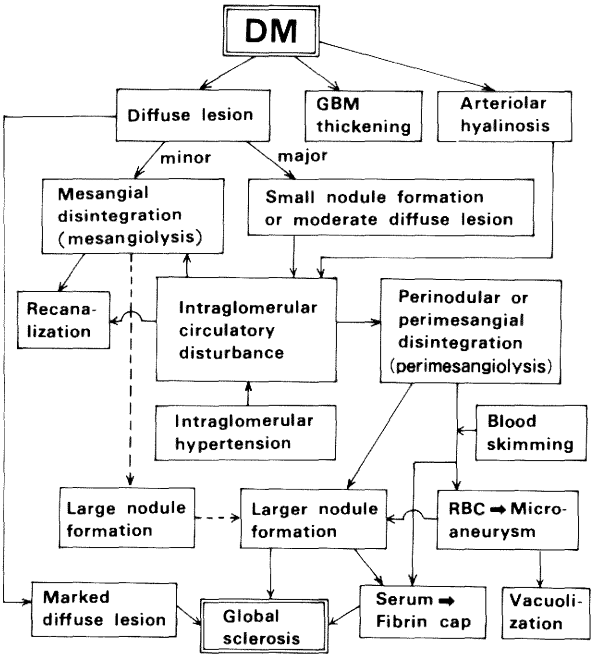


Fig. 13. The pathomorphogenesis of diabetic glomerulosclerosis. The dotted lines denote little, if any, probability.

prevalent like other diabetic lesions (Fig. 12, lower).

DISCUSSION

Based on the present and previous studies, the pathomorphogenesis of diabetic glomerulosclerosis can be depicted in Fig. 13. Diffuse mesangial expansion (the diffuse lesion), thickening of GBM and TBM and arteriolar hyalinosis constitute three major pathologic alterations of diabetic glomerulosclerosis[10-12]. Brownlee et al.[13] have emphasized that the central pathologic features of diabetic microvascular complications are caused by the hyperglycemia-accelerated formation of nonenzymatic advanced glycosylation end-products. A variety of proteins including hemoglobin A_{1c}. collagen fiber, albumin and some serum proteins, have been shown to undergo the glycosylation. Therefore, GBM thickening can be regarded as *in situ* glycosylation, and it is highly probable that the strongly PAS positive-substances causing the diffuse lesion and diabetic arteriolar hyalinosis are glycosylated serum proteins, i.e., glycoproteins. They remain unidentified and are unlikely to be albumin or its variants, since our preliminary study using immunofluorescence techniques was negative for albumin (unpublished data).

The present study focused on sequential features in the progression of the diffuse lesion. As it progresses to grades II and III, segmental accentuation of the mesangial expansion often occurs, leading to nodule formation. The same sequence of events has been observed in the cases of light chain nodular glomerulosclerosis and amyloid nephropathy[14-16]. The mechanisms of nodule formation as the segmental accentuation of deposition have not been clarified, although the molecular quality of amyloid protein was suggested to be a determinant in amyloid nephropathy[16,17]. In any events, a comparison with these diseases will lend support to the concept that diabetic glomerular lesions are under the rule of glomerular injuries induced by the substances of metabolic nature.

The expanded mesangium appears to impede the intraglomerular circulation, since adaptatory processes are soon set in motion. The initial step involves cracking or splitting of the thickened mesangium to

open new circulatory routes (this degree of mesangial splitting does not merit description morphologically as mesangiolysis), and this seems to act as an inhibitory agent to further nodule formation. However, once the nodule is established, or as the mesangial expansion progresses, despite such adaptatory efforts, the blood flow of the surrounding capillaries is further impaired. On rare occasions, although for unknown reasons, the expanded mesangium or small nodules may disintegrate (mesangiolysis) and, subsequently, be recanalized. It is unlikely that nodular formation develops from this type of mesangiolysis. On the other hand, in a majority of cases, segmentally stagnant circulation with increased capillary pressure can occur, thereby resulting in the formation of capillary microaneurysms or the detachment of capillary walls from the anchor points (peri-mesangiolysis). We call it segmental circulatory turbulence. The circulation within the widened perinodular or perimesangial space will remain slowed or stagnant, since, at this point, the glomerular architecture has been considerably distorted. Such stagnation or turbulence will enhance the precipitation in a layer pattern of the increased glycosylated serum proteins upon the original nodule or mesangium. Thus, the larger or gigantic nodules are formed.

Fibrin cap, which is structurally identical to the capillary microaneurysm, has a high prevalence at the end-stage of diabetic kidney disease. This finding is regarded as evidence that diabetic renal disease is prone to induce the microaneurysmal structure. However, there is difference in the contents between the capillary microaneurysm and fibrin cap; the former with red blood cells and the latter with serum protein components. This has not been well explained. From the present studies, it is suggested that there may be blood skimming phenomena within the glomeruli in view of the segmentally turbulent circulation as well as the highly distorted glomerular architecture. If an entering orifice of the blood flow is patent with a markedly stenosed outflow exit, the circulating blood will be separated, leaving red blood cells behind. This will develop the capillary microaneurysms. On the other hand, if an entering portion is already narrowed with a more stenosed exit, the skimmed plasma, with little, if any, red blood cells, will accumulate over a certain length of glomerular capillary, with a resultant fibrin cap. As a mechanism of the autoregulation of renal circulation, Pappenheimer and Kinter[18] in 1956 proposed the plasma skimming or cell separation theory, but this has not been widely accepted. Nevertheless, the blood skimming processes appear to reasonably explain the pathomorphogenesis of the capillary aneurysm and fibrin cap.

The glomerular capillaries are exposed to high blood pressure in the range of one-half of systemic arterial pressure. Therefore, a further rise in the intraglomerular pressure will have more impact on kidney function and structure than on other capillary systems. The intraglomerular hypertension theory proposed by Brenner et al.[19] has greatly contributed to our understanding of the pathophyshology of various glomerular diseases or disease states including diabetic glomerulosclerosis. The intraglomerular hypertension raises GFR and FF, and enhances the permeability of the capillary walls with consequent microalbuminuria. Capsular drop, protein substances deposited along Bowman's capsule, has been shown to reflect the increased permeability[14]. An earlier occurrence of capsular drop and a similar trend of capillary microaneurysms appear to be related to a tendency of higher FF in IDDM patients, although the latter is not statistically supported. Anyway, the intraglomerular hypertension seems to participate in onset and progression of diabetic glomerulosclerosis.

However, the intraglomerular hypertension theory is unable to point out the importance of segmental circulatory turbulence, since it does not take account of the role of mesangial deposition independent of the intraglomerular hypertension. The primary mesangial deposition of unidentified glycoproteins equally leads to the development of diffuse and nodular lesions in NIDDM patients in whom there is no or little elevation of FF[10,12,20], and these lesions cause the segmental circulatory perturbation to which simultaneous hyalinosis of the afferent and efferent arterioles may contribute considerably. Therefore, therapeutical trials in the future should be aimed at suppressing the production of glycosylated proteins, since angiotensin converting enzyme inhibitors to improve the intraglomerular hypertension have been effective to some degree[21,22]. The preliminary results of aminoguanidine, which binds preferentially to the reactive precursor of advanced glycosylation end-products and forms unreactive substituted products, have been promising on experimental diabetic rats[13,23].

Acknowledgment. This work was supported by a research grant for intractable diseases from the Ministry of Health and Welfare of Japan, 1988-1989. We thank Miss Miyuki Hoshi for typing the manuscript.

REFERENCES

1) Parving H-H, Rutili F, Granath K, Noer I, Deckert T, Lyngsoe J, Lassen NA: Effect of metabolic regulation on renal leakiness to dextran molecules in short-term insulin-dependent diabetics. *Diabetologia* 17: 157-160, 1979.
2) Hostetter TH, Troy JL, Brenner BM: Glomerular hemodynamics in experimental diabetes. *Kidney Int* 19: 410-415, 1981.
3) Mogensen, CE: Microalbuminuria as a predictor of clinical diabetic nephropathy. *Kidney Int* 31: 673-689, 1987.
4) Kimmelstiel P, Wilson C: Intercapillary lesions in the glomeruli of the kidney. *Amer J Pathol* 12: 83-98, 1936.
5) Bloodworth Jr: A re-evaluation of diabetic glomerulosclerosis 50 years after the discovery of insulin. *Hum Pathol* 9: 439-453, 1978.
6) Nakamoto, Y, Takazakura E, Hayakawa H, Kawai K, Dohi K, Fujioka M, Kida H, Hattori N, Takeuchi J: Intrarenal microaneurysms in diabetic nephropathy. *Lab Invest* 42: 433-439, 1981.
7) Nakamoto Y, Takazakura E, Takeuchi J: Serial biopsy study of diabetic nephropathy: Onset and progression. In *Diabetic Microangiopathy*, edited by H. Abe and M. Hoshi, Tokyo, University of Tokyo Press, 1983, 6. 117-128.
8) Takazakara E, Nakamoto Y, Hayakawa H, Kawai K, Muramoto S, Yoshida K, Shimizu M, Shinoda A, Takeuchi J: Onset and progression of diabetic glomerulosclerosis: A prospective study based on serial renal biopsies. *Diabetes* 24: 1-9, 1975.
9) Gellman DD, Pirani CL, Soothill JF, Muehrcke RC, Kark RM: Diabetic nephropathy: a clinical and pathologic study based on renal biopsies. *Medicine* 38: 321-367, 1959.
10) Schmitz A, Gundersen HJG, Osterby R: Glomerular morphology by light microscopy in non-insulin-dependent diabetes mellitus. Lack of glomerular hypertrophy. *Diabetes* 37: 38-43, 1988.
11) Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74: 1143-1155, 1984.
12) Osterby R, Gundersen HJG, Nyberg G, Aurell M: Advanced diabetic glomerulopathy. Quantiative structural characterization of non-occluded glomeruli. *Diabetes* 36: 612-619, 1987.
13) Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318: 1315-1322, 1988.
14) Nakamoto Y, Hamanaka S, Akihama T, Miura AB, Uesaka Y: Renal involvement patterns of amyloid nephropathy: a comparison with diabetic nephropathy. Clin Nephrol 22: 188-194, 1984.
15) Nakamoto Y, Imai H, Hamanaka S, Yoshida K, Akihama T, Miura AB: IgM monoclonal gammopathy accompanied by nodular glomerulosclerosis, urine-concentrating defect, and hyporeninemic hypoalldosteronism. *Amer J Nephrol* 5: 53-58, 1985.
16) Shiiki H, Shimokama T, Yoshikawa Y, Toyoshima H, Kitamoto T, Watanabe T: Correlations between morphology, chemical types of amyloid protein and clinical features. *Virchows Arch [Pathol Anat]* 412: 197-204, 1988.
17) Westermark P, Sletten K, Eriksson M: Morphologic and chemical variation of the kidney lesions in amyloidosis secondary to rheumatoid arthritis. *Lab. Invest.* 477: 431, 1979.
18) Pappenheimer, JR, Kinter WB: Hematocrit ratio of blood within mammalian kidney and its significance for renal hemodynamics. *Amer J Physiol* 185: 377-390, 1956.
19) Brenner BM, Meyer TW, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med* 307: 652-660, 1982.
20) Tung P, Levin SR: Nephropathy in non-insulin-dependent diabetes mellitus. *Amer J Med* 85 (Suppl. 5A): 131-136, 1988.
21) Marre M, Chatellier G, Leblanc H, Guyene TT, Menard J, Passa P: Prevention of diabetic nephropathy with enalapril in normotensive diabetics with microalbuminuria. *Brit Med J* 297: 1092-1095, 1988.
22) Parving H-H, Commel E, Smidt UM: Protection of kidney function and decrease in albuminuria by captopril in insulin-dependent diabetics with nephropathy. *Brit Med J* 297: 1086-1091, 1988.
23) Nicholls K, Mandel TE: Advanced glycosylation end-products in exprerimental murine diabetic nephropathy. Effect of islet isografting and of aminoguamidine. *Lab Invest* 60: 486-491, 1989.