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Review Articles

Early Functional and Morphologic Vascular Renal Consequences of the Diabetic State

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Summary. Renal hyperfunction and hypertrophy are characteristic findings in the early diabetic state, both in diabetes mellitus and in experimental diabetes. A number of structure-function relationships and their likely mechanisms are discussed. The metabolically induced hypertrophy of glomerular capillaries possibly plays a central role. Its cause is not known, but recent results on its time-course emphasize the probable long-term consequences of the irreversibility of the accumulation of basement membrane material.

Key words: Basement membrane, diabetic nephropathy, glomerular filtration rate, glomerular hypertrophy, glomerulus, glucagon, hypertension, insulin, kidney function, proteinuria, renal plasma flow.

The occurrence of high GFR-values in some diabetic patients had been noted by a number of earlier authors [1, 2], but it was with the studies by Stalder and co-workers [3] that the elevated GFR of diabetic children was established as a characteristic finding in such patients. Some years later a number of studies appeared in which the finding was confirmed in other diabetic populations [4, 5, 6]. The concomitant hypertrophy was, however, not described until 1973 [7]. Almost all our knowledge regarding this entity is therefore due to a number of investigations in the last few years. The fact that a large number of precise and sensitive methods is now available for studying kidney function and morphology means that some of the more tough questions may now be taken up. From a pathophysiological point of view one may ask: "What is the causal factor of glomerular hyperfunction and hypertrophy and by which mechanisms does it exert its influence?"

From a clinical point of view the question is of course: "Is there a *causal* relationship between the early, "benign" changes and the long-term, "malignant" diabetic nephropathy leading to uremia, and – if so – by which means can we prevent the transition?" The following presentation and discussion of our present knowledge aims at elucidating questions like the ones above. Whenever necessary, the large amount of information from studies in the very useful animal model, the diabetic rat, shall also be taken into consideration.

Glomerular Filtration Rate and Filtration Area

Glomerular Filtration Rate

Using classical constant infusion technique and insulin as well as labelled iothalamate as filtration markers the GFR is increased by 40% in newly diagnosed patients and by 25% in young short-term diabetics [8] given standard treatment, see Fig. 1. The values reported by Ditzel et al. [4, 9] are at approximately the same level.

It is important to note that the elevated GFR is a reversible phenomenon [10]. After a few weeks of intensive insulin treatment of newly diagnosed diabetics, the GFR-values are seen to decline to nearly normal values. The elevated GFR is not, however, related to the momentary blood glucose level since high values are also found in patients who happen to have normal blood glucose values during the clearance procedure [11]. The renal hyperfunction in diabetes is not only confined to the glomerulus. Tubular function as measured by maximal tubular reabsorption capacity of glucose (Tm_G) is elevated approximately to the same extent as GFR [12]. Kidney hyperfunction continues to characterize diabetics until the onset of proteinuria, from which point of time GFR decreases by an average of 1 ml/min/month depending upon blood pressure level [13].

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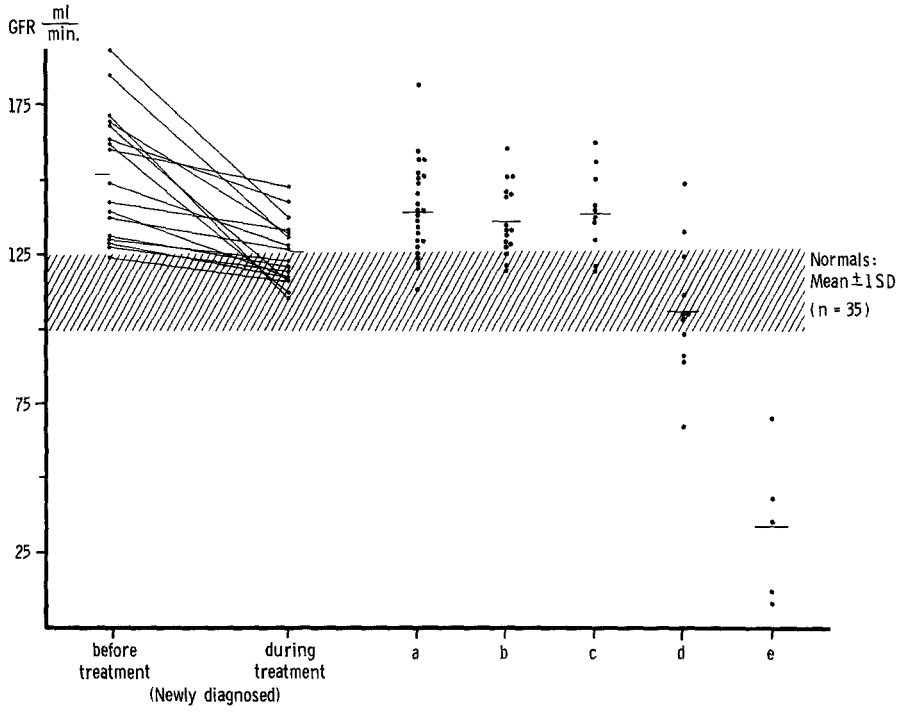

Fig. 1. GFR in newly diagnosed diabetics, before treatment and during treatment, and in diabetics with a) duration 1 to 12 years, b) no proteinuria and duration > 15 years, c) intermittent proteinuria, d) constant and pronounced proteinuria but normal serum-creatinine, e) severe retinopathy and increased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

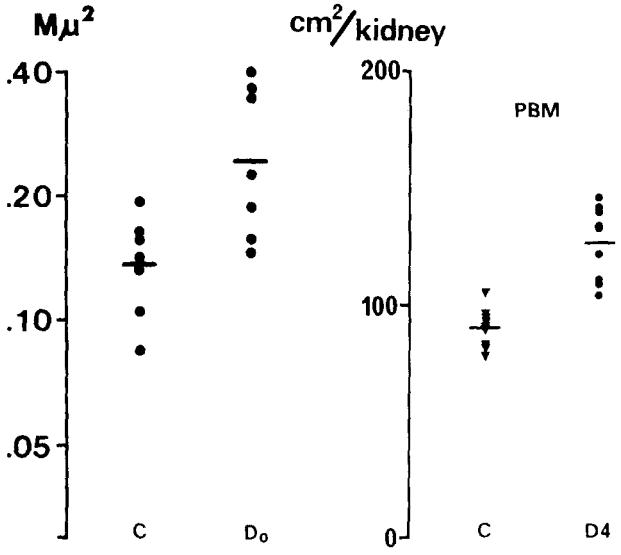

Fig. 2. Left: Area of glomerular capillary surface towards the urinary space in normal control subjects (C) and in juvenile diabetics of short duration (D_o). Millions of μ^2 per mean glomerulus on a log scale. Right: Area of the same-surface in control rats (C) and rats after 4 days of streptozotocin-induced diabetes (D_4). The linear scale is in units of cm^2 per left kidney

Renal Plasma Flow (RPF)

In newly diagnosed patients no deviations from the normal level are observed [10]. During standard treatment we found RPF to be normal or slightly increased depending on the method used [5]. Filtration fraction is always considerably increased. In proteinuric patients RPF declines by a mean of 5 ml/min/month [13].

Mechanisms of the High GFR in Early Diabetes

The first evidence of an expansion of renal tissue in diabetic patients came from radiological demonstration of an increased kidney size [7]. It was shown that the renal size measured after contrast injection is increased to the same extent as GFR in early diabetes, i. e. in patients with diabetes duration of 1 to 12 years. When GFR was expressed per gram calculated kidney weight normal average values were obtained in diabetic patients.

In a recent study it was shown that kidney size in newly diagnosed diabetics was increased approximately to the same extent as in the above-mentioned group of patients. During the first 3 months of strict insulin treatment kidney size as well as GFR fell significantly and to the same extent [14].

The decisive morphological counterpart of the GFR, the filtration surface, has been estimated in stereological studies, see Fig. 2. It was found to be increased by 80% in early diabetes [15]. In experimental diabetes in the rat an increase of 40% is seen after only 4 days of streptozotocin-induced diabetes [16].

Thus, there is a considerable body of evidence supporting the hypothesis that increased filtration surface area may be the main mechanism for the ele-

vated GFR in diabetes. Very recently, a close correlation has been found between individual values of GFR and absolute filtration areas in short-term diabetics [17].

Earlier, increased filtration pressure was suggested from the high FF always found in diabetic patients [18]. However, the demonstration of an increased filtering area in the glomeruli invalidates this inference since it rests on the assumption of an unchanged area available for filtration. The increased filtering area in the glomerulus along with unchanged blood flow would in itself lead to a high FF. On the other hand alterations in filtration pressure are of course not excluded on the basis of the morphological changes. Direct pressure measurements, using micropuncture technique in experimental diabetes in suitable species would be of the greatest interest for a more certain evaluation of this point.

The above-mentioned findings show that it is the acute metabolic changes which cause the enlargement of renal structures which accompany increases in various renal functions. As for GFR the demonstration of an increase in the filtration area is of special importance as probably being the major determinant in producing the high filtration rate. Other factors, e. g. glucagon, producing functional alteration, may play minor roles [19].

Glomerular Filter Properties

From a theoretical point of view one should also consider intrinsic structural alterations in the glomerular basement membrane as a mechanism of increased glomerular function. This possibility has been elucidated using low molecular weight dextran in clearance studies, but no abnormalities in the filtering properties of the glomerulus were demonstrated [10]. This finding together with normal resting urinary albumin excretion in patients in ordinary metabolic control make it unlikely that abnormal filter properties play a role for the elevated glomerular filtration in these patients.

Albumin Excretion

Using a radioimmunological assay [20] we have shown that the albumin excretion is generally normal in young male diabetic patients in whom clinical proteinuria (Albustix (R)) was never seen at the regular visits to the diabetes clinic, [18, 21] and in the series of patients we studied originally there is no rise in the albumin excretion in the interval from 1 to 18 years of diabetes [21]. In a subsequent study a few patients with long-standing diabetes and slightly increased albumin excretion – although Albustix-negative –

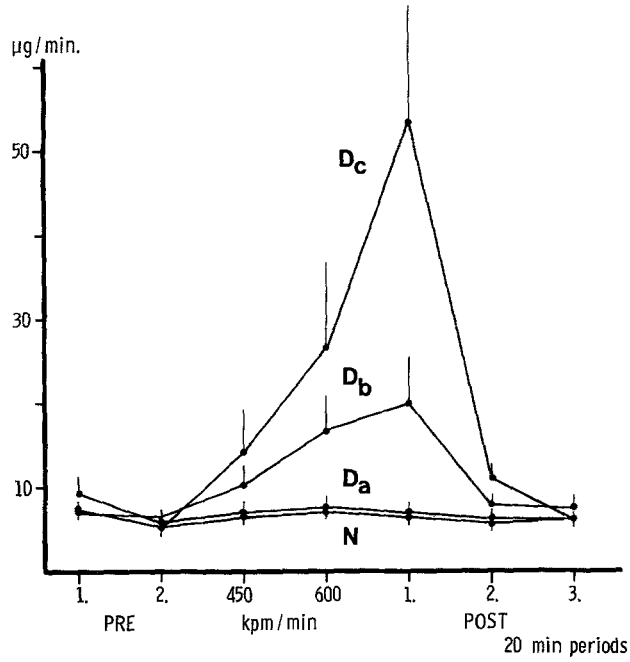

Fig. 3. Urinary albumin excretion during exercise in 11 normals (N), 6 diabetics (D_a = 0 to 1 year diabetes duration (dd)), 18 diabetics (D_b = 2 to 11 years (dd)) and 7 diabetics (D_c = 16 to 20 years (dd)). Mean ± SEM. (Taken from [25])

were identified [24]. Such patients are probably in the process of developing overt proteinuria. However, in the untreated or in the insufficiently treated situation increase in baseline albumin excretion has been demonstrated [18]. This increase is mainly glomerular in origin since beta-2-microglobulin is only moderately increased [22]. Complete normalization is found during regulation of the metabolic derangement [21, 22]. The reduction in albumin excretion during strict metabolic control was also found in a later study by Viberti et al. [23].

As usual, when function is normal in the basal state, it may be appropriate to use provocative tests when looking for abnormalities. Exercise seems a reasonable choice since this is a physiological stimulus which is known in the extreme to produce proteinuria. The idea was that a certain work load might cause an increase in albumin excretion in patients with early glomerular abnormalities, e. g. diabetics who have had the disease for a few years [24]. The results showed that at a work load of 600 kpm for 20 or 30 min. no increase in urinary albumin excretion was found in normal subjects, see Fig. 3, but the excretion in diabetics rose progressively with increasing duration of diabetes. In diabetics with a duration of one year or less no increase is found [25]. In a recent paper Viberti et al. [26] confirmed the abnormal exercise-induced albuminuria in diabetic patients.

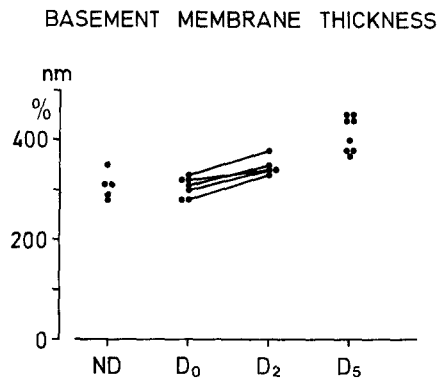

Fig. 4. Harmonic mean thickness of the peripheral glomerular capillary basement membrane in normal controls (ND), juvenile diabetics at the time of diagnosis (D_0), and after 2 (D_2) and 5 years (D_5) of the disease. The lines connect values from individuals rebiopsied after 1.5 to 2.5 years

It is important to notice that in all these patients the pre-values are normal. However, abnormally high excretion rates show up in the exercise periods and in the first post-period. A selected group of patients with elevated baseline values expectedly exhibited still higher increases during exercise [25].

The unchanged excretion of the freely filtered beta-2-microglobulin during exercise [18, 25, 26] indicates that the increased albumin excretion during the test is due to abnormally increased glomerular permeability. The most likely explanation is that the glomerular filter becomes leaky in diabetics during exercise when increased filtration pressure operates, whereas the albumin molecules are retained under the pressure conditions of the resting state.

In a recent study Brøchner-Mortensen et al. [27] found normal base-line albumin and beta-Z-microglobulin excretion in diabetic children with the characteristic elevation of GFR.

Acute Hormonal Effects

Renal Haemodynamics

Insulin: It is now well established that insulin has a marked acute effect on the cardiovascular system in diabetic patients [28, 29]. Along with these changes a rise in plasma noradrenaline is observed [30]. Recent studies have demonstrated that insulin in diabetic patients also exhibits a marked effect on renal haemodynamics [31]. After injecting 7 to 8 IU of insulin intravenously, a highly significant reduction in GFR as well as RPF was noted, 9% and 13%, respectively. It is important to notice that the patients did not develop hypoglycaemia during the procedures. The changes observed in renal haemodynamics may be explained by the concomitant increase in sympathetic nervous activity after insulin administration. They must clearly be distinguished from changes induced by the metabolic regulation of the diabetic state, taking place over days or weeks.

Glucagon: Infusion of small amounts of glucagon induces a moderate increase in GFR in both normal man [19] and diabetic patients (unpublished results) whereas RPF is not substantially altered.

Protein Excretion

Insulin: Besides an effect on renal haemodynamics a marked effect on renal protein excretion has been demonstrated [31]. Insulin has opposite effects on urinary excretion of beta-2-microglobulin and albumin: excretion of beta-2-microglobulin decreases whereas that of albumin increases. Insulin administration thus acutely stimulates both glomerular passage and tubular reabsorption of proteins, a unique response pattern.

Glucagon: The effect of glucagon infusion on kidney function has been studied both in normal young men [19] and in diabetic patients (unpublished results). Albumin excretion is unchanged, but a small increase is found in beta-2-microglobulin excretion.

The Diabetic Microangiopathy

The basement membrane thickening as observed in the peripheral glomerular capillary wall is *the* lesion of diabetic microangiopathy, present in many small blood vessels throughout the body. The diabetic glomerulopathy thereby is an integral part of the widespread microangiopathy [32]. It also shares characteristics with the microangiopathy in general: – Large-scale clinical studies have shown that the development of microangiopathy correlates with the duration of diabetes [33]: the longer the duration in a group of patients the greater the frequency of microangiopathy, and the more severe does it tend to be.

More recently, it has become possible to apply a finer scale for the evaluation of the microangiopathy, namely the basement membrane thickness determined at electron microscopy by precise morphometric or stereological methods [34]. Determination of the thickness of the peripheral glomerular basement membrane in young juvenile diabetics showed that at the onset of disease the thickness does not differ from that of corresponding non-diabetic subjects [35] (Fig. 4). This finding obviously is a prerequisite for accepting the hypothesis of a metabolic genesis of the lesion. Studying basement membrane thickness over the first few years after the acute onset of diabetes it is found that a slight thickening corresponding to about 10–15% is present after two years, and at 5 years' duration a 35% increase in thickness has taken

place [35]. The thickening continues over years, leading to the characteristic diabetic glomerulopathy which can be diagnosed at light microscopy. In experimental diabetes this thickening has been shown – among a number of diabetic kidney abnormalities – to be completely preventible by strict insulin treatment [36].

The augmentation of basement membrane material (BMM) evidently must be an expression of an imbalance of the normal basement membrane metabolism. It has been known for some time that key-enzymes in the synthetic pathway of BMM show increased activity in acute diabetes [37]. Recently, a 40% increase in the amount of peripheral capillary BMM has been found after only 4 days of experimental diabetes [16], and Brownlee & Spiro have demonstrated a doubling of the basement membrane synthesis rate in 9-day alloxan diabetic rats [38]. Correspondingly, a large increase in biochemically determined total glomerular basement membrane material is found in glomeruli from 6-week diabetic rats [39]. The breakdown of BMM apparently is a very slow process, the half life has been too long to be measured so far in rats [40]. Preliminary results from our laboratory indicate that after 4 weeks of complete normalization of the diabetic state by islet transplantation the accumulated BMM does not disappear [41]. Although these findings are restricted to glomeruli, the inability to remove capillary BMM – once accumulated – may well be a much more general phenomenon. Similar observations are made by M. W. Steffes et al. (personal communication).

Diabetes Control and Nephropathy

Today practically all available evidence from human studies and animal experiments indicates that diabetic angiopathy is a consequence of the metabolic derangements characterizing diabetes mellitus [42]. The biochemical abnormalities leading to accumulation of basement membrane material, thickening of the basement membrane, and changes of vascular morphology and function are probably essentially the same in all small blood vessels of the body.

Since the accumulation of BMM clearly can be triggered fast by the metabolic aberrations of diabetes, there is now experimental support for the simple way of considering the development of the slow, irreversible diabetic microangiopathy: whenever metabolic control is poor excess capillary BMM is laid down and in periods of good control *it is not removed again.*

In the practice of daily life – diet, insulin and regulation of exercise – *complete* normalization of the metabolic state is not possible. Periods of more or less pronounced deviations from normal state are unavoidable.

The above mentioned considerations make it highly desirable by all means to stimulate and further the work in the two fields promising complete normalization of the diabetic state, i. e. betacell implantation research and research dealing with an implantable servo-mechanical blood glucose regulator. It may well take some time before one or both of these possibilities are realized. In the meantime another possibility should be considered. Within the broad concept of 'poor metabolic control' only one biochemical factor, viz. growth hormone, has so far been demonstrated to be of isolated importance [43]. Somatostatin is known to be a very efficient suppressor of the growth hormone oversecretion in diabetes [44]. At the moment this compound cannot be used clinically because of its multiple effects and because it is active only when given intravenously. Much effort is applied, however, in various laboratories, to produce acceptable somatostatin analogues, one of which might be useful in the prevention of diabetic vascular disease.

Therefore, the main practical consequence to be derived from all studies is that great efforts should be made to obtain the best possible control in diabetic patients. Apart from that, rigorous control of blood pressure in patients with permanent proteinuria is of specific importance for the life expectancy of diabetic patients [18, 45, 46].

The severe consequences of the diabetic nephropathy were once again stressed by the recent study by Jones et al. [47] who found linear progression in nephropathy in diabetic patients, an observation similar to that of ours [13].

References

1. Amsler, H. A.: Diabetes mellitus und Kapillarpermeabilität (Inaugural-Dissertation). Zürich: Juris Verlag 1952
2. Fiaschi, E., Grassi, B., Andres, G.: La funzione renale nel diabete mellito. Rass. Fisiopat. Clin. Ter. **24**, 373–410 (1952)
3. Stalder, G., Schmid, R., Wolff, M. von: Funktionelle Mikroangiopathie der Nieren beim behandelten Diabetes mellitus im Kindesalter. Dtsch. Med. Wochenschr. **85**, 346–350 (1960)
4. Ditzel, J., Schwartz, M.: Abnormally increased glomerular filtration rate in short-term insulin-treated diabetic subjects. Diabetes **16**, 264–267 (1967)
5. Mogensen, C. E.: Glomerular filtration rate and renal plasma flow in short-term and long-term juvenile diabetes. Scand. J. Clin. Lab. Invest. **28**, 91–100 (1971)
6. Hirose, K., Suzuki, H.: Kidney function in diabetic patients. In: Advances in diabetology. Japanese Diabetes Society (Ed.), p. 155. Tokyo: Shindantuchiryosha 1974
7. Mogensen, C. E., Andersen, M. J. F.: Increased kidney size and glomerular filtration rate in early juvenile diabetes. Diabetes **22**, 706–712 (1973)
8. Mogensen, C. E.: Kidney function and glomerular permeability to macromolecules in juvenile diabetes. With special reference to early changes. Dan. Med. Bull. **19** [Suppl. 3], 1–38 (1972)
9. Ditzel, J., Junker, K.: Abnormal glomerular filtration rate,

renal plasma flow and renal protein excretion in recent and short-term diabetics. Br. Med. J. **1972 II**, 13–19
10. Mogensen, C. E.: Kidney function and glomerular permeability in early juvenile diabetes. Scand. J. Clin. Lab. Invest. **28**, 79–90 (1971)
11. Mogensen, C. E.: Elevated glomerular filtration rate in insulin treated short-term diabetes: Non-dependence of the actual blood sugar level. Acta Med. Scand. **194**, 559–561 (1973)
12. Mogensen, C. E.: Maximum tubular reabsorption capacity for glucose and renal hemodynamics during rapid hypertonic glucose infusion in normal and diabetic man. Scand. J. Clin. Lab. Invest. **28**, 101–109 (1971)
13. Mogensen, C. E.: Progression in nephropathy in long-term diabetics with proteinuria and effect of initial hypertensive treatment. Scand. J. Clin. Lab. Invest. **36**, 383–388 (1976)
14. Mogensen, C. E., Andersen, M. J. F.: Increased kidney size and glomerular filtration rate in untreated juvenile diabetes: Normalization by insulin-treatment. Diabetologia **11**, 221–224 (1975)
15. Kroustrup, J. P., Gundersen, H. J. G., Østerby, R.: Glomerular size and structure in diabetes mellitus. III Early enlargement of the capillary surface. Diabetologia **13**, 207–210 (1977)
16. Østerby, R., Seyer-Hansen, K., Gundersen, H. J. G.: Rapid increase in filtration surface and basement membrane material in acute diabetes. Diabetologia **15**, 259 (1978) (Abstract)
17. Hirose, K., Østerby, R., Gundersen, H. J. G.: A strong structure-function relationship between glomerular filtration rates and filtration surface areas in short-term diabetics. (In preparation)
18. Mogensen, C. E.: Renal function changes in diabetes. Diabetes **25**, 872–879 (1976)
19. Parving, H. H., Noer, I., Kehlet, H., Mogensen, C. E., Svendsen, P. Aa., Heding, L.: The effect of short-term glucagon infusion on kidney function in: normal man. Diabetologia **13**, 323–325 (1977)
20. Miles, D. W., Mogensen, C. E., Gundersen, H. J. G.: Radioimmunoassay for urinary albumin using a single antibody. Scand. J. Clin. Lab. Invest. **26**, 511 (1970)
21. Mogensen, C. E.: Urinary albumin excretion in short-term and long-term juvenile diabetes. Scand. J. Clin. Lab. Invest. **28**, 183–193 (1971)
22. Parving, H. H., Noer, I., Deckert, T., Evrin, P. E., Nielsen, S. L., Lyngsø, J., Mogensen, C. E., Rørth, M., Svendsen, P. Aa., Trap-Jensen, J., Lassen, N. A.: The effect of metabolic regulation on microvascular permeability to small and large molecules in short-term diabetes. Diabetologia **12**, 161–166 (1976)
23. 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 diabetes. N. Engl. J. Med. **300**, 638–641 (1979)
24. Mogensen, C. E., Vittinghus, E.: Urinary albumin excretion during exercise in juvenile diabetes. A provocation test for early abnormalities. Scand. J. Clin. Lab. Invest. **35**, 295–300 (1975)
25. Mogensen, C. E., Vittinghus, E., Sølling, K.: Abnormal albumin excretion after two provocative renal test in diabetes: Physical exercise and lysine injection. Kidney Int. (in press) (1979)
26. Viberti, G. C., Jarrett, R. J., McCartney, M., Keen, H.: Increased glomerular permeability to albumin induced by exercise in diabetic subjects. Diabetologia **14**, 293–300 (1978)
27. Brøchner-Mortensen, J., Ditzel, J., Mogensen, C. E., Rødbro, P.: Microvascular permeability to albumin and glomerular filtration rate in diabetic and normal children. Diabetologia **16**, 307–311 (1979)
28. Gundersen, H. J. G., Christensen, N. J.: Intravenous insulin causing loss of intravascular water and albumin and increased adrenergic nervous activity in diabetics. Diabetes **26**, 551–557 (1977)
29. Mackay, J. D., Hayakawa, H., Watkins, P. J.: Cardiovascular effects of insulin: Plasma volume changes in diabetics. Diabetologia **15**, 453–457 (1978)
30. Christensen, N. J.: Plasma norepinephrine and epinephrine in untreated diabetics during fasting and after insulin administration. Diabetes **23**, 1–8 (1974)
31. Mogensen, C. E., Christensen, N. J., Gundersen, H. J. G.: The acute affect of insulin in renal hemodynamics and protein excretion in diabetes. Diabetologia **15**, 153–157 (1978)
32. Østerby, R.: Early phases in the development of diabetic glomerulopathy. A quantitative electron microscopic study. Acta Med. Scand. [Suppl.] **574**, 3–82 (1975)
33. Lundbæk, K.: Long-term diabetes. Copenhagen: Munksgaard 1953
34. Jensen, E. B., Gundersen, H. J. G., Østerby, R.: Determination of membrane thickness distribution from orthogonal intercepts. J. Microsc. **115**, 19–37 (1979)
35. Østerby, R.: Morphometric studies of the peripheral glomerular basement membrane in early juvenile diabetes. I. Development of initial basement membrane thickening. Diabetologia **8**, 84–92 (1972)
36. Rasch, R.: Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment: Kidney size and glomerular volume. Diabetologia **16**, 125–128 (1979)
37. Spiro, R. G.: Search for a biochemical basis of diabetic microangiopathy. Diabetologia **12**, 1–14 (1976)
38. Brownlee, M., Spiro, R. G.: Glomerular basement membrane metabolism in the diabetic rat. Diabetes **28**, 121–125 (1979)
39. Cohen, M. P., Klein, C. V.: Glomerulopathy in streptozotocin diabetes. Accumulation of glomerular basement membrane analogous to human diabetic nephropathy. J. Exp. Med. **149**, 623–631 (1979)
40. Price, R. G., Spiro, R. G.: Studies on the metabolism of the renal glomerular basement membrane. J. Biol. Chem. **252**, 8597–8602 (1977)
41. Gøtzsche, O., Gundersen, H. J. G., Østerby, R.: Reversibility and non-reversibility of renal morphological changes after islet transplantation. (In preparation)
42. Steffes, M. W., Mauer, S. M., Brown, D. M.: The development, enhancement and reversal of the secondary complications of diabetes mellitus. Hum. Pathol. (in press)
43. Østerby, R., Seyer-Hansen, K., Gundersen, H. J. G., Lundbæk, K.: Growth hormone enhances basement membrane thickening in experimental diabetes. A preliminary report. Diabetologia **15**, 487–489 (1978)
44. Lundbæk, K.: Somatostatin: Clinical importance and outlook. Metabolism **27**, 1463–1469 (1978)
45. Mogensen, C. E.: High blood pressure as a factor in the progression of diabetic nephropathy. Acta Med. Scand. [Suppl.] **602**, 29–32 (1976)
46. Mogensen, C. E.: Diabetes and hypertension. Lancet **1979 I**, 388
47. Jones, R. H., Hayakawa, H., Mackay, J. D., Parsons, V., Watkins, P. J.: Progression of diabetic nephropathy. Lancet **1979 I**, 1105–1106

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