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REVIEW ARTICLE

Search for a Biochemical Basis of Diabetic Microangiopathy*

Claude Bernard Lecture

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Summary. Diabetic microangiopathy, particularly as seen in the renal glomerulus, is characterized by morphological and biochemical alterations of the capillary basement membrane. Observations from a number of disciplines have indicated that the microvascular disease is not a separately inherited entity but a true consequence or "complication" of insulin deficiency. An evaluation of the biochemical events which could be responsible for the basement membrane lesions of diabetes indicates that the hyperglycemia or plasma somatotropin elevation of this disease alone, or in combination, may play an important role.

Key words: Diabetic microangiopathy, diabetic nephropathy, basement membrane biochemistry, renal glomerulus, glycoproteins, glucose metabolism, somatotropin, diabetic complications.

While the discovery of insulin over half a century ago led to the successful therapy of the acute diabetic syndrome and greatly extended the useful life of diabetics, it has so far not resolved the problem of microangiopathy which now without doubt constitutes the most threatening aspect of the disease. Because of the devastating consequences of this capillary disease, it is a topic which continues to stir interest, as well as controversy, in those who deal with the problems of the diabetic, whether in the clinic or in the laboratory.

A rational, generally accepted therapeutic or preventive approach to diabetic small blood vessel disease must be rooted in a firm knowledge of the metabolic sequence of events leading to the vascular alterations and the role which hormones such as insulin and somatotropin play in their genesis. Indeed the conceptual basis for developing sensitive insulin delivery systems by mechanical devices or B-cell transplantation must rest on a clear demonstration that this hormone can influence the biochemical steps which lead to capillary disease in the diabetic.

Morphological Aspects of the Diabetic Microangiopathy

The foundation for an understanding of diabetic microangiopathy was provided in the late 1950's when electron microscopic studies by Bergstrand and Bucht [1] in Sweden and Farquhar et al. [2] in the United States revealed that the capillary disease of the renal glomerulus is characterized by thickening of the basement membrane. Subsequently basement membrane changes were observed in the capillaries of a variety of other tissues of the diabetic including muscle [3], skin [4], retina [5] and ciliary processes of the eye [6].

The pathological picture has been carefully studied in the kidney glomerulus, the site of the most serious clinical consequences, and thickening of the basement membrane of the capillary loops as well as accumulation of similar material in the mesangial region have been noted [7]. In some cases the mesangial deposition may lead to the appearance of the distinctive Kimmelstiel-Wilson nodules, which react intensely with the periodic acid-Schiff (PAS) stain when viewed by light microscopy, although in most instances this progression will not take place and only more diffuse PAS reactive lesions will be evident. An important aspect of the diabetic glomerular pathology is its focal nature; variation in the degree of basement

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membrane thickening is evident not only among the glomeruli of the same kidney but also from one capillary loop to another in the same glomerulus [2].

Electron microscopic studies carried out on the renal glomerulus by Østerby [8] as well as on muscle by Williamson and his collaborators [9] have convincingly shown that thickening of the basement membrane follows the onset of the metabolic disturbances

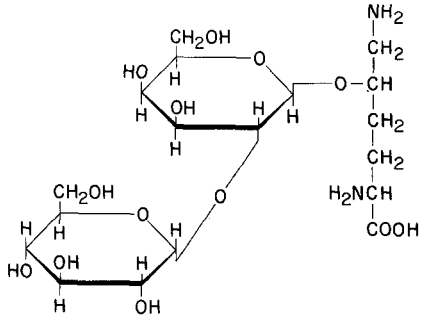

Fig. 1. Structure and peptide attachment of the disaccharide unit of the glomerular basement membrane (From Spiro [16])

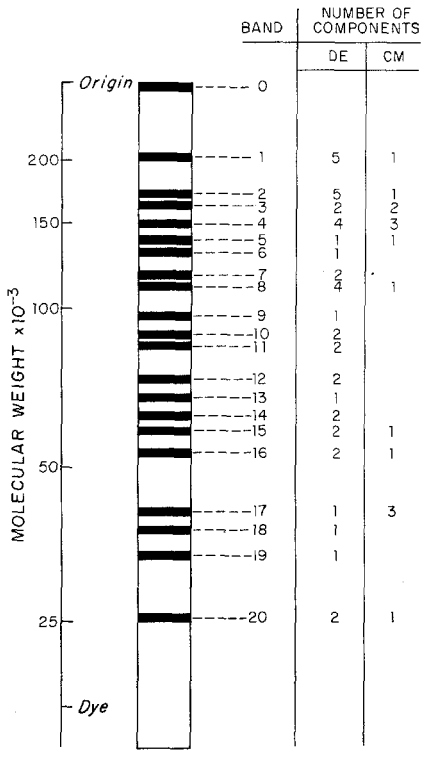

Fig. 2. Diagramatic representation of molecular weight distribution of glomerular basement membrane subunits obtained by polyacrylamide gel electrophoresis and ion exchange chromatography. Fractionation of the reduced alkylated basement membrane by DEAE-cellulose (DE) and CM-cellulose (CM) chromatography followed by electrophoresis has yielded 58 distinct polypeptide components in the 26 000 to 205 000 molecular weight range. These subunits migrated on electrophoresis to the 20 band positions represented in the drawing. (From Sato and Spiro [20])

of diabetes and progresses with the duration of the disease. In the glomerulus these morphological alterations can lead to a defect in its capacity to serve as a selective filter, presumably due to structural changes in the basement membrane which, as the only complete anatomical barrier between the plasma and its filtrate, is believed to be a major component of this apparatus.

These morphological aspects of diabetic microangiopathy strongly suggest that an understanding of their genesis depends to a large measure on information in regard to the chemical structure and metabolism of the basement membrane.

Chemistry of the Glomerular Basement Membrane

Composition

Basement membranes have been obtained in purified form from the isolated renal glomeruli of man and several other mammalian species by ultrasonic disruption and differential centrifugation [10]. The membrane obtained in this manner is amorphous under the electron microscope and reacts with the PAS stain. Compositional analyses have indicated that the basement membrane consists of collagen-like glycoprotein material (Table 1). Although the substantial content of hydroxyproline and hydroxylysine clearly places the membrane into the collagen family of proteins, it differs from fibrillar collagens in several important aspects. In particular its high carbohydrate content (about 10 per cent by weight) and its large number of half-cystine residues differentiate the membrane from interstitial fibrillar collagen (Table 1). However the composition of the basement membrane is remarkably similar to the Clq component of complement, a collagen-like protein of plasma (Table 1).

The glycoprotein nature of the basement membrane is in accord with its strong PAS reactivity [13] and it was indeed this staining property of the glomerular and retinal lesions which prompted Friedenwald as early as 1950 to suggest that the microangiopathy of diabetes could be the result of an abnormality in polysaccharide metabolism [14].

Nature of the Carbohydrate Units

From a detailed study of glycopeptides obtained after collagenase digestion of the glomerular basement membrane it has become evident that its sugar constituents are distributed about evenly by weight between two distinct types of carbohydrate units [15, 16]. One is a disaccharide (2-0-α-D-glucosyl-D-galactose) which is attached in β-glycosidic linkage to

the hydroxyl group of about 80% of the membrane's hydroxylysine residues (Fig. 1) while the other is a branched heteropolysaccharide consisting of sialic acid, fucose, galactose, mannose and N-acetylglucosamine, which is linked to 6% of the asparagine residues on the peptide chain. There are approximately 10 disaccharides for every heteropolysaccharide in the membrane. The hydroxylysine-linked glucosylgalactose unit is stable to strong alkaline hydrolysis and can be measured on the amino acid analyzer after such treatment, permitting comparison of the amount of this constituent in normal and pathological basement membranes to be made [16, 17].

Table 1. Comparison of composition of glomerular basement membrane, skin tropocollagen and Clq protein of complement

Component[a]	Glomerular basement membrane-bovine [11]	Skin tropocollagen-calf [10]	Clq protein of complement-human [12]
	residues per 1000 total amino acid residues		
Glycine	208	320	173
Hydroxyproline	68	94	39
Hydroxylysine	22	7	19
Aspartic acid	68	45	85
Half-cystine	31	–[b]	25
Tyrosine	18	3	30
Glucose	16	1	16
Galactose	20	2	17
Mannose	5	–	6
Fucose	2	–	1
Hexosamines	11	–	9
Sialic acid	4	–	1

[a] Only values for key components are given
[b] Dash indicates that less than 1 residue per 1000 amino acid residues is present

Table 2. Composition of several subunits of the glomerular basement membrane[a]

Component[b]	1[c] (205000)		4 (158000)		10 (93000)		16 (53000)	
	A[d]	D	B	E	B	D	B	C
	residues/1000 total amino acid residues							
Glycine	309	216	288	160	259	104	210	95
Hydroxyproline	126	65	109	39	96	15	58	20
Hydroxylysine	46	29	42	14	31	4	16	5
Lysine	9	23	14	34	19	39	20	48
Aspartic acid	50	64	48	83	53	92	65	83
Tyrosine	7	15	9	18	27	18	20	22
Half-cystine	9	22	11	39	15	35	29	21
Glucosamine	4	12	4	24	4	26	[e]	29

[a] Data from Sato and Spiro [20]
[b] Only values for key components are given
[c] The numbers refer to the position to which the subunits migrate on polyacrylamide gel electrophoresis while the values in parentheses give their molecular weight (Fig. 2)
[d] Letters refer to DEAE-cellulose from which the subunits were obtained by polyacrylamide gel electrophoresis
[e] Glucosamine analysis not performed

Subunit Structure

Although the glomerular basement membrane is essentially insoluble in salt solutions at physiological pH, it can be effectively solubilized and resolved into peptide subunits after reduction and alkylation of its disulfide bonds in the presence of sodium dodecyl sulfate or urea [18–20]. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of the reduced or reduced-alkylated membrane has revealed a multiplicity of polypeptide components ranging from about 25,000 to 220,000 in molecular weight [18–20]. By employing a combination of gel filtration, ion exchange chromatography and polyacrylamide gel electrophoresis it has been possible to fractionate the reduced-alkylated membrane according to size and charge into a large number of subunits. Nearly 60 distinct polypeptide components have now been isolated in this manner and analyzed (Fig. 2). Pronounced compositional diversity among these subunits was evident even when those of apparently identical molecular weight were compared (Table 2). The peptide subunits differed greatly in their content of the amino acids characteristic of collagen, namely hydroxyproline, hydroxylysine and glycine and these three constituents varied in a parallel manner. In contrast a reciprocal relationship between the number of lysine and hydroxylysine residues was observed so that the sum of these two remained fairly constant. The more collagen-like subunits had a relatively low content of half-cystine, tyrosine and aspartic acid, while these amino acids were more abundant in subunits with compositions less similar to collagen. As indicated by their hydroxylysine and glucosamine contents (Table 2) most of the subunits contained carbohydrate in the form of both disaccharides and heteropolysaccharides, although in quite different proportions, with the latter type of saccharide unit prevailing in the more polar subunits and the former being abundant in the collagen-like polypeptide components.

The basement membrane subunit polydispersity, which has now been observed in a number of investigations [19–22], constitutes one of the most striking structural features of this membrane. The proposal that basement membrane contains a collagen consisting of three identical $\alpha 1$ chains, which are 108,000 in molecular weight and devoid of heteropolysaccharide

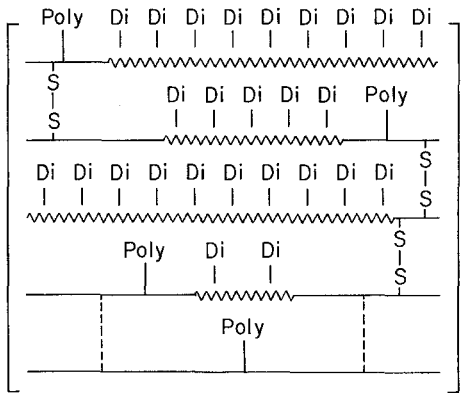

Fig. 3. Schematic representation of a portion of the glomerular basement membrane. The relation of the disaccharide (Di) and heteropolysaccharide (Poly) units to each other and to the peptide chains are indicated. The jagged lines represent helical (collagen-like) portions of the peptide chains while the straight lines represent the more polar segments. The relative proportion of these two different regions can be seen to vary greatly among the chains. Interchain disulfide bonds are shown; however, a small number of chains polar in nature are attached to the membrane by noncovalent bonds (dashed lines) that can be broken by urea or sodium dodecyl sulfate

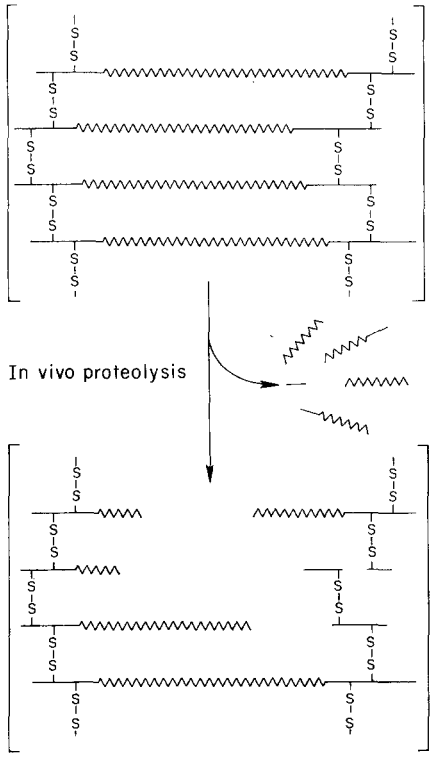

Fig. 4. Hypothetical representation of limited in vivo proteolysis of the glomerular basement membrane to account for the observed subunit polydispersity. The action of proteases on biosynthetic subunits held together by interchain disulfide bonds is depicted. Limited proteolytic cleavage may be seen to yield variously sized polypeptide segments still held together by the disulfide crosslinks as well as some diffusable peptide fragments. Helical (collagen-like) portions of the peptide chains are depicted by the jagged lines while the straight lines represent the more polar segments of the peptide chains

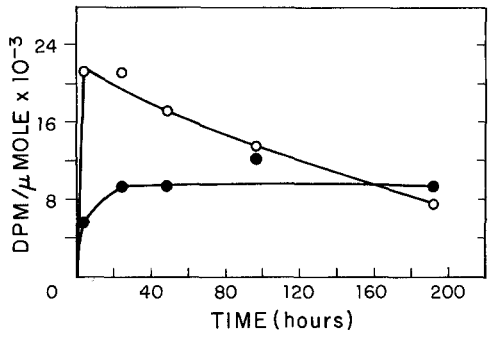

Fig. 5. Comparison of the turnover of the glomerular basement membrane with other glomerular proteins of the rat. The specific activity of renal glomerular basement membrane hydroxyproline (•) and the proline component of non-basement membrane glomerular protein (o) is shown at various times after injection of [^{3}H]proline into rats. Data from Price and Spiro [33]

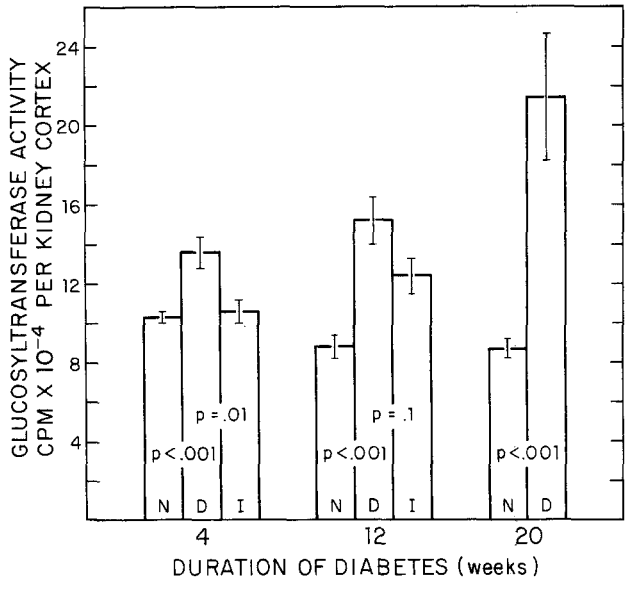

Fig. 6. Effect of diabetes and insulin therapy on the activity of rat kidney cortex glucosyltransferase (UDP-glucose: galactosylhydroxylysine-basement membrane glucosyltransferase). The diabetic animals (D) are compared to age-matched controls (N) and to insulin-treated diabetic rats (I). The mean values are plotted and the standard error of the mean as well as the p values between two adjoining groups are given. Data taken from Spiro and Spiro [35]

units [23], is not based on convincing experimental evidence. Since proteolytic digestion, which removes the polar sequences from the peptide chains, is used in the preparation of this "collagen", it could not be a natural component of the basement membrane and at the best represents a resistant fragment of this structure. Indeed it has been determined by us that the procedure for preparing "basement membrane collagen" by pepsin treatment yields material which represents less than 1% of the membrane's weight and is nevertheless polydisperse on polyacrylamide gel electrophoresis [24].

Structural Model

The information on basement membrane structure which has been obtained so far can be represented by a model in which cross-linked peptide chains varying greatly in length as well as in the proportion of collagen-like (helical) segments which they contain are layered over each other (Fig. 3). Disulfide bonds appear to constitute the major interchain links, although a small number of chains attached to the membrane by noncovalent bonds are also present. The numerous bulky carbohydrate substituents may play an important role in determining the packing of the peptide chains and thereby help to determine the porosity of the membrane. Indeed the amorphous nature of the membrane under the electron microscope may be due to the steric hindrances which the closely spaced saccharide units have on fibril formation [10].

The occurrence of substantial polar segments in the basement membrane subunits is not unknown in other proteins which are members of the collagen family. From a number of recent investigations it is becoming evident that most interstitial collagens in their procollagen stage also contain large polar domains which may include as many as several hundred amino acid residues [26]. Moreover studies on the Clq protein of complement (Table 1) have shown the presence of substantial polar sequences outside the collagen-like region, which itself constitutes only 78 of the 191 amino acid residues of the peptide subunits [27].

The physiological basis of the glomerular basement subunit polydispersity is not yet understood, although it seems unlikely that each one of the identified polypeptides could be a distinct biosynthetic component. While the postribosomal modifications, such as hydroxylation and glycosylation, which the basement membrane undergoes could contribute to the heterogeneity, the pronounced differences noted between peptide subunits cannot be solely brought about by these biosynthetic steps. It would appear more likely that the subunit polydispersity is the result of a limited physiological proteolysis such as is operative in the processing of procollagens [26]. Since the basement membrane is held together as an insoluble structure primarily by its numerous disulfide bonds many of the products of limited cleavage of the peptide chains could still remain connected after such scission occurs (Fig. 4). A limited number of biosynthetic subunits could then yield variously sized peptide segments which would be liberated from the isolated basement membrane upon reduction of the disulfide bonds.

The investigations of Lerner and Dixon have shown that soluble glomerular basement membrane antigens can normally be found in urine [28] and these fragments could very well be the diffusable products of proteolytic modification of this membrane (Fig. 4). The *in vivo* proteolysis of the membrane could be brought about by the neutral proteases of polymorphonuclear leukocytes, which are known to be capable of digesting this structure [29], or by enzymes from the glomerular cells themselves.

Metabolism of the Glomerular Basement Membrane

The biosynthesis of the glomerular basement membrane involves several steps after completion of the peptide chains. Specific enzymes modify these chains by hydroxylating lysine and proline and attaching the sugar residues of both types of carbohydrate units [30]. The hydroxylysine-linked disaccharide is assembled through the action of a galactosyltransferase and a glucosyltransferase which have been isolated from renal cortex and characterized [31, 32]. These enzymes transfer the sugar directly from its respective nucleotide to the nascent polypeptide in the presence of manganese. The more complicated heteropolysaccharide unit bound to asparagine is probably put together by a group of glycosyltransferases such as have been described for the assembly of a similar unit in thyroglobulin [30] and the attachment of its mannose-N-acetylglucosamine core portion to the peptide chain may involve lipid (polyisoprenol)-saccharide intermediates [30].

The postribosomal, enzyme-mediated events in basement membrane assembly are not under direct genetic control and are susceptible to environmental influences, such as substrate and cofactor availabilities. They can therefore serve as points of regulation of the rate of basement membrane synthesis and also as loci for the influence of pathological processes, such as diabetes, on the nature of the product formed.

Recent studies performed in the rat on the metabolism of the glomerular basement membrane have indicated that the turnover of that structure is

exceedingly slow (Fig. 5) and similar to that of insoluble fibrillar collagen [33]. In comparison to other glomerular proteins both the synthesis and degradation of the basement membrane polypeptide proceeds at a much more gradual pace (Fig. 5). The slow rate of basement membrane turnover would tend to explain the difficulty of resolving diabetic basement deposits and would have to be taken into account in evaluating any therapeutic approach employed to accomplish this aim.

Biosynthesis of the Glomerular Basement Membrane in Diabetes

The levels of activity of the glycosyltransferases involved in the synthesis of the hydroxylysine-linked disaccharide unit of the basement membrane appear to mirror the overall rate of membrane synthesis under various conditions [34]. In the kidneys of alloxan diabetic rats a highly significant elevation of the glucosyltransferase over normal age-matched controls has been noted which increased with the duration of the disease [35] (Fig. 6). Careful treatment of the diabetic animals with insulin resulted in a restoration of the enzyme to normal values if the therapy was instituted early in the course of the disease (Fig. 6). However when insulin was administered to animals whose disease had continued for a longer time it did not completely restore blood glucose levels to normal and also resulted in only a partial lowering of the glucosyltransferase activity.

The recent finding of an increased level of lysyl hydroxylase in the kidneys of streptozotocin treated rats further indicates that the various components of the basement membrane synthesizing machinery are overactive in the diabetic state [36].

Chemistry of the Glomerular Basement Membrane in Diabetes

Chemical analyses of human glomeruli from individuals with long-term diabetes and microscopic evidence of glomerular lesions have indicated the presence of increased basement membrane-like material over that of normal [37, 38]. Moreover the basement membranes isolated from the diabetic glomeruli were found to have a composition distinctly different from those obtained from control subjects (Figs. 7 and 8). The diabetic membranes showed a marked increase in their hydroxylysine content and in the glucose and galactose components which are associated with the disaccharide linked to this amino acid (Fig. 7). A reciprocal decrease in the number of lysine residues present in the membrane was noted so that the sum of the lysine and hydroxylysine remained constant. Smaller, but still statistically significant, differences were observed in the hydroxyproline, glycine, valine and tyrosine contents of diabetic and nondiabetic membranes (Fig. 8).

The compositional data suggest that in the diabetic membrane there is an increased presence of some hydroxylysine-rich subunits which, by being more collagen-like in composition than the whole membrane, could be responsible for the amino acid and sugar alterations observed. Since analyses were performed on the unfractionated membranes evidence for a subunit derangement in the diabetic mem-

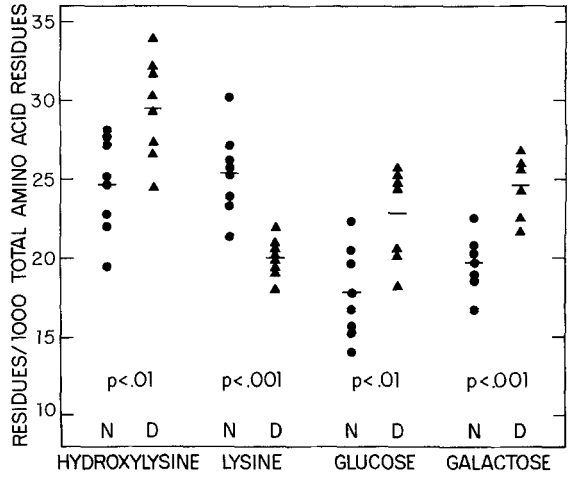

Fig. 7. Comparison of the hydroxylysine, lysine, glucose and galactose contents of basement membranes from nondiabetic (N) and diabetic (D) human subjects. Individual values from nondiabetic (•) and diabetic (▲) membranes are plotted. The mean is indicated for each group and the p value between the normal and diseased membrane for each component is shown. Data taken from Beisswenger and Spiro [38]

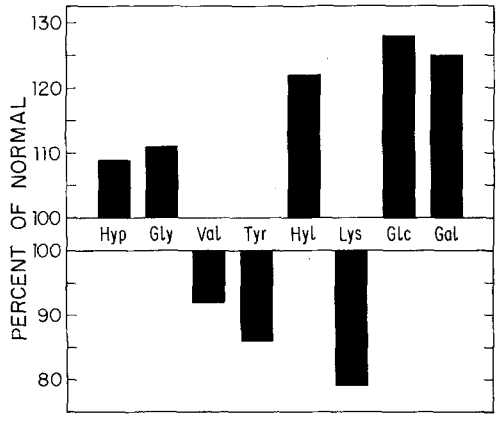

Fig. 8. Effect of diabetes on the composition of the human glomerular basement membrane. The percent change of those constituents which differ significantly from the normal is indicated by the bars. Data taken from Beisswenger and Spiro [38]

Table 3. Relationship of overproduction of hydroxylysine-rich subunits to glomerular basement membrane alterations in diabetes

Amino acid	Whole membrane[a]	Hydroxylysine-rich subunit[b]	Subunit/ Whole[c]	Diabetic/ Normal[d]	Hypothetical[e]/ Normal
	residues per 1000 total amino acid residues				
Hydroxyproline	81	126	1.56	1.09	1.14
Glycine	214	309	1.44	1.11	1.11
Valine	37	24	0.65	0.92	0.91
Tyrosine	16	7	0.44	0.87	0.86
Hydroxylysine	25	46	1.84	1.23	1.21
Lysine	25	9	0.36	0.78	0.87
Alanine	63	36	0.57	0.98	0.89
Half-cystine	20	9	0.45	0.96	0.86
Other amino acids			0.72–1.07	0.90–1.06	0.93–1.02

[a] Refers to normal human glomerular basement membrane [38]
[b] Refers to hydroxylysine-rich basement membrane subunit A-1 (Table 2)
[c] Ratio of hydroxylysine-rich subunit to whole normal membrane
[d] From analyses of normal and diabetic human basement membranes [38]; hydroxyproline, glycine, valine, tyrosine, hydroxylysine, and lysine are the only amino acids which in the diabetic are significantly different from normal
[e] Hypothetical refers to a membrane in which 75% of the amino acid residues are contributed by the normal whole membrane and 25% by the hydroxylysine-rich subunit

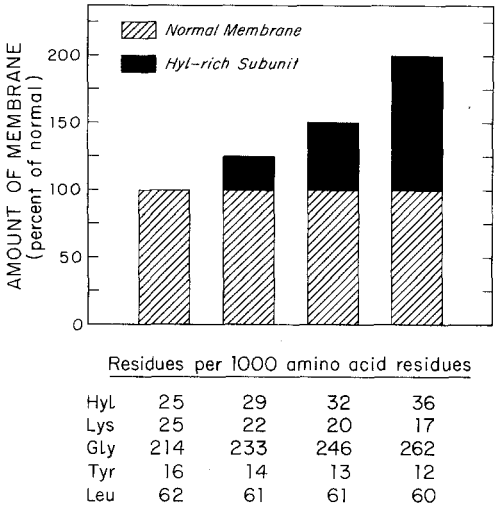

Fig. 9. Effect of proposed overproduction of a hydroxylysine-rich subunit on the composition of the human glomerular basement membrane. Bar graph shows normal membrane (100%) with increasing amounts of hydroxylysine-rich subunit added to it. The composition of the membrane is given for various degrees of subunit overproduction for some amino acids which change in diabetes (hydroxylysine, lysine, glycine and tyrosine) and for a component which does not alter with the disease (leucine). The partial composition of the hydroxylysine-rich subunit (A-1) is given in Table 3 (it has a leucine content of 59 residues per 1000 total amino acid residues)

brane would only be noted in those constituents which differ most strikingly among subunits. Moreover, the slow turnover of the basement membrane would cause the abnormal membrane produced in the diabetic state to be diluted by normal membrane formed prior to the onset of the disease. A similar dilution by normal membrane would result as a consequence of the focal nature of the glomerular basement membrane alterations.

To test whether the hypothesis of an overproduction of hydroxylysine-rich, collagen-like subunits could account for the analytical changes observed in the diabetic membrane, the composition of a hypothetical membrane, in which 25% of the total amino acid residues are contributed by such subunits, was calculated. Such a hypothetical membrane would be expected to differ significantly from the normal membrane only in those constituents with a subunit to membrane ratio significantly above or below unity (Table 3). It should be noted that even in these components the hypothetical membrane showed only modest changes from normal. When the ratios of hypothetical to normal membrane are compared to the actual, experimentally determined, diabetic to normal values a striking similarity is evident in all amino acids except alanine and half-cystine.

That a very substantial subunit derangement has to take place to bring about even modest changes in the amino acid analyses of the whole membrane can

be appreciated from the diagramatic representation in Fig. 9. Due to the focal nature of the disease and the slow removal of membrane synthesized before the onset of diabetes it may be expected that only cases with pronounced glomerulopathy will manifest significant compositional differences from the normal. Indeed a study of basement membranes from subjects with diabetes of short duration and no microscopic evidence of glomerular disease has failed to reveal the chemical changes observed in membranes from long-term diabetics [39]. Total compositional studies of glomerular basement membranes must therefore be considered to be a not very sensitive index of diabetic capillary disease.

Since a number of investigators have in recent years performed compositional analyses of the human diabetic basement membrane with variable results [38, 40, 41] it should be pointed out that there are a number of pitfalls in such an undertaking. For the reasons already stated, the kidneys to be analyzed for basement membrane changes must be limited to those which have microscopic evidence of significant diabetic glomerular pathology. Care must be taken that pathological glomeruli are actually obtained in the isolation technique, that they are properly disrupted during the subsequent sonication step and that they are not lost by filtration. The procedure of ultrasonication of glomeruli is a critical step and must be so standardized in regard to the length of treatment that contamination with cell membranes is avoided. Once cellular contamination has been removed, extension of the sonication time should not lead to any change in the composition of the membrane [24]. Membranes from diabetics should be compared to age-matched nondiabetic controls and if individual subunits of the diabetic and normal membrane are to be compared proteolytic treatment as a step in their isolation must be avoided, as native polypeptide components cannot be obtained in this manner.

Metabolic Hypothesis of Diabetic Microangiopathy

Although the pathogenesis of diabetic capillary disease is still far from understood a large number of observations have emerged in recent years to support a metabolic basis for this disorder. Before evaluating the evidence in support of the metabolic hypothesis the possibility of hereditary or an immunologic cause for the microangiopathy should be considered (Table 4).

The concept of a direct hereditary cause for the diabetic microvascular disease has been primarily espoused by Siperstein [42] who contends that the capillary abnormality is inherited independently from the insulin deficiency and the carbohydrate derangement. Indeed he has proposed that the basement membrane defect antedates and may even cause the hormonal disturbance by interfering with the normal diffusion of insulin. The hypothesis of Siperstein is based primarily on measurements of basement membrane thickening in diabetic muscle which were found by this investigator to be unrelated to the duration of the diabetes and to occur also in a large proportion of "prediabetic" subjects. Although these observations on muscle capillary basement membranes have been effectively challenged by the work of Williamson and his collaborators [9] and although it has been found that diabetic membranes are actually more porous than normal [43], Siperstein's theory has discouraged good control of the diabetic patient, which is a risky policy in the absence of complete knowledge as to the cause of the microangiopathy.

An immunological mechanism leading to diabetic microangiopathy has been suggested for some time [44], but has been brought into serious doubt by the studies of Westberg and Michael [45] who demonstrated that immunoglobulins found in the glomerular basement membrane were present along with other plasma proteins, not as a result of immune processes but probably due to structural alterations in the basement membrane or mesangium. These investigators, moreover, showed that immunoglobulins eluted from diabetic basement membranes did not react with nor-

Table 4. Possible causes of diabetic microangiopathy[a]

1. Hereditary
2. Immunological
A. Insulin antibodies
B. Basement membrane antibodies
3. Metabolic – consequences of insulin deficiency
A. Hyperglycemia
B. Growth hormone excess

[a] See text for references and discussion.

Table 5. Relationship of diabetic microangiopathy to insulin deficiency[a]

1. Less retinopathy and nephropathy with good control [48–53].
2. Correlation of basement membrane thickening with known duration of metabolic disturbance [8, 9, 54].
3. Less basement membrane thickening with good control [55].
4. Microangiopathy in patients with secondary diabetes [56–58].
5. Microangiopathy in various types of experimental diabetes [59–64].
6. Less microangiopathy and basement membrane thickening in well controlled diabetic dogs [65].
7. Increased kidney glycosyltransferase activity in experimental diabetes and reversal by insulin treatment [35].
8. Regression of glomerular mesangial lesions in diabetic rats after islet transplantation [66].

[a] See text for discussion

mal membrane and that little reactivity between insulin or antibodies to insulin and diabetic basement membrane occurred [45].

The metabolic hypothesis holds that diabetic microangiopathy is a true consequence or "complication" of insulin deficiency. Either the lack of insulin itself or secondary phenomena, such as hyperglycemia and/or somatotropin elevation, would be responsible for the development of the capillary alterations (Table 4).

An impressive number of observations from quite different disciplines have supported the metabolic hypothesis by demonstrating a relationship between insulin deficiency and diabetic microangiopathy (Table 5). During the last three decades many clinical investigations have been undertaken in various parts of the world to determine if the incidence of microangiopathy is related to the degree of control of the diabetes [46]. With only a few exceptions [47] these studies have indicated that microvascular disease is less common in well controlled diabetics than in those in whom a less careful regimen of therapy is maintained [48–52]. Perhaps foremost in these clinical assessments is the ambitious longterm prospective study of Pirart and his collaborators [52] which has shown that diabetic capillary disease is closely related to the degree of glycemic control. In view of the extreme difficulty of achieving physiological control of blood glucose with exogenous insulin it is not surprising that therapeutic regimens which utilize multiple injections of this hormone have been the most successful in reducing the incidence of microvascular disease [49, 53].

In kidney [8], muscle [9] and skin [54] the degree of capillary basement membrane thickening has been clearly related to the known duration of diabetes. In those rare cases where minimal thickening of this membrane has been observed in prediabetics this finding should be ascribed to the difficulty of dating the exact onset of metabolic disturbances. The work of Jackson et al. has, moreover, related the degree of control of the diabetes to the extent of the membrane thickening [55].

The occurrence of microangiopathy in patients with secondary diabetes and in laboratory animals with experimental forms of the disease has strongly supported the concept that the capillary disorder is a result of insulin deficiency. Both retinal and glomerular capillary lesions have been found in cases of diabetes due to hemochromatosis or chronic pancreatitis [56–58]. Although the incidence of microvascular disease of patients with secondary diabetes has often not been as great as in those with idiopathic diabetes this can be attributed to the shorter survival of individuals with the primary pancreatic disease. Evidence of microvascular disease in kidney, retina or muscle has now been observed in chemically or hormonally induced diabetes of dogs, rats, and rhesus monkeys as well as in spontaneous diabetes of Chinese hamsters, KK mice and Celebes apes [59–64]. In the Celebes apes, in which the diabetes appears to be the result of infiltration of amyloid into the islets of Langerhans, the extent of muscle capillary basement membrane thickness has been correlated in an impressive manner to the degree of serum insulin decrease as well as to the serum glucose elevation [64]. Bloodworth and Engerman have contributed in an important way to establishing a link between microangiopathy and insulin deficiency by demonstrating that the retinal and glomerular capillary lesions which developed in their diabetic dogs over a period of 5 years could be prevented or minimized by careful administration of this hormone [65]. The biochemical studies already discussed further indicate that the hyperactive enzymatic machinery of basement membrane synthesis can be returned toward normal by treatment with insulin [35].

Mauer et al. have observed that the mesangial lesion which develops in the glomeruli of rats with streptozotocin-induced diabetes could be reversed or arrested by pancreatic islet isotransplantation [66]. Since the glomerular alterations observed in these diabetic rats primarily involved deposition of immunoglobulins and complement in the mesangium rather than accumulation of basement membrane material, the authors justifiably attributed the effect

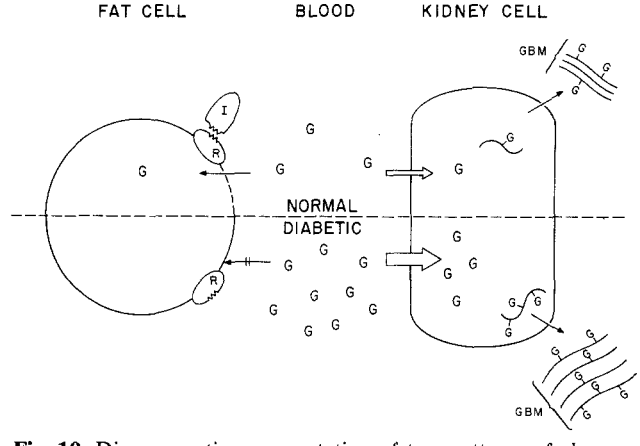

Fig. 10. Diagrammatic representation of two patterns of glucose utilization by cells in the normal and diabetic state. The insulin dependent fat cell requires interaction of insulin (I) with a cell surface receptor (R) to permit glucose (G) entry while the non-insulin requiring kidney cell is freely permeable to this sugar. The hyperglycemia of diabetes causes more glucose to enter the kidney cell with a resultant enhanced production of carbohydrate-rich basement membrane subunits which assemble to form a glomerular basement membrane (GBM) which is more porous than normal due to the steric hindrances imposed by the extra saccharide units on peptide chain packing

brought about by the transplantation to an improvement in mesangial cell function rather than a resolution of matrix material.

Role of Hyperglycemia

Hyperglycemia is one of the obvious consequences of insulin deficiency and it is therefore appropriate to consider in what manner the elevated blood glucose per se could play a controlling role in the metabolic sequence of events leading to the basement membrane lesions. Although diabetes has classically been considered a disease of glucose underutilization, in 1959 we observed that the synthesis of the carbohydrate of glycoproteins from glucose, unlike some other pathways of its metabolism, was not decreased by insulin deficiency [67]. This prompted us to hypothesize that in diabetes a shunting of glucose from insulin dependent pathways to those not requiring this hormone might take place with consequences which could influence the synthesis of the complex saccharide units of glycoproteins and thereby lead to some complications of the disease such as microangiopathy [68, 69].

Kidney cells [70] in common with many other cells, but unlike adipocytes or muscle cells, appear to be freely permeable to glucose so that the concentration of this sugar in the extracellular fluid to a large measure controls its utilization by these cells (Fig. 10). Contrary to fat and muscle, which require interaction of insulin with cell surface receptors to permit glucose penetration, the glomerular cells appear not to respond to this hormone. Thus glucose or one of its metabolites, which may be present in elevated amounts in the diabetic state, could increase basement membrane synthesis by influencing one or more of the post-ribosomal steps involved in the assembly or export of its subunits.

Some metabolic routes leading from glucose to products which are synthesized in excess in diabetes are shown in Fig. 11. In addition to the overproduction of saccharide enriched basement membrane several other pathways of enhanced synthesis should be noted. The deposition of glycogen has been observed in the renal tubules of uncontrolled human diabetics [71] and alloxan diabetic rats [72]. In the latter it could be demonstrated that the severity of the glycogen nephrosis reflected the degree of hyperglycemia and could be reversed by lowering of the blood glucose level.

An elevation of a glycosylated hemoglobin (Hb A_{Ic}) has been observed in the blood of human diabetics [73] as well as in diabetic mice [74]. This variant of hemoglobin A appears to be characterized by the presence of a glucose residue attached to NH_2-terminus of each β chain through a Schiff base linkage [75]. While in normal subjects Hb A_{Ic} constitutes about 5% of their total hemoglobin a two-fold increase of that volume has been observed in diabetic patients [73]. The glycosylation of hemoglobin appears to be a postsynthetic modification of Hb A which is dependent on the concentration of blood glucose and may in fact proceed without enzymatic mediation [76]. An environmental rather than primary genetic basis for the diabetic Hb A_{Ic} elevation is indicated by the recent observation that both members of sets of identical twins concordant for diabetes had elevated levels of this hemoglobin, while in twins discordant for the disease the Hb A_{Ic} increase was generally only observed in the diabetic individual of the pair [77]. Furthermore it has been noted that mouse red cells from either normal or diabetic donors show an accelerated synthesis of Hb A_{Ic} when circulating in a diabetic but not a normal host [74]. The decreased reactivity of Hb A_{Ic} with 2,3-diphospho-

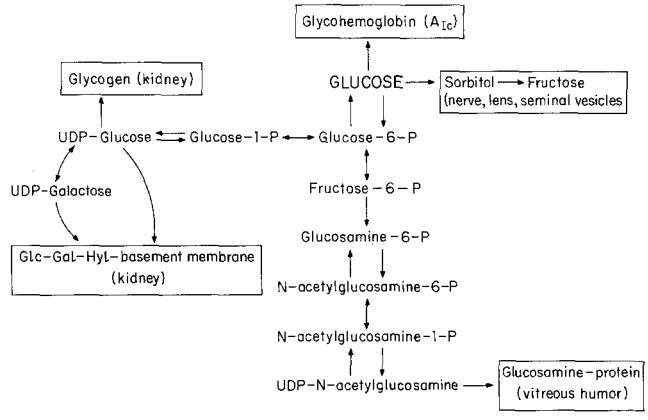

Fig. 11. Some pathways of glucose overutilization by non-insulin-requiring cells in diabetes

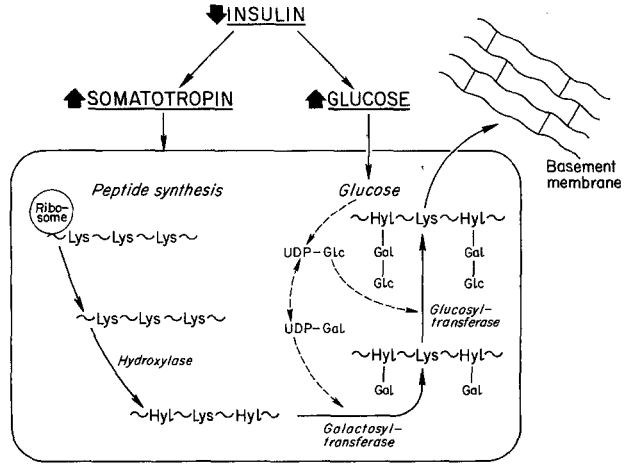

Fig. 12. Potential stimulatory influences on glomerular basement membrane synthesis by kidney cells in diabetes

glycerate [78] causes it to have a high oxygen affinity which might impair oxygen unloading in diabetic subjects and thereby contribute to the tissue anoxia which has been reported to be present in this disease [79].

The sorbitol pathway which is responsible for the formation of fructose in a number of tissues through the sequential action of aldose reductase and sorbitol dehydrogenase [80] responds to elevated levels of blood glucose with a resultant accumulation of the products of these two enzymes. Sorbitol and fructose levels have been shown to be increased in the lens of human diabetics [81] and in the lens and peripheral nerves of alloxan as well as streptozotocin diabetic animals [80, 82, 83]. The fructose content in the semen of diabetic rabbits was found to be considerably higher than normal and to be substantially decreased along with blood glucose upon the administration of insulin [84]. The elevated glucosamine content of rabbit vitreous glycoproteins in diabetes could likewise be lowered toward normal values upon treatment with insulin [85].

Since most products of glucose metabolism have a considerably faster turnover than basement membrane it might be expected that lowering of the blood glucose would accomplish a more rapid resolution of their accumulation than of the membrane material in the capillary lesions.

Role of Somatotropin

In addition to glucose, somatotropin can be considered as a potential factor which can influence basement membrane synthesis in the insulin deficient state (Fig. 12). Hansen has shown that somatotropin is elevated in the blood of juvenile diabetics especially after exercise, but can be brought to normal values by very careful control of the patients with insulin [86]. On the basis of these and other observations Lundbaek has proposed the provocative theory that somatotropin may be involved in the pathogenesis of diabetic microangiopathy [87]. While the reported beneficial effect of pituitary ablation on diabetic retinopathy [88, 89] and reduced skin capillary resistance [90] as well as the apparent absence of microangiopathy in diabetic, somatotropin-deficient dwarfs [91] has supported the "growth hormone hypothesis", no clear biochemical basis for an action of somatotropin on capillary basement membrane synthesis has yet been established. It is known, however, that somatotropin generally stimulates protein synthesis and that diabetic kidney polysomes, contrary to those of liver and muscle, have increased protein synthesizing activity over that of normal [92]. Indeed it is possible that the insulin-reversible increased kidney size and glomerular filtration rate observed in juvenile diabetics could be a reflection of the elevated plasma somatotropin levels which are found in these patients [93]. Since basement membrane abnormalities have not been reported to occur in acromegaly one may surmise that somatotropin needs to act in conjunction with a high glucose, or low insulin, level to bring about increased basement membrane synthesis.

Therapeutic Considerations

On the basis of a simplified scheme of the metabolic events involved in basement membrane synthesis and some of the regulatory factors which impinge on this process it may be worthwhile to consider what therapeutic measures could be employed now and in the future to depress the overactive process of membrane synthesis (Fig. 12).

The metabolic hypothesis of diabetic microangiopathy has as its cornerstone the view that the capillary alterations are in the final analysis a consequence of insulin deficiency. It is evident, therefore, that physiological replacement of this hormone should result in the prevention of the vascular lesions if therapy is instituted prior to the deposition of significant amounts of the rather inert basement membrane material. Since delivery systems which mimic the natural state, whether mechanical or B-cell transplants, seem to be a rather distant practical feasibility, conventional administration of insulin by multiple daily injections seems to hold out the best hope at the present time.

As meticulous treatment of the diabetic patient is not always a practical possibility alternate or ancillary therapeutic tools have to be considered. Although the suppression of somatotropin secretion by the administration of a long-acting analogue of somatostatin [94] has been considered as a possible adjunct to insulin treatment [88], reports of somatostatin's numerous other inhibitory effects [95, 96] and the obvious drawback of suppressing a growth-promoting substance in young patients has diminished the prospect of its therapeutic use. In the more distant future one could visualize the development of agents which could interfere with some of the postribosomal steps of basement membrane assembly such as hydroxylation, glycosylation, export and cross-link formation.

In conclusion it may be stated that although we are still far from having fully deciphered the biochemical events which lead to microangiopathy in the diabetic, the contributions of a number of disciplines have made it possible to view this dreaded complication in a more rational manner than was possible only a decade ago. Moreover, it has been possible to draw up a preliminary metabolic map which should ultimately lead to a fuller understanding of the pathogenesis of

diabetic capillary disease and its effective therapy or prevention.

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