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Morphometric Studies of the Peripheral Glomerular Basement Membrane in Early Juvenile diabetes

I. Development of Initial Basement Membrane Thickening*

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Summary. An extension of a previously reported quantitative electronmicroscopic study of the glomerular basement membrane in juvenile diabetes is presented. The initial phase in the development of basement membrane thickening in diabetic glomeruli has also been studied. — Measurements of the basement membrane were obtained from photomontages of glomerular cross sections produced from electron micrographs. A total of 16 glomeruli from five non-diabetics and 83 glomeruli from diabetics were measured. — The results showed that the peripheral glomerular basement membrane is normal at the onset of acute, juvenile diabetes, but a thickening is demonstrable in patients with a duration of the disease of about 2 years. — These findings support the hypothesis that diabetic angiopathy is a consequence of the metabolic derangement in diabetics.

Etudes morphométriques de la membrane basale glomérulaire périphérique chez le diabétique jeune. I. Développement de l'épaississement initial de la membrane basale

Résumé. Le complément d'une étude quantitative au microscope électronique dont le rapport a été fait préalablement est présenté. Cette étude portait sur la membrane basale glomérulaire chez le diabétique jeune. La phase initiale du développement de l'épaississement de la membrane basale dans les glomérules diabétiques a également été étudiée. — Les mesures de la membrane basale ont été faites à partir du photomontage de coupes à travers le glomérule provenant de clichés obtenus au microscope électronique. Une totalité de 16 glomérules venant de 5 non-diabétiques et de 83 glomérules venant de diabétiques a été mesurée. — Les résultats ont montré que la membrane glomérulaire périphérique est normale dans le diabète jeune aigu, mais un épaississement apparaît chez les malades diabétiques depuis deux ans environ. — Ces constatations appuient l'hypothèse que l'angiopathie diabétique est la conséquence d'une perturbation du métabolisme chez les diabétiques.

Morphometrische Studien an peripheren glomerulären Membranen bei frühem jugendlichen Diabetes. I. Entwicklung der Initialphase von Verdickungen der Basalmembran

Zusammenfassung. Es wird eine Erweiterung einer früher veröffentlichten quantitativen elektronenmikroskopischen Studie über die glomeruläre Basalmembran im jugendlichen Diabetes vorgelegt und auch die Initialphase in der Entwicklung von Verdickungen der Basalmembran untersucht. — Messungen der Basalmembran wurden auf Grund von Photomontagen der glomerulären Querschnitte von elektronenmikroskopischen Bildern vorgenommen. Insgesamt 16 Glomerula von fünf nicht diabetischen und 83 Glomerula von Diabetikern wurden untersucht. Das Ergebnis zeigt, daß die periphere Glomerulusmembran bei Ausbruch eines akuten juvenilen Diabetes normal ist, aber Verdickungen bei Patienten mit einer etwa 2 Jahre dauernden Erkrankung nachweisbar sind. Diese Befunde unterstützen die Hypothese, daß die diabetische Angiopathie Folge von metabolischen Veränderungen beim Diabetiker ist.

Key words: diabetic glomerulosclerosis, diabetic microangiopathy, glomerular ultrastructure, basement membrane, quantitative stereology, early diabetes, kidney, kidney biopsy

In almost every patient with long-term diabetes a highly characteristic glomerular lesion is found. The involvement of glomerular capillaries is considered to be part of a generalized diabetic angiopathy and is probably the most characteristic component of it. For the patient it is of great significance since he may eventually die from renal failure due to diabetic glomerulopathy.

With the introduction of electron microscopy a new field of study attracted the attention of many investigators, namely the early diabetic lesions. The great increase in resolution and magnification that was obtained with the electron microscope was believed to provide a readily obtainable and precise detection of very small initial changes. However, at that point of time it was not realized how difficult it is to establish slight deviations from normal of basement membrane thickness. In some of the early studies it was claimed that the glomerular basement membrane is thickened at the onset of diabetes. In later quantitative studies, on the other hand, it was found that the thickness of the glomerular basement membrane is normal at the onset of juvenile diabetes [13, 35].

The present study is an extension of that previously published [35] and includes measurements from several more glomeruli in each patient. In addition the material has been extended to include groups of patients with a few years' duration of the disease with the aim of searching for the initial lesion of the glomerular basement membrane in juvenile diabetes using a quantitative method.

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Material

The patients. Three groups of diabetics were studied. They were all young; their ages ranging from 13—34 years and there was a preponderance of males to females (Table 1). Results obtained in *group I* have previously been reported [35]. This group comprised seven diabetics with quite recent onset. Kidney biopsy was obtained on admission to hospital before institution of therapy and again after normalization of blood sugar and cessation of glucosuria resulting from intensive treatment with insulin and diet in hospital for about one month. The duration of symptoms was from a few weeks to three months in five cases having a well defined, acute onset. Two patients, however, gave a history of vague symptoms for about a year. In five of the patients (cases 1, 2, 4, 6, 7) a third biopsy was obtained after another $1^1/_2$—$2^1/_2$ years. These patients constitute *group II*. In *group III* are eight patients with a well-defined duration of the disease of $3^1/_2$—5 years. Diabetes began as an acute disease with marked symptoms in these patients. Diagnosis was made after symptoms had been present for a few weeks in six of the patients and for a few months in two cases (cases no. 9 and 12).

All of the diabetics were insulin dependent. The daily insulin requirement at the time of biopsy is given in Table 1.

The urinary sediment was normal in all of the patients, and none had proteinuria. All were normotensive and serum-creatinine was within the normal range. Endogenous creatinine clearance was performed in some of the patients (4 controls, all patients in group I, and 4 in group III) and was within the normal range.

Intravenous urography was performed prior to biopsy in all of the patients except one diabetic (case no. 3) and three controls (cases 16, 17, 18), an open biopsy being taken in these patients. Pyelograms were normal in all cases.

The diabetic patients were up and about. The patients in groups II and III were admitted for the biopsy procedure.

The controls represented the same age-groups as the diabetics. All of the controls had normal fasting blood glucose and oral glucose tolerance tests. They had no history of kidney disease, and at the time of biopsy the usual parameters of kidney function (those mentioned for the diabetics) were normal.

One control patient, 28 years old, was excluded because the ultrastructure of the glomeruli from this patient differed markedly from that seen in the remaining control patients. Abnormalities of the arterioles were observed, suggesting a significant degree of arteriosclerosis. No glomeruli were present in the section of the biopsy prepared for light microscopy. There were no clinical signs of renal involvement. The suspected diagnosis on admission was hyperthyroidism, but it was not confirmed.

The case histories of the patients that served as controls are, in brief:

case no. 16: admitted for cholecystolithiasis. Four years previously an attack of acute hepatitis with icterus.

Table 1. *Patients included in the present material*

Group	Case no.	Age	Sex	Duration [b] months/ years	Average fasting blood glucose at biopsy	Average urinary glucose g/ 24 h	Insulin doses IU/24 h
Diabetics group I	1	18	M	1	318—117[c]	303— 0[c]	0— 8[c]
	2	25	M	3	252— 89	112— 2	0—12
	3	16	F	1	207—106	60— 0	0—12
	4	32	M	12 (?)	211— 65	115— 6	0—84
	5	19	M	$^3/_4$	237— 93	202— 2	0—84
	6	21	M	12	255—129	253—12	0—44
	7	13	F	$^3/_4$	274— 94	132— 1	0—36
Diabetics group II	1a[a]	21	M	$2^2/_3$	158	0	24
	2a	27	M	2	123	9	20
	4a	34	M	$1^3/_4$	167	71	56
	6a	22	M	$1^1/_2$	149	27	40
	7a	15	F	$1^1/_2$	101	10	44
Diabetics group III	8	17	M	5	126	126	64
	9	20	F	$4^1/_2$	118	41	44
	10	17	F	4	164	48	48
	11	23	M	$5^3/_4$	230	302	64
	12	17	M	5	90	49	88
	13	28	M	$3^1/_2$	124	32	44
	14	31	M	$4^1/_2$	123	14	48
	15	27	M	4	121	37	24
Non-diabetics	16	19	M		111	0	
	17	12	M		88	0	
	18	28	F		83	0	
	19	22	M		85	0	
	20	17	F		100	0	

[a] Diabetics in group II are identical with the patients in group I with corresponding case-numbers.

[b] In group I: duration of symptoms in months before admission. In group II—III: duration of diabetes in years from diagnosis.

[c] The two values were obtained at the time of 1st and 2nd biopsy, i.e. before and after treatment

An estimate of the severity of the diabetic state at the time of biopsy is given in Table 1, based on the average fasting blood glucose and urinary glucose output during 2—5 days.

No signs of diabetic retinopathy were found on ophthalmoscopy in any of the patients.

No intervening disease until the actual acute attack. At operation a stone-containing gall bladder was removed. It might be added that five years later the patient was re-admitted to the surgical ward with ureterolithiasis.

case no. 17: no significant previous disease. The actual disease started acutely 2 weeks before admission with abdominal pain and fever. Preoperatively treatment with penicillin and streptomycin was given for 5 days, followed by normalization of temperature. The diagnosis was "abscessus periappendicularis".

case no. 18: two years previously a cervical cancer, stage no. I, was diagnosed and treated with radium and x-ray. Prior to the present admission a local recurrence was found, and the patient was admitted for hysterectomy.

case no. 19: previously well except for a tendency to sore throat. Gradual increase in size of neck glands last five months. Glandular biopsy one month previously showed malignant lymphogranulomatosis. During the week preceding biopsy raised temperature treated with terramycin. For one week, x-ray treatment to the neck. Laparotomy was performed to obtain an abdominal lymph node biopsy.

case no. 20: admitted to the medical department because of secondary amenorrhoea, weight gain and low basal metabolic rate. She had previously been well. Evaluation revealed normal thyroid function.

Methods

Percutaneous kidney biopsies were obtained using the technique of Brun and Iversen [5] in most patients. In four control patients (cases 16, 17, 18, 19) a needle biopsy was obtained from the exposed kidney during laparotomy. In two diabetics in whom percutaneous biopsy had been unsuccessful (cases 9, 10), needle biopsy was performed under light anaesthesia through a short incision in the lumbar region which permitted palpation of the kidney [10]. In all biopsies, percutaneous as well as open ones, a cylinder of kidney tissue was drawn with the Iversen-Roholm needle by the application of low pressure suction.

Part of the biopsy was prepared for light microscopy. Fixation with OsO_4 of small blocks of tissue was undertaken at the bed-side immediately after the biopsy was obtained. The small blocks were dehydrated in acetone and embedded in Vestopal W. Sections were cut with an LKB-Ultrotome. The first glomerulus observed, which was located entirely within the tissue, was selected for thin sectioning. Thin sections were picked up on copper grids (no. 100) and counter stained with either uranyl acetate [28] or uranyl-lead citrate [9]. Photographing was done with a Philips EM 200.

The total cross section of all glomeruli was photographed, and the micrographs (magnification 13500 ×) were pasted together as a photomontage representing a total cross section [20]. The thickness of the basement membrane was measured at regular intervals along the total length of the peripheral basement membrane as described in detail elsewhere [35, 37]. Only cross sections from which at least 400 measurements were obtained are included in the present report. The average number of measurements per cross section was 972 for all 99 glomeruli (range 425—2146), 1103 for the controls and 941 for the diabetics.

Sections containing the vascular pole were selected for thin sectioning whenever possible. This was done especially with the purpose of obtaining an estimate of the mesangial areas [39]. The present material includes 80 glomerular cross sections containing the vascular pole and 19 sections located outside the pole-region. The variation in basement membrane thickness at different levels within the same glomerulus has been studied and the results will appear in another paper [38].

From one biopsy (case no. 1) no acceptable glomerulus for electron microscopy was obtainable. The patient is included in the material since a second and third biopsy were studied (Table 1). From the remaining biopsy specimens between 2—7 glomerular cross sections were produced and the peripheral basement membrane was measured.

Measurements from each cross section were plotted as a frequency distribution curve, providing a visual impression of basement membrane thickness.

The frequency distribution curves are always skewed to the right. To obtain normal distribution some type of reciprocal function might be used. The useful application of one reciprocal function, membrane thickness $^{-1}$, was shown for the air-blood barrier in the lung by Weibel *et al.* [29]. As shown in another paper [11] the frequency distribution curves from measurements of glomerular basement membrane can be transformed to normal distribution, replacing basement membrane thickness (T) with $\sqrt{\frac{1}{T}}$. Statistical evaluation of the results in various groups was done by testing the differences in mean values, T_h, obtained from the distributions of $\sqrt{\frac{1}{T}}$ applying a 5% limit of significance.

Results

The distribution curves of basement membrane thickness were skewed in the controls and in the diabetics as well. The modal values for 16 glomeruli measured from control patients ranged between 2200 and 3300 Å (rounded figures) with a mean of 2800 Å. Since the curves are skewed the arithmetic mean values of basement membrane thickness in individual glomeruli are higher, between 2700 and 3500 Å in 15 glomeruli, and at the somewhat higher level of 4100 Å in the 16th.

The number of glomeruli that were measured and the number of measurements obtained from each biopsy appear in Table 2.

A curve representing the distribution of the total number of measurements was drawn for each biopsy. In Fig. 1, a—b, examples of such curves from a control patient and a 5 years' diabetic are presented together with the distribution curves of individual glomerular cross sections. The interglomerular variation was small in the non-diabetic cases, and in the diabetics in groups

I and II. However, as appears from Fig. 1b, it was rather large in some cases in group III.

Comparison between the frequency distribution curves obtained in controls and in recently diagnosed juvenile diabetics shows that the position as well as the shape of the curves are identical (Fig. 2). This result confirms that obtained in an earlier study [35], where a single glomerulus was measured in each of the biopsies.

thickness is identical in the diabetics in group I in the untreated and in the strictly normalized state.

The difference between controls and diabetics in group III (Fig. 3) is highly significant ($2\ p < 0.001$). A significant increase in basement membrane thickness is also found when diabetics in group II are compared with those in group III ($2\ p < 0.005$).

Since the patients in group II are identical with 5 of the patients in group I, mean values of basement

Table 2. *Results of measurements of basement membrane thickness on total glomerular cross sections. In diabetics in group I results from the 1st and 2nd biopsy are given separately*

Group	Case no.	No. of glomeruli measured	Total number of measurements	Bm-thickness, Å arithmetic mean	Bm-thickness, Å T_h
D, I	1	0—5	—4523	—3300	—3100
	2	2—3	1764—2747	3100—3300	3200—3200
	3	7—2	6992—2346	3000—3000	2900—2800
	4	2—2	2767—2266	3600—3200	3400—3300
	5	2—2	1251—1929	3500—3400	3300—3200
	6	2—2	2727—1740	3500—3100	3300—3000
	7	3—4	2478—3156	2700—2900	2600—2800
D, II	1a	4	3646	3700	3500
	2a	3	2494	3600	3400
	4a	3	2903	4000	3800
	6a	4	4138	3600	3400
	7a	3	2758	3000	3300
D, III	8	5	3439	4100	4000
	9	2	3531	4100	3800
	10	3	2773	4000	3700
	11	4	2664	4700	4500
	12	3	2636	4700	4400
	13	3	2587	4800	4400
	14	2	2008	4100	3800
	15	6	6160	4700	4500
ND	16	2	1525	3800	3500
	17	5	6703	3400	3100
	18	2	2876	3000	2900
	19	3	2450	3200	3100
	20	4	4622	3000	2800

On studying glomeruli from diabetics in group III it was found that in some of the biopsies (cases no. 9, 11—15) all of the curves obtained from various glomeruli were obviously different from the normal curves; whereas curves within the normal range as well as abnormal curves were found in others (cases no. 8, 10). Comparison of the curves obtained by plotting the total number of measurements performed in all the glomeruli of the individual biopsies from controls and from diabetics in group III (Fig. 3) shows that the curves from the diabetics are placed to the right of the curves from the controls, and all of the peaks are at a lower level.

Mean values (T_h) calculated from the transformed distributions $\left(\sqrt{\frac{1}{T}}\right)$ appear in Table 2 for individual biopsies, and Table 3 shows the mean values for the various groups. The mean values in the group of controls and in recently diagnosed diabetics are identical, confirming the impression obtained by direct inspection of the curves representing the actual measurements (Fig. 2). It is also seen that basement membrane

Table 3. *Mean values, T_h, of basement membrane thickness in the groups with SD of individual biopsies. 2nd column shows interglomerular variation expressed as SD of individual glomeruli*

Group	Bm-thickness, Å $T_h \pm$ SD	SD of individual glomeruli, Å
D, I	3100 ± 313[a] 3100 ± 216[b]	175[a] 205[b]
D, II	3500 ± 175	228
D, III	4100 ± 351	406
ND	3100 ± 244	219

[a] 1st biopsy, before treatment
[b] 2nd biopsy, after treatment

membrane thickness can be compared at different points of time in the same individual. A paired comparison in these patients between the 2nd biopsy (in the treated state shortly after onset) and the 3rd biopsy (after 1½—2½ years) shows a highly significant increase in basement membrane thickness over the first 2

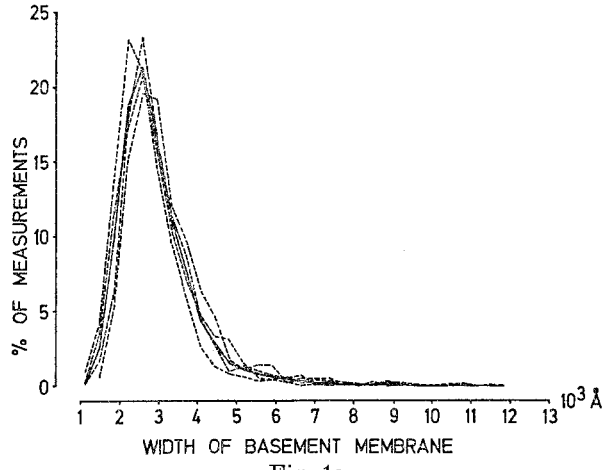

Fig. 1a

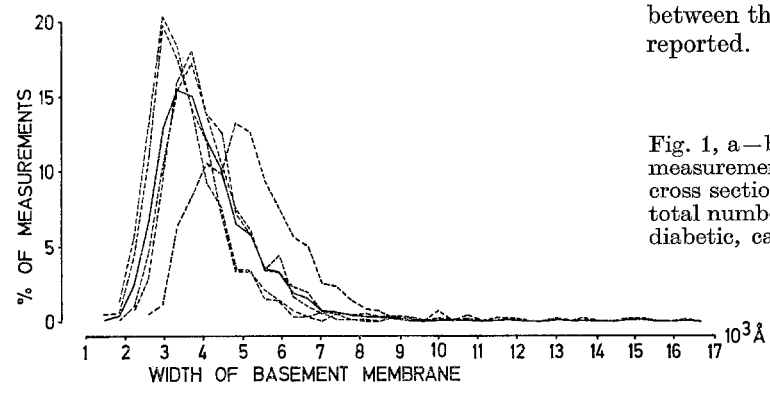

Fig. 1b

Fig. 1, a—b. Distribution curves of basement membrane measurements. Each dotted line represents one glomerular cross section, the solid line shows the distribution of the total number of measurements from each biopsy. a) non-diabetic, case no. 20; b) diabetic, group III, case no. 8

years of diabetes ($2\ p < 0.005$). The increase is illustrated in Fig. 4.

The degree of interglomerular variation was evaluated with the standard deviation of T_h of glomeruli within individual biopsies. This SD for the various groups is shown in Table 3, second column. It was larger in diabetics in group III than in any of the other groups. Only the difference between group III and group I a was significant ($2p < 0.05$).

A correlation between basement membrane thickness and duration of diabetes is thus obtained within the first 5 years of diabetes. This correlation is illustrated in Fig. 5 in which the mean basement membrane thickness (T_h) for each patient is plotted for the various groups. It is noteworthy that all of the values obtained in the females in the present material are found at the lowest range in each of the groups. When mean values for female patients are expressed as per cent of mean values for the males in each group, they are on the average 90%. This difference in basement membrane thickness between males and females is significant ($2\ p < 0.001$).

When the sex-difference is taken into consideration the figure shows that there is no correlation between basement membrane thickness and age in any of the groups for the age interval considered.

Discussion

Comparison of exact quantitative morphological data obtained in one laboratory with those from another should always be done with great reservation. A large number of variables, e.g. methods of preparation of tissue, definition of object to be measured, and methods of measurements may influence results significantly. Searching in the literature for values for normal glomerular basement membrane thickness, one, therefore, finds a large diversity of results. Mean values obtained in individual subjects range from 1440 Å (12) to 5822 Å [8], and mean values for groups of normals comprising from 3 to 24 patients range from 2200 Å to 4106 Å [1, 2, 8, 12, 14, 15, 22, 27]. If the figures for normal basement membrane thickness obtained in the present study are compared with this wide range it may merely be stated that they fall in between the upper and lower extremes that have been reported.

It has been suggested [30] that the skewed distribution of glomerular basement membrane thickness is due to the inclusion of measurements obtained at places where the section is not perpendicular to the basement membrane. However, as published in another paper [37] it has been shown that the skewness is an expression of a biological feature of the basement membrane, since it could be demonstrated that with the technique employed measurements were taken only at places where the angle between the section and the basement membrane was very low, between 0 and about 13—20°.

The total group of controls studied at various laboratories is very heterogeneous. In some studies, even autopsy material has been included [2, 22, 27]. This fact, of course, is due to the great difficulties in obtaining kidney biopsies from normal subjects, which also played a role in the present material. The size of the control material as well as the degree of normality of the individuals was not optimal. The history of previous X-ray treatment, of hepatitis, and febrile periods immediately preceding the biopsy, as well as taking the biopsy during an operation are certainly disadvantages.

However, the controls were of the same age as the diabetics and they all fulfilled the criteria of no clinical

or laboratory signs of kidney disease and no derangement of carbohydrate metabolism as based upon fasting blood sugars, glucose tolerance tests and tests of urinary glucose. On the basis of these criteria they were accepted as the best non-diabetic control group obtainable for the present study.

The inter-individual variation in basement membrane thickness for the control group was somewhat larger than expected. This shows the great importance of carefully studying normal variations.

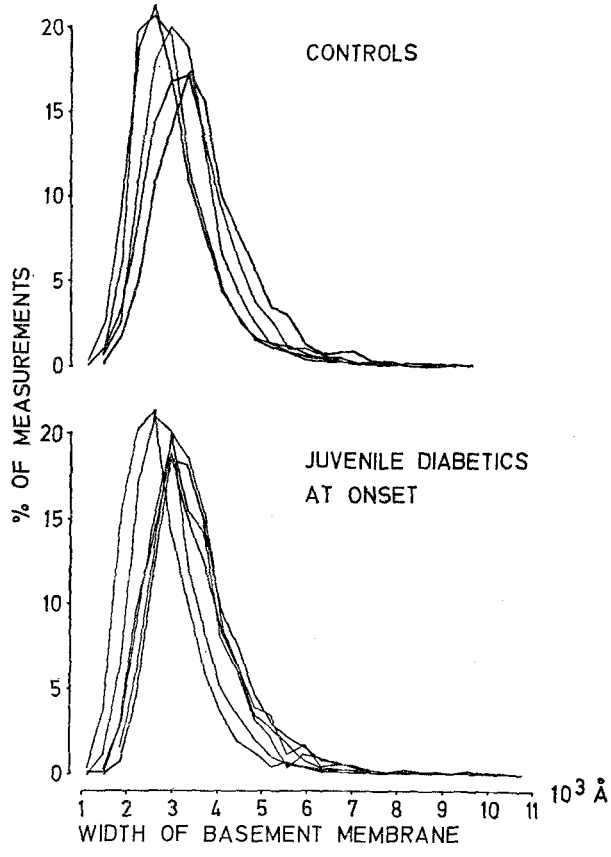

Fig. 2. Basement membrane thickness in the controls and recently diagnosed juvenile diabetics. Distribution curves represent total number of measurements obtained in each of the patients

Although the number of female patients in the present study is only low, the difference in basement membrane thickness between the sexes was shown to be significant. The female basement membrane thickness varied in the narrow range of 86.1 to 93.9% of that of the average value obtained in males. A significant sex-difference in the basement membrane of muscle capillaries was similarly found by Williamson [31, 32].

The present series represents purposely only a narrow age-range. It is therefore not suited to answer the question of an age-dependent thickening of the

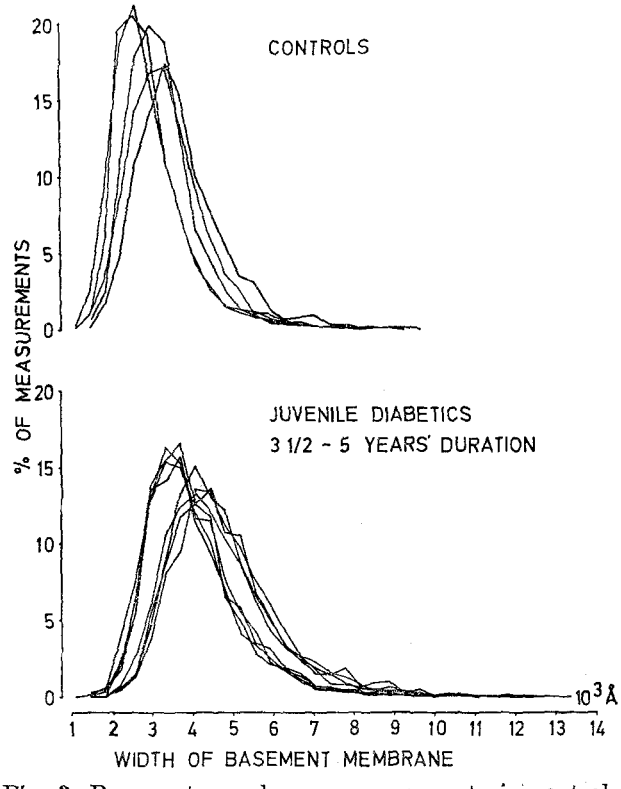

Fig. 3. Basement membrane measurements in controls and diabetics with $3^1/_2-5$ years' duration

The correlation between basement membrane thickness and age and sex has been studied by only a few investigators. In Vernier's autopsy material [27] no increase in basement membrane thickness was found after the age of 3 years. In Jørgensen's series [14] most of the patients were older than 40 years. The author concludes that there is no correlation with age and sex in his series, and the same conclusion was drawn by Osawa *et al.* [22]. However, picking out the lowermost range of basement membrane thickness in the series of Jørgensen and Osawa and those of the present study, it is found that all of the subjects with the thinnest basement membranes were females.

glomerular basement membrane. Within the two decades represented here no increase in basement membrane thickness was discernible (Fig. 5).

The question of how representative one glomerular cross section is of one particular glomerulus has been studied. The results which show a slight systematic change to thicker basement membranes when approaching the vascular pole will appear in another paper [38].

The number of glomeruli that should be measured per case depends on the inter-glomerular variation. From the results it was apparent that in the case of "normal basement membranes" the interglomerular

variation was small. However, even in control cases, one may select a glomerulus that deviates from the remainder of the group (case no. 16). It is known from light microscopy that an occasional ischaemic glomerulus may be observed in a normal kidney from a young person. It seems likely that the basement membrane of a glomerulus in the very first stage of ischaemic change may show thickening in comparison with other "normal" glomeruli. This may, of course, take place in glomeruli from young diabetics as well. Should these glomeruli be included in the control group and what should the criteria of exclusion be ? No clear-cut answer can be given. In the present study it was decided to exclude glomeruli from one control patient that clearly fell outside the group, whereas, both glomeruli from another patient (case no. 16) were included, although one of them differed somewhat from the rest of the group.

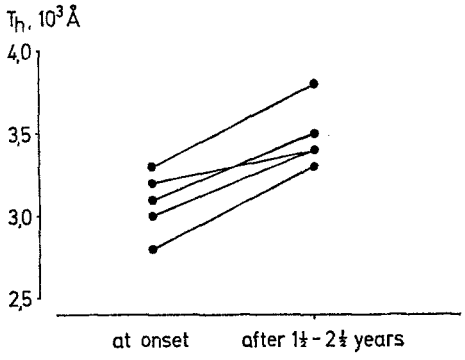

Fig. 4. Basement membrane thickness in five diabetics followed with serial biopsies

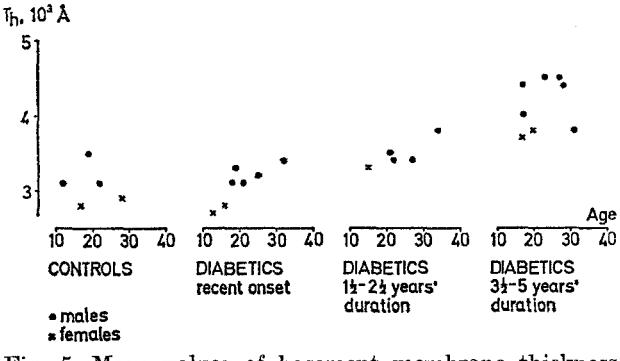

Fig. 5. Mean values of basement membrane thickness according to age and sex

The finding of a normal glomerular basement membrane in juvenile diabetics at onset, as published in a preliminary report [35] was confirmed in the present extension of the study. Results contrary to these were reported some years ago [6, 17, 21, 23, 24]. Some of the studies did not contain control patients and in some cases conclusions were drawn without measurements. However, with the knowledge of the great intraglomerular range of basement membrane thickness it is evident that unless a great number of unselected measurements form the basis of the conclusions they are invalid.

Extensive quantitative evaluation of the glomerular basement membrane has only been performed on a limited number of juvenile diabetics at the time of onset or shortly thereafter. Both Ireland [13] studying four patients of this category, and Kimmelstiel *et al.* [16], studying another four patients, found normal values in all of the cases. In Lazarow's publication [18] of the relationship between duration of diabetes and basement membrane thickness two patients are included with about 1 year's duration. Both of these are in the normal range. Urizar *et al.* [26] reported quantitative data on five children with coincidental diabetes and idiopathic nephrotic syndrome. Four of the patients had a duration of diabetes of one year or less, and in all of them the basement membrane was of normal thickness. The results published have therefore confirmed those obtained in this laboratory.

The finding of basement membrane thickening after a duration of diabetes of about 2 years shows that this lesion is present several years before vascular abnormalities can be demonstrated with any other method. The thickening found in the diabetics in group II at about 2 years post-onset compared with their own values at onset suffers to some extent from the lack of a corresponding study of the control patients with the same interval. However, since no correlation with age was found in the group of controls, and since an evident thickening was found in the diabetics of group III, it seems justified to suggest that the slight thickening after 2 years is caused by the diabetic state.

Although all of the diabetics in groups II and III showed basement membrane thickening, this, of course, does not indicate the prevalence in the total diabetic population of this category. However, positive findings in thirteen of thirteen patients suggest that the lesion is found with a very high prevalence. These findings do not correspond to those reported by Kimmelstiel *et al.* [16] who found the peripheral basement membrane of normal thickness in diabetic patients without nodules, and even in long-term diabetics with nodular lesions. Since, however, measurements in these last-mentioned cases were obtained from several glomeruli, this conclusion should perhaps be restricted to the statement that in single capillary loops the basement membrane may have a thickness within the normal range, whereas the distribution of measurements from the entire cross section may show quite a different result. In their group of diabetics without nodules there are only two patients with more than 5 years' duration. One of these patients had a mean value of 5097 Å. These authors, however, accept very high values of normal width, i.e. up to 5300 Å. The high normal values may perhaps, as pointed out by Lazarow [18], be explained by the composition of

their control material, which included alcoholics some of whom had cirrhosis. Kimmelstiel *et al.* concluded that the primary lesion is to be found in the mesangial regions, and thickening of the peripheral basement membrane occurs as a secondary phenomenon. Other investigators have suggested the same sequence of events [7, 23, 26], but their conclusions were not based on quantitative data. The interrelationship between abnormalities in the peripheral basement membrane and in mesangial regions has been studied in this laboratory. The results, which will appear in another paper [39] showed, that on the contrary thickening in the peripheral basement membrane precedes the increase in size of mesangial regions.

Diabetic glomerulopathy is generally considered a part of the generalized diabetic angiopathy. The best characterized and most closely studied part of this disease is that located in the eyes and the kidney. From a great number of clinical and histological studies, a correlation between the duration of diabetes and the development of angiopathy has been well established. From the present investigation of glomerular abnormalities which involves quite another scale, both as to time and to the structure studied, a similar conclusion regarding the correlations between duration of diabetes and vascular abnormalities can be drawn. Since no abnormality was demonstrated at the clinical onset of diabetes, and since there was no reason to suspect other kidney disease to develop in these young patients in the course of the years covered in the present study, there is no problem about the "specificity" of the changes observed .This observed correlation is in full agreement with the hypothesis that angiopathy develops as a consequence of the metabolic derangement. The finding of basement membrane thickening in animals with experimental diabetes [3, 4, 19, 36], and in human subjects with secondary diabetes [12] also supports the hypothesis.

In an extensive study of the capillary basement membrane from striated muscle Siperstein *et al.* [25] found a slight thickening in a group of prediabetic patients, and no correlation to duration of disease in their group of diabetics. However, the finding of significantly lower values in the group of prediabetics compared with the diabetics and lack of correlation to duration of diabetes suggests a very peculiar development. The report of Siperstein has been criticized [20, 34] and contrary results have been published [31, 32, 33].

Taking together the results from various types of studies it may be concluded that the most reliable evidence available to-day supports the view that the development of angiopathy depends on the duration of diabetes.

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