Studies of Muscle Capillary Basement Membranes in Normal Subjects, Diabetic, and Prediabetic Patients

M. D. SIPERSTEIN, R. H. UNGER, and L. L. MADISON

From the Department of Internal Medicine, The University of Texas Southwestern Medical School at Dallas, and Veterans Administration Hospital, Dallas, Texas 75235

ABSTRACT A technique is described for the measurement of muscle capillary basement membranes by electron microscopic examination of needle biopsies of the quadriceps muscle. With this procedure it has been possible to obtain an objective evaluation of the significance of capillary basement membrane hypertrophy in diabetic microangiopathy. The results of such studies of muscle capillary basement membrane thickness in 50 normal, 51 diabetic, and 30 prediabetic patients have demonstrated the following. First, that the average capillary basement membrane width of diabetic patients is over twice that of normal subjects; moreover, such basement membrane thickening is a very constant finding among overtly diabetic patients, in that approximately 98% of individual diabetic subjects demonstrated this lesion. The degree of basement membrane thickening in diabetic patients is, however, unrelated to age, weight, severity, or duration of diabetes. Second, capillary basement membrane hypertrophy has been found in approximately 50% of patients who are genetically prediabetic but who have not yet demonstrated evidence of the manifest carbohydrate disturbances of diabetes mellitus. Third, in contrast to the results obtained in genetically diabetic patients, subjects with severe hyperglycemia due to causes other than genetic diabetes only infrequently show basement membrane hypertrophy.

These results indicate that thickening of the muscle capillary basement membranes is a characteristic of genetic diabetes mellitus, and further, that the hyperglycemia of diabetes is probably not the factor responsible for the microangiopathy characteristic of diabetes mellitus. Finally, the discovery of thickened capillary basement membranes in prediabetic patients suggests that basement membrane hypertrophy is a relatively early lesion of the diabetic syndrome and provides further support for the conclusion that this vascular defect is independent of carbohydrate derangements of diabetes mellitus.

INTRODUCTION

With the prolongation of life that insulin and subsequently antibiotic therapy have made possible for the diabetic patient, it has become apparent that the vascular complications of diabetes mellitus constitute the major causes of morbidity and death in this disease (2). Despite increasing interest in these vascular manifestations of diabetes mellitus, neither the basic pathology of diabetic microangiopathy nor the relationship of this vascular disease to the carbohydrate derangements of diabetes has yet been resolved.

A major insight into this problem was provided when Friedenwald (3, 4), on the basis of light microscopic studies, first suggested that a specific abnormality of the capillary basement membrane

This work was presented in part at the Seventy-Ninth Session of the Association of American Physicians, Atlantic City, 3–4 May, 1966, and published in the Transactions (1).

Received for publication 28 December 1967.

might underlie both the renal and the retinal complications of diabetes. This suggestion was strongly supported by the electron microscopic observations of Bergstrand and Bucht (5), and of Farquhar, Hopper, and Moon (6), each of whom independently provided evidence that an accumulation of basement membrane or of basement membrane–like material in the capillaries of the glomerulus may represent the earliest and perhaps the underlying vascular abnormality of diabetic renal disease. In subsequent years, hypertrophy of the capillary basement membranes has also been observed in a number of other diabetic tissues, including retina (7), skin (8, 9), nerve (10), muscle (7, 11–14), and placenta (15). On the basis of these observations several investigators have raised the possibility that widespread thickening of the capillary basement membranes may in fact represent the single lesion responsible for all of the vascular complications of diabetes (7, 11, 15). Despite its potential importance, it should be noted that such a concept of diabetic vascular disease has not to date been supported by any systematic study either of the actual incidence or of the degree of basement membrane thickening in diabetic patients. Moreover, in recent years several reports using objective measurements of capillary basement membrane width in kidney (16, 17), muscle (18), and skin (19) have raised serious doubts as to the validity of the earlier, more subjective impressions that basement membrane hypertrophy represents the underlying lesion of diabetic microangiopathy.

Finally, there are as yet few data that can serve to resolve the important question of whether capillary basement membrane thickening, *even when present,* is the consequence of the carbohydrate abnormalities of diabetes, or whether on the other hand such basement membrane hypertrophy might represent a primary lesion of diabetes mellitus.

The present study has attempted to answer these questions by employing a simple needle biopsy technique that permits examination of muscle capillaries in large numbers of patients on an outpatient basis. This technique, used in connection with an objective and reproducible quantitative measurement of capillary basement membrane width, has made it possible to evaluate the significance of muscle capillary basement membrane thickness in normal, diabetic, and genetically prediabetic subjects.

An examination of capillary basement membrane width in such patients has demonstrated first, that basement membrane thickening is a very constant finding in the diabetic muscle capillary, the lesion being present in 98% of patients with diabetes mellitus. The degree of this basement membrane hypertrophy, however, bears little relationship to the age or weight of the diabetic patient or to the duration or severity of the carbohydrate abnormalities of the diabetes. Second, in contrast to the findings in genetically diabetic patients, severe hyperglycemia of nondiabetic origin is rarely accompanied by basement membrane thickening. Finally, significant basement membrane hypertrophy could be demonstrated in approximately one-half of a series of prediabetic patients with no detectable evidence of carbohydrate abnormalities. These latter two findings taken together strongly suggest that thickening of capillary basement membranes, and hence the microangiopathy of diabetes, are not the result of the hyperglycemia of diabetes mellitus, but may represent a distinct lesion of the diabetic syndrome.

METHODS

Biopsy technique. Except where specifically noted, muscle biopsies throughout this study were obtained from the lateral aspect of the quadriceps muscle at a point midway between the knee and the hip. After infiltration of the skin, underlying tissue, and muscle with 1% Xylocaine (Astra Pharmaceutical Products, Inc., Worchester, Mass.), a 10–20 mg specimen of muscle was obtained with a modified Franklin-Silverman biopsy needle. The most satisfactory results were noted with a needle in which a plug, 2 mm in length, was silver-soldered into the tip of the needle (Fig. 1).[1] With this needle, biopsies could be readily obtained as an outpatient procedure with minimal discomfort to the patient. The only complications encountered in the biopsies performed to date at this site have consisted of three hematomas, two of which were sufficiently severe to cause discomfort for about 1 wk. An identical technique was employed to obtain biopsies of deltoid and gastrocnemius muscles and adipose tissue. The site of the adipose tissue biopsies was the lateral aspect of the thigh. Renal tissue was obtained by percutaneous biopsy[2] with a Vim Silverman needle.

[1] These modifications were made for us by the Travenol Laboratories, Morton Grove, Ill., and are designated as Vim No. VT131 Silverman Biopsy Needle with modified cannula for cutting and holding specimen.

[2] We wish to thank Dr. Wadi N. Suki for making these renal biopsies available to us.

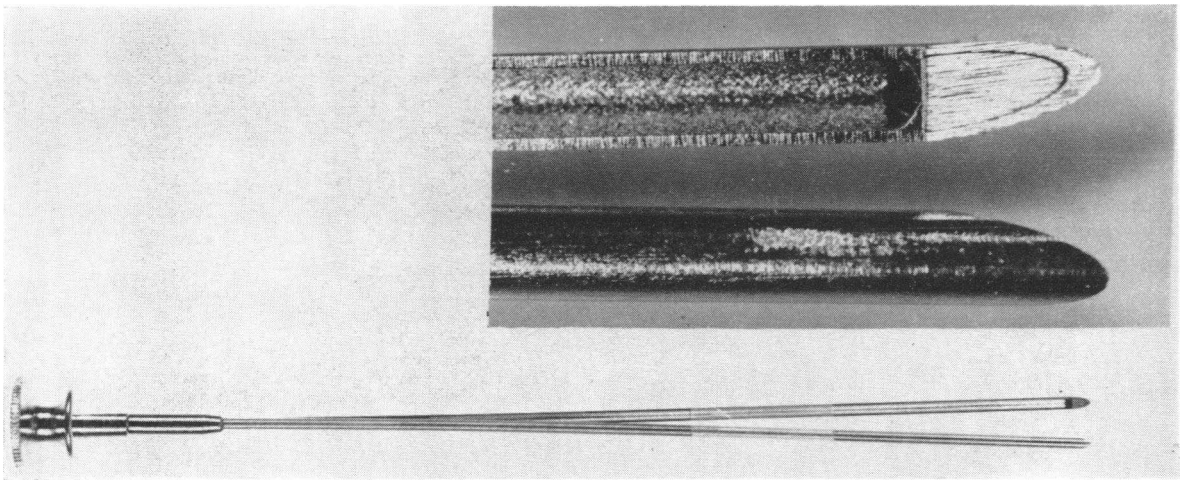


FIGURE 1 Modified Franklin-Silverman needle adapted for muscle biopsies.

Electron microscopy and measurement of capillary basement membrane width. All muscle biopsies were obtained in duplicate and fixed in Palade's buffered osmium tetroxide fixative (20). After 60 min of fixation, the specimens were placed in 3.7% formaldehyde for 30 min and then were dehydrated successively through 70%, 95%, and 100% ethanol, and two changes of propylene oxide. The tissue was next embedded in Maraglas (Marblette Corp., Long Island City, N. Y.) according to the procedure of Spurlock, Kattine, and Freeman (21), and sections approximately 300–500 A thick were cut with a glass knife on a Porter-Blum microtome. All electron micrographs were obtained with an RCA EMU-3 Electron Microscope. The instrument was calibrated at monthly intervals with a carbon grid (2160 lines/mm), and when necessary, suitable corrections were made in the calculated basement membrane widths. All electron micrographs used in this study were obtained at a single tap setting of 6 (approximately × 8200) to eliminate possible errors in recording the magnification.

The routine technique for assaying the basement membrane width employed the following procedure, which was adhered to strictly: without knowledge of the source of the biopsy, a technician obtained electron micrographs of the first 15 vessels observed on random scanning of successive tissue sections. No selection of vessels or photographs for quality, sharpness of focus, or detail was permitted, and every vessel that could be included completely within the frame at the single tap setting was photographed.

A second technician, again without knowledge of the diagnosis of the individual patients, was responsible for measuring the width of the basement membranes of the 15 capillaries. Extensive preliminary trials were carried out in an attempt to find the most accurate method of measuring basement membrane width. Procedures involving planimetry, or weighing of the appropriate portions of the electron micrographs to obtain estimates of basement membrane area, proved to be highly subjective and in practice were not reproducible. The most satisfactory and reproducible technique for quantitation of basement membrane widths consisted of overlaying the electron micrograph with a transparent plastic sheet on which were etched 20 radiating lines exactly 18° apart and measuring the basement membrane widths with a ruler at the 20 points of intersection of these lines with the basement membrane (Fig. 2). Measurements were not taken when a radiating line intersected a pericyte because such pericytes are typically surrounded by basement membrane, and as a result the total width of the basement membrane at these sites tends to be twice that seen at other locations. If for any reason at least 10 measurements could not be obtained from a given photograph, the picture was discarded, and an additional capillary was photographed. In this manner between 190 and 275 measurements of basement membrane width were obtained from each muscle biopsy, and from these data the average basement membrane width of the 15 vessels was calculated for each patient in the study. An identical procedure was employed to determine basement membrane widths of adipose tissue capillaries and of the capillaries from the peripheral portions of the renal glomeruli. Further justification for this technique and its reproducibility will be presented in the Results section.

Patients. Four groups of patients comprised the subjects of the major portion of the study[3]: (*a*) normal, nondiabetic patients were defined as those subjects having a normal fasting plasma sugar and a normal oral glucose tolerance test, as defined below. In addition, each normal subject was required to have a negative family history for diabetes in a family sufficiently large to give this negative history validity, that is, a total of at least eight brothers, sisters, aunts, and uncles; (*b*) prediabetic

[3] This study was reviewed and approved by the Human Research Review Committee of the University of Texas Southwestern Medical School, and written consent was obtained from both the normal and prediabetic patients to carry out the biopsy procedures.

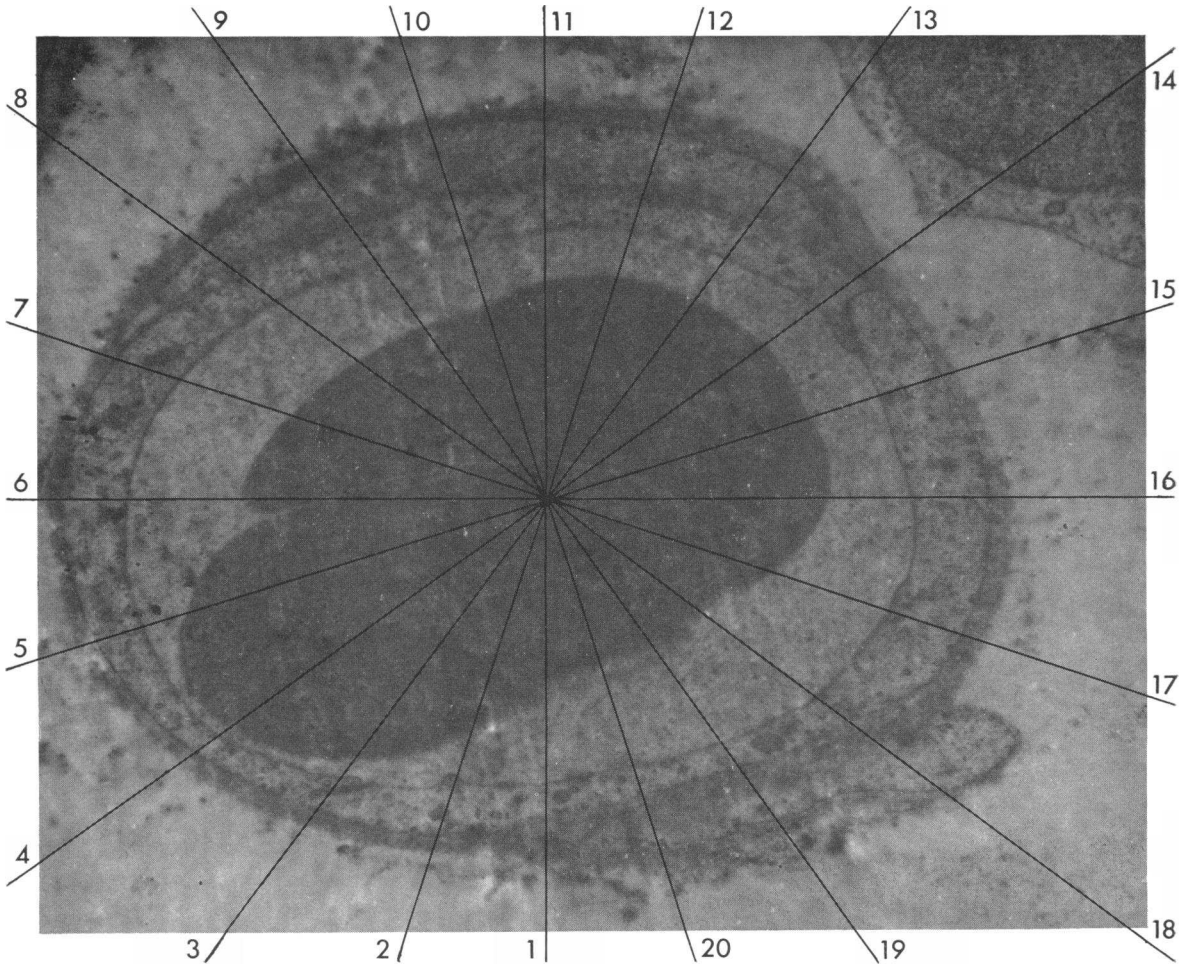


FIGURE 2 Electron micrograph of a muscle capillary overlaid with 20 equidistant radiating lines to illustrate the method used for quantitating basement membrane width.

patients were defined as patients both of whose parents by history had diabetes mellitus, and who themselves had a normal fasting plasma sugar and a normal glucose tolerance test; (*c*) patients with diabetes mellitus, defined as patients with at least two fasting plasma sugars in excess of 140 mg/100 ml not obviously due to causes other than diabetes mellitus, i.e., pancreatitis, Cushing's disease, etc.; (*d*) patients with fasting hyperglycemia ascribable to causes other than diabetes mellitus. Details as to age, sex, race, and weight of these patients, and where relevant estimated duration and therapy of diabetes, are presented in Tables III–V. In addition, a small group of six patients with nephrosis as evidenced by clinical history and a 24-hr urine albumin excretion of over 3 g was examined for possible muscle capillary basement membrane thickening.

Animals. The inbred hyperglycemic Chinese hamsters and normal control animals were kindly loaned to us for quadriceps biopsy by Dr. William E. Dulin of The Upjohn Company, Kalamazoo, Mich. The hyperglycemic and normal KK mice were the gift of Dr. James H. Birnie, Department of Biochemistry, Smith Kline & French Laboratories, Philadelphia, Pa. The normal and hyperglycemic Egyptian sand rats were given to us by Dr. George F. Cahill of the Joslin Research Laboratory, Harvard Medical School, Cambridge, Mass.

Analytical methods. Glucose tolerance tests were performed after the oral administration of 100 g of glucose to patients after an overnight fast. All patients were instructed to eat a high carbohydrate, preparative diet for at least 3 days before the test. Blood samples were obtained before glucose administration, at 1 hr and 2 hr thereafter. Because of their greater reliability (22), plasma glucose determinations were employed throughout this study. All analyses were carried out on the Technicon AutoAnalyzer, by the method of Hoffman (23). The normal upper limits for blood glucose defined by Conn and Fajans (24), but corrected by a factor of

TABLE I

Basement Membrane Widths of Duplicate Muscle Biopsies

Patient	Basement membrane width average 15 vessels	Per cent difference	Patient	Basement membrane width average 15 vessels	Per cent difference
	A			*A*	
K. H. a	776	3.7	S. W. a	1135	12.7
b	748		b	1289	
S. J. a	992	11.8	M. C. a	1287	11.9
b	1117		b	1450	
B. B.*a	1211	8.3	P. B. a	1450	1.0
b	1114		b	1436	
L. H. a	1470	17.0	D. D. a	1514	5.1
b	1240		b	1439	
N. F. a	1406	7.1	B. S. a	1358	3.5
b	1309		b	1406	
J. L. a	1226	6.1	G. G. a	1066	3.8
b	1303		b	1026	
B. S. a	1450	9.0	F. N. a	1002	1.0
b	1586		b	992	
D. G. a	1038	1.9			
b	1058		Average difference of duplicates		6.9 ± 1.24

* Biopsies a and b in this patient were obtained 3 months apart.

16%[4] for plasma glucose values, i.e. fasting, 128 mg/100 ml (110 + 18); 1 hr, 186 mg/100 ml (160 + 26); and 2 hr, 139 mg/100 ml (120 + 19), were employed to exclude abnormalities in carbohydrate metabolism in the nondiabetic and prediabetic patients. However, in contrast to the criteria of Conn and Fajans, subjects were excluded from this study if the plasma glucose at any *single* time interval was above the upper limit of normal. Immunoreactive insulin (25) and growth hormone levels were obtained during the glucose tolerance tests on 15 normal and on 27 of the 30 prediabetic subjects. The results of the growth hormone analyses have been presented elsewhere (26).

RESULTS

Validation of technique of measuring basement membrane width. Since the muscle biopsy technique involves an assessment of basement membrane width in only a small, 10–20 mg sample of muscle, there would appear to be a strong possibility both of sampling errors as well as of technical errors in the procedure. For this reason, preliminary studies were undertaken to determine the validity and the reproducibility of the technique for measuring basement membrane width. By redirecting the biopsy needle at the time of the initial biopsy, duplicate samples were obtained from the adjacent portions of the quadriceps muscle. In the 15 cases shown in Table I, these duplicate biopsies were fixed, embedded separately, and independent measurements of basement membrane widths were made of each of the two biopsies. As shown in Table I, the average error in measurement of basement membrane thickness of duplicate biopsies is 6.9%. In the case of one patient, B.B.,

[4] Plasma glucose levels in our laboratory have been found to average 16% higher than whole blood values obtained on the same blood samples.

TABLE II

Relationship between Capillary Size and Basement Membrane Width

Diagnosis	Basement membrane		Correlation coefficient*	Significance
	Perimeter	Width		
	μ	*A*		
Normal	14.9	1080	0.031	>0.05
Diabetic	15.0	2403	0.017	>0.05
Prediabetic	15.0	1373	−0.055	>0.05

* Based on regression analysis of 747 normal, 765 diabetic, and 450 prediabetic capillaries.

TABLE III
Normal Subjects

Subject	Age	Race	Sex	Weight	GTT			Capillary	
					F	1 hr	2 hr	Perimeter	Basement membrane width
	yr			*lb.*	*mg/100 ml*			*avg μ ± SE*	*avg A ± SE*
J. B.	19	W	M	143	88	138	79	16.6 ± 1.2	852 ± 54
K. H.	21	W	F	135	66	67	55	13.9 ± 1.7	691 ± 51
P. L.	22	W	F	130	92	136	106	12.9 ± 1.0	775 ± 57
J. S.	22	W	M	140	106	110	119	17.4 ± 0.8	797 ± 36
S. J.	22	W	F	125	94	105	121	12.9 ± 1.0	962 ± 66
B. B.	23	W	M	172	84	134	107	10.8 ± 0.5	1076 ± 65
L. H.	23	W	F	126	79	100	75	15.8 ± 0.9	1092 ± 50
B. H.	23	W	F	115	87	143	110	16.3 ± 0.6	1167 ± 124
L. H.	23	W	M	180	89	125	93	12.9 ± 1.0	1111 ± 63
N. F.	24	W	F	140	99	113	78	14.3 ± 1.0	1252 ± 89
L. S.	25	W	M	173	72	96	57	12.3 ± 0.8	1019 ± 75
J. L.	25	W	M	155	85	115	86	16.6 ± 0.9	1145 ± 61
B. S.	25	W	M	158	85	96	108	16.5 ± 1.3	1445 ± 100
B. S.	25	W	F	107	104	105	88	14.7 ± 0.8	1381 ± 119
D. G.	26	W	F	145	85	139	84	13.3 ± 0.7	939 ± 61
S. W.	26	W	M	194	90	157	80	14.6 ± 1.2	1010 ± 56
McF.	26	W	M	150	100	107	93	16.0 ± 1.1	1145 ± 94
B. H.	26	W	M	160	89	132	103	13.1 ± 1.5	1241 ± 61
J. J.	27	W	M	165	112	184	63	12.9 ± 0.8	949 ± 72
P. B.	27	W	M	195	101	135	111	14.7 ± 0.6	1278 ± 78
D. D.	27	W	M	185	95	107	84	14.3 ± 0.8	1347 ± 110
D. H.	28	W	M	210	91	151	96	13.8 ± 0.9	1092 ± 64
R. K.	28	W	M	187	86	84	187	13.1 ± 1.1	1065 ± 83
A. W.	29	W	M	178	108	163	111	17.4 ± 1.0	1116 ± 64
D. F.	30	W	M	170	86	92	98	13.4 ± 0.6	967 ± 94
A. W.	30	W	F	143	89	92	75	14.4 ± 1.0	993 ± 70
B. S.	30	W	F	102	100	102	94	15.2 ± 1.4	1161 ± 84
E. A.	30	W	M	153	77	113	80	13.4 ± 0.7	1273 ± 84
G. G.	32	W	F	135	83	68	81	12.4 ± 1.1	967 ± 51
E. S.	32	W	F	95	102	151	86	14.6 ± 0.9	940 ± 49
B. B.	32	W	F	95	94	70	106	19.3 ± 0.5	976 ± 33
V. H.	34	W	F	112	83	129	107	14.3 ± 1.0	748 ± 50
M. F.	34	W	F	125	93	108	119	15.0 ± 0.8	840 ± 50
G. H.	34	W	M	240	97	131	114	15.6 ± 0.6	1233 ± 73
J. G.	34	W	M	160	78	54	60	13.8 ± 0.7	1722 ± 87
A. W.	37	W	F	134	93	86	94	14.8 ± 0.7	793 ± 60
J. G.	37	W	M	170	99	138	94	16.6 ± 1.0	1002 ± 46
R. M.	37	W	M	179	103	137	98	14.3 ± 0.7	1240 ± 84
D. H.*	37	W	F	140	97	121	92	16.2 ± 1.4	1049 ± 86
W. O.	38	W	M	165	105	163	120	14.8 ± 0.9	1151 ± 78
R. S.	38	W	M	204	106	148	119	13.2 ± 0.8	1150 ± 50
J. F.	38	W	M	176	90	143	91	16.1 ± 0.8	961 ± 67
M. M.	39	W	M	193	107	134	107	13.0 ± 0.6	1243 ± 92
M. M.	39	W	F	123	91	96	90	20.2 ± 1.1	1023 ± 59
F. N.	45	W	F	125	100	97	103	16.4 ± 1.2	1060 ± 66
T. W.	45	W	F	137	96	152	89	16.2 ± 0.6	1094 ± 74
S. H.	45	W	M	160	77	82	90	16.7 ± 0.9	1314 ± 76
O. B.	54	W	M	145	106	146	113	10.8 ± 0.4	1085 ± 71
D. H.	54	W	F	139	82	160	108	20.3 ± 0.8	1020 ± 65
V. W.	62	W	F	133	104	96	136	16.5 ± 0.8	1029 ± 113
Average ± SE	32 ± 1			152 ± 4	Fasting	93 ± 1		14.9 ± 0.3	1080 ± 27
					1 hr	120 ± 4			
					2 hr	97 ± 3			

GTT, glucose tolerance test; F, fasting; W, white.
* In this subject 12 rather than 15 capillaries were examined.

TABLE IV
Diabetic Patients

Patient	Age	Race	Sex	Weight	Duration	Treatment	Capillary	
							Perimeter	Basement membrane width
	yr			*lb.*			*avg* $\mu \pm SE$	*avg* $A \pm SE$
M. H.	19	B	F	105	6 mo	Tolbutamide	15.7 ± 0.6	3467 ± 201
G. J.	19	B	F	145	15 yr	Insulin	13.6 ± 1.5	2655 ± 171
J. H.	20	W*	F	237	6 mo	Tolbutamide	18.0 ± 1.1	4160 ± 255
J. J.	22	B	M	94	10 yr	Tolbutamide	12.5 ± 1.1	4022 ± 205
B. H.	23	B	F	141	3 yr	Diet	15.3 ± 0.9	1478 ± 102
D. S.	23	B	M	141	6 mo	Insulin	14.7 ± 0.5	2455 ± 183
J. M.	24	W	M	145	13 yr	Insulin	14.9 ± 1.2	1991 ± 147
B. R.	27	B	F	140	9 yr	Insulin	12.9 ± 0.8	4625 ± 272
W. B.	31	W	M	165	15 yr	Diet	13.4 ± 0.8	1341 ± 81
J. B.	32	B	F	205	20 yr	Tolbutamide	15.7 ± 1.1	1779 ± 129
D. G.	34	W	M	176	1 wk	Tolbutamide	15.1 ± 0.7	4237 ± 302
A. R.	35	W	F	181	9 yr	Insulin	14.6 ± 0.9	2614 ± 302
B. H.	37	B	F	196	3 yr	Tolbutamide	14.7 ± 0.9	1474 ± 82
J. R.	37	W*	M	158	2 yr	Tolbutamide	15.2 ± 0.6	1905 ± 147
J. R.	37	B	M	138	10 yr	Tolbutamide	16.0 ± 0.5	2121 ± 179
C. W.	38	W	M	150	20 yr	Insulin	17.6 ± 0.9	2210 ± 178
H. W.	39	W	M	155	18 yr	Insulin	14.4 ± 1.0	1564 ± 191
G. A.	40	W	M	133	20 yr	Insulin	10.5 ± 0.6	2214 ± 254
E. G.	40	B	F	159	3 yr	Tolbutamide	11.4 ± 0.6	2383 ± 227
P. M.	41	W	F	146	8 yr	Tolbutamide	12.6 ± 0.8	1909 ± 154
A. G.	41	B	F	172	17 yr	Tolbutamide	15.2 ± 1.2	1668 ± 87
E. B.	41	W	M	182	14 yr	Insulin	13.8 ± 1.0	2117 ± 230
K. G.	41	W	M	155	15 yr	Insulin	16.1 ± 0.8	1274 ± 54
L. H.	42	B	M	178	1 wk	Diet	14.0 ± 1.0	2171 ± 208
C. G.	42	B	F	229	1 yr	Tolbutamide	15.7 ± 1.2	1691 ± 134
L. T.	44	B	F	169	20 yr	Tolbutamide	15.9 ± 0.6	2101 ± 205
A. G.	44	W	F	118	33 yr	Insulin	16.5 ± 1.0	3482 ± 334
J. H.	44	W*	F	178	10 yr	Tolbutamide	16.9 ± 0.8	3752 ± 182
E. B.	45	B	M	172	5 yr	Insulin	14.8 ± 0.7	2303 ± 156
D. T.	46	W	F	158	5 yr	Tolbutamide	14.2 ± 0.9	2082 ± 253
B. O.	46	W	M	181	1 wk	Insulin	11.1 ± 0.8	2212 ± 208
V. R.	47	B	F	145	22 yr	Insulin	18.0 ± 1.6	1413 ± 175
M. B.	47	B	M	136	2 yr	Insulin	15.2 ± 0.8	2063 ± 178
R. C.	48	B	F	197	1 wk	Tolbutamide	15.0 ± 0.7	3425 ± 222
R. J.	50	W*	F	173	14 yr	Tolbutamide	13.4 ± 0.7	2307 ± 138
E. L.	54	B	F	150	4 mo	Insulin	14.6 ± 1.2	2098 ± 136
M. S.	55	W	F	155	7 yr	Tolbutamide	14.3 ± 1.6	2822 ± 222
M. C.	56	W	F	187	1 wk	Tolbutamide	16.5 ± 1.0	1388 ± 81
A. H.	56	B	M	191	2 wk	Tolbutamide	13.0 ± 0.8	1626 ± 171
E. B.	57	W	F	336	1 wk	Diet	12.6 ± 1.1	1541 ± 91
E. T.	57	W	M	150	1 mo	Insulin	15.6 ± 1.2	1931 ± 180
L. B.	57	B	F	116	23 yr	Insulin	12.6 ± 1.1	4207 ± 487
R. S.	58	B	F	134	3 yr	Tolbutamide	17.6 ± 0.7	2691 ± 208
M. L.	59	W	M	146	13 yr	Insulin	14.9 ± 0.5	2048 ± 141
R. B.	61	W	F	222	6 mo	Tolbutamide	14.2 ± 1.0	2748 ± 250
A. M.	61	B	F	190	6 yr	Tolbutamide	18.7 ± 1.7	2883 ± 212
R. S.	62	W	M	145	46 yr	Insulin	16.5 ± 0.8	2566 ± 213
L. J.	62	W*	F	150	4 yr	Insulin	17.9 ± 0.8	2751 ± 166
A. T.	70	B	M	140	1 wk	Tolbutamide	12.9 ± 0.6	3182 ± 285
M. J.	73	W*	M	165	10 yr	Diet	13.7 ± 1.0	1530 ± 106
H. R.	92	W	M	132	25 yr	Insulin	17.0 ± 1.3	1898 ± 136
Average ± SE	45 ± 2			164 ± 5	112 month ± 16 month		14.9 ± 0.3	2403 ± 119

W, white; B, black.
* Latin American.

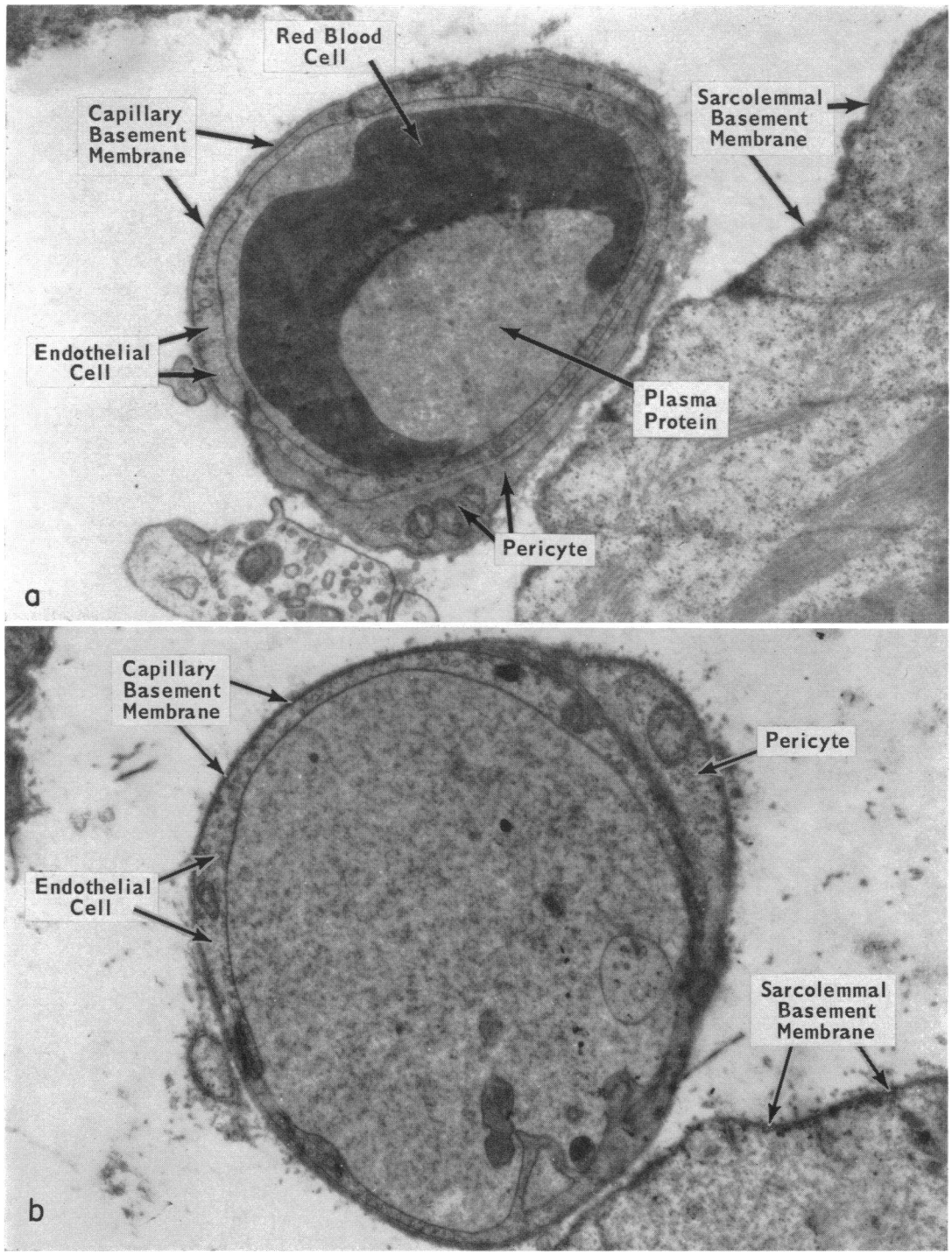
the second biopsy was obtained after an interval of 3 months, despite which the values for basement membrane width agreed within 9%. This relatively small variation between biopsies indicates that both the sampling and measuring techniques were sufficiently reproducible for the purposes of this study.

Another potential source of error in assessing basement membrane width is a possible variation of the calibre of the vessels among the groups of patients being studied. It has, for example, been suggested without supporting data (18) that basement membrane width is proportionate to capillary size, a suggestion that would imply that larger capillaries would have relatively thicker basement membranes. That this is not the case is conclusively demonstrated by the data in Table II, which are derived from the detailed figures given in Tables III, IV, and V. In none of the three types of capillaries examined in this study is there a positive correlation between capillary perimeter and basement membrane width. Actually, a small but statistically significant negative correlation between these parameters was noted in the prediabetic

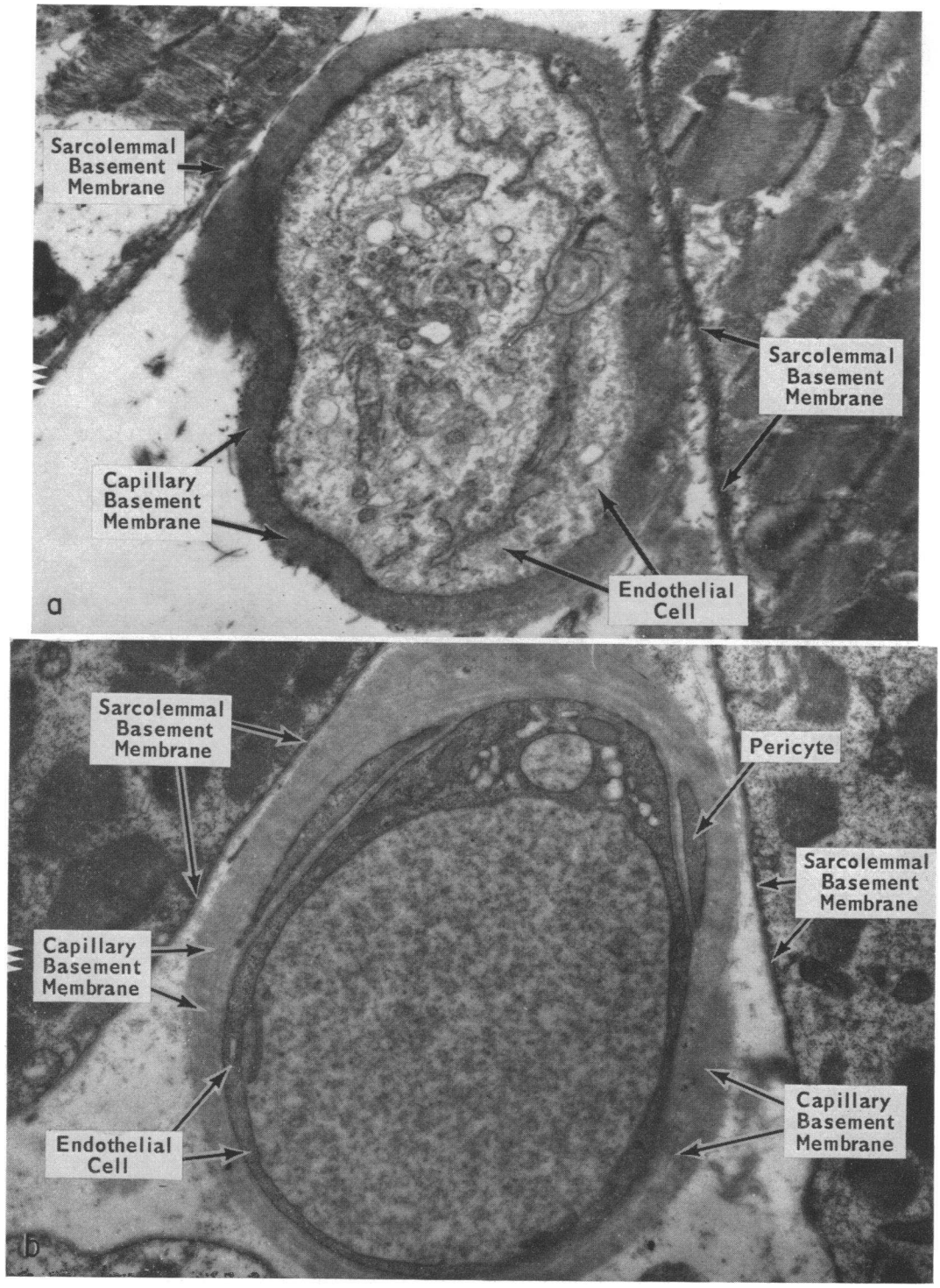
TABLE V
Prediabetic subjects

Subject	Age	Race	Sex	Weight	GTT			Capillary	
					F	1 hr	2 hr	Perimeter	Basement membrane width
	yr			*lb.*	*mg/100 ml*			*avg* $\mu \pm$SE	*avg* $A \pm$SE
N. J.	27	W*	M	205	88	96	110	13.1 ± 1.0	1529 ± 94
N. B.	28	W	F	150	84	98	121	11.6 ± 1.5	1568 ± 90
W. G.	28	W	F	118	92	126	94	15.0 ± 1.0	1248 ± 152
G. L.	29	W	F	154	84	147	65	15.1 ± 1.2	1229 ± 64
I. F.	29	W	F	226	114	150	141	15.2 ± 1.2	1690 ± 77
C. H.	31	W	F	147	95	98	117	17.0 ± 0.7	1473 ± 84
E. D.	33	W*	F	143	93	119	118	16.1 ± 1.1	1250 ± 86
R. K.	34	W	M	185	98	97	87	17.0 ± 1.0	1276 ± 113
M. L.	37	W	F	121	100	130	78	17.1 ± 0.7	976 ± 102
C. H.	38	W	M	145	74	157	100	14.5 ± 0.6	1078 ± 79
M. E.	40	W	F	129	96	128	92	15.2 ± 1.0	1355 ± 122
S. J.	40	W	M	159	103	183	135	15.8 ± 0.5	1302 ± 188
M. S.	41	W	F	132	90	144	120	14.0 ± 0.7	1236 ± 101
M. D.	43	W	F	123	92	153	132	16.4 ± 0.9	1088 ± 95
N. G.	43	W	M	214	112	148	102	13.1 ± 0.8	1633 ± 88
P. M.	43	W	F	146	75	182	118	15.5 ± 1.2	1417 ± 148
C. C.	46	W	M	196	94	126	76	15.6 ± 1.0	1352 ± 96
D. T.	46	W	F	140	107	152	110	14.4 ± 1.1	1308 ± 90
L. K.	48	W	F	132	92	152	121	15.7 ± 0.9	1436 ± 97
R. S.	50	W	F	164	100	94	105	16.1 ± 0.6	1195 ± 57
A. G.	51	W	M	218	112	122	106	13.7 ± 1.2	1552 ± 128
D. M.	51	W	F	147	80	88	73	13.9 ± 0.9	1135 ± 115
C. M.	51	W	M	149	92	83	102	14.8 ± 0.5	899 ± 66
V. J.	52	W	F	200	94	125	109	14.1 ± 1.5	1893 ± 159
S. M.	53	W	M	146	112	161	124	14.2 ± 1.2	1480 ± 78
E. M.	55	W	F	115	117	172	104	15.3 ± 0.8	1181 ± 91
J. M.	57	W	M	146	120	158	133	18.0 ± 1.1	1430 ± 69
R. B.	58	W	M	215	83	97	89	14.0 ± 1.1	1999 ± 158
R. E.	58	W	F	153	87	118	84	15.5 ± 0.7	1404 ± 88
C. B.	60	W	M	160	93	154	54	15.6 ± 1.1	1458 ± 144
Average ± SE	43 ± 2			159 ± 6	Fasting		96 ± 2	15.0 ± 0.2	1373 ± 44
					1 hr		132 ± 5		
					2 hr		104 ± 4		

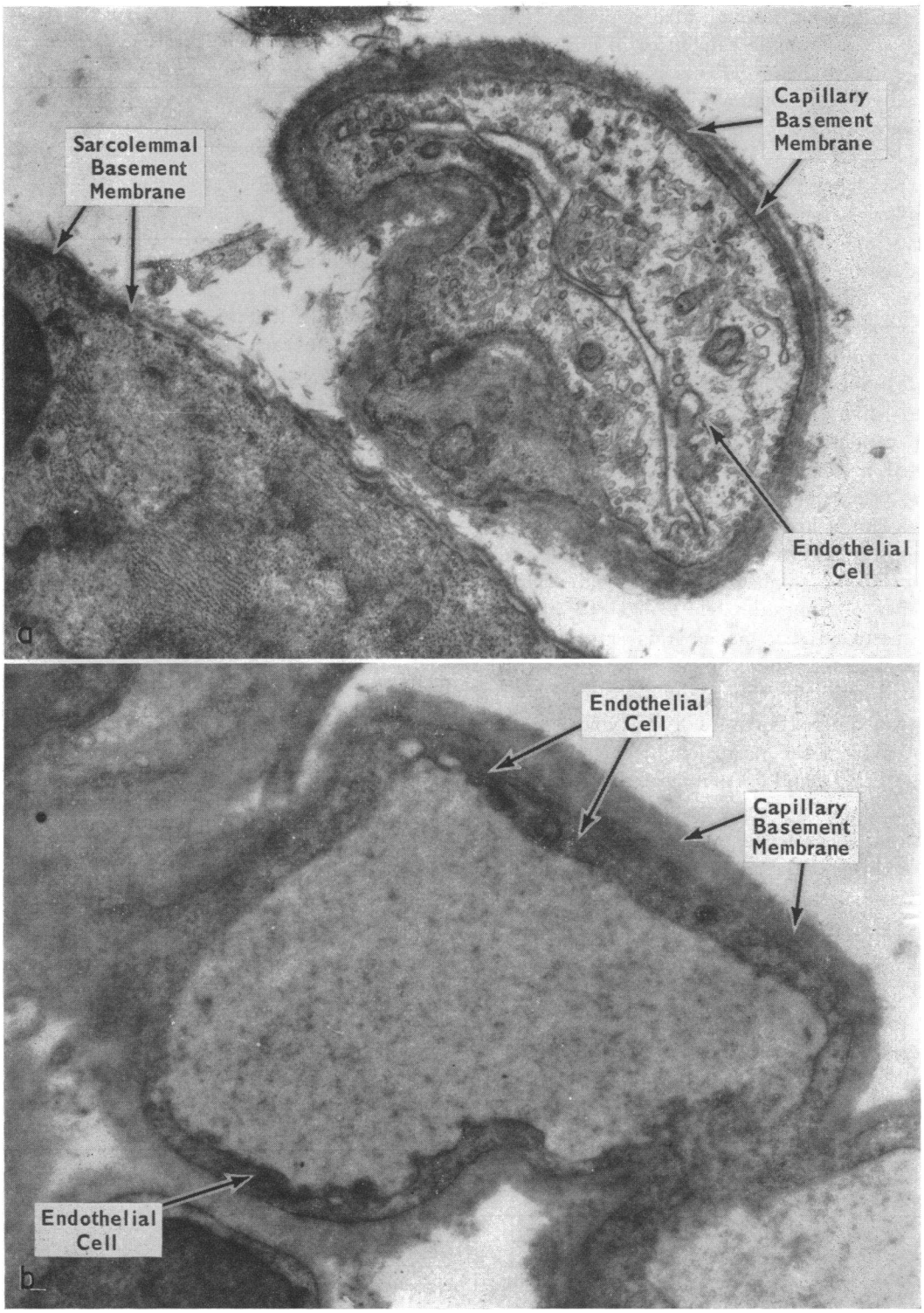
GTT, glucose tolerance test; F, fasting; W, white.
* Latin American.



FIGURES 3 *a* and *b* Examples of normal muscle capillary basement membranes. Note in Fig. 3 *a* and *b* that the muscle capillary basement membrane has a width approximately equal to that of the sarcolemmal basement membrane, the electron micrographs are, respectively, from patients T. W. (× 11,600), Table III, and A. C. (× 8,965), **Table XV.**



FIGURES 4 *a* and *b* Examples of muscle capillary basement membranes from diabetic patients. Note that while capillary basement membranes are greatly thickened, the sarcolemmal basement membranes are not affected. The electron micrographs are, respectively, from patients R. S. (× 8,396) and N. J. (× 12,864), Table IV.



FIGURES 5 *a* and *b* Examples of thickened basement membranes in prediabetic patients. The electron micrographs are, respectively, from patients V. J. (× 12,008) and R. B. (× 8,477), Table V.

group. In addition, as indicated in Table II, the average size of muscle capillaries in normal, diabetic, and prediabetic patients is almost identical, further indicating that a bias in size of vessels studied could not have played a role in the results obtained.

The possible effect of the Xylocaine infiltration on capillary basement membranes was determined by immersing fresh biopsy specimens in 1% Xylocaine for 5 min before fixation. This treatment was found to have no significant influence upon basement membrane width.

Muscle capillary basement membrane width in normal subjects. The initial aim of this study was to define the basement membrane width of muscle capillaries of a nondiabetic population. As noted in the Methods section, this group of subjects was rigorously selected on the basis of a negative family history and a normal oral glucose tolerance curve. Nonetheless, in view of a probable incidence of diabetes mellitus in the general population of approximately 2–6% (27, 28) it can be assumed that despite this screening procedure, a similar percentage of this "normal" group will in fact be genetically diabetic.

Electron micrographs of two typical muscle capillaries from the normal subjects studied are shown in Fig 3 *a* and *b*. As indicated, in these electron micrographs the basement membrane of muscle capillaries from nondiabetic patients form single, thin, relatively homogeneous structures completely surrounding the endothelial cell. It should also be noted that in a normal patient the width of the capillary basement membrane is very similar to that of the adjacent sarcolemmal basement membrane.

TABLE VI

Average Muscle Capillary Basement Membrane Width in Normal, Diabetic, and Prediabetic Patients

Subjects	Number of subjects	Number of capillaries	Average basement membrane width	Significance
			$A \pm SE$*	
Normal	50	747	1080 ± 27	
				<0.01
Diabetic	51	765	2403 ± 119	<0.01
				<0.01
Prediabetic	30	450	1373 ± 44	

* SE based on number of subjects.

TABLE VII

Incidence of Basement Membrane Thickening in Normal, Diabetic, and Prediabetic Patients*

Diagnosis	Total patients	Incidence of thickening	Per cent
Normal	50	4	8
Diabetic	51	50	98
Prediabetic	30	16	53

* Average of 15 vessels >1325 A.

The detailed data dealing with the weights and glucose tolerance tests as well as the capillary sizes and basement membrane widths of the normal subjects are presented in Tables III and VI. The average muscle capillary basement membrane width of the 747 capillaries examined in these nondiabetic subjects was 1080 A, with a standard error of the mean (SE) of 27 A. The values for the basement membrane width of individual capillaries are shown in Fig. 6 and illustrate that the variation within a single subject's capillary basement membrane is relatively small, with only 6% of individual capillaries having basement membrane widths in excess of 1500 A. Moreover, as indicated by the dotted line in Fig. 6, if an upper limit of normal for the average basement membrane width based on the point of optimum discrimination is set at 1325 A, 46 of the 50 or 92% of the nondiabetic subjects have average basement membrane widths that fall within this normal range (Table VII).

Within the age range of 19–62 yr examined in this study, there is no correlation between the subjects' ages and their muscle capillary basement

TABLE VIII

Correlation between Capillary Basement Membrane Width and Age, Weight, and Duration of Diabetes

	Age	Weight	Duration of diabetes
	Correlation coefficient	Correlation coefficient	Correlation coefficient
Normal	+0.063	+0.319*	—
Diabetic	+0.108	−0.214	−0.030
Prediabetic	+0.137	+0.679‡	—

* Significant correlation at the 0.05 level.
‡ Significant correlation at the 0.01 level.

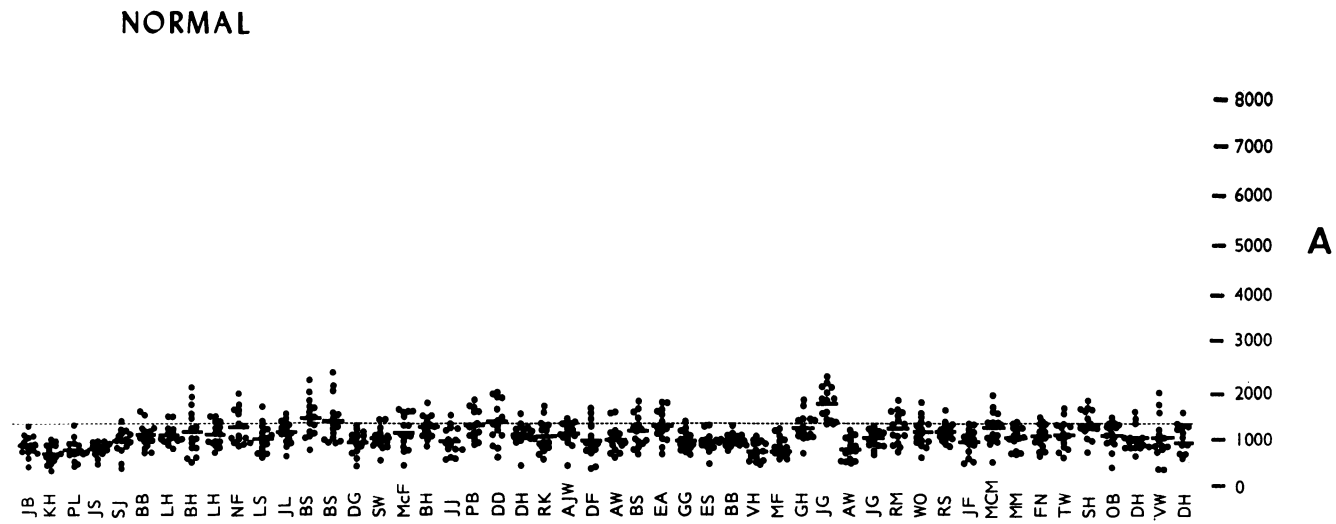


FIGURE 6 Plot of individual capillary basement membrane widths from each of 50 Normal patients. The bars indicate the average (A) value for the basement membrane width of the 15 capillaries. The horizontal dotted line indicates the upper limit of normal for average capillary basement membrane width.

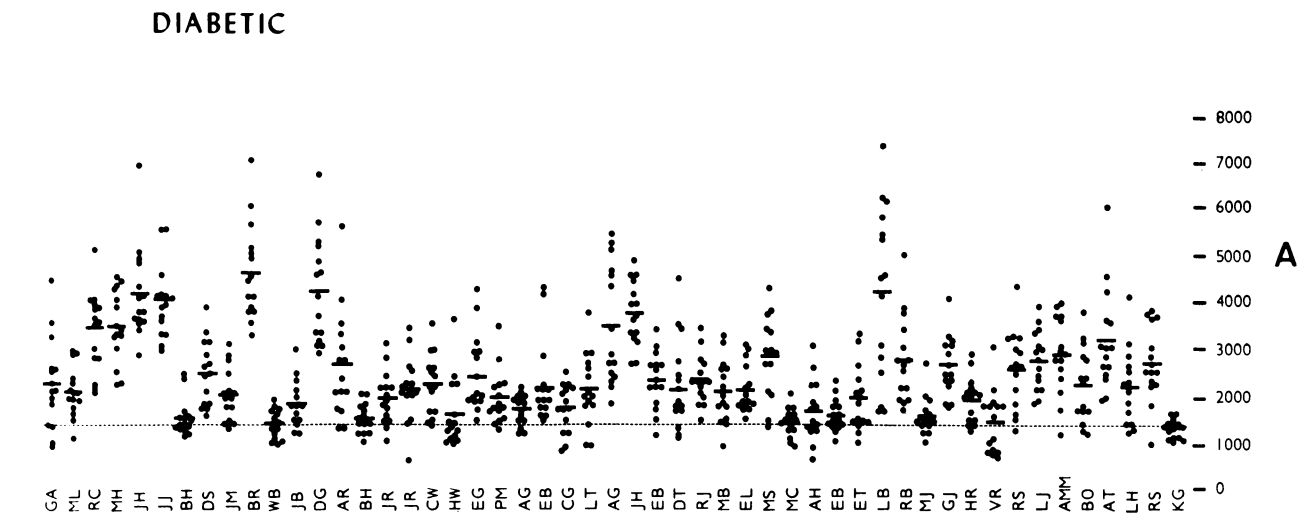


FIGURE 7 Plot of individual capillary basement membrane width from each of 51 Diabetic patients. See Fig. 6.

membrane widths (Table VIII). Moreover, as shown in Fig. 9 *A*, there is also no relationship between the glucose tolerance, i.e. fasting, 1- or 2-hr plasma glucose values, and basement membrane width. On the other hand, a slight but statistically significant ($P < 0.05$) correlation between body weight and basement membrane width was observed in the nondiabetic subjects (Table VIII). In addition, normal men were found to have somewhat thicker basement membrane than women, 1144 A vs. 998 A, $P < 0.05$ (Table IX).

Muscle capillary basement membrane width in diabetic subjects. Typical electron micrographs of two diabetic capillaries are presented in Fig. 4 *a* and *b*. It is readily apparent from these examples that marked basement membrane hypertrophy can be subjectively recognized in many diabetic muscle capillaries. The density of the diabetic basement membrane does not differ detectably from that of normal capillaries nor can any difference in internal structure of the membrane be detected. Finally, the endothelial cells surrounding the capillaries of diabetic patients show no obvious alterations from those of normal subjects.

The measurements of basement membrane width presented in Tables IV and VI, and Fig. 7, clearly demonstrate that the muscle capillary basement membranes of the 51 diabetic patients are in fact much thicker than those of the nondiabetic subjects, the average basement membrane width of the 765 diabetic capillaries being 2403 A (SE ± 119), a value that is over twice that of the normal subjects of 1080 A. As indicated in summary Table VI, the difference between the means of the normal and diabetic basement membrane widths is significant at the < 0.01 level.

The incidence of capillary basement membrane hypertrophy in the diabetic group is presented in Table VII. Utilizing the upper limit of normal of 1325 A, as shown in Fig. 7, all but one of the 51 diabetic patients demonstrated capillary basement membrane thickening. These results therefore demonstrate not only that basement membrane hypertrophy of muscle capillaries can readily be documented on the basis of the average widths of large numbers of diabetic capillaries, but that in addition this lesion is an almost constant feature of individual cases of diabetes.

Relation of basement membrane width to age, sex, weight, duration, and severity of diabetes.

TABLE IX
Basement Membrane Width in Men and Women

Subjects		Number	Basement membrane width	Significance
			A ± *SE*	
Normal	Men	28	1144 ± 35	$P < 0.05$
	Women	22	998 ± 35	
Diabetic	Men	23	2217 ± 152	$P > 0.05$
	Women	28	2557 ± 174	
Prediabetic	Men	12	1415 ± 80	$P > 0.05$
	Women	18	1345 ± 51	

Attempts were next made to relate the degree of hypertrophy of the diabetic basement membrane to a number of characteristics of the diabetic patients that might logically be expected to correlate with the severity of this vascular lesion. The results, summarized in Table VIII, demonstrate that, somewhat unexpectedly, there is no correlation within the diabetic population between the age of the subjects and the degree of muscle capillary basement membrane hypertrophy; young diabetics have as thick basement membranes as do diabetic patients in their 7th, 8th, and even 10th decades. In this connection, as noted in Tables III and IV, the average age of the diabetic patients in this study was somewhat greater than that of normal subjects. However, in view of the lack of an effect of age on basement membrane width in either normal or diabetic groups, this factor could obviously not have influenced the marked differences in basement membrane width that exist between the two groups. As the data in Table VIII also demonstrate, there is no relationship between the weight of the diabetic patients and the degree of thickening of their basement membrane. Most surprisingly, however, basement membrane thickening in the diabetic patients becomes no worse with increasing duration of known diabetes mellitus, and in fact, as was frequently apparent, severe degrees of basement membrane hypertrophy were present at the time that overt carbohydrate abnormalities were first detected.

In an attempt to determine whether this lesion is related to the *severity* of the carbohydrate derangements of diabetes, the 51 diabetic subjects were subdivided into those whose diabetes could

be controlled with either diet alone or with oral hypoglycemic agents, and those who require insulin to maintain reasonable control of their diabetes. As shown in Table X, there is no relationship between the degree of basement membrane hypertrophy and the severity of the carbohydrate disturbance of diabetes.

Finally, a similar analysis of diabetic patients who had developed their disease before age 21 as compared to those with a later onset of diabetes again demonstrates no significant difference in the degree of basement membrane hypertrophy between the two groups (Table XI). These results therefore indicate that "juvenile" and "adult" onset diabetics have equally severe thickening of muscle capillary basement membrane, a finding that leads to the general conclusion that neither severity of carbohydrate disorder nor age of onset influences the severity of the muscle capillary lesion of the diabetic.

Basement membrane width in patients with nondiabetic hyperglycemia. The lack of any relationship between the severity of the carbohydrate derangements of diabetes and basement membrane thickening raises the question of whether the carbohydrate abnormalities of diabetes in fact play a significant role in the development of the microangiopathy of diabetes mellitus. To answer this question, a study was undertaken to determine whether hyperglycemia in the absence of genetic diabetes mellitus will result in basement membrane hypertrophy. As demonstrated in Table XII, we have to date studied a total of nine patients with acquired hyperglycemia secondary to alcoholic pancreatitis. Of these nine subjects, seven had normal basement membranes, one (R.M.) had minimal basement membrane thickening, and one (B.R.) had severe hypertrophy of his capillary basement membrane. It should be emphasized that with one exception each of the patients with pancreatitis and normal capillary basement membranes had severe fasting hyperglycemia of over 200 mg/100 ml. Moreover, in the case of W.K., marked hyperglycemia even of 13 yr duration did not result in basement membrane hypertrophy.

TABLE X

Basement Membrane Width in Mild and Severe Diabetes

Treatment	Number of patients	Basement membrane width	Significance
		$A \pm SE$	
Diet or tolbutamide	29	2410 ± 167	>0.05
Insulin	22	2395 ± 173	

TABLE XI

Basement Membrane Widths in Juvenile Onset and Adult Onset Diabetic Patients*

Onset	Number of patients	Basement membrane width	Significance
		$A \pm SE$	
Juvenile*	13	2766 ± 298	>0.05
Adult*	38	2284 ± 119	

* Juvenile onset diabetics are arbitrarily defined as patients having known disease before age 21; adult onset refers to those who developed diabetes after age 21.

Several other selected cases of hyperglycemia of nondiabetic origin are presented in Table XIII. Severe hyperlipidemia may be associated with fasting hyperglycemia (29), but as illustrated by cases 1–3, Table XIII, basement membrane hypertrophy need not result from such an elevation in blood sugar even though the hyperglycemia has been present for as long as 27 yr. One case of lipodystrophic diabetes,[5] case 6, despite 5 yr of documented hyperglycemia, has shown no evidence of basement membrane thickening. Finally, in one patient with a pheochromocytoma and in another patient with iatrogenic Cushing's syndrome leading to hyperglycemia, basement membranes were normal.

In summary, these findings would strongly suggest that in man, hyperglycemia, even though severe and of long duration, does not cause basement membrane thickening in the absence of genetic diabetes mellitus.

Basement membrane width in hyperglycemic animals. The inability of hyperglycemia to produce muscle capillary basement membrane hypertrophy has been confirmed in several animal models. Spontaneous hyperglycemia occurs in inbred strains of Chinese hamsters (30, 31). How-

[5] We wish to thank Dr. Richard J. Havel of the University of California San Francisco Medical Center, Department of Internal Medicine, for arranging the biopsy on this patient.

TABLE XII
Basement Membrane Width in Pancreatic Diabetes

Patient	Age	Sex	Diagnosis	FBS	Basement membrane width
	yr			*mg/100 ml*	*A* ±SE
T. H.	24	M	Chronic pancreatitis, steatorrhea, insulin dependent	531	1219 ± 80
M. S.	30	M	Chronic pancreatitis, calcification, insulin dependent	272	1302 ± 60
S. H.	31	M	Chronic pancreatitis	400	1028 ± 89
E. W.	41	M	Acute pancreatitis	453	1039 ± 35
W. K.	45	M	Chronic pancreatitis, insulin dependent	200–300*	796 ± 38
B. R.	45	M	Chronic pancreatitis, insulin dependent	298	2344 ± 114
J. M.	46	M	Chronic pancreatitis, steatorrhea	364	1000 ± 50
R. M.	49	M	Chronic pancreatitis	320	1428 ± 167
H. M.	49	M	Chronic pancreatitis, with fibrosis	236 (2 hr PP)	1167 ± 85
Average					1258 ± 149

* Fasting hyperglycemia has been documented in this patient for 13 yr.
FBS, fasting blood sugar.

ever, as shown in Table XIV, basement membrane width is no greater in such hyperglycemic hamsters than in their normal litter mates. Moreover, a Golden hamster, which does not develop hyperglycemia, had a capillary basement membrane width that was not significantly different from that of the Chinese hamster with spontaneous hyperglycemia. As shown in Table XIV, the KK mouse with spontaneous hyperglycemia (32) manifests no evidence of basement membrane thickening. Finally, the one example of a hyperglycemic Egyptian sand rat examined to date had basement membranes that did not differ significantly from those of a nonhyperglycemic sand rat.

Basement membrane width in prediabetic subjects. The failure of hyperglycemia of nondiabetic origin to cause basement membrane thickening

TABLE XIII
Basement Membrane Width in Nondiabetic Hyperglycemia

Patient	Age	Sex	Diagnosis	Clinical and laboratory data	FBS	Basement membrane width
	yr				*mg/100 ml*	*A* ±SE
R. J.	35	M	Hyperlipidemia	Triglyceride, 1200 mg/100 ml	294	1032 ± 75
E. G.	31	F	Hyperlipidemia	Triglyceride, 17,500 mg/100 ml	245	1364 ± 107
D. S.	72	F	Hyperlipidemia	Triglyceride, 900 mg/100 ml	200 (27 yr)	1190 ± 75
S. P.	49	F	Cushings (iatrogenic)	Wegner's granulomatosis; Prednisolone, 100 mg/day	130	861 ± 90
F. W.	51	M	Pheochromocytoma	Normal FBS after surgery	184	1190 ± 92
K. K.	40	F	Lipodystrophic diabetes	Requires 250 U of insulin per day	330 (5 yr)	972 ± 68
Average						1102 ± 74

FBS, fasting blood sugar.

TABLE XIV

Capillary Basement Membrane Width in Diabetic Chinese Hamsters and Hyperglycemic Rodents

Experiment	Diagnosis	Muscle capillary width*
		A ± *SE*
Chinese hamsters		
Male	Normal litter mate (1)	656 ± 22
	Hyperglycemic litter mate (1)	620 ± 19
Female	Normal litter mate (1)	568 ± 30
	Hyperglycemic litter mate (1)	719 ± 37
Golden hamster	Normal (1)	618 ± 18
KK mouse	Normal (3)	731 ± 40
	Hyperglycemic (5)	573 ± 17
Sand rat	Normal (1)	843 ± 73
	Hyperglycemic (1)	1023 ± 58

* Basement membrane widths represent averages from 15 capillaries obtained from the quadriceps muscle. Numbers in parenthesis indicate numbers of animals.

either in man or in the few examples of hyperglycemic animals studied raises the possibility that in patients with overt genetic diabetes mellitus hyperglycemia may not be the factor responsible for the basement membrane lesions and hence the microangiopathy of diabetes. To evaluate this possibility further, we have determined muscle capillary basement membrane widths in a series of 30 prediabetic subjects both of whose parents were overtly diabetic but who themselves had normal fasting plasma sugars and normal glucose tolerance tests as defined in Methods. It should be emphasized that the glucose tolerance tests in this group of prediabetic patients were not only normal, but did not differ significantly from those of the normal subjects (Tables III, V, and Fig. 10).

Representative examples of capillaries from these prediabetic subjects are shown in Fig. 5 *a* and *b*, and, as is evident from these electron micrographs, the basement membranes of such capillaries appear thicker than those from nondiabetic patients (Fig. 3 a and b). This impression is confirmed by the quantitative data presented in Tables V and VI, and illustrated in Fig. 8. The average basement membrane width of the prediabetic group is 1373 A (SE ± 44), a figure that is significantly greater than that of the normal subjects. The actual incidence of basement membrane hypertrophy within the prediabetic group bears out this conclusion: that of the 30 prediabetic patients, 16 or 53% were found to have demonstrable basement membrane hypertrophy at the time of the study (Table VII). This finding clearly indicates that basement membrane hypertrophy may precede the onset of abnormalities of

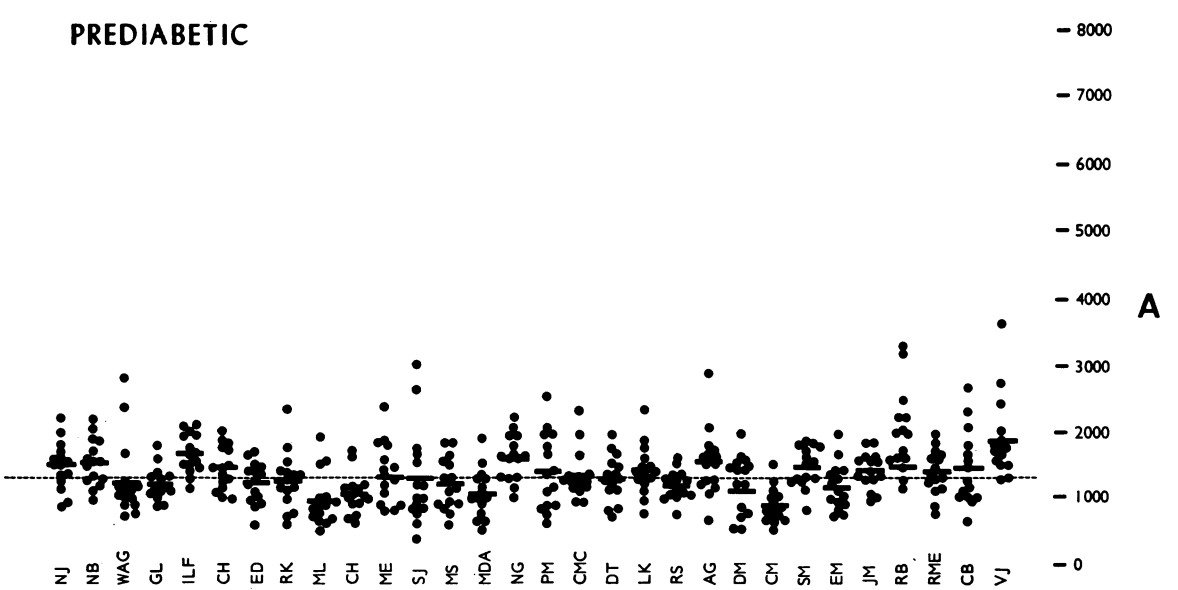


FIGURE 8 Plot of individual capillary basement membrane width from each of 30 prediabetic subjects. See Fig. 6.

glucose metabolism in the individual who is destined to become diabetic.

Relationship of basement membrane width to glucose tolerance in prediabetic subjects. As noted above, the prediabetic patients in this study were found to have glucose tolerance tests that did not differ significantly from those of the normal group. Nonetheless, if subtle abnormalities in carbohydrate metabolism were responsible for basement membrane thickening in diabetes mellitus, it is still possible that, despite the normal glucose tolerance tests of the prediabetic group, those prediabetic patients with relatively poorer glucose tolerance might show the thicker basement membranes. The regression analyses in Fig. 9 *A* and *B* in fact demonstrate however that within the prediabetic subjects, there is no significant relationship between fasting, 1-hr, or 2-hr glucose levels, and basement membrane width. In other words, thickening of capillary basement membranes was as frequently observed in those prediabetic patients with flat glucose tolerance tests as in those with tolerance curves that are in the upper range of normal.

Two other variables could conceivably have played roles in yielding the increased basement membrane thickness noted in the prediabetic group. As noted earlier, a small but significant difference in basement membrane width was observed between men and women in the normal group. As shown in Table IX this difference is not, however, found in the prediabetic population.

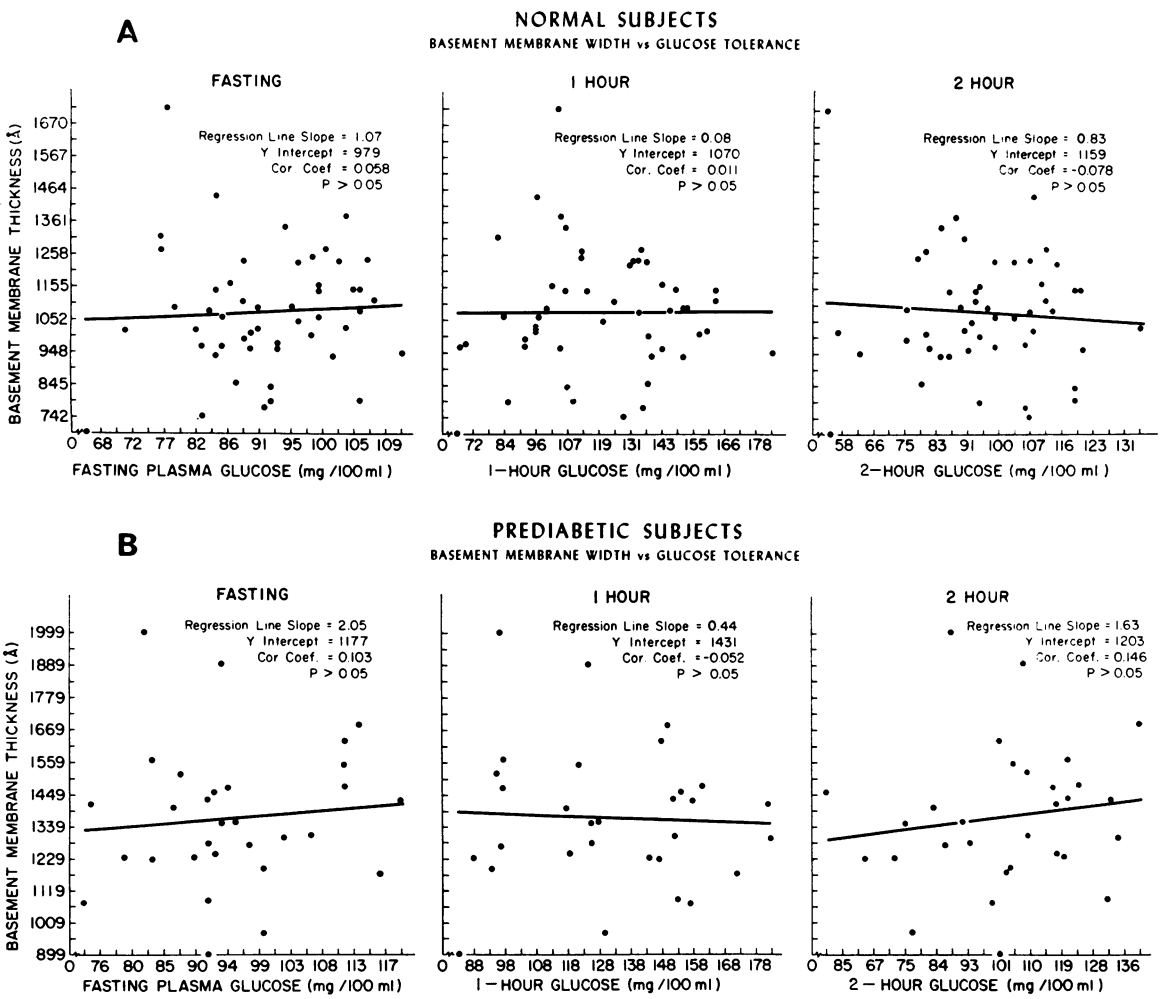


FIGURE 9 Regression analyses of relation between glucose tolerance tests and basement membrane width in normal subjects (9 *A*), and prediabetic subjects (9 *B*).

Moreover, since normal men have somewhat thicker basement membranes than do normal women, the greater proportion of women in the prediabetic group would, if anything, decrease the difference in basement membrane width between the two populations. Finally, when analyzed separately by sex, the difference between the normal and prediabetic groups remains highly significant for both sexes.

A correlation between weight and basement width was observed both in the normal and in the prediabetic groups, Table VIII, and conceivably therefore, differences in weights between the two groups might have influenced the average basement membrane widths. To eliminate this possibility, the data were analyzed in selected groups of normal and prediabetic subjects that were equalized for weight and for weight/height. Even when evaluated in this manner, the difference in basement membrane width between normal and prediabetic subjects remains highly significant (equalized for weight, 1083 A ± 28 vs. 1373 A ± 44 [$P < 0.01$]; equalized for weight/height, 1064 A ± 41 vs. 1369 A ± 45 [$P < 0.01$]).

Comparison of capillary basement membrane thickening in deltoid, quadriceps, and gastrocnemius. While all biopsies used in this study were obtained from a relatively restricted site in the quadriceps muscle, a limited study of basement membrane width in two other muscle groups was carried out to determine whether capillary basement membrane thickening is detectable in other muscles of the diabetic patient. The data in Table XV indicate that both in normal and in diabetic subjects there tends to be a progressive increase in basement membrane width from deltoid to quadriceps to gastrocnemius muscles. It should, however, be emphasized that when compared to capillary basement membrane widths at *comparable* sites, basement membrane thickening is apparent in the diabetic subject regardless of the biopsy site chosen. These data also indicate, however, that basement membrane widths based on measurements from one group of muscles cannot be directly applied to muscle capillaries obtained at other sites.

Capillary basement membrane width in adipose tissue of diabetic patients. During the procedure employed to obtain muscle biopsies, it is relatively simple to biopsy subcutaneous adipose tissue as well. A comparison of basement membrane widths of such adipose capillaries in a small series of patients (Table XVI) demonstrates that these capillaries have basement membranes that are not statistically different in thickness from those of underlying muscle. While adipose tissue biopsies may therefore be utilized to determine basement membrane hypertrophy in diabetes, the lesser frequency with which capillaries are found in sections of adipose tissue makes this tissue far less practical than muscle for this purpose.

Capillary basement membranes in the kidneys

TABLE XV

Capillary Basement Membrane Width in Deltoid, Quadriceps, and Gastrocnemius Muscles

Patient	Diagnosis	Deltoid	Quadriceps	Gastrocnemius
		A ±SE	A ±SE	A ±SE
A. C.	Normal	818 ± 74	1126 ± 59	1218 ± 56
N. M.	Normal	1168 ± 109	1217 ± 116	1651 ± 127
M. T.	Normal	1057 ± 66	1192 ± 101	1255 ± 88
Average	Normal	1014 ± 103	1178 ± 27	1374 ± 138
M. B.	Diabetic	1796 ± 165	2063 ± 178	2737 ± 261
R. S.	Diabetic	1696 ± 196	2691 ± 208	1521 ± 110
C. R.	Diabetic	—	1998 ± 174	1403 ± 172
K. G.*	Diabetic	1103 ± 84	1274 ± 54	1986 ± 165
G. A.	Diabetic	1938 ± 230	2214 ± 254	2860 ± 419
M. J.	Diabetic	1406 ± 166	1943 ± 281	2089 ± 215
Average	Diabetic	1588 ± 149	2031 ± 187	2099 ± 246

* K. G. is the only presumably diabetic patient biopsied to date who has a normal average basement membrane width in his quadriceps muscle. It may be of significance that the capillaries of his gastrocnemius muscle have basement membranes that are probably thickened.

TABLE XVI

Capillary Basement Membrane Width in Diabetic and Normal Adipose Tissue

Patient	Diagnosis	Muscle	Adipose tissue
		A ± *SE*	*A* ± *SE*
A. C.	Diabetic	1437 ± 87 N = 15	1476 ± 122 N = 11
A. G.	Diabetic	3519 ± 252 N = 11	2790 ± 307 N = 5
A. R.	Diabetic	2292 ± 165 N = 14	1879 ± 113 N = 25
V. S.	Normal	1077 ± 32 N = 15	1174 ± 105 N = 10

N, number of capillaries measured.

and muscles of nephrotic patients. While extensive studies on the specificity of basement membrane thickening of the muscle capillaries have not been carried out, preliminary observations have clearly shown that malignant hypertension and atherosclerosis do not lead to basement membrane hypertrophy. Because of the severe basement membrane thickening of the renal capillary that characterizes the nephrotic syndrome, a comparison was made between muscle and renal capillaries from a series of nephrotic patients. The data in Table XVII demonstrate that there is no correlation between the width of renal capillary basement membranes and that of muscle, and, in fact, despite severe hypertrophy of the renal capillary basement membrane, in only one of the patients with the nephrotic syndrome was some thickening of the muscle capillary basement membrane noted. It is therefore apparent that the renal capillary

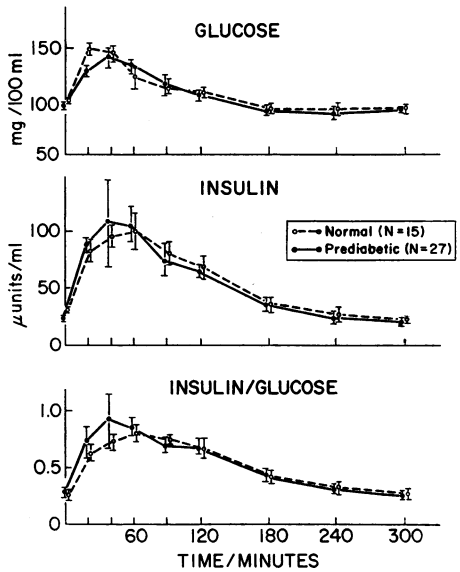


FIGURE 10 Insulin secretion and insulinogenetic index during glucose tolerance test in normal and prediabetic patients. Values represent mean ± standard error.

basement membrane hypertrophy that is characteristic of the nephrotic syndrome does not affect muscle capillaries; and it follows that thickening of the muscle capillary basement membrane is a more specific lesion of diabetes mellitus than are the proliferative changes of renal glomerulus, which, while characteristic of diabetes mellitus, are also observed in numerous other conditions.

Insulin levels in prediabetic patients. As demonstrated in Fig. 10, in the 27 out of 30 prediabetic patients in whom both glucose tolerance tests and insulin concentrations were determined, serum insulin levels did not differ significantly

TABLE XVII

Muscle Capillary Basement Membrane Width in Patients with Thickened Renal Capillary Basement Membranes

Patient	Age	Race	Sex	Muscle	Kidney	Diagnosis
				A ±SE	*A* ±SE	
S. C.	29	W	F	1286 ± 117	4478 ± 510	Nephrosis, cause unknown
M. W.	15	B	M	860 ± 63	5186 ± 486	Nephrosis, chronic glomerulonephritis
M. W.	32	B	F	1476 ± 87	6910 ± 408	Nephrosis, cause unknown
T. O.	53	B	F	1330 ± 68	4530 ± 376	Nephrosis, chronic glomerulonephritis
G. H.	38	B	F	1043 ± 119	4481 ± 405	Nephrosis, cause unknown
H. S.	32	B	M	926 ± 60	9795 ± 1531	Membranous glomerulosclerosis
C. G.	51	B	M	801 ± 39	3541 ± 265	Probable glomerulonephritis
Normal upper limit				1325	3200*	

W, white; B, black.
* This value is based on the data of Vernier (33).

from those of the 15 normal subjects studied. Moreover, when insulin/glucose ratios were calculated, no decrease in insulin response was observed at any interval during the glucose tolerance test. It is apparent therefore that, at least as indicated by this type of challenge, the prediabetic patients studied demonstrated no abnormalities of insulin secretion.

DISCUSSION

The primary objective of the present study of basement membrane width of muscle capillaries was twofold: first, to determine whether thickening of the capillary basement membranes is a consistent lesion in patients with overt diabetes mellitus, and second, to evaluate the possibility that such basement membrane thickening may precede and be independent of the carbohydrate abnormalities of the diabetic syndrome.

There have appeared four previous electron microscopic studies that have employed quantitative methods in attempts to demonstrate basement membrane thickening in the muscle capillaries of diabetic patients. The number of patients in each case has been small, and the results have been contradictory. Zacks, Pegues, and Elliott (12), confirming the older light microscopic studies of Goldenberg, Alex, Joshi, and Blumenthal (11), were able to demonstrate that of five diabetic patients studied, four showed detectable basement membrane thickening of the muscle capillaries. Bloodworth has reported thickening of the basement membrane of muscle capillaries in two diabetic patients (7), and most recently, Fuchs studied three patients with diabetes and reported thickening of the muscle capillary basement membrane in each (13). Except for the study by Fuchs, neither the sites of the muscle biopsies nor the methods of quantitation of basement membrane width were reported in these earlier studies. Bencosme, West, Kerr, and Wilson (14), while not reporting quantitative data, state that basement membrane thickening of muscle capillaries was found in the capillaries of 12 of 16 diabetic patients studied, and in 3 of 19 nondiabetic subjects. More recently, Vracko and Strandness (18) have seriously criticized these earlier reports of thickened muscle capillary basement membranes in diabetic patients. Utilizing a well-described method for measuring basement membrane area, these investigators have examined capillaries of abdominal muscle obtained at surgery, and report that, in contrast to earlier studies, they were unable to detect hypertrophy of capillary basement membrane in diabetic patients. It should, however, be noted that these conclusions are based on studies of a total of only four overtly diabetic patients; moreover, one of these subjects showed an average basement membrane width that was probably significantly thickened. The reason for the failure of Vracko and Strandness to detect more consistent basement membrane thickening in the abdominal muscle capillaries of their four diabetic subjects is not apparent.

In contrast to previous studies, the present investigation has employed a simple needle biopsy technique, which when coupled with a standardized and reproducible method of quantitating the width of basement membranes, has made it possible to determine basement membrane thickness reliably on over 1500 normal, diabetic, and prediabetic capillaries in a total of 131 patients. This quantitative approach has furthermore permitted detection of a more subtle thickening of basement membrane than has been possible with smaller numbers of samples or less rigorous quantitation.

The results of this study have demonstrated that capillary basement membrane width of diabetic muscle averages over twice that of a normal nondiabetic population. Moreover, it is clear that basement membrane thickening is a remarkably constant finding in diabetic subjects, being present in 98% of the 51 diabetic patients examined. By contrast, basement membrane thickening was observed in only 8% of nondiabetic subjects. These presumably false positive results may represent in part the approximately 2–6% of the general population, who are estimated to be genetically prediabetic; or on the other hand, this result may simply indicate that the criterion employed overestimates the incidence of significant basement membrane thickening by approximately 8%. Indeed, even with the relatively close agreement between duplicate measurements, the data in Table II illustrate that in the case of borderline values occasionally one sample may show basement membrane widths greater than 1325 A, whereas its duplicate may be within normal range.

The advantages of utilizing muscle as the tis-

sue in which to study diabetic microangiopathy is well illustrated by the report of Friederici, Tucker, and Schwartz (19), that thickening of capillary basement membranes cannot be objectively demonstrated in skin biopsies even in overtly diabetic patients. As we have previously noted (1, 34), changes in the dermal capillaries of diabetics, while subjectively apparent, are difficult to quantitate objectively due to the normal fragmentation and reduplication that characterize the basement membrane of skin capillaries. It is for this reason that, after an unsuccessful preliminary attempt to employ dermal biopsies, we have made use of muscle capillaries as offering a far more reliable means of measuring basement membrane width. In this regard, it should also be noted (Fig. 7) that even in the case of those diabetic patients in whom the *average* basement membrane width is markedly increased, individual capillaries will have basement membranes that are of normal width. These results demonstrate that even in patients with long-standing diabetes, diabetic microangiopathy need not involve all muscle capillaries; but in addition, this observation further serves to emphasize the need for rigorous adherence to a quantitative procedure of basement membrane measurement that is both "blind" and uses large numbers of electron micrographs taken at random and unselected for quality.

The quantification of basement membrane width has also made possible an examination of the possible relationship of the degree of basement membrane thickening to several factors that might influence microangiopathy in the overtly diabetic patients. As demonstrated in Tables VIII and IX, no correlation was observed between basement membrane width and age, sex, weight, severity of carbohydrate abnormalites, or duration of diabetes. Moreover, (Table XI) patients with juvenile diabetes mellitus have a similar degree of basement membrane hypertrophy as do those whose disease became manifest in adulthood. The absence of a correlation between severity of the carbohydrate derangements and thickness of basement membrane is not unexpected and simply gives quantitative support to the clinical impression that there is little or no relationship between severity of such carbohydrate abnormalities and the time of onset or progression of the vascular complications of diabetes (35, 36). It was, however, somewhat more surprising to find that there is also no relationship between either the age of the diabetic patients or the duration of known carbohydrate abnormalities and muscle capillary basement membrane width. Though duration of the diabetic state can obviously not be fixed with certainty, the severity of basement membrane thickening was as great in those patients who were examined within days of the onset of overt clinical diabetes as in those who had their disease for many years. These results, as well as those in the prediabetic patients, would indicate that muscle basement membrane thickening must occur very early in the development of the diabetic syndrome and reaches a stable and presumably maximum degree of involvement by the time that hyperglycemia first becomes apparent. By contrast, clinical and light microscopic experience (2, 35, 36) indicates that the microangiopathy of the diabetic kidney and retina is clearly related to duration of overt diabetes. Moreover, progressive proliferation of basement membrane or basement membrane-like material in the renal capillary as demonstrated by electron microscopy is probably also a function of duration of diabetes (37). We would conclude, therefore, that whereas muscle capillary basement membrane thickening is an early and sensitive indication of microangiopathy, it is probably poorly correlated with comparable basement membrane thickening at other sites in the body.

The present studies have not attempted to establish the absolute specificity of muscle capillary basement membrane thickening for diabetes mellitus. Another study from this department has in fact shown that slight basement membrane thickening of muscle capillary can occur in several collagen diseases[6]; however, preliminary studies have demonstrated that severe atherosclerosis or hypertension does not lead to an increase in basement membrane width. It is of interest, as shown in Table XVII, that nephrosis, which characteristically produces thickening in the glomerular capillary basement membrane, does not cause thickening of the basement membranes of muscle capillaries. It is apparent, therefore, that the basement membrane hypertrophy of the muscle capillary of the diabetic is a more specific indication of

[6] Norton, W. L., D. C. Lewis, and Morris Ziff. 1968. Evidence of microvascular injury in scleroderma, and systemic lupus erythematosis. *J. Lab. Clin. Med.* **71**: 919.

diabetic vascular disease than is the comparable lesion in the diabetic kidney.

The second question that this study has attempted to answer is whether the characteristic microangiopathy of the diabetic syndrome is secondary to or independent of the carbohydrate manifestations of diabetes. This problem was investigated by first determining whether severe hyperglycemia in the absence of genetic diabetes would produce basement membrane thickening in animals and in man, and secondly, by attempting to determine whether genetically prediabetic patients who have neither hyperglycemia nor an abnormal glucose tolerance test will demonstrate basement membrane hypertrophy.

The results of the former studies (Tables XII, XIII, and XIV), clearly demonstrate that, in contrast to the almost constant finding of basement membrane hypertrophy in patients with genetic diabetes, hyperglycemia of nondiabetic origin, even though of many years duration, seldom leads to hypertrophy of muscle capillary basement membranes. The low incidence of basement membrane thickening in patients with acquired pancreatitis noted in this study is particularly striking in view of the fact that pancreatitis is known to occur more commonly in patients with genetic diabetes mellitus (38). This finding therefore provides strong evidence that hyperglycemia per se need not cause basement membrane hypertrophy.

The inability of severe pancreatogenous hyperglycemia to cause thickening of capillary basement membranes leads to the related conclusion that it is highly unlikely that the hyperglycemia of genetic diabetes is the factor in the diabetic syndrome responsible for diabetic vascular disease. It is, of course, conceivable that given the genetic predisposition to the diabetic syndrome, the presence of deranged carbohydrate metabolism might influence the rate of development of diabetic microangiopathy. An obvious argument against even such a secondary role for hyperglycemia in the genesis of diabetic vascular disease is the lack of any correlation in the present study (Table X) between severity of the carbohydrate abnormalities and the degree of microangiopathy within the diabetic population.

In general, previous clinical and experimental studies also support the concept that nondiabetic hyperglycemia does not cause the characteristic vascular lesion of diabetes. Lonergan and Robbins (39), confirming Bell's earlier study (35), were unable to find nodular intercapillary glomerulosclerosis in any of the 62 cases of hemochromatosis that they examined. Similarly, on the basis of renal biopsy studies, Kark and Gellman have stated that they have never observed intercapillary glomerulosclerosis in nondiabetic hyperglycemia (40). The only reports of a significant incidence of glomerular changes in secondary hyperglycemia are those of Becker and Miller (41), who noted that at autopsy 7 of 22 cases of hemochromatosis demonstrated basement membrane thickening of glomerular capillaries; and of Ireland, Patnaik, and Duncan (42), who observed basement membrane thickening in 7 of 10 renal biopsies from patients with probable nondiabetic hyperglycemia. It should be emphasized, however, that in only four of the patients of Becker and Miller, and in only one of the patients examined by Ireland, et al., were specific Kimmelsteil-Wilson nodules observed. Finally, Marble and Steinke (43) have reported the finding of funduscopic lesions in only 2 of a series of 42 patients with hemochromatosis. While the true incidence of retinopathy may have been underestimated in the latter study,[7] the occasional case of vascular disease observed in such reports is readily explainable on the basis of an incidence of genetic diabetes mellitus in the general population of approximately 2–6%. Finally, the cirrhosis that accompanies hemochromatosis is itself well known to produce nonspecific basement membrane thickening of renal capillaries (44).

Consistent with the evidence in man that hyperglycemia is not responsible for diabetic renovascular disease is the observation that in animals, hyperglycemia, either induced or spontaneous, does not result in the small vessel disease observed in human diabetes mellitus. Attempts to produce specific small vessel disease in various animals made hyperglycemic with alloxan (45), pituitary extract (46), cortisone (47), or pancreatectomy (46, 48), have been almost uniformly unsuccessful. The early claims that such vascular lesions have been produced in experimental animals have probably been due to misinterpretation of nonspecific renal changes (49). Recently, Bloodworth has reported the production of diabetic renal lesions in dogs made hyperglycemic with alloxan or

[7] Alexander Marble (personal communication).

growth hormone (50). However, the specificity of this lesion must seriously be questioned in view of the fact that as many as 25% of his nondiabetic control dogs showed similar glomerular lesions.

It should finally be noted that *spontaneous* hyperglycemia in rodents has not produced diabetic vascular changes (30), a finding that is consistent with our inability (Table XIV) to find basement membrane thickening of muscle capillary in the three examples of spontaneous hyperglycemia of rodents examined in the present study.

The observation that severe and prolonged hyperglycemia need not cause basement membrane hypertrophy in nondiabetic subjects raises the critical question of whether this vascular lesion in patients with diabetes mellitus may actually be independent of the characteristic carbohydrate derangement of diabetes. Previous suggestions that the small vessel disease of diabetics may precede obvious hyperglycemia have been based on rather fragmentary evidence. There have appeared occasional individual reports in which clinical vascular disease, either glomerulosclerosis (51, 52) or neuropathy (53), has apparently preceded overt carbohydrate disturbances. The sole previous study of this question consists of a report by Camerini-Davalos et al. (54), who examined two renal biopsies from genetically prediabetic subjects. While irregular thickening of the glomerular basement membrane was described in both renal biopsies, interpretation of this finding is difficult since neither control biopsies nor quantitative data were included in the report. Moreover, as noted earlier, proliferative changes in the basement membrane of the glomerular capillaries are quite nonspecific and can be produced by a number of nondiabetic diseases.

In the present study we have approached this problem by quantitating muscle capillary basement membrane width in a series of 30 genetically prediabetic patients. The results clearly demonstrate that the average basement membrane width in such subjects is significantly greater than that of normal subjects. Furthermore, thickening of the capillary basement membrane can be shown to be present in over one-half of genetically prediabetic patients.

This finding, we believe, provides the first definitive evidence that the basement membrane lesion of diabetes mellitus can precede the hyperglycemia of diabetes, and this observation, coupled with the further evidence (Table IV) that hyperglycemia almost never precedes the characteristic basement membrane thickening of diabetes mellitus, strongly supports the possibility that this vascular derangement represents a distinct lesion which is clearly independent of the carbohydrate abnormalities of diabetes mellitus.

It is noteworthy that, as demonstrated in Table XV, such capillary basement membrane thickening is demonstrable in every skeletal muscle group examined in this study. Moreover, in contrast to a previous report (7), this lesion definitely can be shown to involve the capillaries supplying adipose tissue. On the other hand, our findings would indicate that the laying down of the collagenous protein (55–58) responsible for basement membrane thickening must be relatively specific for vascular tissue since, as demonstrated in Fig. 4 a and b, the sarcolemmal basement membrane is completely unaffected in diabetic subjects despite the fact that the adjacent capillary basement membrane a few angstroms away may be massively thickened. Whether this specificity is due to differences in chemical composition of the collagen at these two sites, or due to differences in the localization of the enzymes responsible for deposition or removal of such collagen, remains to be determined.

In drawing the major conclusions of this study, two important assumptions have been made in defining the prediabetic subjects. First, it has been considered most likely that diabetes mellitus is inherited as a simple autosomal recessive trait with a high degree of penetrance, and as a result, the offspring of two diabetic parents should have a high probability of ultimately developing overt diabetes mellitus. The data of Steinberg (59, 60), of Post (61), and most recently of Nilsson (62) are consistent with an autosomal recessive inheritance of diabetes. Neel, Fajans, Conn, and Davidson (63, 64), however, have concluded that diabetes is probably inherited as a multifactorial trait in which case approximately 75% of the offspring of two diabetic parents would be expected to develop diabetes. Regardless of the mode of inheritance therefore, it can be concluded that between 75 and 100% (65) of children both of whose parents are diabetic will develop diabetes. If the lower figure proves to be correct, it would follow from our data that over 70 rather than 53%

of the prediabetic patients studied had thickened basement membranes at the time of biopsy. In other words, were diabetes inherited with variable expressivity, or as a multifactorial trait, the major conclusions of this portion of the study would be strengthened rather than weakened. In this regard, it will obviously be necessary to follow those prediabetic patients who did not have basement membrane thickening when first studied to determine whether they, too, will ultimately develop this vascular lesion before the onset of the gross carbohydrate abnormalities of diabetes.

Second, the possibility clearly exists that despite the normal fasting plasma sugars, normal glucose tolerance tests, and normal serum insulin levels employed to rule out abnormalities of glucose and insulin metabolism in the prediabetic patients, subtle but pathologically significant derangements of carbohydrate metabolism as suggested by Fajans and Conn (66), and Camerini-Davalos (67), or of insulin secretion as proposed by Cerasi and Luft (68),[8] may be actually present. It should, however, be noted that the oral glucose tolerance test, especially with the strict criteria employed in this study, greatly overdiagnoses carbohydrate abnormalities (72); indeed, over one-half of normal individuals age 40 yr or more would be excluded from both the "normal" and "prediabetic" groups by the rigid criteria used in this study (73). Further evidence against a pathologically *significant* derangement in carbohydrate metabolism being present in the prediabetic group is the observation that the glucose tolerance tests of the prediabetics were not only normal, but moreover, did not differ from those of the normal subjects; and most important, even within the prediabetic group there was no relationship between degree of glucose tolerance and basement membrane thickening (Fig. 9 b). Finally, it should be emphasized that, as shown in Tables XII and XIII, even marked abnormalities in carbohydrate metabolism with exposure of capillaries to many years of both gross hyperglycemia and insulin deficiency will not cause basement membrane hypertrophy in the absence of genetic diabetes mellitus. Taken together, these findings indicate that subtle abnormalities of insulin secretion or of carbohydrate metabolism, even if ultimately they should be demonstrated to be present in prediabetic patients, are unlikely to be the factors responsible for the basement membrane thickening and hence the microangiopathy of diabetes mellitus.

[8] Two additional studies have suggested that absolute insulin levels may be depressed in prediabetic patients (69–70); however, in both of these studies, patients with abnormal glucose tolerance tests were included in the prediabetic group. By contrast, in the study by Soeldner et al. (71), in which only prediabetic patients with normal oral glucose tolerance were examined, no abnormality in the absolute level of plasma insulin was observed during the glucose tolerance test, and finally, in the study of Camerini-Davalos (67), prediabetic patients were found to have an *elevation* in plasma insulin during a glucose tolerance test.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. Walter L. Norton and Mr. Henry Burns for their valuable advice and assistance in carrying out the electron microscopy, to Mrs. Mary Jo Sill, Mrs. Irene Batlle, and Mrs. Dixie Jo Porcher for their technical assistance, and to Mrs. Joan Reisch for her statistical analysis of the data. We are very grateful to Dr. Kelly M. West and to Dr. John W. Farquhar for allowing us to carry out a portion of these studies on patients under their care.

This study was supported by U. S. Public Health Service Grants CA-08501 and AM-05864, and by research grants from The Dallas Diabetes Association, Merck, Sharp and Dohme, Abbott Laboratories, Pfizer Laboratories, and The Ames Company. Dr. Siperstein is a recipient of the Research Career Award, National Heart Institute, National Institutes of Health, U. S. Public Health Service.

REFERENCES

1. Siperstein, M. D., W. Norton, R. H. Unger, and L. L. Madison. 1966. Muscle capillary basement membrane width in normal, diabetic and prediabetic patients. *Trans. Assoc. Am. Physicians.* **79:** 330.
2. Joslin, E. P., H. F. Root, P. White, and A. Marble. 1959. The Treatment of Diabetes Mellitus. Lea & Febiger, Philadelphia. 10th edition.
3. Friedenwald, J. S. 1949. A new approach to some problems of retinal vascular disease. *Am. J. Ophthalmol.* **32:** 487.
4. Friedenwald, J. S. 1950. Diabetic retinopathy. *Am. J. Ophthalmol.* **33:** 1187.
5. Bergstrand, A., and H. Bucht. 1957. Electron microscopic investigations on the glomerular lesions in diabetes mellitus (diabetic glomerulosclerosis). *Lab. Invest.* **6:** 293.
6. Farquhar, M. G., J. Hopper, Jr., and H. D. Moon. 1959. Diabetic glomerulosclerosis: electron and light microscopic studies. *Am. J. Pathol.* **35:** 721.
7. Bloodworth, J. M. B., Jr. 1963. Diabetic microangiopathy. *Diabetes.* **12:** 99.

8. Aagenaes, O., and H. Moe. 1961. Light- and electron-microscopic study of skin capillaries of diabetics. *Diabetes.* **10**: 253.
9. Banson, B. B., and P. E. Lacy. 1964. Diabetic microangiopathy in human toes. With emphasis on the ultrastructural change in dermal capillaries. *Am. J. Pathol.* **45**: 41.
10. Fagerberg, S-E. 1956. Studies on the pathogenesis of diabetic neuropathy. *Acta Med. Scand.* **154**: 145.
11. Goldenberg, S., M. Alex, R. A. Joshi, and H. T. Blumenthal. 1959. Nonatheromatous peripheral vascular disease of the lower extremity in diabetes mellitus. *Diabetes.* **8**: 261.
12. Zacks, S. I., J. J. Pegues, and F. A. Elliott. 1962. Interstitial muscle capillaries in patients with diabetes mellitus: a light and electron microscope study. *Metab. Clin. Exptl.* **11**: 381.
13. Fuchs, U. 1964. Elektronenmikroskopische Untersuchungen menschlicher Muskelcapillaren bei Diabetes mellitus. *Frankfurter Z. Pathol.* **73**: 318.
14. Bencosme, S. A., R. O. West, J. W. Kerr, and D. L. Wilson. 1966. Diabetic capillary angiopathy in human skeletal muscles. *Am. J. Med.* **40**: 67.
15. Burstein, R., S. D. Soule, and H. T. Blumenthal. 1957. Histogenesis of pathological processes in placentas of metabolic disease in pregnancy. II. The diabetic state. *Am. J. Obstet. Gynecol.* **74**: 96.
16. Kimmelstiel, P., G. Osawa, and J. Beres. 1966. Glomerular basement membrane in diabetics. *Am. J. Clin. Pathol.* **45**: 21.
17. Hansen, R. 1965. A quantitative estimate of the peripheral glomerular basement membrane in recent juvenile diabetes. *Diabetologia.* **1**: 97.
18. Vracko, R., and D. E. Strandness, Jr. 1967. Basal lamina of abdominal skeletal muscle capillaries in diabetics and nondiabetics. *Circulation.* **35**: 690.
19. Friederici, H. H. R., W. R. Tucker, and T. B. Schwartz. 1966. Observations on small blood vessels of skin in the normal and in diabetic patients. *Diabetes.* **15**: 233
20. Palade, G. E. 1952. A study of fixation for electron microscopy. *J. Exptl. Med.* **95**: 285.
21. Spurlock, B. O., V. C. Kattine, and J. A. Freeman. 1963. Technical modifications in maraglas embedding. *J. Cell. Biol.* **17**: 203.
22. Zalme, E., and H. C. Knowles, Jr. 1965. A plea for plasma sugar. *Diabetes.* **14**: 165.
23. Hoffman, W. S. 1937. A rapid photoelectric method for the determination of glucose in blood and urine. *J. Biol. Chem.* **120**: 51.
24. Conn, J. W., and S. S. Fajans. 1961. The prediabetic state. *Am. J. Med.* **31**: 839.
25. Yalow, R. E., and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39**: 1157.
26. Unger, R. H., M. D. Siperstein, L. L. Madison, A. M. Eisentraut, and N. Whissen. 1964. Apparent growth hormone hyperresponsiveness in prediabetes. *J. Lab. Clin. Med.* **64**: 1013. (Abstr.)
27. Knowles, H. C., Jr. 1960. The incidence and development of diabetes mellitus. *In* Diabetes, Robert H. Williams, editor. Paul B. Hoeber, Inc., New York. 360.
28. Steinberg, A. G., and R. M. Wilder. 1962. A study of the genetics of diabetes mellitus. *Am. J. Human Genet.* **4**: 113.
29. Fredrickson, D. S., and R. S. Lees. 1960. Familial hyperlipoproteinemia. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. Blakiston Division, McGraw-Hill Book Company, New York. 2nd edition. 429.
30. Meier, H., and G. A. Yerganian. 1959. Spontaneous hereditary diabetes mellitus in Chinese hamsters (Cricetulus griseus). I. Pathological findings. *Proc. Soc. Exptl. Biol. Med.* **100**: 810.
31. Meier, H. 1964. Hereditary diabetes mellitus in the Chinese hamster: certain aspects of the prediabetic state in the hamster and reference to the obese-hyperglycemic mouse. *In* Small Blood Vessel Involvement in Diabetes Mellitus. M. D. Siperstein, A. R. Colwell, Sr., and K. Meyer, editors. American Institute of Biological Sciences, Washington, D. C. 303.
32. Nakamura, M., and K. Yamada. 1967. Studies on a diabetic (KK) strain of the mouse. *Diabetologia.* **3**: 212.
33. Vernier, R. L. 1964. Electron microscopic studies of the normal basement membrane. *In* Small Blood Vessel Involvement in Diabetes Mellitus. M. D. Siperstein, A. R. Colwell, Sr., and K. Meyer, editors. American Institute of Biological Sciences, Washington, D. C. 57.
34. Siperstein, M. D. 1964. Skin and renal basement membrane changes at onset of diabetes mellitus. *In* Small Blood Vessel Involvement in Diabetes Mellitus. M. D. Siperstein, A. R. Colwell, Sr., and K. Meyer, editors. American Institute of Biological Sciences, Washington, D. C. 125.
35. Bell, E. T. 1952. A postmortem study of vascular disease in diabetics. *Arch. Pathol.* **53**: 444.
36. Hatch, F. E., M. F. Watt, N. C. Kramer, A. E. Parrish, and J. S. Howe. 1961. Diabetic glomerulosclerosis. *Am. J. Med.* **31**: 216.
37. Hartmann, J. F., F. C. Goetz, and A. Lazarow. 1958. Electron microscopy of the human glomerulus in diabetes. *In* Proceedings of the 3rd International Diabetes Congress. Georg Thieme Verlag, Stuttgart. 189.
38. Blumenthal, H. T., J. G. Probstein, and A. W. Berns. 1963. Interrelationship of diabetes mellitus and pancreatitis. *Arch. Surg.* **87**: 844.
39. Lonergan, P., and S. L. Robbins. 1959. Absence of intercapillary glomerulosclerosis in the diabetic patient with hemochromatosis. *New Engl. J. Med.* **260**: 367.
40. Kark, R. M., and D. D. Gellman. 1960. Renal disease in diabetes. *In* Diabetes. Robert H. Williams, editor. Paul B. Hoeber, Inc., New York. 563.
41. Becker, D., and M. Miller. 1960. Presence of diabetic glomerulosclerosis in patients with hemochromatosis. *New Engl. J. Med.* **263**: 367.

42. Ireland, J. T., B. K. Patnaik, and L. J. P. Duncan. 1967. Glomerular ultrastructure in secondary diabetics and normal subjects. *Diabetes.* **16:** 628.
43. Marble, A., and J. Steinke. 1959. Hämochromatose. Bericht uber 42 Fälle mit Diabetes. *Medizinische Nr.* **1:** 19.
44. Bloodworth, J. M. B., Jr., and S. C. Sommers. 1959. "Cirrhotic glomerulosclerosis," a renal lesion associated with hepatic cirrhosis. *Lab. Invest.* **8:** 962.
45. Mann, G. V., J. W. Goddard, and L. Adams. 1951. The renal lesions associated with experimental diabetes in the rat. *Am. J. Pathol.* **27:** 857.
46. Rickets, H. T., C. E. Test, E. S. Petersen, H. Lints, N. Tupikova, and P. E. Steiner. 1959. Degenerative lesions in dogs with experimental diabetes. *Diabetes.* **8:** 298.
47. Bloodworth, J. M. B., Jr., and G. J. Hamwi. 1956. Experimental diabetic glomerulosclerosis. *Diabetes.* **5:** 37.
48. Foglia, V. G., R. E. Mancini, and A. F. Cardeza. 1950. Glomerular lesions in the diabetic rat. *Arch. Pathol.* **50:** 75.
49. Warren, S., P. M. LeCompte, and M. A. Legg. 1966. The Pathology of Diabetes Mellitus. Lea & Febiger, Philadelphia. 4th edition. 326.
50. Bloodworth, J. M. B., Jr. 1965. Experimental diabetic glomerulosclerosis. II. The dog. *Arch. Pathol.* **79:** 113.
51. Freedman, L. F. 1957. Inapparent diabetes mellitus as a cause of renal insufficiency due to Kimmelstiel-Wilson lesions. *Bull. Johns Hopkins Hosp.* **199:** 132.
52. Ellenberg, M. 1962. Diabetic nephropathy without manifest diabetes. *Diabetes.* **11:** 197.
53. Ellenberg, M. 1958. Diabetic neuropathy presenting as the initial clinical manifestation of diabetes. *Ann. Intern. Med.* **49:** 620.
54. Camerini-Dávalos, R. A., J. B. Caulfield, S. B. Rees, O. Lozano-Castaneda, S. Naldjian, and A. Marble. 1963. Preliminary observations on subjects with prediabetes. *Diabetes.* **12:** 508.
55. Lazarow, A., and E. Speidel. 1964. The chemical composition of the glomerular basement membrane and its relationship to the production of diabetic complications. *In* Small Blood Vessel Involvement in Diabetes Mellitus. M. D. Siperstein, A. R. Colwell, Sr., and K. Meyer, editors. American Institute of Biological Sciences, Washington, D. C. 127.
56. Kefalides, N. A., and R. J. Winzler. 1966. The chemistry of glomerular basement membrane and its relation to collagen. *Biochemistry.* **5:** 702.
57. Kefalides, N. A. 1966. A collagen of unusual composition and a glycoprotein isolated from canine glomerular basement membrane. *Biochem. Biophys. Res. Comm.* **22:** 26.
58. Spiro, R. G. 1967. Studies on the renal glomerular basement membrane. Preparation and chemical composition. *J. Biol. Chem.* **242:** 1915.
59. Steinberg, A. G. 1961. Heredity in diabetes mellitus. *Diabetes.* **10:** 269.
60. Steinberg, A. G. 1965. Genetics and diabetes. *In* On the Nature and Treatment of Diabetes (International Diabetes Federation Fifth Congress). B. S. Leibel and G. A. Wrenshall, editors. Excerpta Medica Foundation, New York. 601.
61. Post, R. H. 1962. An approach to the question, does all diabetes depend upon a single genetic locus? *Diabetes.* **11:** 56.
62. Nilsson, S. E. 1964. On the heredity of diabetes mellitus and its interrelationship with some other diseases. *Acta Genet. Statist. Med.* **14:** 97.
63. Neel, J. V. 1964. The genetic basis for diabetes mellitus. *In* Small Blood Vessel Involvement in Diabetes Mellitus. M. D. Siperstein, A. R. Colwell, Sr., and K. Meyer, editors. American Institute of Biological Sciences, Washington, D. C. 295.
64. Neel, J. V., S. S. Fajans, J. W. Conn, and R. T. Davidson. 1965. Diabetes mellitus. *In* Genetics and Epidemiology of Chronic Diseases, a Symposium, June 17–19, 1963, Ann Arbor, Mich. J. V. Neel, M. W. Shaw, and W. J. Schull, editors. U. S. Public Health Service Publication No. 1163. 105.
65. Neel, J. V. 1966. Discussion of muscle capillary basement membrane width. *Trans. Assoc. Am. Physicians.* **79:** 345.
66. Fajans, S. S., and J. W. Conn. 1965. Prediabetes, subclinical diabetes and latent clinical diabetes: interpretation, diagnosis and treatment. *In* On The Nature and Treatment of Diabetes (International Diabetes Federation Fifth Congress). B. S. Leibel and G. A. Wrenshall, editors. Exerpta Medica Foundation, New York. 641.
67. Camerini-Dávalos, R. A. 1965. Biochemical and histological aspects of prediabetes. *In* On The Nature and Treatment of Diabetes (International Diabetes Federation Fifth Congress). B. S. Leibel and G. A. Wrenshall, editors. Exerpta Medica Foundation. 657.
68. Cerasi, E., and R. Luft. 1967. "What is inherited—what is added" hypothesis for the pathogenesis of diabetes mellitus. *Diabetes.* **16:** 615.
69. Colwell, J. A., and A. Lein. 1967. Diminished insulin response to hyperglycemia in prediabetes and diabetes. *Diabetes.* **16:** 560.
70. Pyke, D. A., and K. W. Taylor. 1967. Glucose tolerance and serum insulin in unaffected identical twins of diabetics. *Brit. Med. J.* **4:** 21.
71. Soeldner, J. S., R. E. Gleason, R. F. Williams, M. J. Garcia, D. M. Beardwood, and A. Marble. 1968. Diminished serum insulin response to glucose in genetic prediabetic males with normal glucose tolerance. *Diabetes.* **17:** 17.
72. Unger, R. H. 1957. The standard two-hour oral glucose tolerance test in the diagnosis of diabetes mellitus in subjects without fasting hyperglycemia. *Ann. Intern. Med.* **47:** 1138.
73. Hayner, N. S., M. O. Kjelsberg, F. H. Epstein, and T. Francis, Jr. 1965. Carbohydrate tolerance and diabetes in a total community, Tecumseh, Mich. *Diabetes.* **14:** 413.