DIABETIC MICROANGIOPATHY IN HUMAN TOES

With Emphasis on the Ultrastructural Change in Dermal Capillaries

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Few observations have been published on the histologic changes in capillaries of patients with diabetes mellitus [1–8] aside from those of the kidneys [9–17] and retina.[17–23] Handelsman, Morrione and Ghitman [2] examined dermal capillaries in the forearm of human diabetic patients by conventional microscopy and found conspicuous thickening of the wall and endothelial proliferation. Aagenaes and Moe [3] examined 24 specimens of the toe and finger pulp in diabetic patients and similar specimens from 9 nondiabetic patients. A PAS-positive material was found deposited beneath the endothelium in many of the capillaries from diabetic subjects, particularly in those whose disease began before the age of 40 years. They also studied specimens from the pulp of fingers in 4 diabetic patients (below 40 years of age) electron microscopically. In these 4 cases, they observed thickening of the capillary basement membrane, which was not present in 2 control specimens.

In the present study, specimens from the dermis of toes of patients with and without maturity-onset diabetes were examined by light and electron microscopy. This location was selected because of its accessibility and its possible relation to diabetic gangrene of the foot since gangrene starts at about this area.

Material and Methods

Material from 18 diabetic and 17 nondiabetic patients was obtained at necropsy and from amputation specimens. The cases comprised 4 groups: group I, amputation specimens from 11 adult diabetic patients; group II, 7 necropsied adult cases of diabetes; group III, amputation specimens from 7 arteriosclerotic nondiabetic individuals; group IV, 10 necropsied nondiabetic persons of comparable age. The postmortem intervals in groups II and IV ranged from 2½ hours to 12 hours. Gangrene was not present in any of the necropsied diabetic cases (group II). Tissues from amputation specimens were obtained soon after the operation. The specimens obtained were results of toe, transmetatarsal, below-the-knee and above-the-knee

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amputations for diabetic gangrene of the foot (group I) or for gangrene due to vascular insufficiency but without diabetes (group III).

For light microscopy, sections of the skin were taken from the pulp of nongangrenous toes and were fixed in 10 per cent neutral buffered formaldehyde solution or Carnoy's alcoholic fixative. Sections were stained with hematoxylin and eosin (H and E) and by the periodic acid-Schiff (PAS) method. Some sections from the diabetic and nondiabetic groups were stained with Congo red and crystal violet stains to determine if amyloid was present in the area of the capillary basement membrane.

Stained sections were examined without knowledge as to what group they belonged. The capillary basement membrane thickness was noted and the degree of thickness arbitrarily graded from 1+ to 4+ (Figs. 1 to 6). Those with grades of 3+ to 4+ were considered significantly thickened.

For electron microscopy, tissues were cut into 1 mm. cubes while immersed in 1 per cent osmium tetroxide buffered with White's saline to a pH of approximately 7.4. Blocks were then further fixed in this solution for 1½ hours. The tissues were then dehydrated and embedded in both Epon and methacrylate. Thin sections were cut on a Porter-Blum microtome and stained with a saturated solution of uranyl acetate. These were examined in an RCA EMU 3C or 3F electron microscope. Electron micrographs of dermal capillaries were enlarged photographically as desired. Sections for phase contrast microscopy were also made and stained with PAS and PAS-methylene blue.

From the enlarged electron micrographs measurements of the thickness of the capillary basement membrane were taken in 3 portions of the capillary wall not tangentially cut, avoiding areas where pericytes were present. The average of these 3 was taken to represent the measurement of the basement membrane of that capillary. At least 10 capillaries per case were examined and treated in this manner, and the average of the measurements along with the standard error of the mean expressed in angstroms (Å) were recorded (Tables I to IV). Thus, a total of at least 30 measurements were obtained in each case.

Observations

The basement membrane thickening was evident in H and E stained sections although it was more clearly delineated in those stained with PAS (Figs. 1 to 4). In group III (arteriosclerotic nondiabetic amputations), significant basement membrane thickening (3+ to 4+) was present in only 2 of 7 cases (28 per cent), 1 with a 15-year history of hypertension and the other with hypertensive cardiovascular disease. In group IV (necropsied nondiabetic persons), significant capillary basement membrane thickening was noted in only 2 of 10 cases (20 per cent), one of which had generalized arteriosclerosis (Table IV). In these two control groups significant basement membrane thickening was present in 4 of 17 cases (23 per cent). In the diabetic cases, 15 of 17 (88 per cent) showed significant capillary basement membrane thickening (Tables I and II). The distribution of the lesions in the sections was usually patchy. Uninvolved capillaries were interspersed with those surrounded by thickened basement membrane. Sections stained for amyloid were negative.

Endothelial proliferation was not observed in capillaries in any of the 4 groups. Some capillaries were found to contain several nuclei within their walls (Fig. 7); these appeared to be surrounded by the PAS-positive material on all their surfaces, suggesting that they were nuclei of pericytes rather than of proliferating endothelial cells.

TABLE I

SUMMARY OF DATA ON DERMAL CAPILLARIES. GROUP I: DIABETES (AMPUTATION)

Patient	Age, sex	Color	Duration	Blood pressure	Treatment	BMT *	BMT †
EE	52 F	W	10 yr.	150/90	Diet	3+	11,200 ± 3,100
HE	55 M	W	6 yr.	140/90	Insulin and orinase	4+	15,300 ± 3,500
TD	55 M	C	15 yr.	174/90	Orinase	3+	9,900 ± 2,100
MD	59 F	W	12 yr.	130/80	Insulin	3+	15,200 ± 4,300
BW	59 F	C	20 yr.	190/75	Insulin	3+	5,800 ± 1,000
EH	66 F	C	10 yr.	185/90	Orinase	4+	14,400 ± 3,000
AF	68 M	W	3–4 mo.	135/80	Orinase	4+	26,400 ± 2,800
LM	70 M	W	6 yr.	180/110	Orinase	4+	15,000 ± 1,800
IP	72 F	W	4 yr.	200/100	Insulin	3+	18,600 ± 3,200
RG	73 M	W	2–3 yr.	130/85	Orinase	2+	11,500 ± 1,400
DB	74 F	W	8 yr.	130/70	Insulin	3+	17,200 ± 2,700

BMT: mean capillary basement membrane thickness.
* Criteria for grading given in text (by light microscopy).
† In angstroms (by electron microscopy), ± standard error of mean.

TABLE II

SUMMARY OF DATA ON DERMAL CAPILLARIES. GROUP II: DIABETES (NECROPSY)

Patient	Age, sex	Color	Duration	Blood pressure	Treatment	BMT *	BMT †
FG	46 M	W	13 yr.	210/100	Insulin	3+	12,100 ± 1,000
HM	48 M	W	3 yr.	140/96	None	2+	3,500 ± 500
OH	50 M	C	5 yr.	185/95	Insulin	3+	16,400 ± 3,400
LP	53 F	C	7 yr.	260/150	Insulin	3+	14,300 ± 1,300
JS	55 M	W	10 yr.	160/110	Diet	3+	13,200 ± 2,400
AG	63 F	W	3 yr.	140/80	Orinase	‡	8,300 ± 1,100
KN	63 F	W	6 yr.	150/80	Insulin	3+	11,700 ± 1,700

BMT: mean capillary basement membrane thickness.
* Graded as indicated in the text (by light microscopy).
† In angstroms (by electron microscopy), ± standard error of mean.
‡ Section not taken for light microscopy.

In the groups that had amputation because of gangrene (groups I and III), almost 50 per cent of the cases in the nondiabetic group were over 80 years of age, with an average of 71 years. In the diabetic group, the average age was 64 years, ranging from 52 to 74 years.

By electron microscopy, the components of the capillaries were the endothelial cells, a basement membrane external to the endothelium and pericytes encased in the basement membrane (Fig. 8). The general ultra-

structural features of the capillaries were identical in both necropsy and surgical specimens. The endothelial cytoplasm contained a large number of vacuoles near the plasma membranes as well as in the cytoplasm. These measured approximately 650 Å in diameter and probably represented pinocytotic vesicles believed to be a transport system for the conveyance of fluid across the cell.[24] The cytoplasm also contained scattered mitochondria and a small amount of lamellar ergastoplasm.

Pericytes were observed external to the endothelium and were completely enveloped on both their inner and outer surfaces by basement membrane. Portions of one or two pericytes were usually observed in the wall of a capillary. The cytoplasm of pericytes was indistinguishable from that of endothelial cells.[25] The complete enclosement of pericytes by basement membrane was a characteristic differentiating them from endothelium. The latter was bordered by basement membrane on only one surface.

The ultrastructure of the material comprising the basement membrane was similar in both the diabetic and nondiabetic groups. It appeared as an amorphous substance with exceedingly fine short, filamentous structures (Fig. 9).

The basement membrane in the diabetic groups differed from that in the controls primarily by its thickness and by the form of its thickening. In both, the thickening occurred in 3 forms: (a) homogeneous, as a single layer of amorphous material (Figs. 9 to 11); (b) stratified, comprised of several strips or layers of basement membrane (Figs. 12 and 13). In the Epon-embedded tissues, the laminated basement membrane appeared rigid and almost circular (Fig. 12); in methacrylate-embedded tissues, it appeared to be slightly wavy (Fig. 13); (c) mixed, a combination of the first two types. This combination of homogeneous and stratified forms might occur in the same capillary (Fig. 14) or could be found in different capillaries in the same case.

Remarkably good preservation and fixation of the different capillary structures were obtained in groups II and IV, even though the tissues were taken several hours after death. Figure 11 is an electron micrograph of a dermal capillary obtained 8 hours after death. Among the structures, the basement membrane appeared to be the most resistant to postmortem changes.

Tables III and IV show the basement membrane thickness in angstroms in capillaries from nondiabetic individuals. Four cases, 2 in group II (KN and NS) and 2 in group IV (FF and HB), obviously had higher measurements (ranging from 10,000 to 20,000 Å) than the other 13 cases in these groups (ranging from 900 to 6,400 Å). The measurements in these 4 cases were not considered to be within normal range. Values

greater than 6,500 Å were considered to indicate significant thickening.

In the diabetic groups (groups I and II), basement membranes exhibited significant thickening ranging from 8,300 to 26,400 Å (Tables I and II). A large gap of 1,900 Å with no overlapping existed between the lowest value (8,300 Å) among the significant basement membrane thickness measurements and the highest value (6,400 Å) among the measurements which were not significant.

Table V gives the mean values of the basement membrane measurements in each of the 4 groups, as well as the mean value for the entire diabetic groups (I and II) and the nondiabetic groups (III and IV). The thickness of the basement membrane in the diabetics was 13,000 Å

TABLE III

SUMMARY OF DATA ON DERMAL CAPILLARIES. GROUP III: ARTERIOSCLEROSIS (AMPUTATION)

Patient	Age, sex	Color	Blood pressure	BMT *	BMT †
AS	52 M	W	110/70	2–3+	6,300 ± 700
KR	62 F	W	190/110	2–3+	11,000 ± 1,700
BM	64 M	W	150/65	1+	2,700 ± 500
OW	71 F	W	170/90	3+	5,100 ± 600
NS	81 F	W	210/100	3+	20,900 ± 2,200
CH	81 M	W	150/84	2+	5,800 ± 1,000
MM	84 M	W	130/80	2+	4,100 ± 500

BMT: mean capillary basement membrane thickness.

* Graded as indicated in text (by light microscopy).

† In angstroms (by electron microscopy), ± standard error of mean.

TABLE IV

SUMMARY OF DATA ON DERMAL CAPILLARIES
GROUP IV: CONTROL (NONDIABETIC NECROPSIED CASES)

Patient	Age, sex	Color	Disease	Blood pressure	BMT *	BMT †
GW	49 F	W	Hepatoma	144/80	2+	1,800 ± 400
GH	52 M	W	Retroperitoneal abscess	140/90	2+	1,800 ± 700
JB	53 M	W	Subacute bacterial endocarditis	110/70	2+	1,700 ± 500
NM	57 M	W	Aplastic anemia	125/90	1+	4,500 ± 800
CF	63 M	W	Infarct of small intestine	112/96	2+	6,400 ± 1,300
FF	63 M	W	Mesenteric vascular occlusion	200/100	2–3+	10,000 ± 1,400
RF	64 M	W	Prostatic hyperplasia	140/90	2+	5,900 ± 700
HB	68 M	W	Generalized arteriosclerosis	130/90	3+	10,000 ± 1,400
OS	72 M	W	Carcinoma of colon	106/60	2+	900 ± 200
GT	80 F	W	Carcinoma of lung	130/70	3+	1,200 ± 300

BMT: mean capillary basement membrane thickness.

* Graded as indicated in text (by light microscopy).

† In angstroms (by electron microscopy), ± standard error of mean.

as compared to 5,900 Å in the nondiabetics. The difference between these means was significant, with a P value less than 0.001.

In the nondiabetic groups, the basement membranes with significant thickening (4 cases) were of the stratified type (Table VI). In approximately 50 per cent of the cases in the diabetic groups, the basement membrane thickenings were also of the stratified type (Table VI).

TABLE V

MEAN MEASUREMENT OF BASEMENT MEMBRANE

Group I Diabetic (amputation)	Group II Diabetic (necropsy)	Group III Nondiabetic (amputation)	Group IV Nondiabetic (necropsy)
14,500 * ± 1,500	11,400 ± 1,500	8,000 ± 2,200	4,400 ± 1,100
13,300 ± 1,200 ←		P < 0.001	→ 5,900 ± 1,200

* In angstroms, ± standard error of mean.

TABLE VI

DISTRIBUTION OF TYPES OF BASEMENT MEMBRANE THICKENING

	No. of cases	Stratified	Homogeneous	Mixed
Group I	10	5	1	4
Group II	6	2	2	2
Group III	2	2	0	0
Group IV	2	2	0	0
Total	20	11	3	6

Occasionally, the findings by light microscopy did not correlate with those by electron microscopy with regard to basement membrane thickness. In group III, OW had a 3+ degree of thickening as evidenced by light microscopy; by electron microscopy, there was no significant thickening, the measurement being only 5,100 Å. Similarly, in group IV, GT had 3+ degree of thickening by light microscopy but none by electron microscopy.

Hypertension (blood pressure greater than 150/95) was present in 6 of 18 cases (33 per cent) in the diabetic groups and in 3 of 17 cases (18 per cent) in the nondiabetic groups. Three of the 4 cases with hypertension in group II (necropsied diabetics) showed diabetic glomerulosclerosis microscopically. Significant basement membrane thickening occurred in the diabetic groups whether or not there was hypertension. In these groups significant basement membrane thickening was evident in 6 patients with hypertension and 10 patients without it. In the control groups 3 cases had hypertension with significant basement membrane thickening; in 1 case there was basement membrane thickening without hypertension.

Discussion

Aagenaes and Moe[3] reported basement membrane thickening in dermal capillaries in the pulp of fingers of 4 diabetic patients below the age of 40 years. Measurements of the membrane thickness were not given. Zacks, Pegues and Elliott[5] reported that thickness of the capillary basement membranes in the interstitial tissues of muscles in nondiabetic human subjects was 2,000 to 4,000 Å.

In the present study, the average basement membrane thickness in the nondiabetic groups was 5,900 Å while in the diabetic groups it was 13,300 Å (Table V). An arbitrary value of 6,500 Å was selected as the upper limit of normal basement membrane thickness since 13 of 17 nondiabetic patients had measurements less than this value whereas 4 had values ranging from 10,000 to 20,900 Å. With 6,500 Å as the upper limit of normal, two among the diabetic cases had no significant basement membrane thickening. The values for these were 5,800Å and 3,500 Å. An explanation for this lack of thickening is not apparent. It did not appear to be related to the type of treatment or duration of diabetes; one patient had diabetes for 20 years. It is possibly attributable to a sampling error. As stated previously, the thickening as observed by conventional microscopy was not uniform in distribution but appeared patchy. It is possible that the samples examined by electron microscopy were from unaffected portions of capillaries.

In the nondiabetic groups, 3 of the 4 cases with basement membrane thickness greater than 6,500 Å were obtained from patients with hypertension. Further clinical studies to exclude the possibility of latent diabetes were not done. It is possible that the thickened basement membrane might be associated with hypertension. A larger number of nondiabetic hypertensive individuals must be studied in a thorough clinical and anatomic manner in order to determine the role of hypertension in the change of the basement membrane of the capillaries of the dermis. In the diabetic groups, hypertension was not necessarily associated with basement membrane thickening since in 10 cases without hypertension the basement membranes were significantly thickened.

The different forms of basement membrane thickening (homogeneous, stratified and mixed) were not correlated with the degree of thickening or the duration of diabetes. These types could simply represent different stages in the formation or deposition of basement membrane material.

The basement membrane thickening in peripheral capillaries is similar in appearance to that seen in capillaries in the retina,[17,22,23,26] ciliary process of the eye,[27] and in renal glomeruli[11-17] in diabetes.

This study supports the concept that microangiopathy in human dia-

betes is not confined to glomerular and retinal lesions, but is rather a generalized process. The factor or factors responsible for the vascular changes, however, remain obscure. The basement membrane substance is known to consist essentially of glycoproteins.[28,29] It has been suggested that the basement membrane lesions in diabetes, on the basis of their histochemical properties, are the result of some defect in the metabolism of glycoproteins or related substances.[19,30,31] Whether or not the basement membrane changes represent a primary or secondary alteration in diabetes mellitus is unknown.

It is possible that the basement membrane thickening in diabetes may impede the rate of transfer of nutrients to the surrounding tissue and then interfere with the normal defense mechanism in inflammation such as the migration of leukocytes into the interstitial tissue. This may be a contributory factor to the greater incidence of necrosis and gangrene following minor injury or infection of the skin in diabetes. It may also explain the frequent occurrence of gangrene in diabetes [32] and why it occurs at a relatively younger age in this condition.

Although the mean measurement of the capillary basement membrane in group I (diabetic amputations) was higher (14,500 Å) than in group II (diabetic necropsied cases), no truly significant difference was present. Thus no correlation could be made between the degree of thickening and diabetic gangrene.

Some investigators [1,2] have described endothelial proliferation in diabetes. This was not seen in any of the capillaries examined in this study. At times, several nuclei were observed in the capillary wall (Fig. 7); on closer examination these were found to be pericytes encased in the basement membrane. They did not protrude into the capillary lumen.

Summary

Dermal microangiopathy in the toes of 18 human diabetics was examined by light and electron microscopy. By the latter method, marked thickening of the capillary basement membrane was found in 88 per cent of individuals with diabetes and in 23 per cent of those without diabetes. The degree of basement membrane thickening showed no correlation with the duration of the diabetes or with the form of therapy.

Basement membrane thickening appeared as a stratified form, a homogeneous form and a mixed (stratified and homogeneous) type. These types exhibited no correlation with the degree of basement membrane thickening. They probably represented different stages in the deposition of basement membrane substances.

The findings suggested that diabetic microangiopathy was not limited to organs such as the kidneys and eyes, but was rather a generalized process.

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LEGENDS FOR FIGURES

FIG. 1. A 72-year-old white man (OS), group IV, nondiabetic, necropsy. The capillary basement membrane is not thickened (arrow). This represents a 1+ degree of thickening. Periodic acid-Schiff (PAS) stain. × 2,000.

FIG. 2. Capillary basement membrane exhibits 2+ thickening. PAS stain. × 2,000.

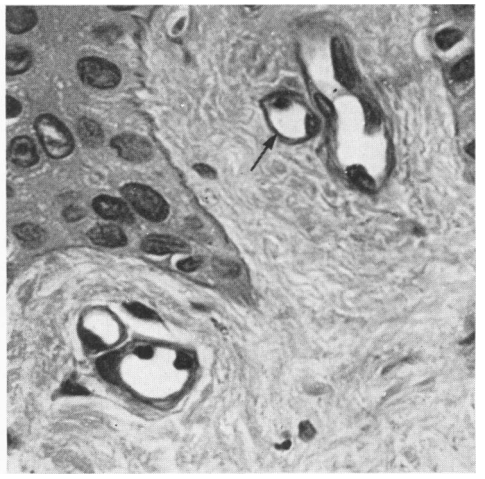
FIG. 3. Diabetic patient (group I). Two capillaries in the center show 3+ thickening of the basement membrane. Nearby capillaries are not involved. PAS stain. × 2,000.

FIG. 4. Dermal capillary from a diabetic patient (AF), group I. Marked thickening of the basement membrane (4+) is a feature. PAS stain. × 2,000.

FIG. 5. Capillaries in a nondiabetic necropsied individual (group IV) exhibit no (1+) basement membrane thickening (arrow). Osmium tetroxide fixation, methacrylate embedding. PAS-methylene blue stain. × 600.

FIG. 6. Dermal capillary in an amputation specimen from a patient (LM) with diabetes. Marked thickening of the basement membrane (4+) is a feature. Tissue prepared as in Figure 5. × 600.

FIG. 7. Several nuclei of pericytes in a capillary wall are surrounded by a basement membrane. Hematoxylin and eosin stain. × 2,100.



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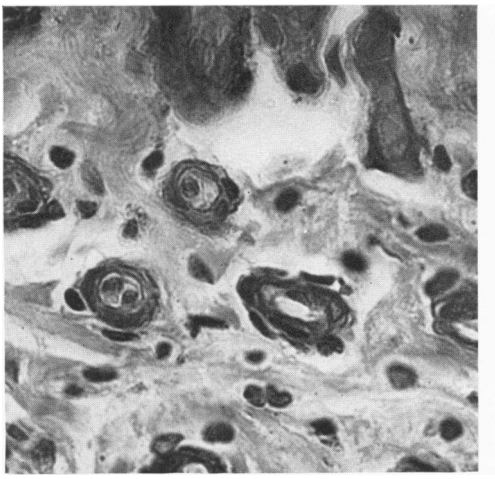

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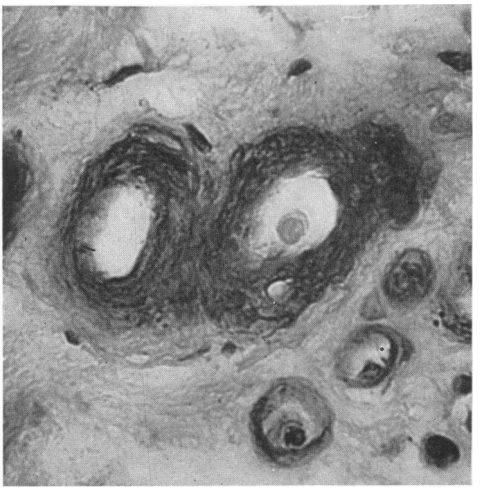

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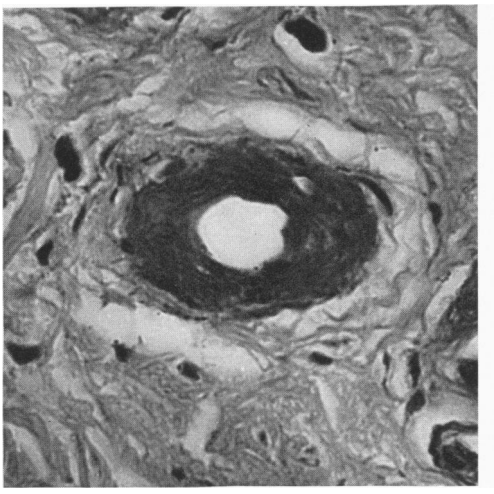

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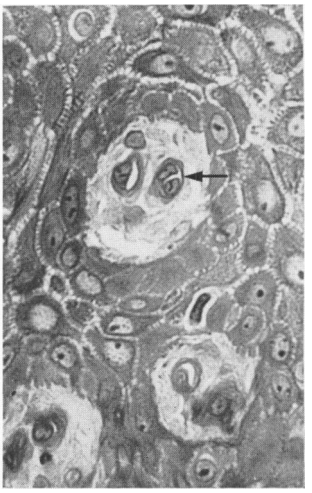

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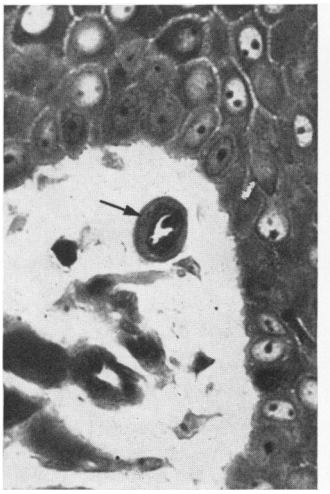

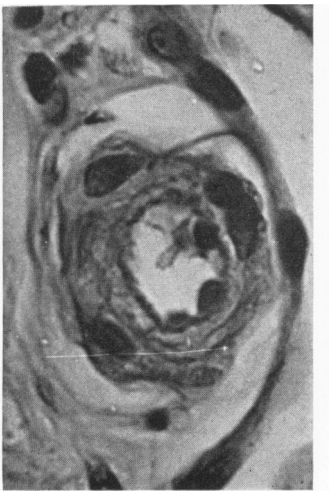

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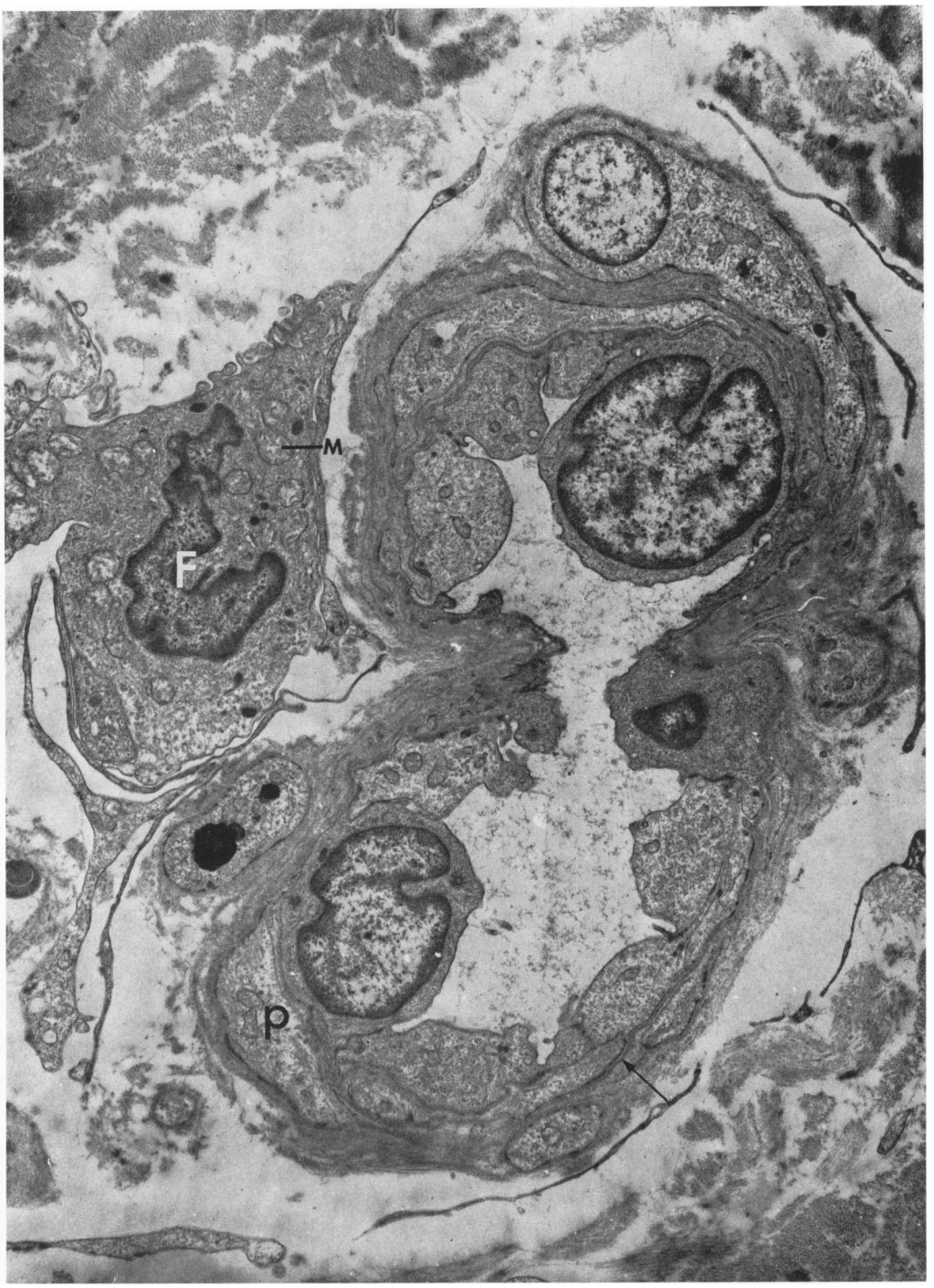

FIG. 8. Control, group IV, nondiabetic necropsy, 3 hours postmortem. This is probably a section through a bifurcation. The capillary lumen is empty. Endothelial nuclei protrude slightly into the lumen. A fairly thin basement membrane (arrow) lies on the external surface of the endothelium and envelops so-called "pericytes" (p). A fibrocyte (F) is adjacent to the capillary. Mitochondria (M) appear in the cytoplasm of fibrocytes, pericytes and endothelial cells. Epon embedding. Approximately × 7,000.

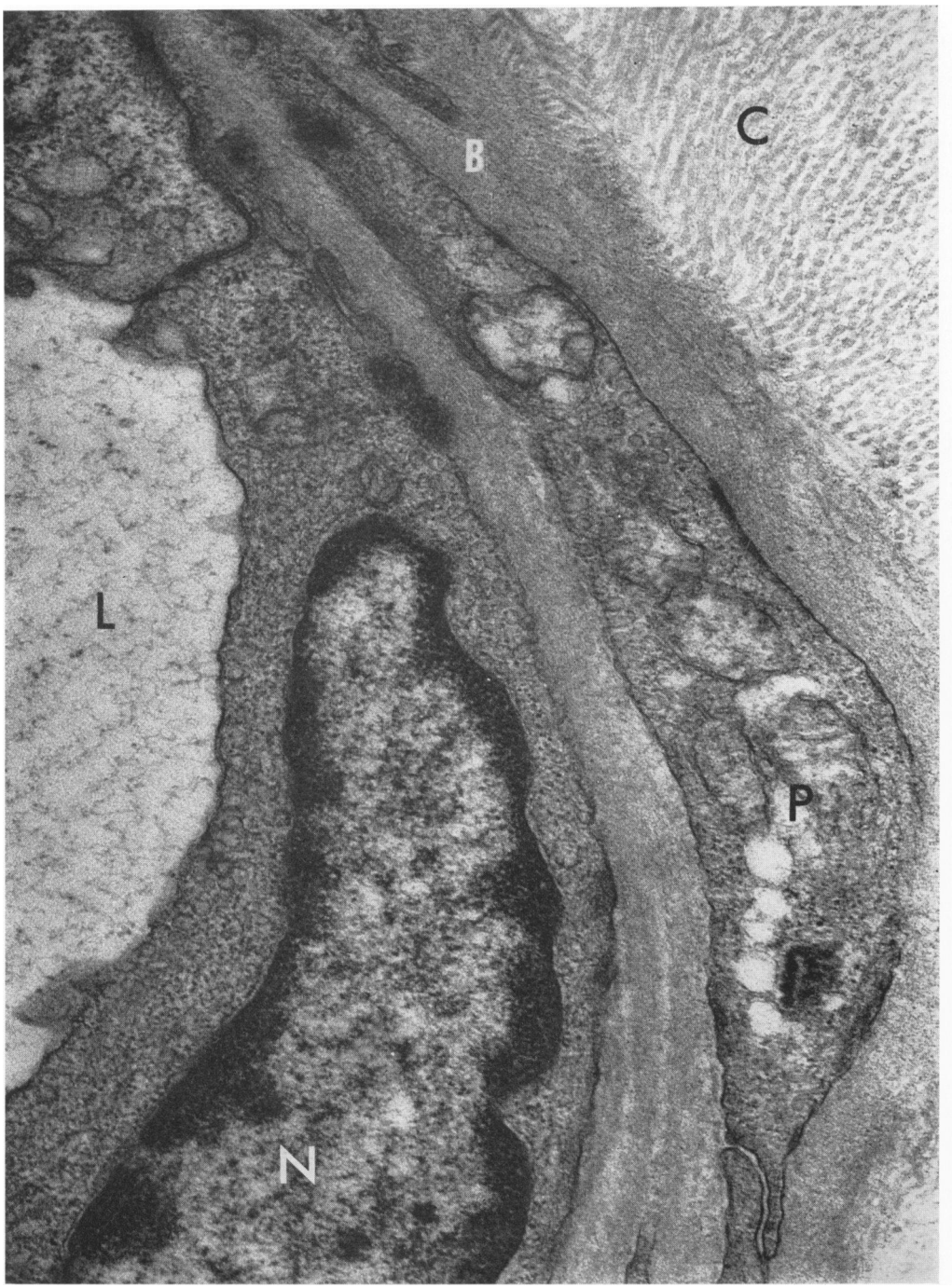

FIG. 9. Portion of a capillary, diabetic, necropsy (group II). A thickened basement membrane (B) is made up of an amorphous material with exceedingly fine and short filamentous structures. Collagen fibrils (C) are seen on the peripheral side of the basement membrane. L, lumen of capillary; N, nucleus of endothelial cell; P, pericyte. Epon embedding. Approximately × 34,600.

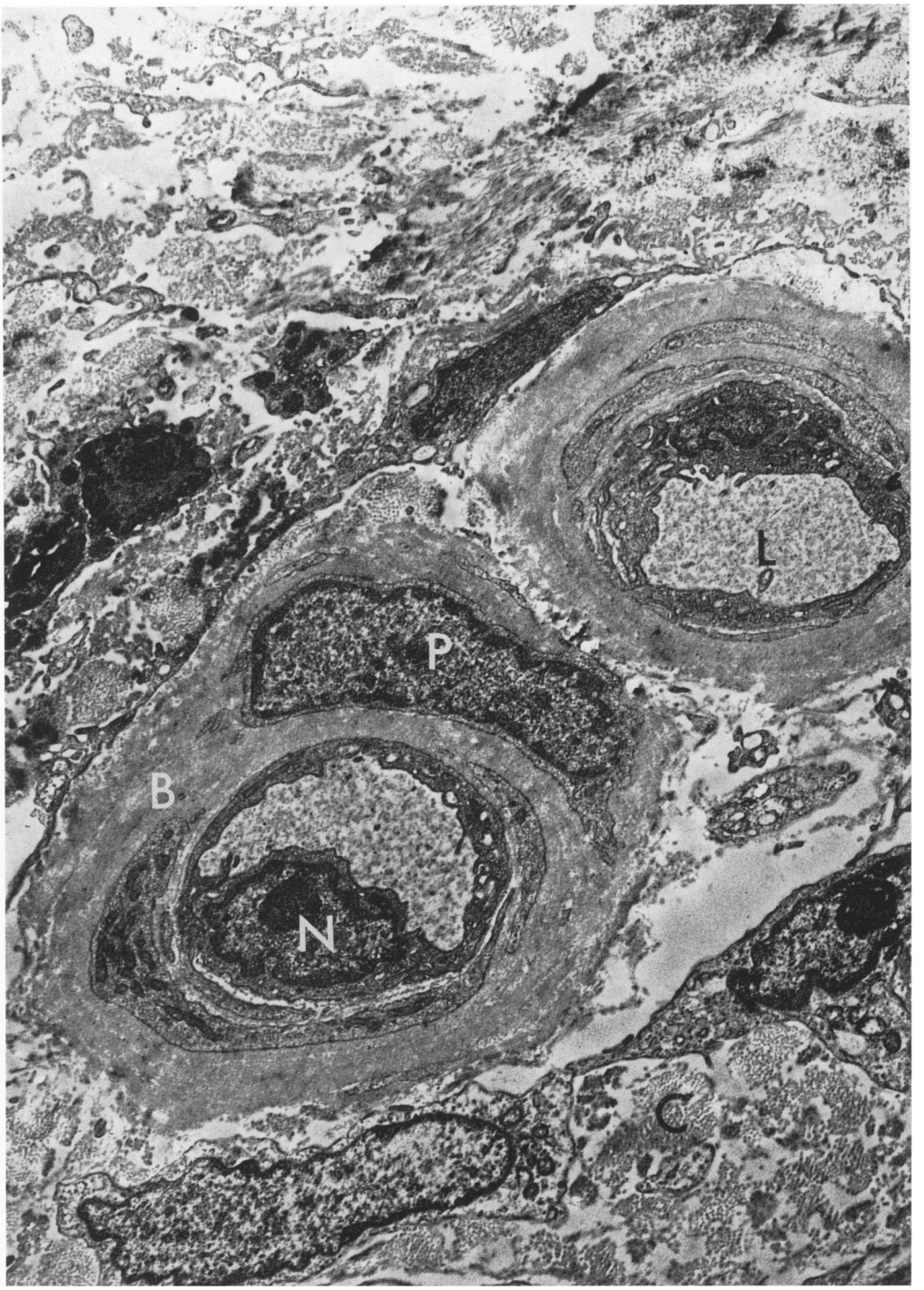

FIG. 10. Capillaries in a diabetic amputation specimen (group I). The basement membrane exhibits a homogeneous type of thickening (B) and is made up of amorphous material. P, pericyte; C, collagen fibrils. Methacrylate embedding. Approximately × 8,200.

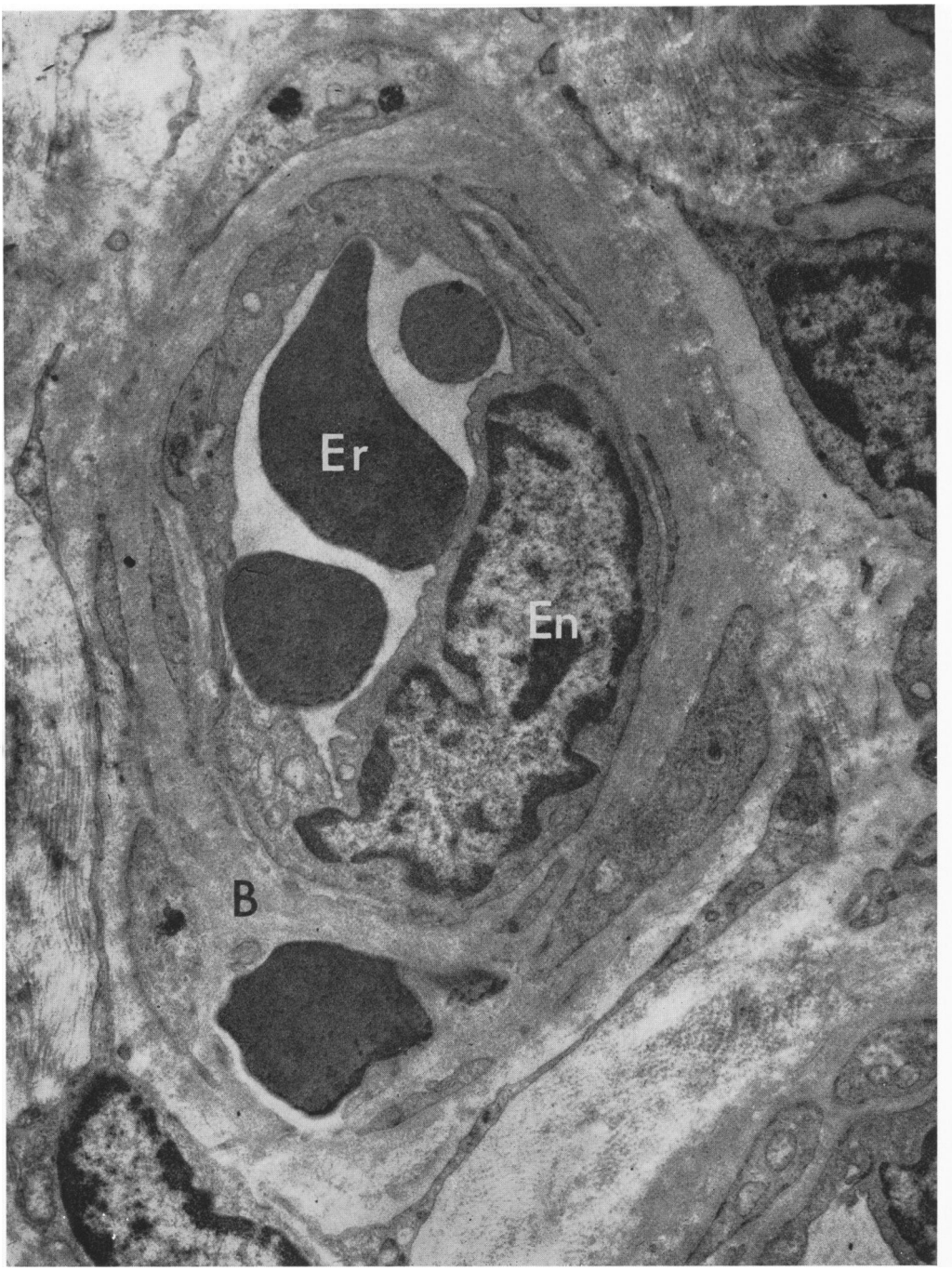

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FIG. 11. Diabetes, necropsy specimen (group II). The postmortem interval was 8 hours. There is excellent preservation of the tissue. The capillary basement membrane (B) is thickened. An erythrocyte within the basement membrane in the lower center portion of the capillary is probably passing into the extravascular area. A portion of another capillary is seen in the lower right-hand corner of the micrograph. Er, erythrocyte; En, endothelial nucleus. Epon embedding. Approximately × 11,100.

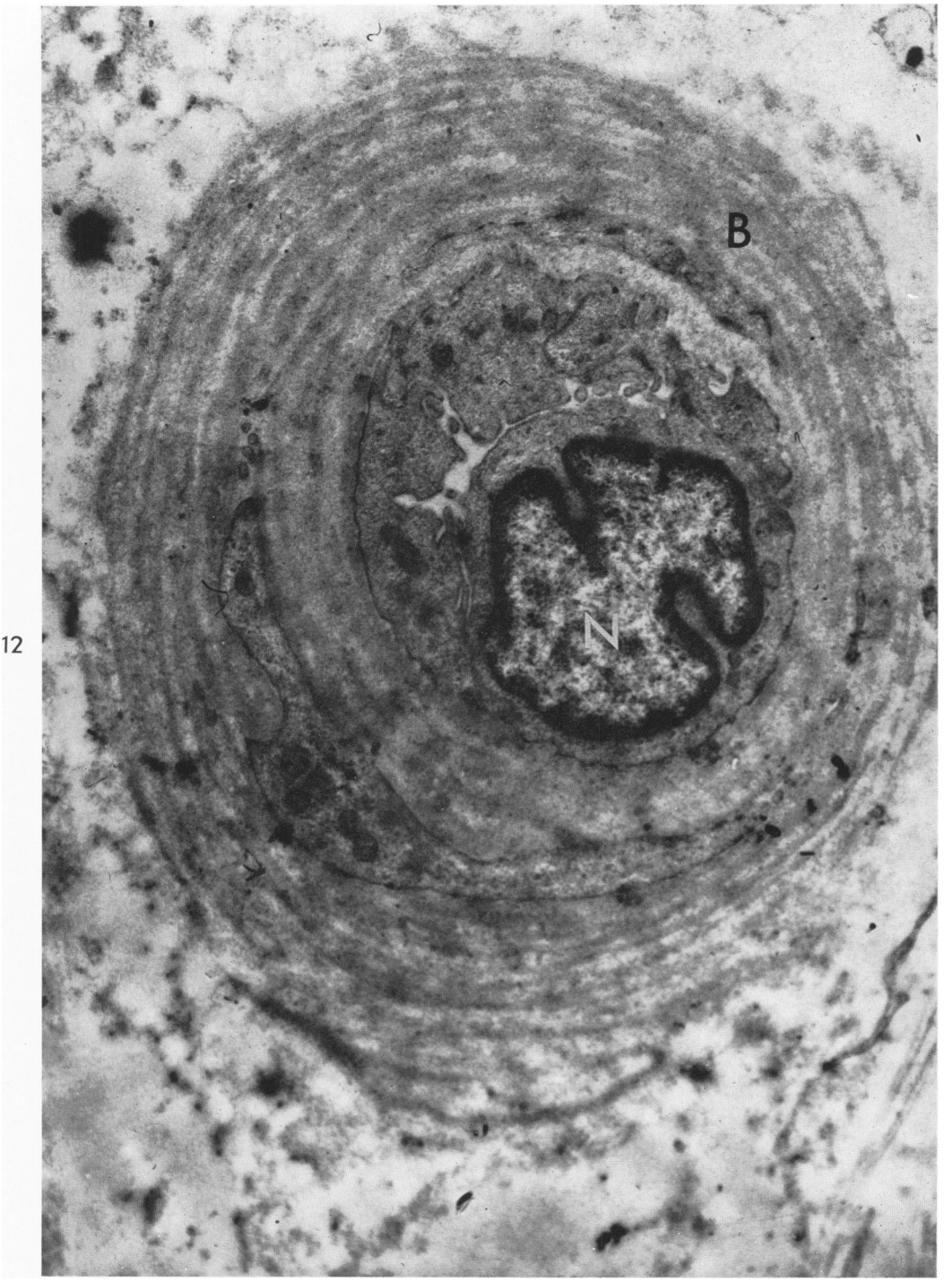

FIG. 12. Diabetes, amputation specimen (group I). Basement membrane thickening is of the stratified type (B). Its layers appear almost circular and rigid. The lumen is collapsed. N, endothelial nucleus. Epon embedding. Approximately × 13,700.

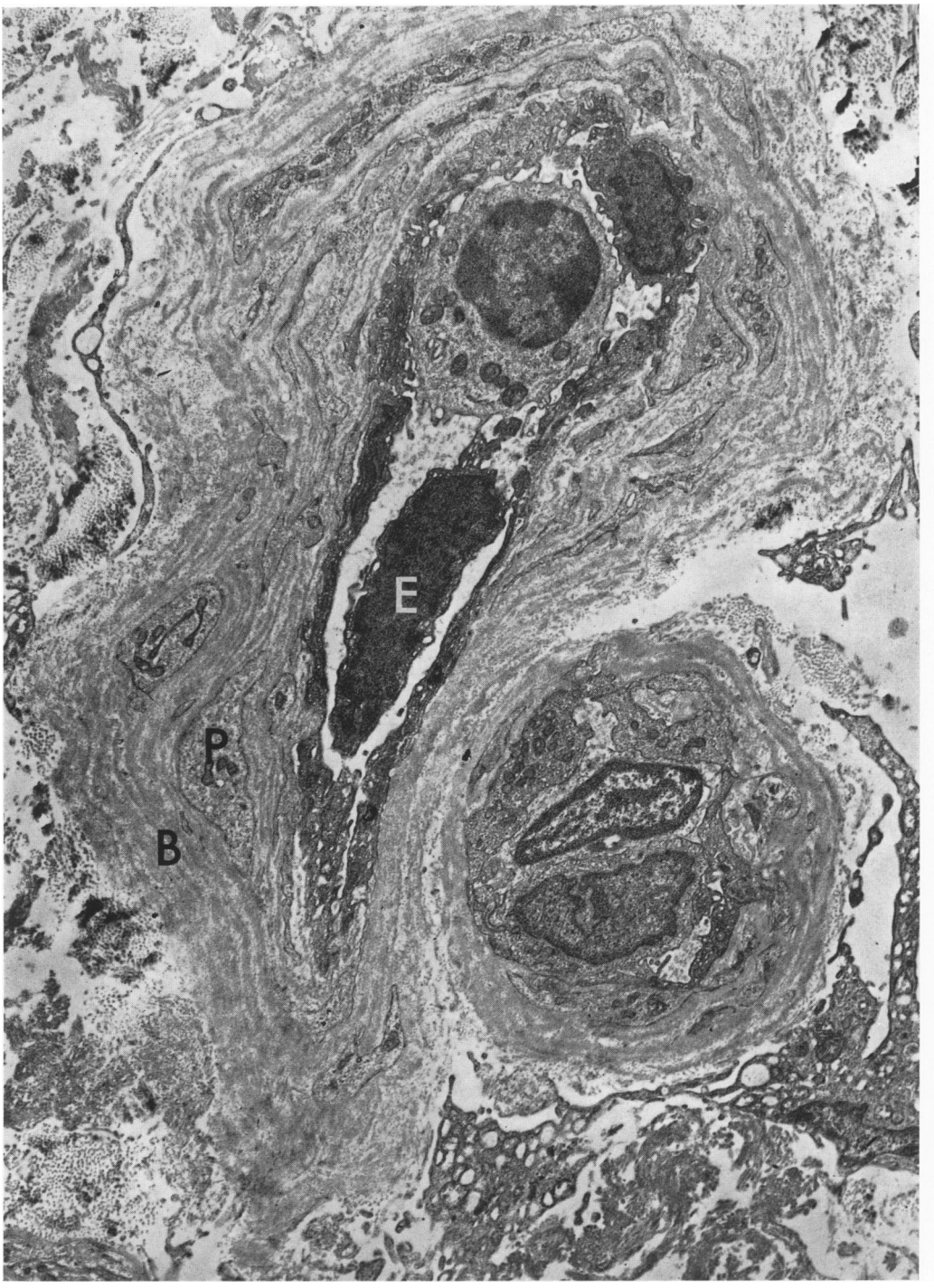

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FIG. 13. Diabetes, amputation specimen (group I). The markedly thickened basement membrane (B) exhibits layers or strips arranged in a laminated pattern with a slightly wavy appearance. Pericytes (P) are embedded in the basement membrane substance. E, erythrocyte. Methacrylate embedding. Approximately × 6,400.

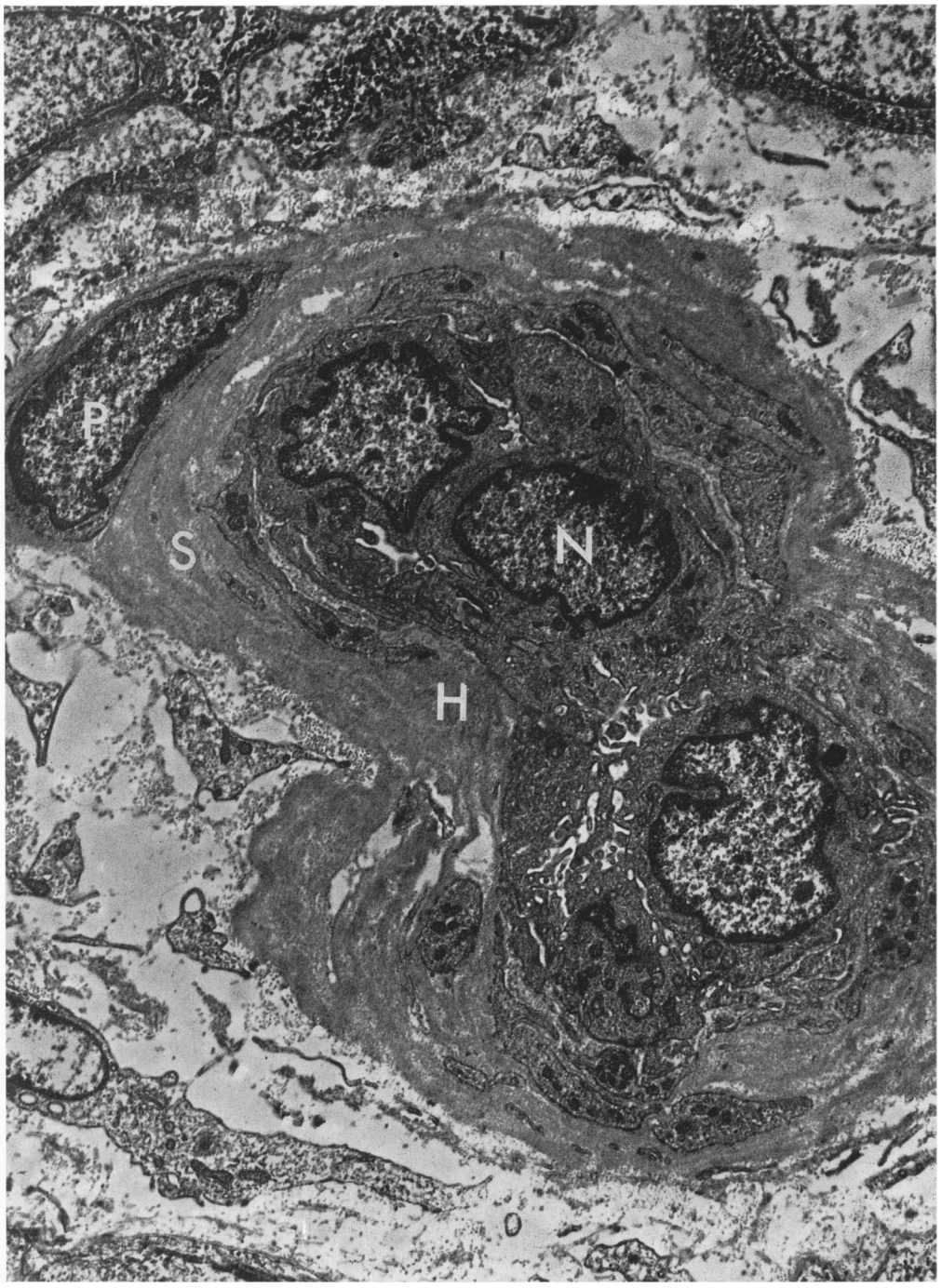

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FIG. 14. Diabetes, amputation specimen (group I). Basement membrane thickening is marked. In some areas (H) it appears to be homogeneous, while elsewhere (S), usually in the peripheral portions, it appears in layers. This represents a mixed type of thickening occurring in the same capillary. The capillary lumen is collapsed. N, nucleus of endothelial cell; P, pericyte. Methacrylate embedding. Approximately × 7,400.