

The Incubation of Laminin, Collagen IV, and Heparan Sulfate Proteoglycan at 35°C Yields Basement Membrane-like Structures

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Abstract. Three basement membrane components, laminin, collagen IV, and heparan sulfate proteoglycan, were mixed and incubated at 35°C for 1 h, during which a precipitate formed. Centrifugation yielded a pellet which was fixed in either potassium permanganate for ultrastructural studies, or in formaldehyde for Lowicryl embedding and immunolabeling with protein A-gold or anti-rabbit immunoglobulin-gold. Three types of structures were observed and called types A, B, and C.

Type B consisted of 30–50-nm-wide strips that were dispersed or associated into a honeycomb-like pattern, but showed no similarity with basement membranes. Immunolabeling revealed that type B strips only contained heparan sulfate proteoglycan. The structure was attributed to self-assembly of this proteoglycan.

Type A consisted of irregular strands of material that usually accumulated into semisolid groups. Like basement membrane, the strands contained laminin,

collagen IV, and heparan sulfate proteoglycan, and, at high magnification, they appeared as a three-dimensional network of cord-like elements whose thickness averaged ~3 nm. But, unlike the neatly layered basement membranes, the type A strands were arranged in a random, disorderly manner.

Type C structures were convoluted sheets composed of a uniform, dense, central layer which exhibited a few extensions on both surfaces and was similar in appearance and thickness to the lamina densa of basement membranes. Immunolabeling showed that laminin, collagen IV, and proteoglycan were colocalized in the type C sheets. At high magnification, the sheets appeared as a three-dimensional network of cords averaging ~3 nm. Hence, the organization, composition, and ultrastructure of type C sheets made them similar to the lamina densa of authentic basement membranes.

BASEMENT membranes are thin extracellular layers which underlie epithelia and surround muscle and fat cells as well as the nervous system (1, 2, 31). The major and specific components of basement membranes are collagen IV, the glycoprotein laminin, and heparan sulfate proteoglycan (14, 19, 25, 29–32). Basement membranes also contain other glycoproteins, particularly entactin (28) and fibronectin, but the latter may not be an integral matrix component (1).

The use of rotary shadowing and optical density measurements has revealed that some of the component molecules are able to self-assemble. Thus, collagen IV molecules in monomeric and dimeric forms undergo spontaneous aggregation to give rise to orderly networks, which may provide the framework for basement membrane formation (3, 33, 35–37). Self-assembly has also been observed with laminin (3, 10) and heparan sulfate proteoglycan (6, 27, 38). In addition, basement membrane components can interact with one another. In particular, laminin, heparan sulfate proteogly-

can, and fibronectin can bind to specific sites along the length of collagen IV (3, 22, 36).

The affinity of the components for one another is likely to be a major factor in their assembly into a basement membrane. The assembly must occur in an ordered manner since all basement membranes appear to have the same ultrastructure, that is, a network of tridimensional “cords” that are 3–4-nm thick on the average (15, 16, 24). Experiments with mixtures of basement membrane components also suggest that they interact specifically in an ordered manner (19). Thus, when the components are mixed and incubated at 35°C, a precipitate is formed. The best conditions for precipitation occur when type IV collagen, laminin, and heparan sulfate proteoglycan are mixed in a molar ratio of 1:1:0.1 at a pH of 7.4 at 35°C (19). We, therefore, incubated these three components of basement membrane at 35°C in various concentrations and studied the ultrastructure of the resulting precipitate. Three main types of structures were observed in

the electron microscope. Moreover, the distribution of the three components within these structures was examined by colloidal gold immunohistochemistry.

Materials and Methods

Basement Membrane Components

Laminin and collagen IV were obtained from Bethesda Research Laboratories, Gaithersburg, MD, as frozen solutions containing ~1 mg per ml. Laminin was in 0.05 M Tris buffer, pH 7.2, containing 0.15 M NaCl, while type IV collagen was in 0.5 M acetic acid. Before use, 0.5 M Tris buffer containing 1.5 M NaCl was added to the type IV collagen in a volume equal to 10% of the collagen solution and the neutralization was completed to pH 7.2 with 1.0 N NaOH. The large form of heparan sulfate proteoglycan was extracted from the mouse Englebreth-Holm swarm (EHS) tumor (14, 18) with 6 M urea, pH 6.8, purified on a DEAE-Sephadex column followed by cesium chloride density-gradient centrifugation and chromatography on a Sepharose CL-4B column (17, 26); it was stored in 0.05 M Tris buffer containing 0.15 M NaCl, pH 7.2.

The purity of laminin, heparan sulfate proteoglycan, and collagen IV was tested by 5–15% SDS-PAGE (11, 14, 18). After staining with Coomassie brilliant blue (Fig. 1), laminin samples showed characteristic bands at 200 and 400 kD. While there was no distinct band of entactin, some low molecular mass bands were present and, on densitometric scans of the gels, represented ~4% of total band proteins. However, examination of laminin preparations by rotary shadowing, kindly carried out by Dr. Gordon Laurie (University of Virginia, Charlottesville, VA), showed the presence of a small number of entactin molecules among the numerous laminin molecules. The collagen IV samples displayed the two characteristic bands at 185 and 170 kD. The heparan sulfate proteoglycan, after heparitinase digestion, exhibited a single band (26). Western blots confirmed that collagen IV and the proteoglycan were not contaminated by each other (11, 14, 18).

Incubation of the Mixture of Basement Membrane Components

Various amounts of laminin, collagen IV, and proteoglycan were incubated in the proportions shown in Table I. In each experiment, the solutions containing the individual substances were slowly thawed at 4°C for 4–6 h before use, then vortexed and centrifuged to remove any particulate material. Such

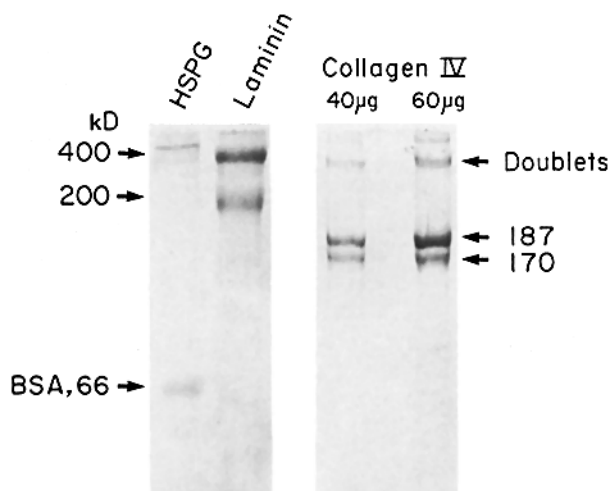


Figure 1. 7% SDS-PAGE. In the first lane, 30 µg of heparan sulfate proteoglycan (HSPG) after heparitinase digestion in the presence of BSA (BSA, 66 kD) yield a single band located just above 400 kD. In the second lane, 25 µg of laminin (Bethesda Research Laboratories) yield two bands at 400 and 200 kD, respectively. In the last lanes, 40 and 60 µg of collagen IV (Bethesda Research Laboratories) yield two bands at 187 and 170 kD, respectively, as well as doublets representing dimers of collagen IV.

Table I. Experimental Summary

Experiment	Amounts used			Identification of types [‡]		
	Laminin µg/ml	Collagen IV µg/ml	HSPG* µg/ml	A	B	C
1	100	200	40	++++	+	+
2	100	200	40	++++	+	+
3	100	200	40	++++	+	+
4	200	200	200	++++	++	++
5	100	200	167	+++	++++	+

* HSPG, heparan sulfate proteoglycan.

[‡] The number of +'s indicates the relative abundance of each type as assessed subjectively.

material, when present, was estimated by protein assay to be <5% of the sample. The solutions were then combined in a 1.5-ml Eppendorf centrifuge tube (Brinkman Instruments Co., Westbury, NY) maintained in ice so that self-assembly would not be favored. Laminin was aliquoted first, followed by collagen IV. The tube was then vortexed for 3 s. Finally, heparan sulfate proteoglycan was added and the tube was vortexed for another 6 s. In each case, the total volume in the centrifuge tube was 1 ml. The four to eight tubes used in each experiment were placed in a water bath at 35°C and rocked gently for 1 h. A flocculent precipitate appeared. The tubes were spun at 10,000 rpm for 10 min in a table top Eppendorf centrifuge (Brinkman Instruments Co.) at room temperature and the supernatant fraction was decanted, leaving a pellet consisting of a gellike precipitate adherent to the bottom and sides of the tube. The pellet was processed for electron microscopy in Epon or Lowicryl K4M as described below.

As controls, 1-ml solutions of laminin (200 µg) or collagen IV (200 µg) were prepared in individual Eppendorf tubes (Brinkman Instruments Co.) and incubated for 1 h at 35°C. No precipitate was thus observed with laminin, but a small one was obtained with collagen IV; it was pelleted by centrifugation. In another control experiment, two batches of 200 µg each of laminin, collagen IV, and heparan sulfate proteoglycan were mixed in the cold as indicated above. One batch was kept for 1 h at 4°C and the other at 35°C. In this control experiment only, the fixative (3% potassium permanganate at 4°C) was added before centrifugation in the hope of eliciting precipitation of any protein that had remained in solution.

Epon Processing of the Pellets for Ultrastructural Study

The pellets obtained in the main experiments (Table I) were fixed directly in the tube by adding freshly made 3% potassium permanganate dissolved in Krebs-Henseleit buffer, pH 7.4, at 4°C for 30 min (33). After washing for 20 min in several changes of buffer and enbloc staining for 45 min at 4°C in 1% uranyl acetate dissolved in the same buffer, the samples were briefly washed with distilled water and dehydrated in acetone, infiltrated with Epon 812, and polymerized at 68°C for 3 d. Epon sections were stained for 5 min with Reynolds' lead citrate for electron microscopic examination. The thickness of the structures referred to as type C sheets was measured on 10 different specimens, with at least five recordings on each, using narrow regions that appeared to be cut in cross section. Measurements done at high magnification were carried out on only one or two specimens, with 50 randomly selected areas being recorded in each.

Lowicryl Processing of the Pellets for Immunohistochemistry

The pellets were fixed in the tube by addition of 4% formaldehyde in 0.1 M phosphate buffer, pH 7.4, at 4°C for 30 min. Further fixation was then carried out in 4% formaldehyde, pH 9.0, for ~16 h. The samples kept in the tubes were washed three times for 2 h at 4°C with 0.15 M phosphate buffer containing 4% sucrose. Then dehydration was effected through 30% methanol for 5 min at 4°C, 50% for 10 min at -10°C, 75% for 10 min, and 90% for 15 min (twice) at -20°C. Lowicryl infiltration was carried out in the tube placed in a rotating device within a freezer compartment at -20°C, successively using a 1:1 and then 2:1 Lowicryl to 90% methanol mixture for 1 h each before transfer to 100% Lowicryl (with one change) for 1 h. The pellet was embedded by completely filling the Eppendorf tube with

fresh precooled Lowicryl at -35°C . Each tube was slipped into holes drilled in a thin plastic tray, and the resin was slowly polymerized at -35°C by exposure to two ultraviolet lamps set 15 cm away.

Antisera and Antibodies

The antisera and antibodies used for immunohistochemistry were prepared by the injection into rabbits of either laminin (5), type IV collagen (18), or heparan sulfate proteoglycan (14), all extracted from EHS tumors grown in lathyrus mice. The antilaminin antiserum was tested in immunoprecipitation assays on extracts of the radiolabeled EHS tumor, with only the two laminin bands being observed in SDS-PAGE; the results were confirmed using Western blots and ELISA (11). The antiserum to collagen IV was partially affinity purified by passage over laminin- and entactin-affinity columns. Immunoprecipitation tests, Western blots, and ELISA demonstrated the antiserum specificity (18). The antiserum to the proteoglycan was purified by adsorption to a column composed of the protein core of heparan sulfate proteoglycan coupled to Sepharose CL-4B (17). The titer of the resulting antibodies was low, but they showed a strong reaction with the heparan sulfate proteoglycan and none with either laminin, type IV collagen, or proteoglycan extracted from cartilage (14, 26).

Immunohistochemical Procedure

Immunolocalization of the weak antibodies against heparan sulfate proteoglycan was done by an amplified protein A-gold technique. The plastic Eppendorf tube containing the Lowicryl-embedded pellet was cut away. Straw to gold sections were mounted on Formvar-coated, 200-mesh nickel grids. The technique was done in three steps. (a) The grids were preincubated on drops of 0.01 M Dulbecco's PBS containing 1% BSA for 5 min, and then floated for 1 h over a drop of antibodies against heparan sulfate proteoglycan or of nonimmune serum for control, either of which was diluted at 1:100. The grids were then passed sequentially across three wells filled with PBS. (b) In the second or amplification step, the grids were successively floated on PBS containing 1% BSA for 5 min, a 1:100 dilution of goat anti-rabbit immunoglobulin in PBS (Cooper Biomedical, Inc., Malvern, PA) for 5 min, six sets of PBS-filled wells, and two drops of PBS containing 1% BSA. (c) For the third or gold-labeling step, the protein A-gold complex was prepared as previously described (13) and diluted 1:15. The grids were incubated on this solution for 1 h and washed by streaming first PBS containing 1% BSA, then PBS alone, and finally distilled water. The backs of the grids were blotted with filter paper; the sections were allowed to air dry and were then stained for 4 min with uranyl acetate and 15 s with lead citrate.

The immunolabeling for laminin and collagen IV was done by the Auroprobe method as follows. Lowicryl sections mounted on nickel grids were prepared as above and exposed to the strong antisera against these substances for 25 min. They were then exposed to goat anti-rabbit immunoglobulin labeled with 15-nm gold particles (Auroprobe GAR-15; Janssen Pharmaceutica, Beerse, Belgium) as described elsewhere (12). Sections were examined in a Philips Electronic Instruments, Inc. (Mahwah, NJ) 400T electron microscope.

Results

Incubation of Laminin, Collagen IV, and Heparan Sulfate Proteoglycan

When the three substances were incubated together at 35°C in various concentrations (Table I), a substantial precipitate arose which was centrifuged and fixed in either permanganate for Epon embedding or formaldehyde for Lowicryl embedding. In either case, three distinctive structures, designated types A, B, and C were observed (Fig. 2). Type A appeared as semisolid groups of various sizes (Figs. 2 and 3 a). They generally were the most abundant structures throughout the pellet (Table I). Type B consisted of narrow strips of material (Figs. 2 and 3 c) that were abundant in the experiments in which the proportion of heparan sulfate proteoglycan was high (Table I). Type C was in the form of continuous sheets usually cut in cross section (Figs. 2 and 3 e).

These sheets were somewhat less abundant than the other structures, but generally predominated at the periphery of the pellet. Their amount was increased in the experiment in which the three incubated substances were mixed in equal amounts (Table I).

For control, tubes containing only 200 $\mu\text{g}/\text{ml}$ of our laminin were incubated at 35°C for 1 h, but no precipitate was formed. In control tubes containing collagen IV alone and kept at 35°C for 1 h a small precipitate appeared which, on examination in the electron microscope, showed patches of disorganized filamentous material. In a last control, laminin, collagen IV, and the proteoglycan were kept together either at 35 or 4°C for 1 h, and cold potassium permanganate fixative was added before centrifugation. A substantial precipitate arose in the 35°C batch and showed the types A, B, and C structures throughout. In contrast, the 4°C batch yielded only a minute precipitate, which was mainly composed of nondescript material without a definite pattern but which in a few small places displayed characteristic type A and C structures but no type B.

Type A. After permanganate fixation and Epon embedding, the type A structure is composed of irregular strands which may accumulate into large aggregates measuring up to 10 μm (Fig. 2) or may remain separated by interstitial spaces of various sizes (Fig. 3 a). At high magnification, type A strands are composed of fuzzy, anastomosing elements forming a tridimensional network (Fig. 3 b). These elements average 2.9 ± 0.6 nm in thickness; they resemble the 3–4-nm-thick elements observed in basement membranes at high magnification and are referred to as "cords" (15) (Fig. 4 b).

Type B. This structure is composed of 25–35-nm-wide strips. The strips may be scattered individually between the other two types (Fig. 2) or connected to one another in a honeycomb pattern (Fig. 3 c); the intervening spaces measure from 100 to 300 nm. Each strip is seen at high magnification to be formed of a few 4.5-nm-wide sets of two dense parallel lines separated by a light space, while fuzzy material surrounds them (Fig. 3 d).

Type C. This structure consists of slender sheets usually cut in cross section (Figs. 2 and 3 e). These sheets curl and fold on themselves and continue over long distances, while retaining a fairly uniform thickness. Type C sheets are generally composed of an electron dense central layer, with a few extensions protruding on both surfaces (Fig. 3 e). The mean thickness of this central dense layer is 65.2 ± 2.2 nm with a range of 36–108 nm. These values are similar to those observed on measuring the lamina densa in four basement membranes (Table II). The lateral extensions of type C sheets vary widely in both length and width. The length averages 90.2 ± 5.9 nm with a range that may extend to 205 nm. At high magnification, the central layer is composed of anastomosing cords that are 3.0 ± 0.6 nm wide and form a tridimensional network (Fig. 3 f), as in basement membrane (Fig. 4 b).

In Fig. 4 a, the basement membrane of the monkey semiferous epithelium is depicted for comparison with a type C sheet displayed at the same magnification in Fig. 4 c. The basement membrane shows the lamina densa with extensions across the lamina lucida above and into connective tissue below (Fig. 4 a), while the cord network making up the lamina densa is shown at high magnification in Fig. 4 b. A remarkably similar pattern is presented by the dense central layer

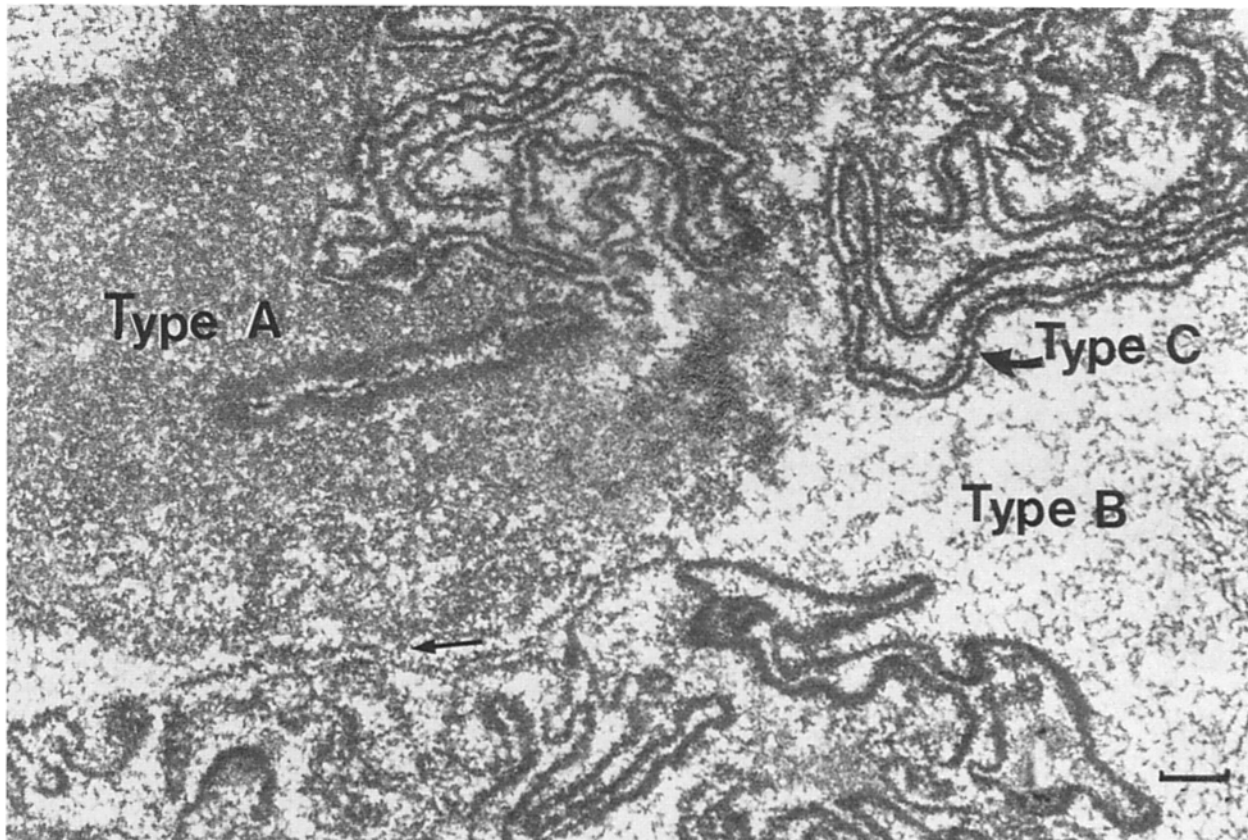


Figure 2. Electron micrograph of the precipitate produced by incubation of preparations of laminin, collagen IV, and heparan sulfate proteoglycan at 35°C for 1 h. Fixation was in permanganate and embedding in Epon. The periphery of the pellet lies to the lower right. Three structures, referred to as types A, B, and C, may be seen. At center left, a mass of type A material is formed of irregular deposits of anastomosing strands separated by narrow spaces. Type B is seen as strips scattered between the other two types, as shown at lower right. The type C structures are the densely stained, thick, wavy lines at upper right and base; they are cross sections of sheets and may be arranged in pairs. The arrow points to material which may be transitional between types A and C. Bar, 1 μ m.

of the C sheet, the extensions on both sides (Fig. 4 c) and, at high magnification, the cord network making up this layer (Fig. 4 d).

Gold Immunolabeling

After formaldehyde fixation and Lowicryl embedding, the types A, B, and C structures have the same overall appearance as after permanganate fixation and Epon embedding, although the thickness of the central region of type C sheets is increased to 72.8 ± 2.0 nm (Table II) while the mean length of the extensions is decreased to 51.5 ± 2.1 nm. Gold particles indicative of immunolabeling were lacking or rare in controls (Fig. 5, a, e, and i). In type A strands, gold particles revealed the presence of laminin (Fig. 5 b), collagen IV

(Fig. 5 c), and heparan sulfate proteoglycan (Fig. 5 d). In type B strips, laminin (Fig. 5 f) and collagen IV (Fig. 5 g) were absent and only the proteoglycan was present (Fig. 5 h). Type C sheets, like type A strands, included the three substances (Fig. 5, i-l).

Discussion

Laminin-enriched extracts of the mouse EHS tumor were previously combined with collagen IV or heparan sulfate proteoglycan or both (18), but electron microscopy of the resulting materials showed various amorphous and irregular elements with no distinctive pattern (13, 20). In the present work, coincubation of preparations of laminin, collagen IV,

Figure 4. (a) Basement membrane of monkey seminiferous epithelium fixed with 3% potassium permanganate and embedded in Epon. At the top of the picture, the epithelium (Ep) is followed by the pale lamina lucida (L) crossed by either single cords (such as the one facing the arrowhead) or small groups of several cords. Below, the thick lamina densa (D) consists of packed cords. On its lower side, a few single cords (arrowheads) and, in center right, a large group of cords extend into connective tissue. (b) Higher magnification of part of the lamina densa. Distinct cords form a tridimensional network. (c) Cross section of type C sheet fixed in 3% potassium permanganate and embedded in Epon. The general appearance is similar to that of the basement membrane in the upper panel. (d) Higher magnification of part of the dense central region. The cord network is similar to that in the basement membrane depicted in b. Bars: (a and c) 100 nm; (b and d) 10 nm.

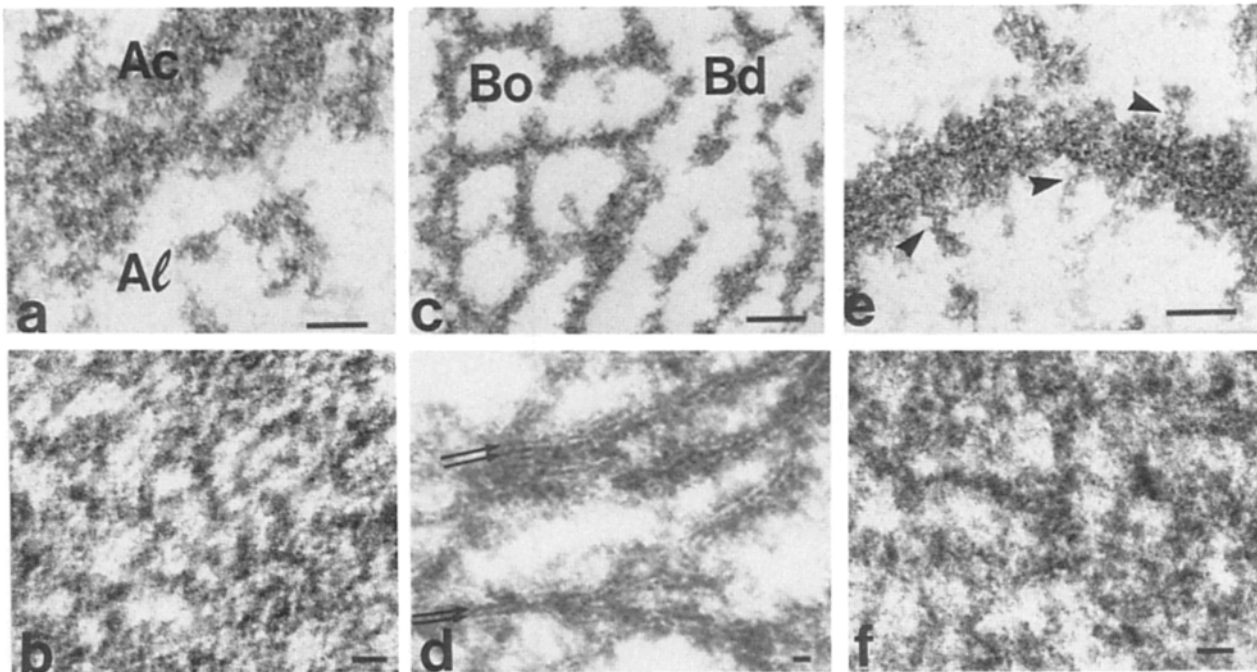
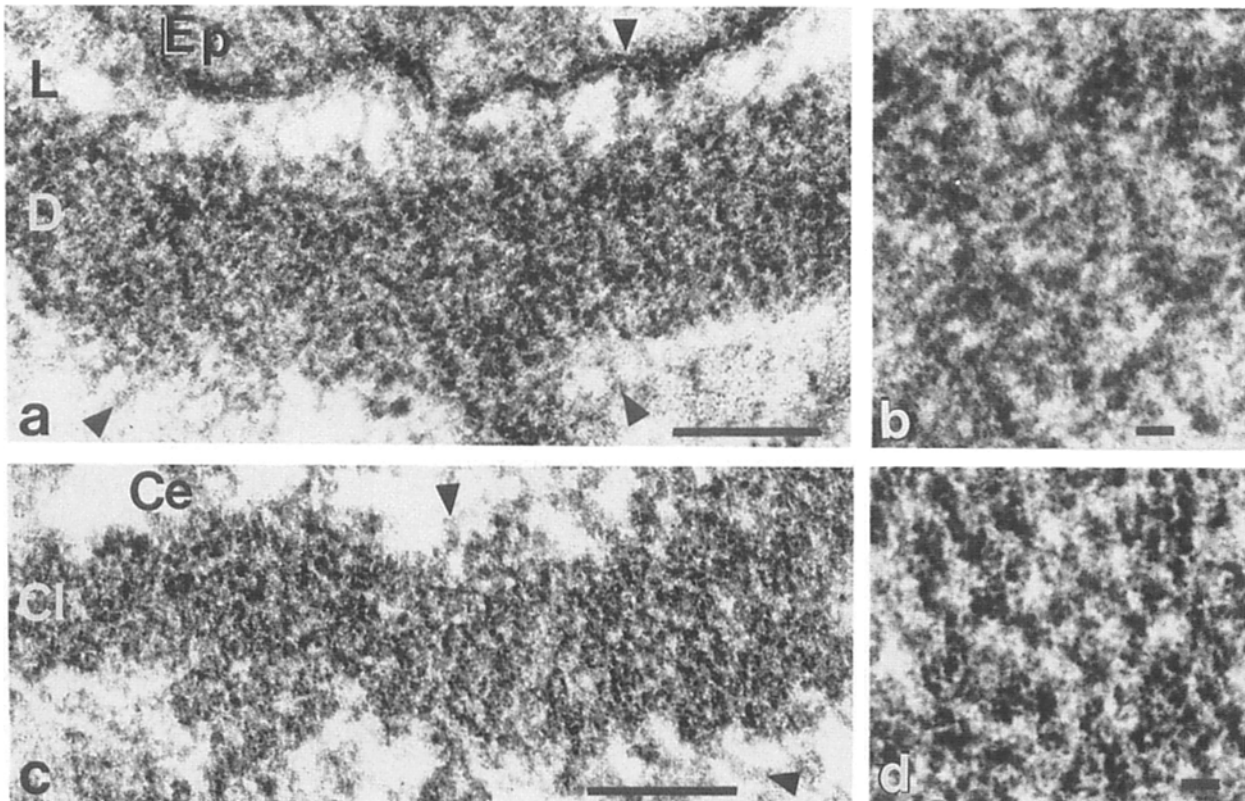


Figure 3. (a) Loose type A strands at lower right (*Al*) are in continuity with a fairly compact group of strands above (*Ac*). (b) At high magnification, the strands are seen to be composed of anastomosing elements referred to as cords. (c) At left, connecting type B strips are organized to form a polygonal pattern (*Bo*). On the right, a few individual strips are dispersed (*Bd*). (d) At high magnification, the strips appear to be composed of ribbons consisting of two parallel lines (*double arrows*) separated by a light space and associated with fuzzy material. (e) From left to right across the figure is a type C sheet composed of a dense central region from which extensions protrude on both surfaces (*arrowheads*). (f) At high magnification, the dense central region of the type C sheet is composed of anastomosing elements referred to as cords. Bars: (a, c, and e) 100 nm; (b, d, and f) 10 nm.



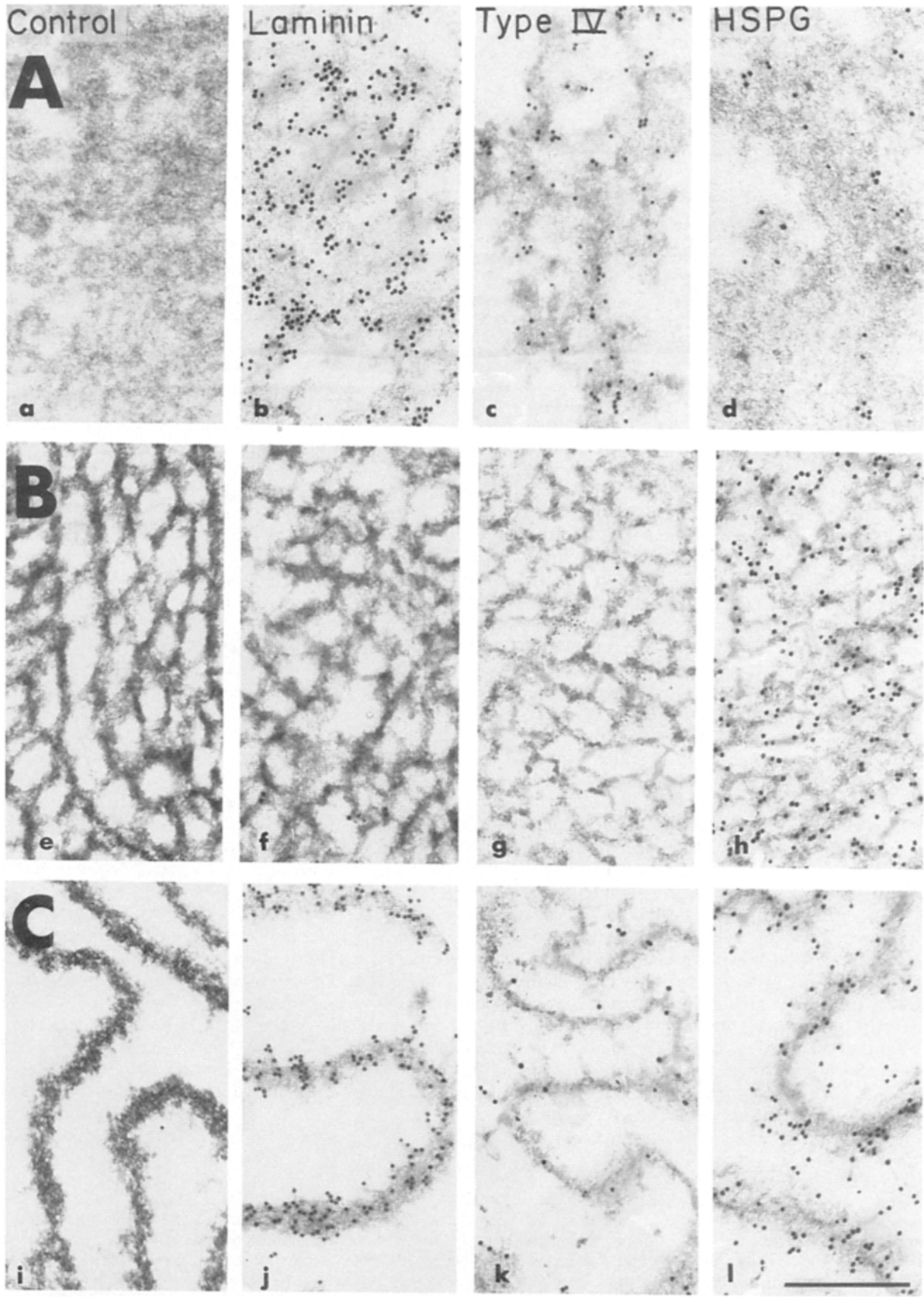


Table II. Comparison of Type C Sheets and Basement Membranes

	Dense central region of type C sheets		Lamina densa of basement membrane	
	Thickness	Range of 50 measurements	Thickness	Range of 30 measurements
	nm	nm	nm	nm
Permanganate fixation and Epon embedding	65.2* ± 2.2	(36-108)	51.8‡	(25-90)
Formaldehyde fixation and Lowicryl embedding	72.8* ± 2.3	(38-108)	53.2§	(21-169)

* The thickness of the dense region is the average from 10 specimens, in each one of which at least five measurements were recorded in perpendicularly cut areas.
 ‡ The thickness of the lamina densa after permanganate fixation is the average of measurements on four basement membranes, those of rat foot pad epidermis, 53 (range 30-80) nm; trachea, 40 (25-50) nm; jejunum, 32 (25-40) nm; and seminiferous tubule, 82 (75-90) nm; see reference 15.
 § The thickness of the lamina densa after formaldehyde fixation is the average of measurements on four basement membranes, those of rat vas deferens, 79 (61-115) nm; outer enamel, 34 (21-46) nm; colon, 37 (31-43) nm, and kidney glomerulus 116 (87-169) nm; see reference 12.

and heparan sulfate proteoglycan at 35°C for 1 h yielded a precipitate exclusively made of three characteristic structures called types A, B, and C. Two of them, type A and type C, differed at low magnification in the electron microscope, but showed a similar ultrastructure at high magnification and, by immunohistochemistry, were found to contain the three substances as do basement membranes (12, 31).

The third structure, type B was completely different from the other two and contained only heparan sulfate proteoglycan. At high magnification, the type B strips were composed of groups of 4-5-nm-wide sets of two parallel lines similar to a structure identified in basement membranes under the name "double tracks" (15). Such paired lines were recently obtained in vitro by incubating the proteoglycan alone at 35°C (14a) and were attributed to its self-assembly (6-9, 27), presumably by disulfide bonding of the core protein (27). At any rate, the overall appearance of type B strips bore no resemblance to the thin basement membranes observed in vivo.

The type A structure showed two similarities to basement membrane: the combined presence of the three initial substances and, at high magnification, a tridimensional network of 3-nm cords. However, at low magnification, type A strands were arranged in a random, unorganized manner and, therefore, were quite different from the neatly layered basement membranes commonly found in tissues.

In type C sheets, as in type A structures, the three initial substances were colocalized and, at high magnification, a three-dimensional cord network was present. But, in addition, the uniform thickness of the central region was in the same range as that of the lamina densa of thin basement membranes (Table II). These observations led to the conclusion that the type C sheet could be considered as a basement membrane assembled in vitro.

While the present results were obtained with preparations of laminin, collagen IV, and heparan sulfate proteoglycan only, these substances might include contaminants that played a role in producing the results. In particular, since entactin was known to be present in basement membranes (1), the small amount of this substance contaminating laminin might be involved in the production of type C sheets. Other components, some of which had not yet been precisely identified (1), might also intervene. In addition, the ability of EDTA to release laminin and entactin from the EHS tumor (29a) suggested that cations might be involved in the assembly of basement membrane components.

Nevertheless, assembly of the components did not seem to require the presence of enzymatic factors, but only of the components themselves. Their ability to interact with one another in vitro was well known (10, 19, 22, 33). Even entactin was recently found to bind to laminin (28) and collagen IV (Kleinman, H. K., and J. R. Hassell, unpublished observations). In particular, collagen IV could self-assemble to form polymers and produce networks (3, 33, 37, 38), which were similar to, but wider-meshed than, the network demonstrated in basement membrane after partial digestion of the cords with either protease (15, 16) or guanidine (37). Thus, for the formation of type C sheets, collagen IV molecules could first assemble into a network along which specific sites served as receptors for laminin and the proteoglycan. In the control experiment in which the three substances were kept for 1 h at 4°C, small but definite amounts of type A and C structures were observed. Hence, the assembly could take place in the cold, although presumably at a slow rate. The full reaction observed at 35°C illustrated the importance of a temperature similar to that of the body for the completion of the assembly.

In summary, we have incubated various amounts of the

Figure 5. Immunolabeling of type A (A), type B (B), and type C (C) after fixation in 4% formaldehyde and embedding in Lowicryl. Sections were first exposed to either nonimmune serum for control (first column), or to antiserum against laminin (second column), or against collagen IV (third column). The sections were then immunolabeled with gold-bound anti-rabbit globulin antiserum (Auroprobe GAR-15), followed by uranyl acetate and lead citrate. Other sections were exposed to antibodies against heparan sulfate proteoglycan (HSPG, fourth column) followed by amplification with goat anti-rabbit immunoglobulin (unlabeled), then protein A-gold, and finally uranyl acetate and lead citrate staining. Type A strands show no gold particles in controls (a) but numerous ones in the tests for laminin (b), collagen IV (c), and heparan sulfate proteoglycan (d). Type B strips lack gold particles in controls (e) and in the tests for laminin (f) and collagen IV (g), but show many particles in the test for the proteoglycan (h). Type C sheets have a negative control (i), but many gold particles on the tests for laminin (j), collagen IV (k), and the proteoglycan (l).

major basement membrane components and assembled them under certain conditions, which resulted in a structure resembling authentic basement membranes. This model system could be useful in sorting out the molecular events involved in the organization of these extracellular matrices.

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