The noncollagenous domain of collagen from three different basement membranes of bovine origin (glomerular, lens capsule, and placental) was excised with bacterial collagenase, purified under nondenaturing conditions, and characterized. In each case the domain existed as a hexamer comprised of four distinct subunits (α1(IV)NC1, α2(IV)NC1, M2*, and M3). Each subunit exists in both monomeric and dimeric (disulfide-cross-linked) forms. Certain dimers also exist which contain nonreducible cross-links. The hexamers from the three membranes differ with respect to stoichiometry of subunits and subunit isoforms and to the degree of cross-linking of monomers into dimers. The minor subunits, M2* and M3, vary in quantity over a 20-fold range relative to the major ones among the three hexamers. The results indicate that: 1) at least two populations of triple-helical collagen molecules, differing in chain composition, exist in each membrane and that their relative proportions are tissue-specific; and 2) the chemical nature of the noncollagenous domain of these populations is tissue-specific with regard to subunit isoforms and relative proportion of reducible and nonreducible cross-links in dimers.

A novel structural feature of the noncollagenous domain of basement membrane collagen was also evinced from these studies. Namely, that each of the four monomeric subunits exists in charge isoforms.

Basement membranes are complex extracellular matrices that play key roles in diverse biological processes such as ultrafiltration of blood (1), orchestration of embryonic development and maintenance of tissue architecture during remodeling and repair (2, 3). Emerging information suggests that their supramolecular structure varies with respect to relative amounts (4, 5) and chemical nature of their known macromolecular constituents (4–11), features which may be of fundamental importance in conferring the diverse functions.

The molecular properties of collagen IV, the major constituent of mammalian basement membranes, are of interest from the standpoints of structure-function relationships and their role in diseases. Collagen IV interacts with laminin, heparan sulfate proteoglycan, and fibronectin and it is proposed to serve as a scaffold for the proper organization of these constituents in basement membranes (12, 13). The noncollagenous (NC1) domain of collagen IV is of particular importance because it is a critical site for cross-linking two triple-chain collagen molecules (14–17) and it seems to be important for the lateral assembly of these molecules to form networks (18). Moreover, it contains the structural epitope which reacts with autoantibodies from patients with Goodpasture syndrome (19).

The NC1 domain is released from collagen IV as a hexamer, composed of monomeric and dimeric subunits, upon digestion of membrane with bacterial collagenase (19–21). In GBM, 3 different subunits (M1, M2*, and M3) were identified by chemical and immunochemical techniques (22). Each occurs in monomer and disulfide-linked forms. The GP epitope is exclusively localized to M2* and is sequenced under non-denaturing conditions.

The collagen chain origins of these subunits from LBM were recently determined (23). M1 comprises two polypeptides, designated α1(IV)NC1 and α2(IV)NC1, which correspond to the noncollagenous segments of the α1 and α2 chains of collagen IV, respectively. M2* and M3 have physicochemical properties remarkably similar to those of α1(IV)NC1 and α2(IV)NC1 but their amino acid sequences differ. Each have Gly-X-Y triplets and hydroxyproline at their amino terminus, reflecting that each has a collagen chain origin, designated α3 and α4, respectively. These new chains may be variants of the α1(IV) and α2(IV) chains in which the NC1 segments are modified, or they may be entirely new chains with distinctive collagenous and noncollagenous sequences (23).

Earlier studies (24) suggested that the absolute amount of the GP antigen, now designated as M2*, varies among basement membrane preparations from different tissues (glomerulus, lung, and placenta). Such differences could reflect: 1) the presence of other contaminating connective-tissue elements in preparations from lung and placenta as compared to glomerulus; or 2) a stoichiometric difference in the subunit

* This work was supported by National Institutes of Health Grants AM 3831, AM 26178, Swedish Medical Research Council Grant 7341 (to J. W.), American Heart Association Kansas Affiliate Grant K8-85-P-3 (to J. L.), Ministry of Education and Science (Spain) Fulbright Fellowship (to J. T.), and by the Instituto de Investigaciones Citologicas-Kansas University Medical Center International Center of Cell Biology. Electron microscopy research support was provided in part by the John W. and Effie E. Speas Foundation and the Electron Microscopy Research Center of the University of Kansas Medical Center. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Present address: Dept. of Nephrology, University Hospital, S-221 85 Lund, Sweden.
2 Present address: Dept. of Biochemistry, School of Pharmacy, University of Valencia, Valencia, Spain.
3 Present address: Dept. of Laboratory Medicine and Pathology, University of Minnesota, Box 491 Mayo, Minneapolis, MN 55455.
composition of the hexamer form of the noncollagenous domain of basement membrane collagen.

The purpose of the present study was to determine whether the subunit composition and other properties of the hexamer varies among different basement membrane. This was accomplished by purifying the hexamer under non-denaturing conditions from three different basement membranes of bovine origin and comparing their molecular properties. The results indicate that the chemical nature of the noncollagenous domain and the relative proportions of at least two distinct populations of triple-helical collagen molecules are specific for a basement membrane of a given tissue.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bovine kidneys were collected as described previously (25). Bovine lenses were obtained from Pel-Freeze Biologicals. Bovine placenta (5–6 months of gestation) was obtained from a local slaughterhouse, transported on ice, and immediately used. The following fine biochemical products are specifically mentioned together with the filtration medium of collagenase (CLSPA) from Worthington, DE-52 cellulose from Whatman, Sephacryl S-200 and S-300 from Pharmacia LKB Biotechnology Inc. C4m columns (201TP, 10-micron) for reverse-phase HPLC from Varian.

**Basement Membrane Preparation**—Basement membrane preparations were carried out under associative conditions essentially as described before (21). For comparable measurements, samples were shadowed together with the method previously used (22) employing a metal-enhanced diaminobenzidine reaction for visualization of anti-immunoglobulin-peroxidase conjugates (28).

**Electrophoresis Techniques**—SDS-PAGE was performed with 1.5-mm thick slab gels of linear gradients of 6–22 or 10–22% polyacrylamide for one and two-dimensional analysis, respectively (23, 29). The amount of hexamer applied on the gels was based on absorbance at 280 nm, considering an absorption coefficient of 2184 g\(^{-1}\) cm\(^{-1}\), in which the mass weight was derived from amino acid analysis of the hexamer from GBM. The value is close to that for the hexamer from Engelbreth-Holm-Swarm tumor (20). Spectrophotometric scanning and Coomassie Blue-stained gels for quantitation of nonreducible dimers was carried out as described previously (30).

In two-dimensional gel electrophoresis, nonequilibrium pH gradient gel electrophoresis as the first dimension was conducted according to O'Farrell et al. (31) with the following modifications. Tube gels of 1.5-mm thickness and 11-cm length consisted of 4% polyacrylamide (5% cross-linker), 2 μm, 25% Noted P-40, 20% glycerol, and 2% ampholine mixture (LKB, equal volumes of pH 5–8 and 7–9). Samples contained 5 μg of hexamer, 1 μm urea, 20% glycerol, 2% ampholines as above, 20 μm β-alanine, and 20 μm 6-aminohecanonic acid, and marker proteins as reference for migration. After application, the samples were covered with overlay solution composed of 10% glycerol and 1% of the above ampholines. Electrophoresis was carried out at 8 °C for 3000 V-h. To check the pH gradient, 5-mm pieces were cut from a tube gel run without protein, incubated in 0.5 ml of degassed distilled water for 12 h, and then measured. Tube gels for the second dimension were incubated for 10-min periods, twice in 50% methanol (twice in water between). When the bands were visible at the top of the second dimension gel, an agarose plug containing molecular weight markers for the second dimension was added at each end. Marker proteins are useful in establishing the pH gradient developed in the first dimension because in nonequilibrium pH gradient gel electrophoresis, proteins, especially alkaline ones, do not reach their final position in this system. Marker proteins when run in the second dimension are helpful in establishing the quality of migration of proteins through the gel and aid in identifying the protein spots originating from the hexamers.

To prepare Western blots, proteins separated by SDS-PAGE or two-dimensional gel electrophoresis were electrophoretically transferred to nitrocellulose papers as described (33).

**RESULTS**

**General Properties of the NC1 Domain (Hexamer) of BM Collagen from Different Tissues**—Analysis of collagenase digests of GBM, PBM, and LBM by SDS-PAGE indicated that the subunit structure of the NC1 domains is very different with respect to relative proportions of subunits and quantity

**Antiserum and Immunochromatographic Techniques**—The antiserum used was either from patients with GP syndrome, verified by immunofluorescence, or from rabbits immunized with the monomeric subunits of type IV collagen from bovine GBM, namely M1, M2*, and M3, raised as described previously (22).

**Competition ELISA** was performed as described previously (19, 21, 22). Coating of antigens was done overnight at 22 °C, either under associative conditions in 0.05 M Tris-HCl, pH 7.5, 0.15 M NaCl, or under dissociative conditions in 6 M guanidine HCl, 0.05 M Tris-HCl, pH 7.5. Samples analyzed under associative conditions were mixed with the antiserum, using the incubation buffer (0.05 M phosphate, pH 7.5, 0.15 M NaCl, 0.05% Tween 20, 0.2% bovine serum albumin) for dilution. Samples analyzed under dissociative conditions were first diluted in 6 M guanidine HCl, 0.05 M Tris-HCl, pH 7.5, followed by incubation for 5 min in boiling water. The samples were then diluted 10 times or more directly with the incubation buffer containing the antiserum. These sample-antibody mixtures were left to stand overnight at 4 °C. The remaining steps were performed as described previously (21).

**Immunoblots** of Western blots with the antiserum were carried out by the method previously used (22) employing a metal-enhanced diaminobenzidine reaction for visualization of anti-immunoglobulin-peroxidase conjugates (28).
Basement Membrane Collagen

Electron microscopic analysis of the globular domain (hexamer) of collagen IV from GBM, LBM, and PBM. Although hexamer from GBM and PBM is spherical in shape, that from LBM shows two distinct particles: spherical and ellipsoid. Note also in the LBM sample, that some of the spherical particles are present as closely associated pairs (double arrowhead). Histograms represent frequency distributions of particle diameters. Average diameters and standard deviations were 14.5 ± 1.6 nm for GBM, 13.8 ± 2.9 nm for LBM, and 14.6 ± 1.7 for PBM, with 140 particles measured in each case. The values are lower than in our previous report (17.5 nm; Ref. 21), which can be ascribed to variability of platinum deposition in separate experiments during rotary shadowing. Bar indicates 200 nm.

Fig. 2. Analysis by SDS-PAGE of the hexamers from GBM, LBM, and PBM.

of GP epitope. This observation poses basic questions about variations in the structural organization of BM-collagen in relation to tissue location and function. Therefore, a more detailed study was undertaken to elucidate the structural differences using purified NCⅠ domains (hexamers) obtained from two mature basement membranes (GBM and LBM), of vascular and of avascular origin, respectively, and from a developing basement membrane-rich tissue, placenta (PBM).

The hexamer from bovine GBM, LBM, and PBM was excised by bacterial collagenase and purified, under non-denaturing conditions, by sequential fractionation on columns of DE52, pH 7.5, DE52, pH 9.0, and Sephacryl S-300 as described for GBM (21). LBM and PBM gave identical results on the Sephacryl S-300 column to that of GBM. Namely, pool I contained 7S collagen, pool II contained hexamer with \( M_r = 160,000 \), and pool III contained polypeptides with the same mobilities, on SDS-PAGE, as those in pool II, indicating that they exist in a species of smaller size than that of the hexamer (data not shown). Pool II from GBM contained about 90% of the total material present in pools II and III as measured by absorbance at 280 nm, whereas for pool II from LBM and PBM this value amounted to 62 and 93%, respectively.

Comparison of the hexamers from LBM and PBM, by inhibition ELISA under non-denaturing conditions, revealed that the GP epitope is sequestered like that found in GBM (21). In each case, only very low levels of GP antibodies bind the GP epitope. Pretreatment of the hexamer with 6 M guanidine HCl at 100 °C causes a 20–40-fold increase in binding of GP antibody (data not shown), while the amount of GP antigen in this pool from the three basement membranes varied in decreasing order of GBM, LBM, and PBM.

Electron micrographs also revealed similarities and distinct differences in size and shape of the hexamers from the three tissues (Fig. 1). The preparations from GBM and PBM appeared homogenous with respect to size and spherical character. However, the sample from LBM differed from both of these aspects. Firstly, the range of particle sizes in the sample from LBM (8–19) was about twice that from GBM and PBM (12–18). The amount of particles in these ranges accounted for 95% of the total number in each population (Fig. 1, histograms). Secondly, two different shapes of particles were observed in the sample from LBM: spherical (diameter range: 8–17 nm) and ellipsoid (range of diameters of the long axis: 16–22). The latter often appeared as closely associated pairs of spherical particles, possibly indicating partial dissociation. The diameter of the spherical particles when measured perpendicular to the long axis ranged from 8 to 10.6 nm. The dissociation phenomenon most likely occurs
Baseline Membrane Collagen

Fig. 3. Identification of subunits of the hexamers from GBM, LBM, and PBM by immunoblotting. The blots were incubated with antibodies to M1, M2*, M3, and with serum from a patient with Goodpasture syndrome, indicated respectively by aM1, aM2*, aM3, and GP. In each of the four blots, hexamer samples from GBM, LBM, and PBM were applied in the same sequence, as indicated by G, L, and P, respectively.

Fig. 4. Separation of monomeric and dimeric subunits of hexamer from GBM, LBM, and PBM by gel filtration HPLC under dissociative conditions. Before application to the column, the samples were kept for 5 min in a boiling water bath in 6 M guanidine HCl, 0.05 M Tris-HCl, pH 7.5. The column (TSK SW 3000) was eluted at 0.5 ml/min with the same solution. The hexamers were completely separated into monomeric (M) and dimeric (D) subunits, as confirmed by SDS-PAGE.

during manipulation in the rotary-shadowing process, because the ellipsoid particles occur throughout pool II. Repeated gel filtration of the LBM hexamer on Sephacryl S-300 and S-200 resulted in a single, symmetrical peak with an elution position corresponding to \(M_r = 160,000\), and inhibition ELISA (see above) demonstrated that the GP epitope was sequestered.

The hexamers from GBM, LBM, and PBM are similar with respect to their banding pattern in the monomer (25-30 kDa) and dimer (43-53 kDa) regions, as shown by SDS-PAGE analyses, but they are dissimilar in their monomer/dimer ratios (Fig. 2). The hexamers from GBM and PBM consist mainly of dimer-size components, whereas, that of LBM consists primarily of monomer-size components. Also, the relative ratio of components in both regions differs among the three tissue sources. Most evident is the difference between components in the dimer-size region. In this region, GBM and PBM contain two intensely staining dimer bands (48.9 and 42.9 kDa) and different amounts of other weakly staining dimers. In LBM the staining of the dimer at 48.9 kDa is more intense than the one at 42.9 kDa, while other weakly staining dimers are also present.

The amino acid composition of the hexamers from GBM, LBM, and PBM is comparable to those reported for monomeric and dimeric subunits (22). Therefore, although there

---

**Table I**

Properties of the noncollagenous domain (hexamer) from different basement membranes

| Basement membrane | Degree of cross-linking | Subunit identity* |
|-------------------|-------------------------|-------------------|
|                   | Total amount in dimer form | Amount of dimer in nonreducible form |
|                   | % | M1 | M2* | M3 |
| GBM               | 64 | 54 | 73  | 16 | 11 |
| LBM               | 15 | 30 | 94  | 3  | 3  |
| PBM               | 80 | 69 | 98  | 1  | 1  |

* Values represent relative amounts of each of the subunit species M1, M2*, and M3 in monomer and dimer form as separated by reverse-phase HPLC of the hexamers as described in Fig. 4. The relative amounts are expressed as percentage of the total amount of protein in pools 1-4, with M1 in pools 1 and 2, M3 in pool 3, and M2* in pool 4.

Values are percentage of protein in dimer peak to total protein in monomer and dimer peak as obtained by gel filtration of hexamers on a TSK SW 3000 column as described in Fig. 4. Protein amounts are based on absorbance at 230 nm. Identical results were obtained when gel filtration was carried out on Sephacryl S-200 in the presence of 6 M guanidine HCl.

Values were calculated from the relative areas presented in Fig. 7.
Basement Membrane Collagen

Fig. 5. Quantitation of hexamer subunits by HPLC analysis. Hexamer samples were acidified to 0.5 percent of trifluoroacetic acid, and applied to a C18 column equilibrated with 0.1% trifluoroacetic acid, 30% acetonitrile. Samples were eluted with a gradient from starting conditions to 39% acetonitrile, 0.1% trifluoroacetic acid, over 30 min at 2.0 ml/min. The sample from LBM contained 0.74 mg of protein, that from GBM 1.61 mg protein. The top inset in A and B represents SDS-PAGE analysis of pools 1-4 stained with silver, and the bottom inset represents Western blot analysis of the corresponding pools reacted with serum of a Goodpasture patient. Similar blots were also reacted with anti-M1-, anti-M2*-, and anti-M3-antibodies to further confirm the identity of the subunits species (not shown). Based on these analyses, M1 elutes in pool 1, D1 in pool 2, M3 and D3 in pool 3, and M2* and D2* in pool 4. Relative amounts of M1 (pools 1 and 2), M2*, and M3 were calculated from the relative areas under the peaks corresponding to the respective pools.

are major differences in the relative amounts of monomer and dimer species which comprise these globules (results are presented below), there are no striking differences in their amino acid composition.

Identification of Subunits—Immunoblotting after SDS-PAGE was used to determine whether the subunits M1, which comprises the α1(IV)NC1 and α2(IV)NC1 domain (23), M2* (GP antigen), M3, and their corresponding dimers D1, D2*, and D3 are constituents of the hexamer from LBM and PBM as was described previously for that from GBM. The results are summarized in Fig. 3, which shows blots of the three hexamers immunostained with antibodies to M1, M2*, and M3 and with GP serum.

In each hexamer, antibodies to subunits M1, M2*, and M3 reveal the presence of all three subunit species in both monomer and dimer forms. With anti-M1 antibodies, the amount of reactivity in the dimer region increases and at the same time in the monomer region decreases in the sequence LBM, GBM, and PBM (Fig. 3). Anti-M2* and anti-M3 antibodies react in the monomer and dimer regions of the GBM and LBM hexamers, with the highest staining in the dimer region in the case of GBM, and in the monomer region in the case of LBM. Anti-M3 antibodies, which had not been characterized previously, permitted the identification of D3. D3 consists of a set of polypeptides with mobilities distinct from D1; however, it was not determined whether D3 polypeptides are distinct from D2*. Reactivity of the hexamer from PBM was weak with anti-M2* and anti-M3 antibodies. For controls, purified monomers M1, M2*, and M3 were run in separate lanes; immunoblots further substantiated the identity of the subunits of the three hexamers and the absence of cross-reactivity of the three antibodies (not shown).

The presence of the GP antigen in the hexamers of LBM and PBM, analogous to GBM, was revealed by Western blotting using patients' sera (Fig. 3). The staining patterns show that for each hexamer the GP epitope is contained in subunit M2*, which is present in both monomer and dimer forms. It is particularly noteworthy that the staining intensity with GP sera is analogous to that of anti-M2*, reflecting a higher concentration of the M2* chain in GBM than in LBM or PBM.

These results indicate that the hexamers from GBM, LBM, and PBM are composed of identical monomer and dimer constituents, although they greatly differ in the amounts of monomers relative to dimers for each of the three monomer-dimer pairs, and in the absolute amounts of M2*, D2*, M3, and D3 subunits. The basis for these distinct differences was further explored by quantitative analysis.

Quantitation of Subunits—To determine the relative amounts of monomers and dimers in the three hexamers, samples were heated to 90-95 °C for 10 min in the presence of 6 M guanidine HCl to obtain complete dissociation, followed by gel filtration, which separates monomers from dimers (Fig. 4). The results indicate that the percentage of subunits in dimer form varies over a 5-fold range among the three hexamers in the increasing order of LBM, GBM, and PBM (Table I).

The relative amounts of subunits M1, M2*, and M3, present in both their monomer and dimer forms, were determined in each of the three hexamer preparations on a chemical basis by reverse-phase HPLC. As shown in Fig. 5, these species resolve in the following sequence: M1 in pool 1 and pool 2, M3 in pool 3, and M2* in pool 4. The relative amount of each subunit in the three tissues was calculated from the relative
areas from the elution profile, and the data are presented in Table I. The major subunit is M1 for each hexamer, while the total amounts of M2* and M3 vary over a 20-fold range among the three hexamers in the decreasing order of GBM, LBM, and PBM. Similar results were obtained using rabbit antibodies specific for the various subunits and GP serum in competitive ELISA (Fig. 6).

The relative amounts of dimers present in nonreducible and reducible forms were also determined for GBM, LBM, and PBM. Previous studies have shown that dimers are held together by both disulfide and nondisulfide cross-links (20, 22). The purified dimers from each of these tissues were reduced and analyzed by SDS-PAGE. The amount of nonreducible dimer was calculated from the relative areas of the profile (Fig. 7), and the data are presented in Table I. As noted the amount of nonreducible dimer varies over a 2-fold range with the highest amount in PBM and the lowest in LBM.

Multiple Charge Forms of Subunits—The hexamer from each of the three basement membranes displays a complex pattern on analysis by nonequilibrium pH gradient gel electrophoresis and SDS-PAGE in a two-dimensional gel system (Fig. 7). Multiple spots exist in both the monomer and dimer regions. There is a striking commonality among the three patterns, as depicted in Fig. 1D. In the monomer region (Mr = 28,000) there are six major and five minor spots in common, and in the dimer region there are at least eight major ones, distributed about the 48.9- and 42.9-kDa positions.

The identity of the various spots in the monomer region was determined by two-dimensional gel analysis of purified subunits. This result is also depicted in Fig. 8D. The three main spots at pH 7-9 correspond to a1(IV)NC1 monomer, and the three at pH 6-7 correspond to a2(IV)NC1 monomer. In addition, M2* and M3 occur at least as two and three spots, respectively. These observations were further confirmed with immunoblots (not shown) of two-dimensional gels of whole hexamers and the isolated monomers as well, using GP serum and specific antisera against M1, M2*, and M3, prepared as described previously (22). The multiple spots for each monomer are designated as charge isoforms, because the unresolved forms of each subunit yield a single amino terminus (23). Furthermore, this designation is substantiated by the finding that up to 5% bacterial collagenase (enzyme/substrate ratio) for 24 or 48 h did not alter the two dimensional profile, which rules out incomplete digestion as a basis for multiple forms. Of particular note, subunits a1(IV)NC1 and a2(IV)NC1, which comprise M1, M2*, and M3, prepared as described previously (22). The multiple spots for each monomer are designated as charge isoforms, because the unresolved forms of each subunit yield a single amino terminus (23). Furthermore, this designation is substantiated by the finding that up to 5% bacterial collagenase (enzyme/substrate ratio) for 24 or 48 h did not alter the two dimensional profile, which rules out incomplete digestion as a basis for multiple forms. Of particular note, subunits a1(IV)NC1 and a2(IV)NC1, which comprise M1, are resolved by the two-dimensional system.

Several distinct differences exist in the monomer region of the two-dimensional patterns of the three hexamers (Fig. 8, A–C). The most alkaline of the three isoforms of the a1(IV)NC1 monomer is the most prominent one in GBM, whereas the most acidic one is prominent in PBM, and an intermediate distribution occurs in LBM. The relative intensity of the a2(IV)NC1 isoforms is similar for both GBM and LBM, but PBM is richer in the more acidic one. The a1(IV)NC1 and a2(IV)NC1 monomers are the predominant ones for each of the three hexamers. In comparison to these,
FIG. 8. Two-dimensional gel electrophoresis of hexamers from GBM (A), LBM (B), and PBM (C). D schematically represents the relative positions of the major spots (closed circles) common for each of the three hexamers and of minor spots of M2* and M3 (open circles) that are common to GBM and LBM. Identification of the spots depicted in D was carried out by two-dimensional electrophoresis of monomers of α1(IV)NC1, α2(IV)NC1, M2*, and M3, isolated from LBM as indicated under "Experimental Procedures." The pH gradient of each gel is given at the bottom. At the right, the positions of molecular mass markers are indicated in kilodaltons, and at the left the region of migration of monomers and dimers is indicated at M and D, respectively. Gels were stained with silver.

the concentrations of M2* and M3 are largest in GBM and decrease in the order GBM, LBM, and PBM. In PBM these latter constituents are barely visible (Fig. 8C). These results confirm those presented above regarding the relative abundance of monomers.

Several distinct differences also exist in the dimers at the region of pH 7-9. The dimers of GBM and PBM show similar intensities at the 48.9- and 42.9-kDa positions, but with LBM the ones at 48.9 kDa are more prominent. The most alkaline dimers are more enriched in GBM than in PBM.

In summary, each of the four monomer subunits exists in charge isoforms, and the relative abundance of isoforms for the respective monomer subunits varies among the membranes. Subunits α1(IV)NC1, α2(IV)NC1, and M3 exist in at least three isoforms, and subunit M2* exists in at least two. This large diversity of monomer forms accounts for the multiplicity of cross-linked dimers that are observed with the two-dimensional analysis.

DISCUSSION

The present study reveals similarities and distinct differences in the structural features of the noncollagenous domain of BM collagen from three different basement membranes of bovine origin (GBM, LBM, and PBM). In each case, after excision by bacterial collagenase, the domain exists in the form of a hexamer under nondenaturing conditions, and it is comprised of four distinct subunits, each of which exists in both monomeric and dimeric forms. The hexamers from the three membranes differ with respect to the stoichiometry of subunits and subunit isoforms, the degree of cross-linking of monomers into dimers, and the relative proportion of nonreducible and reducible cross-links.

Specifically, the hexamers differ in the following ways: 1) the minor subunits, M2* and M3, vary in quantity over a 20-fold range relative to the major ones, α1(IV)NC1 and α2(IV)NC1, in the decreasing order of GBM, LBM, and PBM; 2) the distribution of charge isoforms of α1(IV)NC1 and α2(IV)NC1 varies among the membranes with PBM containing the greatest amount of acidic ones, whereas GBM is enriched in the alkaline form of α1(IV)NC1; and 3) the percentage of subunits in dimer form, reflecting the degree of interchain cross-linking, varies over a 5-fold range in the decreasing order of PBM, GBM, and LBM.

The stoichiometry of hexamer subunits together with our recent identification of their collagen-chain origins (23) lead to the conclusions that at least two different populations of triple-helical collagen molecules, differing in chain composition, exist in each membrane and that their relative proportions are tissue-specific. The predominant subunits, α1(IV)NC1 and α2(IV)NC1, are derived from the α1 and α2 chains of the classical collagen IV molecule, which appears to have a chain composition of (α1)2 α2 (34-37), denoted herein as population A. Subunits M2* and M3, which occur in minor amounts, are derived from two novel chains, α3 and α4, respectively (23), which could exclusively comprise a separate triple-helical molecule(s), denoted as population(s) B. Alternatively, the α3 and α4 chains could substitute for either the α1 or α2 chain in the collagen IV triple-helical molecule, denoted as population(s) C. The theoretical proportions of these populations, computed from the stoichiometric data (Table I), are presented in Table II. These computations show that the relative proportions of populations are tissue-specific.

A novel structural feature of the noncollagenous domain was also evinced from these studies. Namely, that each of the four monomeric subunits exists in charge isoforms and that...
the relative proportions of isoforms are tissue-specific. Monomers α1(IV)NC1, α2(IV)NC1, and M3 exist in at least three isoforms, and M2* exists in at least two. The presence of four distinct monomers and their respective isoforms accounts for the multiplicity of disulfide-cross-linked dimers that are observed with the two-dimensional analyses. The identification of isoforms also provides an explanation for the complex two-dimensional gel patterns observed by others (38, 39). The different isoforms presumably reflect amino acid substitutions or posttranslational modifications, a feature which may be an important structural determinant for the linear and lateral assembly of collagen molecules in the formation of the matrix network.

It is especially noteworthy that the amount of both intermolecular disulfide and nonreducible cross-links is very low in the LBM hexamer in contrast to that of GBM, PBM, and the hexamer from mouse Engelbreth-Holm-Swarm tumor (20). This property may account for the presence of the ellipsoid-shaped particle in the LBM hexamer (Fig. 1) in which the absence of such cross-links would destabilize the hexamer under the conditions used for electron microscopy. The low level of cross-linking in the domain, however, indicates that such bonding is not essential for stabilization of the hexamer from mouse Engelbreth-Holm-Swarm tumor (20). This property may account for the presence of the ellipsoid-shaped particle in the LBM hexamer (Fig. 1) in which the absence of such cross-links would destabilize the hexamer under the conditions used for electron microscopy.

The present study provides the first direct evidence of tissue specificity in the chemical nature of basement membrane collagen. This specificity provides an explanation for differences in staining among basement membranes of different tissues using either GP- serum or antibodies to specific regions of collagen IV (40–44). Conceivably, certain of these structural differences may be of importance in conferring a specific function to a membrane.

Acknowledgments—The skillful technical assistance of Parvin Todd, Anjana De, and Cecilia Johanssen and the typing assistance of Denise Byrd are greatly appreciated. We also recognize Dr. Juan Saus for his participation in detecting the dimer of M3.

REFERENCES
1. Farquhar, M. G., Courtoy, P. J., Lemkin, M. C., and Kanwar, Y. S. (1982) in New Trends in Basement Membrane Research (Kuehn, K., Schoene, H., and Timpl, R., eds) pp. 9–29, Raven Press, New York
2. Hay, E. D. (1984) in The Role of Extracellular Matrix in Development (Trestrail, R. L., ed) pp. 1–32, Alan R. Liss, New York
3. Bernfield, M., Banerjee, S. D., Koda, J. E., and Rapraeger, A. C. (1984) in The Role of Extracellular Matrix in Development (Trestrail, R. L., ed) pp. 545–572, Alan R. Liss, New York
4. Kefalides, N. A., Howard, P., and Ohno, N. (1985) in Basement Membranes (Shibata, S., ed) pp. 73–87, Elsevier Scientific Publishing Company, Amsterdam
5. Mohan, P. S., and Spiro, R. G. (1986) in J. Biol. Chem. 261, 4328–4336
6. Cooper, A. R., Taylor, A., and Hogan, B. L. M. (1983) Dev. Biol. 99, 510–516
7. Cooper, A. R., and MacQueen, H. A. (1983) Dev. Biol. 96, 467–471
8. Ohno, M., Martinez-Hernandez, A., Ohno, N., and Kefalides, N. served a role in the formation of basement membranes (1983) Biochem. Biophys. Res. Commun. 112, 1081–1088
9. Wan, Y.-J., Wu, T.-C., Chung, A. E., and Damjanov, I. (1984) J. Cell Biol. 98, 971–979
10. Kanwar, Y. S., Vea, A., Kimura, J. H., and Jakubowski, M. L. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 762–766
11. Hynes, R. O. (1986) Sci. Am. 254, 42–51
12. Charonis, A. S., Tsilibary, E. C., Yurchenco, P. D., and Furchmayr, H. (1986) J. Cell Biol. 100, 1848–1853
13. Laurie, G. W., Bing, J. T., Kleinman, H. K., Hassel, J. R., Annable, M., Martin, G. R., and Feldmann, H. J. (1986) J. Biol. Chem. 261, 99–104
14. Timpl, R., Wiedemann, H., Van Delden, V., Furchmayr, H., and Kühn, K. (1981) Eur. J. Biochem. 120, 203–214
15. Bächinger, H. P., Fessler, L. I., and Fessler, J. H. (1982) J. Biol. Chem. 257, 9796–9805
16. Yurchenco, P. D., and Furchmayr, H. (1984) Biochemistry 23, 1839–1850
17. Fessler, L. I., and Fessler, J. H. (1982) J. Biol. Chem. 257, 9804–9810
18. Tsilibary, E. C., and Charonis, A. S. (1986) J. Cell. Biol. 105, 2467–2473
19. Wieslander, J., Barr, J. F., Butkowski, R. J., Edwards, S. J., Bygren, P., Heiniegärd, D., and Hudson, B. G. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3838–3842
20. Weber, S., Engel, Jr., Wiedemann, H., Glanville, R. W., and Timpl, R. (1984) Eur. J. Biochem. 139, 401–410
21. Wieslander, J., Langeveld, J., Butkowski, R., Jodlowski, M., Noelken, M., and Hudson, B. G. (1985) J. Biol. Chem. 260, 8564–8570
22. Butkowski, R. J., Wieslander, J., Wisdom, B. J., Barr, J. F., Noelken, M. E., and Hudson, B. G. (1985) J. Biol. Chem. 260, 3739–3747
23. Butkowski, R. J., Langeveld, J. P. M., Wieslander, J., Hamilton, J., and Hudson, B. G. (1987) J. Biol. Chem. 262, 7874–7877
24. Wieslander, J., and Heiniegärd, D. (1985) Ann. N. Y. Acad. Sci. 460, 363–374
25. Freytag, J. W., Ohno, M., and Hudson, B. G. (1976) Biochem. Biophys. Res. Commun. 72, 796–802
26. Pezzon, B. D., McCarthy, C. A., and Merritt, R. B. (1982) Exp. Eye Res. 35, 645–651
27. Shotton, D. M., Burke, B. E., and Branton, D. (1979) J. Mol. Biol. 131, 393–399
28. DeBlas, A., and Cherwinski, H. M. (1983) Anal. Biochem. 133, 214–219
29. Laemmli, U. K. (1970) Nature 227, 680–688
30. Hung, C.-H., Ohno, M., Freytag, J. W., and Hudson, B. G. (1977) J. Biol. Chem. 252, 3996–4001
31. O'Farrell, P. Z., Goodman, H. M., and O'Farrell, P. H. (1977) Cell 12, 1133–1142
32. O'Farrell, P. H., and O'Farrell, P. Z. (1977) Methods Cell Biol. 16, 407–420
33. Burnette, N. (1981) Anal. Chem. 112, 195–203
34. Mayne, R., and Zettergren, J. G. (1980) Biochemistry 19, 4065–4072
35. Mayne, R., Wiedemann, H., Dessau, W., von der Mark, K., and Bruenker, P. (1982) Eur. J. Biochem. 126, 417–423
36. Trubet, G., Grobel, H., Spies, M., Odermatt, B. F., and Winterhalter, K. H. (1982) J. Biol. Chem. 257, 5239–5245
37. Qian, R., and Glanville, R. W. (1984) Biochem. J. 222, 447–452
38. Kleppel, M. M., Michael, A. F., and Fish, A. J. (1986) J. Biol. Chem. 261, 16547–16552
39. Yoshikawa, K., Kleppel, M., and Fish, A. J. (1985) J. Immunol. 134, 3831–3837
40. Fish, A. J., Carmony, K. M., and Michael, A. F. (1979) J. Lab. Clin. Med. 94, 447–457
41. Scheinman, J. T., Foldart, T. J., and Michael, A. F. (1980) Lab. Invest. 43, 373–381
42. Risteli, J., Wick, G., and Timpl, R. (1981) Collagen Relat. Res. 5, 419–432
43. Fitch, J. M., Mayne, R., and Linsenmayer, T. F. (1983) J. Cell. Biol. 97, 940–943
44. Odermatt, B. F., Lang, A. B., Rüttner, J. R., Winterhalter, K. H., and Trubet, B. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7342–7347