Glycoprotein 115, a Glycoprotein Isolated from Chick Blood Vessels, Is Widely Distributed in Connective Tissue

ALFONSO COLOMBATTI,* GIORGIO M. BRESSAN, INES CASTELLANI, and DINO VOLPIN
Institutes of Histology and Pathological Anatomy,* University of Padova, Padova, Italy. Dr. Colombatti's present address is Oncology Reference Center, Aviano, Italy.

ABSTRACT   An extracellular glycoprotein (gp 115) with an apparent $M_r = 115,000$ isolated from chick aortas (Bressan, G. M., I. Castellani, A. Colombatti, and D. Volpin, 1983, *J. Biol. Chem.*, 258:13262–13267), was used to immunize mice. The antisera were shown to specifically recognize gp 115 by numerous criteria: a major band around $M_r = 115,000$ plus minor bands of lower M_r were visible by immunoblotting on aorta extracts, and a similar pattern was observed with a monoclonal antibody; no cross-reactivity was detected by radioimmunobinding with other extracellular proteins, namely, fibronectin, laminin, and collagen types I, III, IV, V, and VI. Antigen distribution on frozen tissue sections from newborn chicks was investigated by using affinity-purified antibody. Strong immunoreactivity was always found in blood vessels. In the digestive tract, the fluorescent staining was localized both at the level of muscular layers and in the stromal matrix of the villi. Within skeletal muscle and myocardium, staining was associated with large connective tissue bundles and the matrix around each muscle fiber. Intense fluorescence was observed in the kidney, in smooth muscle cells rich areas of parabronchi, and within the portal space and along liver sinusoids. The antigen was not detected at the epidermal–dermal junction; immunoreactivity in the dermis was present as a diffuse fibrillar pattern. That the antigen detected by immunofluorescence in the various organs was indeed gp 115 was demonstrated by immunoblotting analysis: as in aorta extracts, a major band around $M_r = 115,000$ was detected in several tissues. Antibody-reacting material was also incorporated into the extracellular matrix produced by embryo smooth muscle cells grown in vitro and was organized as a meshwork of fine fibrils.

There is increasing evidence suggesting that the extracellular matrix plays an important role in establishing and organizing tissue structure (1, 2). This is likely to be the result of multiple interactions between cells and a specific set of extracellular components in the different anatomical districts. The knowledge of tissue distribution of each novel molecular species is the first step toward the understanding of its possible function. Thus, a characteristic pattern of distribution has been detected in the various organs for several components of the connective tissue such as the different collagen types (3, 4), fibronectin (5, 6), laminin (7), entactin (8), vitronectin (9), and proteoglycans (10, 11). In addition, the composition of the matrix is closely dependent on the cell type and the degree of its differentiation (2).

While studying the proteins of chick blood vessels, we have identified a noncollagenous glycoprotein of about $M_r = 115,000$ (gp 115), which was unrelated by biochemical and immunological criteria to the major known extracellular glycoproteins, fibronectin and laminin (12).

The investigation of the distribution of gp 115 in tissues from newborn chicks by using immunofluorescence histochemistry is reported here. We have found that the protein is widely, although not ubiquitously, distributed in the connective tissue. Moreover, formal evidence for the extracellular localization of gp 115 was obtained by investigating its appearance in cultures of embryonal smooth muscle cells.

MATERIALS AND METHODS

Isolation of the Immunogen and Preparation of an Antiserum: Aortas from 2-d-old chicks were sequentially extracted with phosphate-buffered saline (PBS), 6 M guanidine HCl, and 6 M guanidine HCl plus dithioerythritol (DTE).[1] Proteins solubilized by the last procedure were sepa-

[1] *Abbreviations used in this paper:* DTE, dithioerythritol; gp, glycoprotein.

rated by DEAE-cellulose chromatography as previously described (12). The fractions containing gp 115 were pooled and further fractionated by gel filtration in a Bio-Gel A 5-m column (85 × 1.6 cm, Bio-Rad Laboratories, Richmond, CA) in 10 mM Na phosphate buffer, pH 7.2, 1% sodium dodecyl sulphate (SDS), and 10 mM sodium azide (13).

Mouse anti-chick gp 115 antiserum was raised by immunizing subcutaneously C57BL/6 and SJL/J-(v+) mice with purified gp 115 (40 μg/mouse) in complete Freund's adjuvant, followed by an intraperitoneal boost of 40 μg/ mouse in PBS 15 d later. A very strong immune response was elicited, as indicated by solid-phase radioimmunobinding using purified gp 115 (data not shown).

Affinity Purification of Glycoprotein 115 Antiserum: Partially purified gp 115 (peak fraction from the DEAE-cellulose column) was separated by SDS PAGE. Two lateral strips were briefly stained and destained to localize the protein, a sharp central portion of the gp 115 band was excised from the gel, and the protein was transferred onto nitrocellulose paper (14). The filter was then saturated with 2% bovine serum albumin (BSA) in PBS and used to affinity purify gp 115 antibodies (15). Serum (10 μl) diluted 1:50 was absorbed for 2 h at 4°C, and the paper was then washed extensively with PBS. Bound antibody was eluted with 50 mM acetic acid and immediately neutralized. A protein-free polyacrylamide gel sample was similarly blotted onto nitrocellulose paper which was used as negative control for unspecific absorption of antibody.

Monoclonal Antibodies: Mice were immunized subcutaneously with gp 115 and spleen cells fused with BALB/c myeloma (P3-NS1-Ag4-1) cells as reported in a separate paper. Tissue culture supernatants from hybridoma clones were used for immunoblotting and immunofluorescence staining.

Indirect Immunofluorescence on Cryostat Sections: Tissues were removed from 2-7-d-old chicks and 5-8-μm sections were cut on a cryostat at −20°C. The sections were treated for 2 h with a 1:5 or 1:10 dilution of affinity-purified gp 115 antibodies. Controls included sections incubated with (a) preimmune serum diluted 1:100 in PBS, (b) the immune serum eluate that was absorbed to the filter electroblotted with the protein-free polyacrylamide gel sample, and (c) affinity-purified antibodies preabsorbed onto nitrocellulose filter-immobilized gp 115. Sections from all tissues treated with the above reagents were negative. Positive controls for the specificity of staining included monoclonal antibodies against gp 115 (Colombatti, A., G. M. Bressan, D. Volpin, and I. Castellani, manuscript submitted). The sections were washed with PBS and distilled water and dried. They were then incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG for 45 min, and excess antibody was removed by extensive washing with PBS and distilled water. The sections were mounted in 50% glycerol in PBS. All slides were examined with epifluorescence optics (E. Leitz, Inc., Rockleigh, NJ) and photographed with Kodak Tri-X pan films (Eastman Kodak Co., Rochester, NY).

Cell Culture: Aorta cells were isolated from 16-d-old chick embryos using a procedure similar to that described by Chamley-Campbell et al. (16) for pig and monkey aorta. Briefly, aorta and associated blood vessels were cleansed of the surrounding loose connective tissue, cut into ~1-mm pieces, and digested with 3 mg/ml of collagenase type I (Sigma Chemical Co., St. Louis, MO) in Dulbecco's modified Eagle's medium at 37°C with occasional shaking. After 1 h, the medium was discarded, fresh collagenase solution was added, and the digestion was continued for 2-3 h. At the end of this period the suspension was filtered through a double layer of cheesecloth and centrifuged at low speed, and the cells were resuspended in Dulbecco's modified Eagle medium containing 10% horse serum. The cells were then plated into 3.5-cm Petri dishes containing a gelatin-coated cover slip (1-5 × 10⁵ cells/dish). The medium was changed after 24 h. Some plates were fed ascorbic acid (50 μg/ml) every 2 d. Indirect immunofluorescence of cells grown on coverslips was carried out as for tissue sections with the only modification that cell layers were washed in PBS and fixed in picric acid formaldehyde (17) for 10 min before incubation with antibody. To gain access to the intracellular compartment, the cell membranes in some cultures were permeabilized by a 10-min treatment with 0.5% Triton X-100 in PBS and then the coverslips were developed for immunofluorescence.

Preparation of Tissue Extracts and Purification of Other Connective Tissue Proteins: For immunoblotting analysis, tissues were homogenized in PBS containing 25 mM EDTA, 2 mM phenylmethylsulphonyl fluoride, 5 mM N-ethylmaleimide, and 1 mM p-aminobenzamidine HCl and centrifuged at 100,000 g for 1 h, and the supernatant (PBS extract) was saved. The pellet obtained after centrifugation was resuspended in SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.1% bromophenol blue) and boiled for 5 min, and the cleared supernatant (SDS + 2-mercaptoethanol extract) was used in immunoblotting experiments. Fibronectin, laminin, and various collagen types were purified according to several procedures quoted in (12). Collagenous glycoproteins of apparent M_r = 205,000, 195,000, 150,000, and 135,000, which were

identified in SDS gels after DEAE-cellulose chromatography of chick aorta 6 M guanidine HCl + DTE extracts (12), were further purified by gel filtration in Bio-Gel A 5m in the presence of 1% SDS. For the studies on the solubility of gp 115, the procedure for extracting the molecule from various tissues was as described for aorta (see above).

SDS PAGE and Immunoblotting: One-dimensional SDS PAGE was performed according to the discontinuous method of Laemmli (18). The procedures for performing transfer of proteins from gels and incubation of the nitrocellulose blots were essentially the same as described by Towbin et al. (14). The proteins were transferred in the cold and the blots were saturated in 2% BSA. Blots were then incubated at room temperature for 2 h with gentle shaking with a 1:100 dilution of mouse preimmune or immune serum, or affinity-purified antibodies followed by ^{125}I-labeled affinity-purified rabbit anti-mouse IgG. The dried blots were exposed to AR X-Omat Kodak films (Eastman Kodak Co.) with intensifying screen.

Solid-phase Radioimmunobinding: An indirect assay using affinity-purified, ^{125}I-labeled rabbit IgG anti-mouse Ig (10-15 μCi/μg) as a second reagent was utilized to measure antibody titer and the antigen present in tissue extracts (19). Glycoprotein 115 eluted from the Bio-Gel A 5m column was used; in some experiments partially purified gp 115 (peak fraction from the DEAE-cellulose column) was also used.

RESULTS

Preparation of an Immune Serum Specific for Glycoprotein 115

Glycoprotein 115 was solubilized with 6 M guanidine plus DTE from residues left after extraction of aortas with saline and 6 M guanidine HCl and it was further fractionated by DEAE-cellulose chromatography as described (12). The gp 115-containing peak from the ion-exchange column was dialyzed, lyophilized, dissolved in 10 mM Na phosphate, pH 7.2 containing 1% SDS, and separated from high and low molecular weight contaminants through a column of Bio-Gel A 5m. At each purification step pooled fractions were analyzed by SDS polyacrylamide gel electrophoresis (Fig. 1). The fraction used to raise antiserum is shown in Fig. 1A, lane c. As previously reported, the serum displayed no cross-reactivity with human or chick fibronectin nor with mouse laminin purified from EHS sarcoma (12). We further tested, by solid-phase assay, the binding of the antiserum to purified chick collagen types I, III, IV, V, and VI and to four collagenous glycoproteins that, as gp 115, were also present in the 6 M guanidine HCl + DTE extract from chick aortas (12). These four proteins are presently under investigation. They were included in the assay because they could have been contaminants in the preparations of gp 115 used as immunogen. No reactivity was detected with any of the above proteins (Table I).

Given the heavy loading of the Coomassie Blue-stained gel (Fig. 1A, lane c) a few fainter bands were also detectable. These molecules, probably present in low amount in the 6 M guanidine HCl + DTE extract, were not detected by immunoblotting with immune serum (data not shown). Nevertheless, they could be main components in the tissue. To exclude the possibility that the immunohistochemical pattern could be affected by antibodies directed against these contaminants, specific antibodies were affinity purified on electrophoretically pure gp 115 immobilized onto nitrocellulose filters (see Materials and Methods). With these antibodies a major band with the mobility of M_r = 115,000 was identified in immunoblots of 6 M guanidine HCl + DTE (12; and Fig. 1B, lane b) or SDS aorta extracts (see Fig. 8, lane b). In both types of extract additional faint bands of lower M_r were also recognized. We interpreted these bands as degradation products of gp 115 in that a similar pattern of reactivity was observed
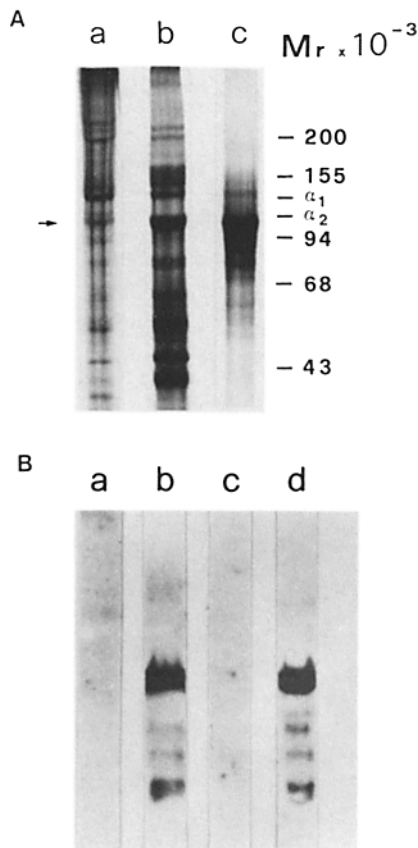
Figure 1   Purification of chick gp 115 and specificity of the antibody. (A) Purification was monitored by SDS PAGE (6%). Samples (100 μg/lane) were reduced before analysis. Lane *a*, 6 M guanidine HCl + DTE aorta extract; lane *b*, gp 115 containing peak from the DEAE-cellulose column; lane *c*, gp 115 containing peak from the Bio-Gel column. Numbers on the right correspond to the mobility of the following molecular weight standards: myosin (200,000). β-subunit of RNA polymerase from *Escherichia coli* (155,000), phosphorilase b (93,000), BSA (68,000), ovalbumin (43,000). α_1 and α_2 indicate the mobility of the α_1- and α_2-chains of acid-soluble bovine skin type I collagen. The arrow on the left indicates the migration of gp 115. (B) Immunoblotting on 6 M guanidine HCl + DTE aorta extract. Aliquots of aorta extract were separated by SDS PAGE (6%) under reducing conditions and transferred onto nitrocellulose filters. The strips were developed with a 1:100 dilution of preimmune serum (*a*), a 1:250 (final dilution) of affinity-purified antibody (*b*), undiluted supernatants containing an unrelated monoclonal antibody (*c*) or the gp 115 monoclonal antibody 142D12 (*d*), and ^{125}I-labeled affinity-purified rabbit IgG anti-mouse Ig. Each strip was incubated with 8.7 × 10⁵ cpm of ^{125}I-labeled IgG.

when a gp 115 monoclonal antibody was reacted with a 6 M guanidine HCl + DTE aorta extract transferred to nitrocellulose filters (Fig. 1 *B*, lane *d*).

Tissue Distribution

Representative immunofluorescence micrographs are shown in Figs. 2–6. Blood vessels displayed strong fluorescence in every tissue examined. In aorta sections, staining was prevalent in the media, where it appeared as concentric rings. These regions correspond to the circular rows of smooth muscle cells. The intima and adventitia were weakly marked and staining was diffuse (Fig. 2).

The digestive tract was extensively stained. In the gizzard,

intense fluorescence was localized in both the basement membrane of smooth muscle cells and the fibrous connective tissue bands running between large muscle bundles (Fig. 3 *a*). A similar pattern of reactivity was found in the small intestine: a pericellular fluorescence was associated with smooth muscle cells of muscolaris mucosae and tonaca muscolaris and was also present as a fibrillar network in the stromal matrix (Fig. 3, *b* and *c*).

In the skeletal muscle immunoreactivity was very strong in the endomysium, perimysium, and epimysium (Fig. 4 *a*). The fluorescence in the cardiac ventricle was localized around muscle fibers, in the connective tissue and, with a stronger reaction, in the walls of large blood vessels (Fig. 4 *b*). Smaller vessels and capillaries were also clearly outlined in both types of muscle.

TABLE I

Absence of Cross-reactivity between Chick Glycoprotein 115 and Extracellular Proteins*

	Preimmune serum	Immune serum
	cpm bound ($\times 10^{-2}$)	
Mouse laminin	12	16
Human plasma fibronectin	15	11
Chick collagen type I	13	14
" " " III	11	13
" " " IV	12	9
" " " V	9	8
" " " VI	14	14
Chick aorta gp 205,000 and 195,000[‡]	17	13
" " gp 150,000 and 135,000[‡]	16	12
" " 6 M guanidine HCl + DTE extract	13	282

* Antibody binding was measured using the solid-phase radioimmunobinding assay with a 1:800 dilution of preimmune or immune serum and 40 μl of ^{125}I-labeled affinity-purified rabbit IgG anti-mouse Ig (0.37 μg/ml, 15 μCi/μg) and different amounts of absorbed proteins. The values reported were those obtained with 2.5 μg of purified proteins and 1 μg of chick aorta 6 M guanidine HCl + DTE extract.

[‡] A preliminary description of these collagenous glycoproteins has been reported (12). The preparations used in the present experiment contained equivalent amounts of the two proteins.
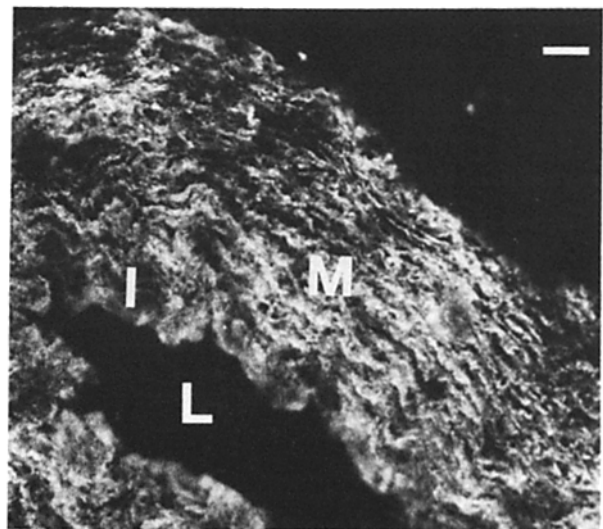

Figure 2   Antigen localization by indirect immunofluorescence on 2-d-old chick aorta section. Strong staining is prevalent in the media (M); a fainter staining is also present in the intima (*I*). *L*, lumen. Bar, 50 μm. × 120.
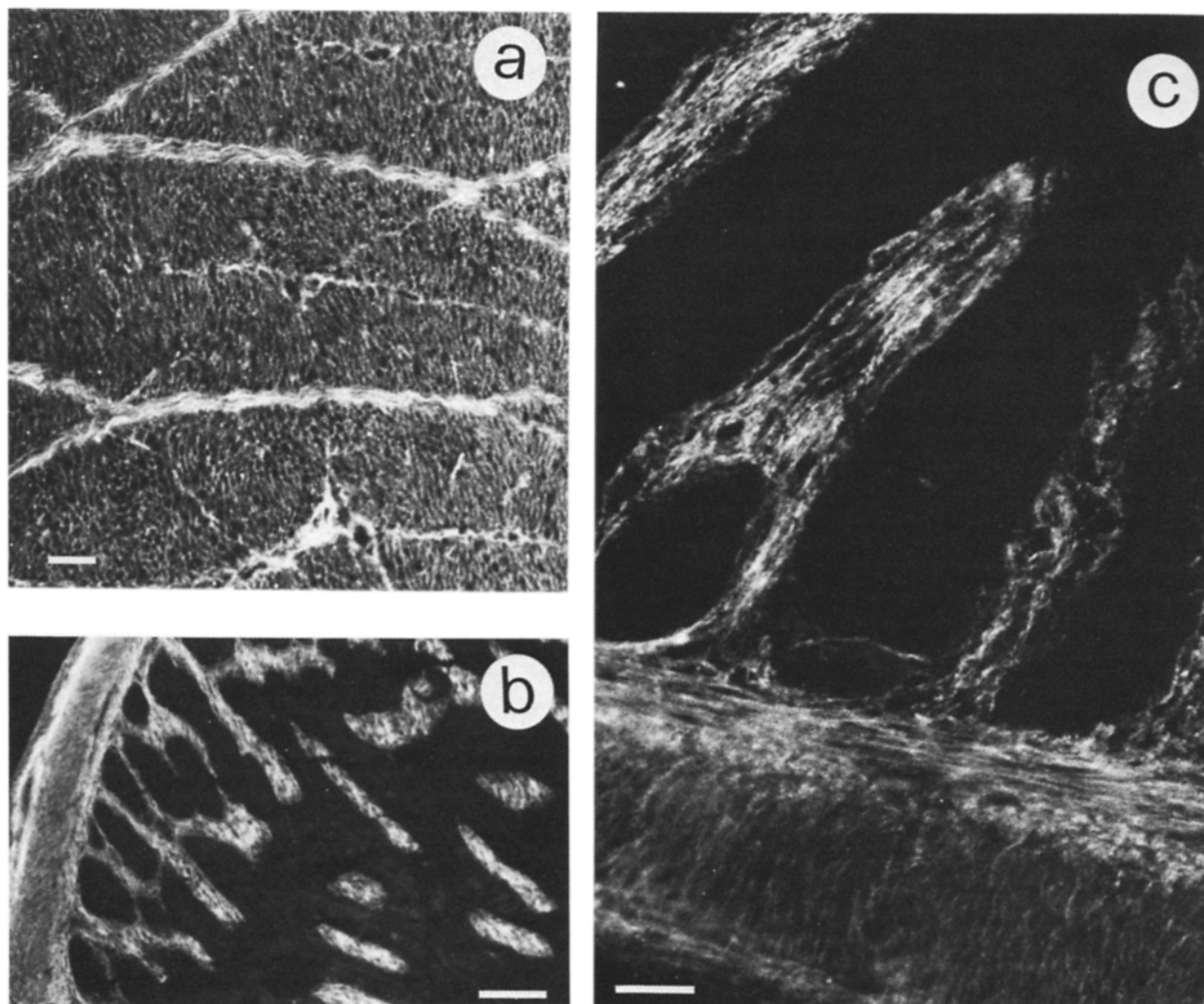
FIGURE 3 Antigen localization by indirect immunofluorescence on 2-d-old chick sections. (a) Gizzard; staining is localized around smooth muscle cells; note that also the septa of connective tissue located between smooth muscle cell bundles are stained. (b) Small intestine; the fluorescence is associated with the smooth muscle cell layers (muscolaris mucosae and tonaca muscolaris). At higher magnification (c) note the fibrillar pattern in the connective tissue core of the villi and the absence of fluorescence in the epithelial basement membrane. Bar (a) 50 μm; (b) 100 μm; (c) 40 μm. (a) × 125; (b) × 90; (c) × 280.

Staining, albeit with different intensities, was associated with the matrix of all the microscopic structures in the kidney (Fig. 5a). The glomeruli, together with large vessels, were the most intensely stained. Brightest fluorescence was localized in the mesangium, but capillaries were also fluorescent, as was the Bowman's capsule connective tissue. Reactivity in the tubules appeared to be associated with basement membranes: the pattern was continuous in the proximal convoluted tubule, while a patchy fluorescence was present in other parts of the tubule. Basement membranes of ducts in the medulla were also positive (data not shown). Bright fluorescence in the lung was localized in smooth muscle cells rich areas (blood vessels and parabronchi) (Fig. 5b). The surrounding parenchima displayed a faint fibrillar staining probably of the matrix of blood and air capillaries. In the liver, vessel walls and the connective tissue comprising the portal space were highly fluorescent. The outline of the synusoids was also clearly visible (Fig. 5c).

In the skin a fine fibrillar network was evident throughout the dermis with distinct brighter areas corresponding to blood vessels and smooth muscle cell clusters around the feather bulbs. No staining was demonstrable in basement membranes

at the dermal–epidermal junction (Fig. 6). Cartilage, bone, and cornea did not react with the antibody even after jaluronidase treatment (data not shown).

An identical distribution pattern was observed using anti-gp 115 monoclonal antibodies (data not shown).

Immunofluorescence Studies on Cell Cultures

The purpose of this experiment was to demonstrate that anti-gp 115 reacting material was synthesized by aorta cells grown in vitro and that it was secreted and organized in the extracellular space. Under phase-contrast microscopy the cells had an elongated shape and were characterized by a pattern of parallel alignment with areas of overlapping. At higher magnification the cells displayed the typical appearance of smooth muscle cells with the perinuclear region full of large phase-dense granules (20). When such confluent cultures were fixed in picric acid formaldehyde and developed with gp 115 antibody, an extracellular staining of a fibrillar nature was observed (Fig. 7a). In other experiments cell cultures were fixed and permeabilized with 0.5% Triton X-100: a prominent pattern of granules of intracellular fluorescence with a peri-
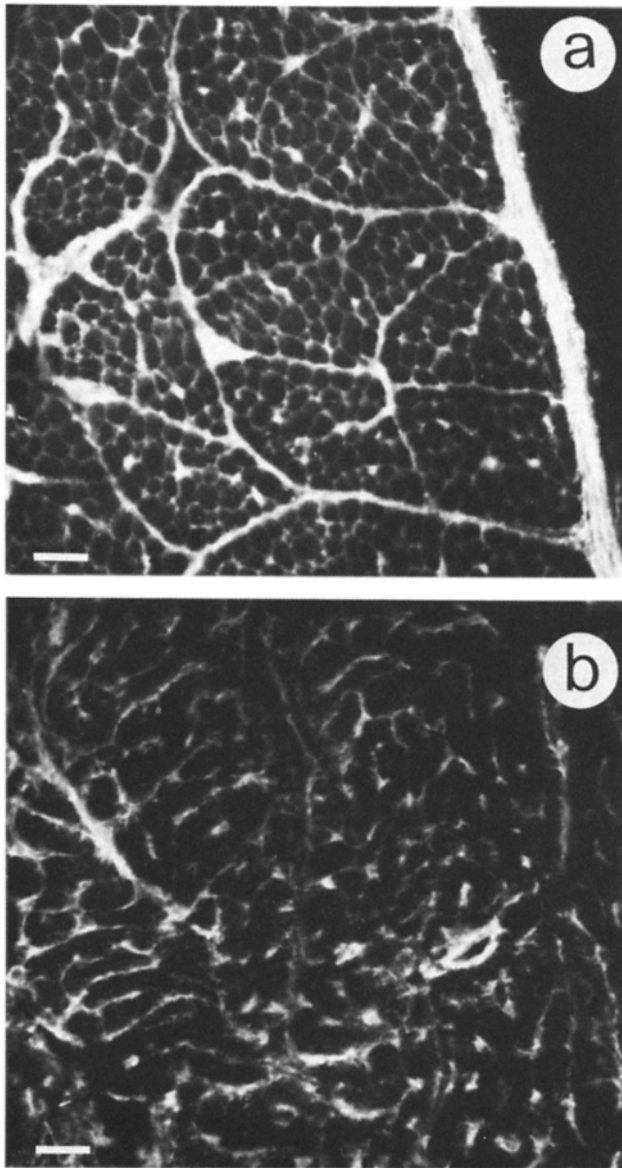
FIGURE 4 Antigen localization by indirect immunofluorescence on 2-d-old chick sections. (a) Skeletal muscle; bright staining is associated with the endomysium and with the connective tissue layers surrounding bundles of muscle fibers (perymisium and epimysium). Note that heavier staining is evident in areas where the interstitial connective tissue matrix is thicker and crossed by small vessels and capillaries. (b) Cardiac ventricle; staining is clearly visible around muscle fibers and in the connective tissue septa. Bars, 50 μm. (a) × 140; (b) × 150.

nuclear distribution was present in addition to the extracellular staining (Fig. 7b). Similar results were obtained with fibroblasts and with cultures grown in the presence or absence of ascorbic acid.

Antigen Detected in Different Tissues Is Glycoprotein 115

The demonstration that gp 115 antibodies reacted extensively with the extracellular matrix and that this reactivity was present in several tissues prompted us to investigate whether the complex pattern of immunofluorescence was due to the existence of a group of immunologically related proteins

or to a single protein species. Therefore, it was necessary to establish the molecular identity of the antigen recognized by the antibodies in tissues other than aorta. This was done by immunostaining of nitrocellulose filters blotted with PBS and SDS + 2-mercaptoethanol extracts that had been separated on SDS gels. The antigen was not detected in PBS extracts. A major labeled polypeptide of apparent M_r = 115,000 was identified in SDS + 2-mercaptoethanol extracts of gizzard, pectoralis muscle, and kidney (Fig. 8, lanes d, f, and h). For comparison a similar extract from aorta was run in parallel and a polypeptide with identical molecular mass but with a much stronger intensity was observed (Fig. 8, lane b). A less intense band of lower M_r was present in the strip of pectoralis muscle. Additional bands of even lower intensity were present in aorta as well as other organs. The presence of these lower M_r bands and their intensity vary depending upon the preparation and the organ examined. We also looked for the presence of soluble gp 115 molecules in plasma and serum, but were unable to detect any band in immunoblots using up to 100 μl of sample (data not shown).

We studied further the solubility properties of gp 115 in different organs. The expression of gp 115 in PBS, 6 M guanidine HCl, and 6 M guanidine HCl + DTE extracts was measured by a solid-phase radioimmunobinding. The data obtained with a matrix-rich (aorta) and a cell-rich (gizzard) tissue are reported in Fig. 9. In both organs the highest antigen activity was found in the 6 M guanidine HCl + DTE extract. However, the amount (dry weight) of gizzard solubilized by 6 M guanidine HCl was seven times that obtained with 6 M guanidine HCl + DTE, suggesting that a small fraction of gp 115 was recovered from the latter extract. The antigen activity measured in the 6 M guanidine HCl + DTE aorta extract in the present study was much higher, thus confirming that a large fraction was extracted from this tissue only in the presence of chaotropic and reducing agents (12). These data suggest a different organization of the protein in the two tissues.

DISCUSSION

This study shows that the newly characterized matrix-associated 115,000-mol-wt glycoprotein gp 115 that was purified from chick aortas (12) has a widespread distribution in the connective tissue. Affinity-purified mouse antibodies raised against gp 115 and specific monoclonal antibodies recognized a major band around M_r = 115,000. These antibodies stained loose and dense connective tissues in several organs and also appeared to be associated with some basement membranes. Smooth muscle cells rich blood vessels were the most reacting structures, but also the muscular layers in the gut and lung were strongly positive. The very intense fluorescence associated with basement membranes of smooth muscle cells might suggest that gp 115 was synthesized and organized there. The finding of a gp 115 meshwork in cultures of smooth muscle cells together with the fact that the protein was not detected in serum or plasma, and therefore, not passively deposited, was in line with the prediction. Other embryo cells grown in vitro such as fibroblasts and myoblasts secreted gp 115 that was organized in a similar fashion (unpublished results). Moreover, the detection of strong immunofluorescence in connective tissue surrounding skeletal, cardiac muscle, and liver sinusoids, interalveolar septa, and along several basement membranes widens the spectrum of cells capable of synthesizing and organizing gp 115.
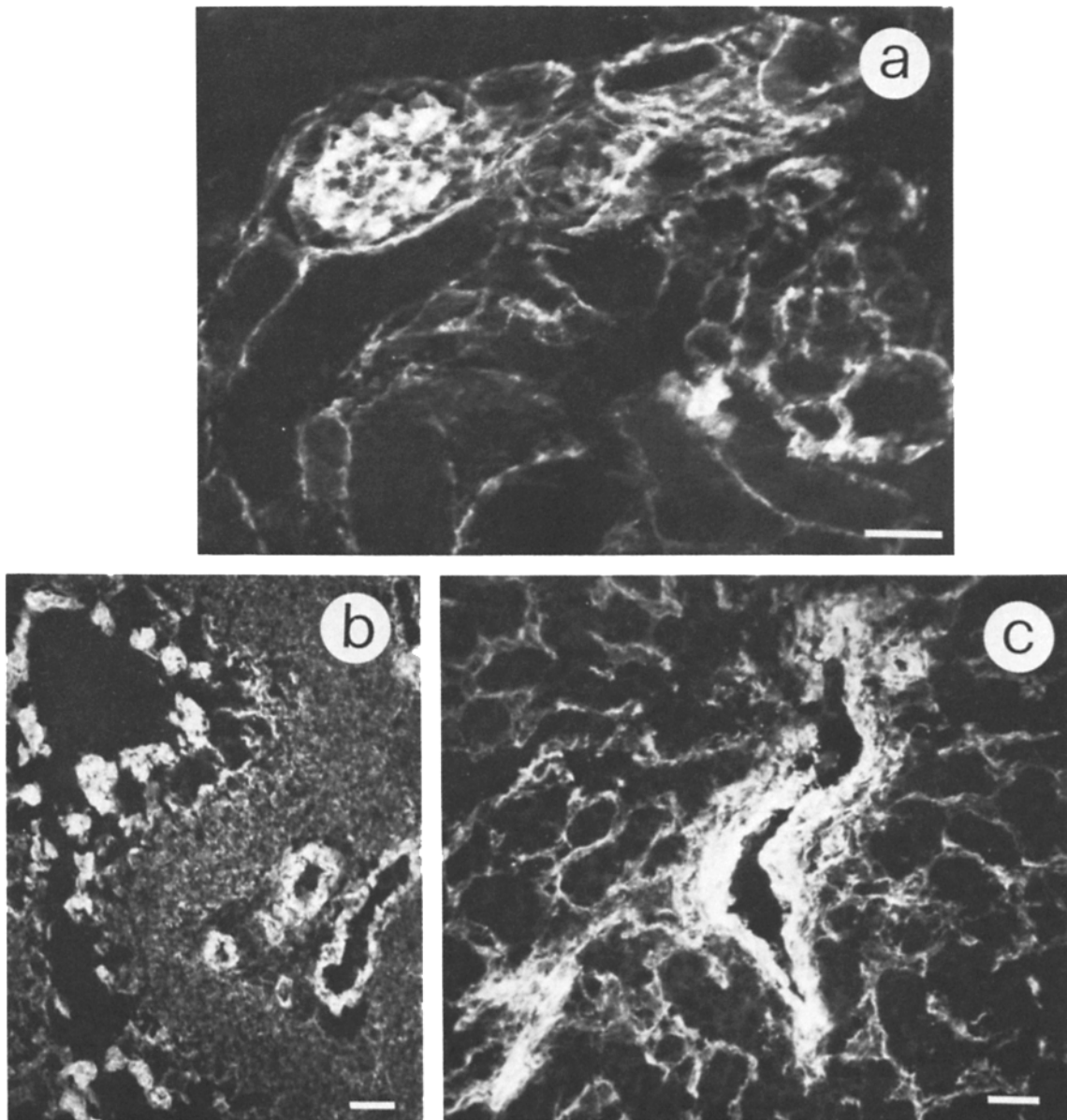
FIGURE 5 Antigen localization by indirect immunofluorescence on 2-d-old chick sections. (a) Kidney; positive reaction is observed in both the vascular and mesangial components of the glomeruli, and in the connective tissue surrounding tubules and the Bowman's capsule. The staining of the capsule apparently continues into that of the proximal tubule. A patchy pattern is also evident at the level of more distal portions of the tubules. (b) Lung; note the very strong staining of the blood vessels and the parabronchial wall areas where smooth muscle cells are located. In the surrounding parenchima, fluorescence shows a trabecular pattern due to the staining of blood and air capillaries matrix. (c) Liver; the blood vessels and the connective tissue of the portal space react very strongly. The outline of the sinusoids is also marked by the immunofluorescent staining. (a) Bar, 40 μm; (b and c) 50 μm. (a) × 270; (b) × 140; (c) × 145.

The antigen identified by immunofluorescence in the different tissues was the same because, after SDS gel electrophoresis and immunoblotting, the reacting molecular species had identical mobility. There were tissue differences in the antigen content, aorta and large blood vessels being largely richer than other tissues: for example, in gizzard the expression of gp 115 was much lower than in aorta. This was also confirmed by the immunoblotting that showed a much more intense band in the case of aorta compared with that of other tissues. It is possible that the organization of the protein changes in the

various connective tissue types or parts, such as fibrous stroma, loose connective tissue, basement membrane, etc. This is suggested by the very large difference in the relative amounts of gp 115 solubilized by different buffers in several organs. Indeed, the antigen activity was higher in the 6 M guanidine HCl + DTE extract than in the 6 M guanidine and PBS extracts in aorta, an organ whose connective tissue is composed mainly of elastic fibers, whereas more antigen was solubilized by 6 M guanidine HCl and PBS than by 6 M guanidine HCl + DTE in gizzard, a cell-rich organ, in which
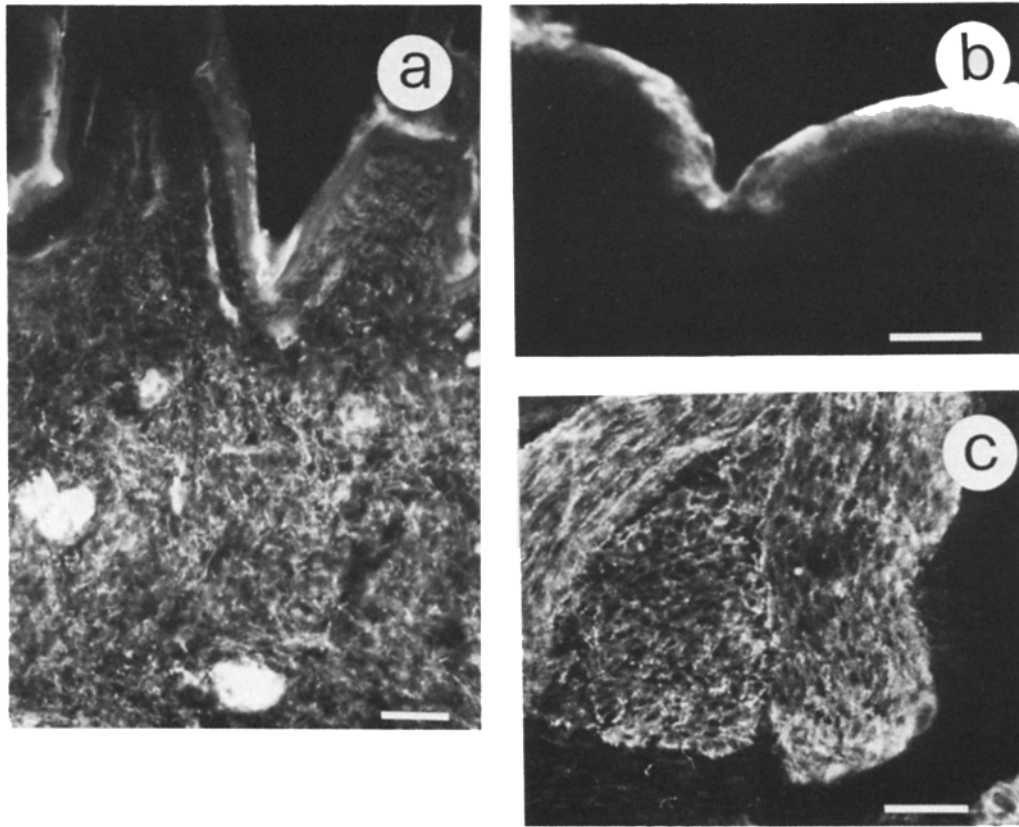
Figure 6   Antigen localization by indirect immunofluorescence on 2-d-old chick sections. Skin sections were incubated with affinity-purified antibody (a and c) or preimmune serum (b). (a) Note the widespread fibrillar staining of the dermis and the lack of staining of the basement membrane of the epidermis. Fluorescence associated with the keratinized portion of the epidermis was not specific. It appeared as a yellow autofluorescence also in sections not treated with antibodies or incubated with preimmune serum. (c) A very intensely stained cluster of smooth muscle cells surrounding a feather's bulb. (a and b) Bar, 40 μm; (c) 20 μm. (a) × 220; (b) × 600; (c) × 270.

basement membranes and pericellular matrix are well represented.

The overall immunofluorescent staining pattern was reminiscent of fibronectin distribution (5, 6, 21). Nevertheless, there were a few significant differences that we could detect by comparing the distribution of fibronectin described in the literature and derived from our own data (unpublished results) with that of gp 115. In blood vessels fibronectin antibodies stained more intensely the intima than the media; the connective stroma of the liver portal space gave a weak reaction with fibronectin and a strong reaction with gp 115 antibodies; in the kidney, gp 115 antibodies consistently stained the peritubular matrix, unlike fibronectin antibodies which reacted irregularly and weakly; finally, in glomeruli, fibronectin reactivity appeared more confined to the mesangial matrix, whereas gp 115 reactivity appeared to be more diffuse, apparently including the capillary loops. Moreover, a fundamental difference between the two proteins was the virtual absence of gp 115 from plasma and serum. This finding distinguishes gp 115 also from vitronectin (serum-spreading factor), a cell-attachment protein that differed in relative molecular weight and antigenicity from fibronectin, but was similarly present both in a soluble form in the plasma and as an insoluble

molecule in the extracellular matrix where it overlapped with the distribution of fibronectin, although with different intensities in some areas (9, 22).

A point that deserves further comment is the presence of gp 115 in association with some basement membranes and its absence from others. Notable was the lack of immunofluorescence in basement membranes of epithelia from skin, bowel, bronchi, and cornea and its presence in the pericellular space of kidney tubules and muscle cells. However, given the low resolution of immunofluorescence, it is not possible to establish whether gp 115 is a true component of basement membranes or is present in the matrix close to it. The precise localization of gp 115 at the electron microscopic level is presently under investigation.
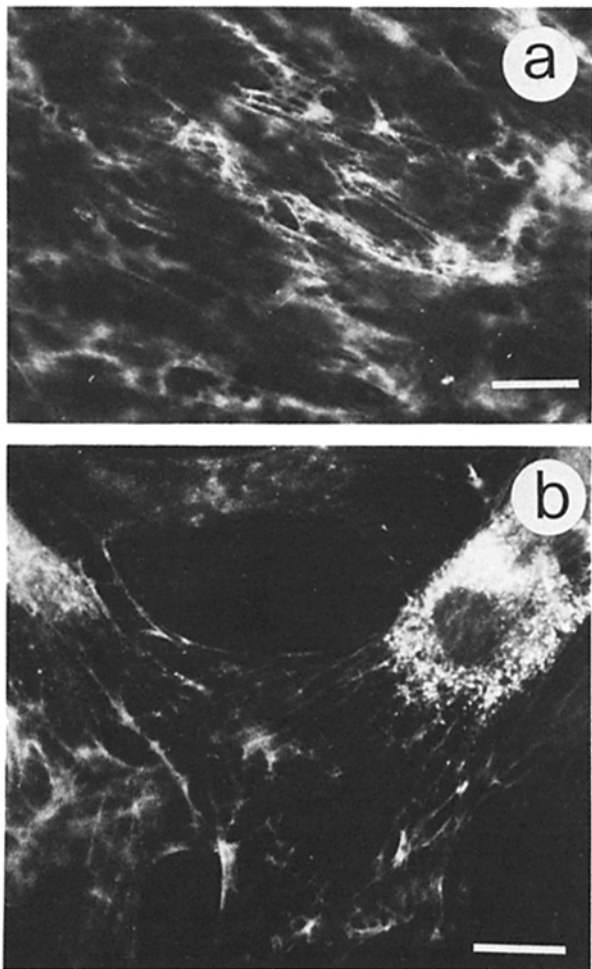
FIGURE 7 Antigen localization by indirect immunofluorescence on aorta embryo smooth muscle cells cultured in vitro. (a) Fixed cells; incorporation of antibody reacting material into extracellular matrix is demonstrated by a meshwork of extracellular fibrils. (b) Fixed and permeabilized cells. In addition to the extracellular staining a granular reaction in the perinuclear area becomes evident. Bar, 20 μm. (a) × 560; (b) × 590.

FIGURE 9 Binding of gp 115 antiserum to aorta and gizzard extracts. Antibody binding was measured by the solid-phase assay using 40 μl of a 1:800 dilution of immune serum and 40 μl of ^{125}I-labeled rabbit IgG anti-mouse Ig (0.28 μg/ml, 13 μCi/μg) and different amounts of protein from 2 d old chick aortas (a) and gizzard (b) extracted with saline (■), 6 M guanidine HCl (▲), and 6 M guanidine HCl + DTE (●).

REFERENCES

1. Hay, E. D. 1981. Extracellular matrix. *J. Cell Biol.* 91(3, Pt.2):205s–223s.
2. Kleinman, H. K., R. J. Klebe, and G. R. Martin. 1981. Role of collagenous matrices in the adhesion and growth of cells. *J. Cell Biol.* 88:473–485.
3. Bornstein, P., and H. Sage. 1980. Structurally distinct collagen types. *Annu. Rev. Biochem.* 49:957–1003.
4. Furthmayr, H., and K. von der Mark. 1982. The use of antibodies to connective tissue proteins in studies on their localization in tissues. In Immunochemistry of the Extracellular Matrix, Vol. II. H. Furthmayr, editor. CRC Press, Boca Raton, FL. 89–117.
5. Stenman, S., and A. Vaheri. 1978. Distribution of a major connective tissue protein, fibronectin, in normal human tissues. *J. Exp. Med.* 147:1054–1064.
6. Linder, W., A. Vaheri, E. Ruoslahti, and J. Wartiovaara. 1975. Distribution of fibroblast surface antigen in the developing chick embryo. *J. Exp. Med.* 142:41–49.
7. Rohde, H., G. Wick, and R. Timpl. 1979. Immunochemical characterization of the basement membrane glycoprotein laminin. *Eur. J. Biochem.* 102:195–201.
8. Carlin, B., J. Ronald, B. Bender, and A. E. Chung. 1981. Entactin, a novel basal lamina-associated sulphated glycoprotein. *J. Biol. Chem.* 256:5209–5214.
9. Hayman, E. G., M. D. Pierschbacher, Y. Öhgren, and E. Ruoslahti. 1983. Serum spreading factor (vitronectin) is present at the cell surface and in tissues. *Proc. Natl. Acad. Sci. USA.* 80:4003–4007.
10. Hassell, J. R., P. Gehron Robey, H-J. Barrach, J. Wilczek, S. I. Rennard, and G. R. Martin. 1980. Isolation of a heparan sulfate-containing proteoglycan from basement
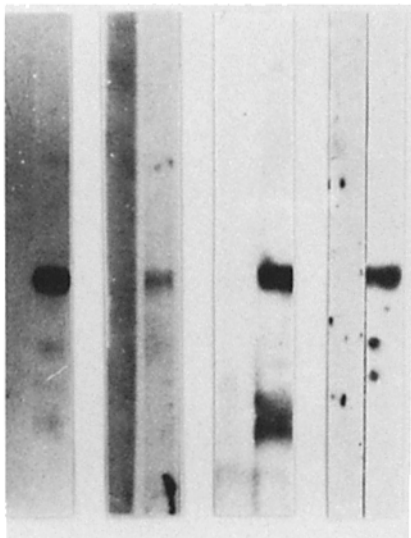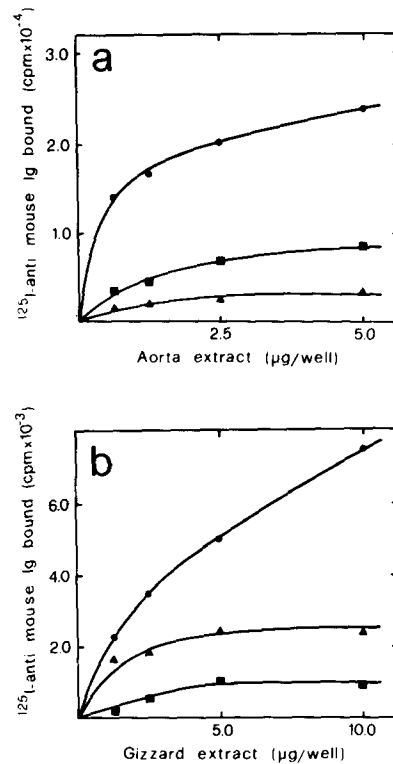
FIGURE 8 Comparison by immunoblotting of anti-gp 115-reacting molecules in several tissues. Aliquots of SDS + 2-mercaptoethanol aorta (a and b), gizzard (c and d), pectoralis muscle (e and f), and kidney (g and h) extracts were separated by SDS polyacrylamide gel (6%) electrophoresis under reducing conditions and transferred onto nitrocellulose filters. The strips were developed with a 1:100 final dilution of preimmune serum (a, c, e, g) or affinity-purified antibodies (b, d, f, h) and ^{125}I-labeled affinity-purified rabbit IgG anti-mouse Ig. Each strip was incubated with 7 × 10⁵ cpm of ^{125}I-labeled IgG.

membrane. *Proc. Natl. Acad. Sci. USA.* 77:4494–4498.

11. Poole, A. R., I. Pidoux, A. Reiner, L. Cöster, and J. R. Hassell. 1982. Mammalian eyes and associated tissues contain molecules that are immunologically related to cartilage proteoglycan and link protein. *J. Cell Biol.* 93:910–920.

12. Bressan, G. M., I. Castellani, A. Colombatti, and D. Volpin. 1983. Isolation and characterization of a 115,000-dalton matrix-associated glycoprotein from chick aorta. *J. Biol. Chem.* 258:13262–13267.

13. Bressan, G. M., and D. J. Prockop. 1977. Synthesis of elastin in aortas from chick embryos. Conversion of newly secreted elastin to cross-linked elastin without apparent proteolysis of the molecule. *Biochemistry.* 16:1406–1412.

14. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.

15. Olmsted, J. B. 1981. Affinity purification of antibodies from diazotized paper blots of heterogenous protein samples. *J. Biol. Chem.* 256:11955–11957.

16. Chamley-Campbell, J. H., G. R. Campbell, and R. Ross. 1981. Phenotype-dependent response of cultured aortic smooth muscle to serum mitogens. *J. Cell Biol.* 89:379–383.

17. Stefanini, M., C. De Martino, and L. Zamboni. 1967. Fixation of ejaculated spermatozoa for electron microscopy. *Nature (Lond.).* 216:173–174.

18. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

19. Colombatti, A., and J. Hilgers. 1979. A radioimmunoassay for viral antibody using binding of 125-I-protein A. *J. Gen. Virol.* 43:395–401.

20. Chamley-Campbell, J., G. R. Campbell, and R. Ross. 1979. The smooth muscle cell in culture. *Physiol. Rev.* 59:1–61.

21. Boselli, J. M., E. J. Macarak, C. C. Clark, A. G. Brownell, and A. Martinez-Hernandez. 1981. Fibronectin: its relationship to basement membranes. I. Light microscopic studies. *Collagen Res.* 5:391–404.

22. Barnes, D. W., and J. Silnutzer. 1983. Isolation of human serum spreading factor. *J. Biol. Chem.* 258:12548–12552.