# DISTRIBUTION OF FIBROBLAST SURFACE ANTIGEN IN THE DEVELOPING CHICK EMBRYO*

By EWERT LINDER, ANTTI VAHERI, ERKKI RUOSLAHTI, AND JORMA WARTIOVAARA

*(From the Department of Serology and Bacteriology, the Department of Virology, and the Third Department of Pathology, University of Helsinki, Helsinki 29, Finland)*

The fibroblast surface (SF)[1] antigen is a major cell surface glycoprotein component of cultured chick fibroblasts. It is shed to the extracellular medium and is also present in the circulation (serum and plasma). The cellular and circulating SF proteins are immunologically indistinguishable and similar also in their polypeptide composition (1, 2). Immunofluorescent staining and scanning electron microscopy have indicated that in normal fibroblasts SF antigen has a highly nonrandom distribution. It is located in discreet cell surface ridges and cytoplasmic extensions, 50-200 nm in diameter (3). SF antigen is absent from the cell surface after malignant transformation by Rous sarcoma virus (4). An analogous antigen is present at the surface of human fibroblasts, in human serum and plasma (5), and human fibroblasts transformed by the oncogenic simian virus 40 do not express the antigen at the cell surface.[2]

SF antigen has been regularly found in fibroblasts, but not in other types of cultured cells with the exception of human glia cells (unpublished observations). This suggests that it is a cell-type-specific marker of fibroblasts. We show here that the tissue distribution of SF antigen as studied by immunofluorescence in chick embryos is compatible with its confinement to fibroblasts and primitive mesenchymal cells in vivo.

## Material and Methods

*Chick Embryos.* Chick embryos (Brown Leghorn) with extraembryonic membranes were collected at different times of incubation, ranging from 2 days (developmental stage 10 days, [6]) to full term. Some tissues from adult animals were also examined. Secondary cultures of chick embryo fibroblasts were prepared from 10-day embryos as described previously (7).

*Immunofluorescence.* Preparation of antiserum against chicken SF antigen in rabbits has been described (1). Papain digests containing SF antigen were prepared using matrix-bound papain (Enzite-EMA, Miles-Yeda) from live fibroblast cultures extensively washed with serum-free cell culture medium and buffered saline. Antisera to these digests were raised in rabbits or sheep and were absorbed with the insolubilized calf serum proteins (calf serum is a constituent of the cell culture medium) and with inactivated (iodoacetamide treated) matrix-bound papain when necessary. Such antisera were specific for SF antigen in immunodiffusion giving a single line of precipitation against

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[1] *Abbreviations used in this paper:* FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; SF, fibroblast surface.

[2] Vaheri, A., and E. Ruoslahti. 1975. Fibroblast surface (SF) antigen produced but not retained by virus-transformed human cells. *J. Exp. Med.* **142**:in press.

papain digests from fibroblasts (immunogen), against fibroblast extracts obtained with urea and the detergent Triton X-100, and against normal chick serum. They have failed to react with similar digests and extracts from other types of cells studied (4). To ensure specificity at the immunofluorescence level, anti-SF antibodies were purified from the antiserum using adsorption to and elution from an immunoadsorbent prepared by coupling (8) normal chick serum proteins including SF antigen to Sepharose 4B (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). For indirect immunofluorescence experiments sheep antirabbit IgG or rabbit antisheep IgG was conjugated with fluorescein isothiocyanate (FITC; Baltimore Biological Laboratories, Cockeysville, Md.) and gave a conjugate with a molar fluorescein to protein ratio of 2.3 (9). Alternatively, the purified anti-SF antibodies were conjugated with FITC to be used in direct immunofluorescence staining. No differences were observed when the direct and indirect immunofluorescence-staining patterns were compared.

Cryostat sections from chick embryo tissues were fixed at −20°C with absolute acetone for 15 min and rinsed in phosphate -buffered saline (PBS), pH 7.2, for 20 min at room temperature. As an alternative procedure, fresh tissue blocks were fixed in 2% paraformaldehyde in PBS for 20 min at room temperature and then rinsed in 30% sucrose overnight at +4°C before cryostat sectioning. These sections were not refixed after sectioning, only washed in PBS. This provided better conservation of the structure but gave some background autofluorescence. The cultured fibroblasts were fixed with acetone or successively with 3.5% HCHO and acetone (10). Tissue sections were incubated with the antisera using standard incubation and rinsing times and employing indirect or direct staining methods. For microscopy, a 100 W 12 V halogen lamp was used as the light source and a narrow band excitation light was obtained by using an FITC-interference filter combination (Optisk Laboratorium A. T. V., Lyngby, Denmark).

## Results

*SF antigen in Loose Connective and Mesenchymal Tissue and Tissue Boundary Membranes.* SF antigen was seen in the loose connective tissue in 2-3 day and older embryos (Fig. 1). It was detected in the unorganized connective tissue surrounding nerves, muscle bundles, and ducts in both embryonic and extraembryonic tissues.

During the organization of tissues and formation of organs accumulation of SF antigen was usually seen at the periphery of the tissue. In the developing kidney (mesonephros stage) condensation of SF antigen-positive material was first seen in the mesonephric mesenchyme (Fig. 1). Along with the appearance of mesonephric tubules (Fig. 2) and glomeruli (Fig. 3) their limiting basement membranes were stained.

Condensation of SF antigen was seen in the mesenchyme of various other organs, e.g., in the submucosa of the developing small intestine (Fig. 4), in the

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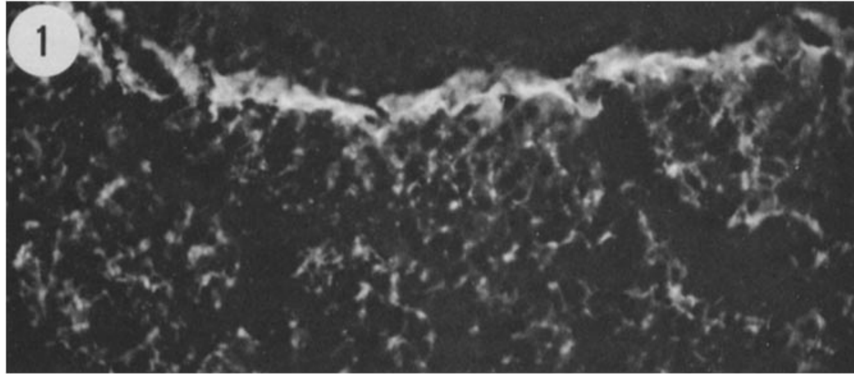
FIG. 1. SF antigen in developing kidney of 5-day old chick embryo. Loose network of SF antigen in nephrogenic mesenchyme. Fixed cryostat section stained with FITC-conjugated anti-SF antibodies. Accumulation of SF antigen is seen at the surface of the developing organ (top). × 800.

FIG. 2. SF antigen in developing kidney of 10-day old chick embryo. Note network of SF antigen in undifferentiated mesenchyme (M) and accumulation of SF antigen in the tubular basement membranes. No staining of tubular epithelia (T). × 400.

FIG. 3. SF antigen in developing kidney of 13-day old chick embryo. Two mesonephric glomeruli at different developmental stages are seen. Undifferentiated glomerulus to the left contains SF antigen in a developing glomerular tuft (GT) and in the Bowman's capsule (BC). In differentiated glomerulus to the right (arrows) SF antigen has accumulated in the glomerular capillary loops and surrounding mesangium. Fluorescence in hilar region is continuous with interstitial connective tissue. Staining of basement membranes around negative tubular epithelium (T). × 800.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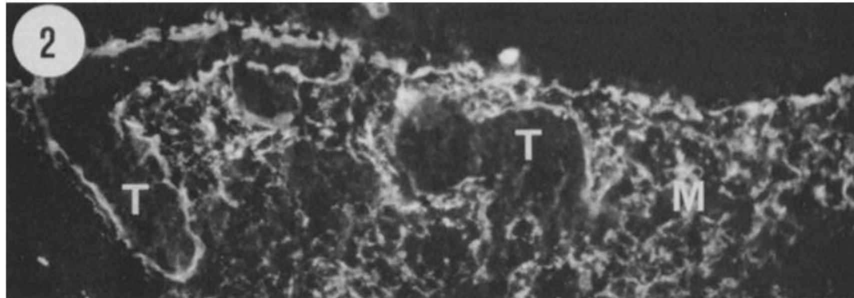
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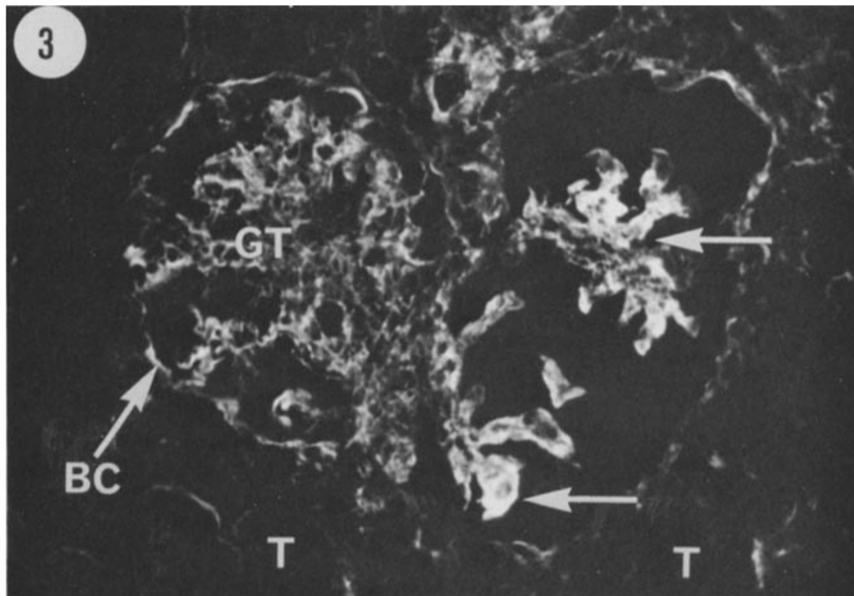
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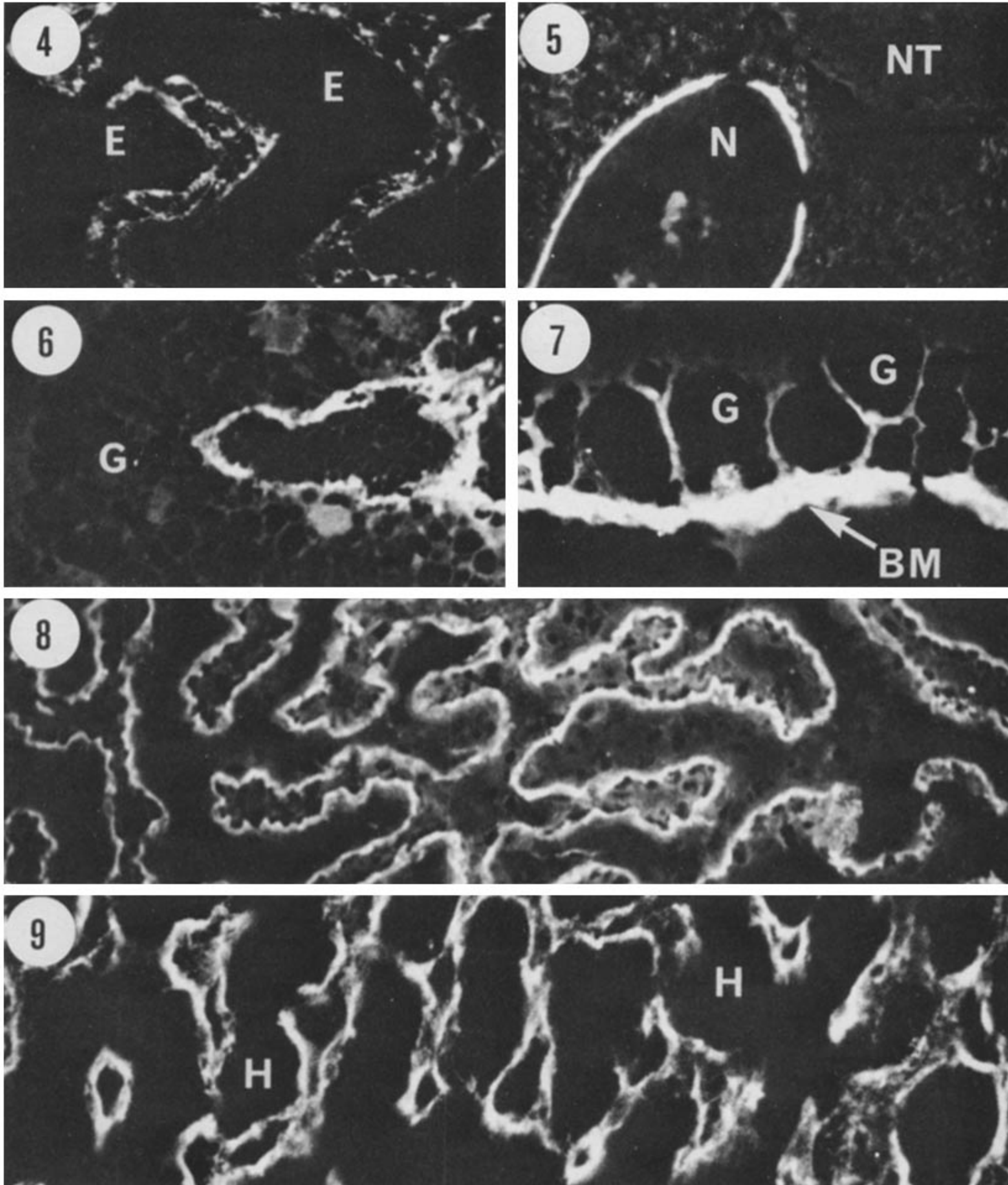
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mesenchyme surrounding the notochord (chorda dorsalis) of the 5-day embryo (Fig. 5), and in the basement membrane of the yolk sac epithelium (Figs. 6 and 7). In the vitelline membrane (Fig. 8) and in the liver (Fig. 9) the fluorescence lined the epithelial cell sheets and strands.

*SF Antigen in Perivascular and Perineural Tissue.* An SF antigen-positive network of extracellular fibers or cells with cytoplasmic fluorescence was also seen around both arteries and veins of different size (Figs. 10 and 12). The walls of capillaries (Fig. 10) appeared to contain large quantities of SF antigen-positive material. The capillaries were readily demonstrated in the brain where narrow tubes were visualized against a negative background. In adult chick brain, SF antigen could be demonstrated with high magnification (Fig. 11) in neural sheaths traversing the glia in various directions. The density of such fluorescence varied considerably in the different parts of the central nervous system; cross-sections of the spinal cord stained almost uniformly positive.

Of the other vascular spaces glomerular capillary basement membranes and Bowman's glomerular capsule also contained SF antigen (Fig. 3). In the liver the staining of the sinusoidal linings with adjoining reticular connective tissue was evident (Fig. 9).

*Cell-Type Specificity of SF Antigen.* SF antigen fluorescence was found in mesenchymal cells at all embryonal stages studied. The antigen was confined to the connective tissue cells and absent from the parenchymal cells of all the various organs as shown above. The antigen was not present in the epithelial cells of the kidney tubules after their differentiation from mesenchymal cells (Figs. 2 and 3). The SF antigen-negative cells included also those of the fetal brain (Fig. 10), bone, and cartilage (not shown). Parenchymal muscle cells remained unstained during differentiation from the primitive myotomes (Fig. 13) to adult striated muscle fiber bundles (Fig. 14).

*Comparison of Cell Surface Distribution of SF Antigen In Vivo and In Vitro.* SF antigen was located both in vivo in the developing mesenchyme (Figs. 1 and 13) and in vitro in embryonic fibroblast cultures (Fig. 15) to thin filamentous structure that formed a fine network. A tendency to the formation of larger fluorescent bundles was observed in fibroblast cultures grown to confluency. A higher degree of organization of SF antigen was seen, e.g., at borders of various parenchymal tissues (Figs. 2, 4, 5, and 7-9).

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FIG. 4. A longitudinal section of intestinal villi of 5-day old chick embryo demonstrating presence of SF antigen in subepithelial stroma but absence from epithelial cell (E). × 800.

FIG. 5. Section through 5-day old chick embryo showing accumulation of SF antigen in the boundary membrane of the notochord (N). Weak fluorescence in surrounding mesenchyme. No staining of the neural tube (NT). × 400.

FIG. 6. SF antigen in yolk sac basement membrane. No SF antigen in clusters of trophoblastic giant cells (G) in primitive yolk sac in 3-day old chick embryo. × 400.

FIG. 7. A stained yolk sac basement membrane (BM) is seen in a 13-day old chick embryo. Note interposition of SF antigen between trophoblastic giant cells (G). × 800.

FIG. 8. SF antigen in vitelline membrane of 13-day old chick embryo forms a continuous subepithelial layer. × 400.

FIG. 9. SF antigen in liver sinusoids of 13-day old chick embryo. Bright fluorescent staining of sinusoidal walls and no staining of hepatocytes (H). × 800.

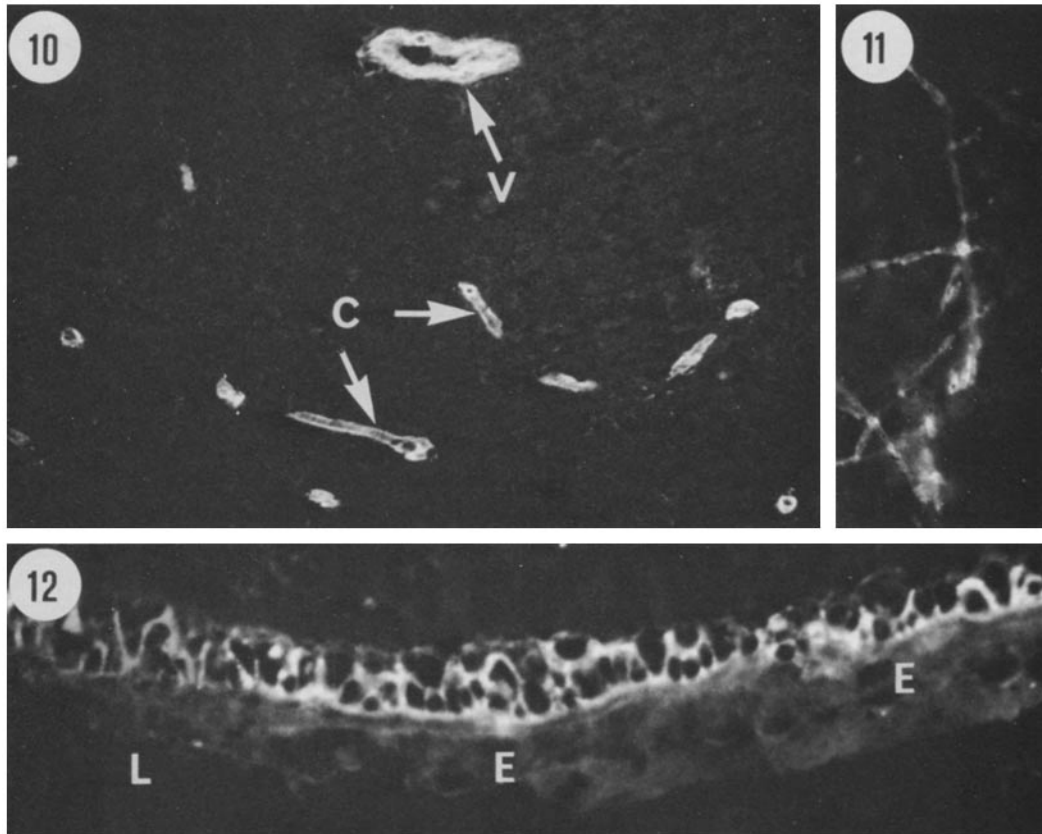


FIG. 10. Localization of SF antigen in brain vessels surrounded by negative remaining neuroglia in a 7-day old chick embryo. Capillaries (C) and the wall of a larger vessel (V) have cells containing SF antigen. × 400.

FIG. 11. SF antigen in neural sheaths in adult chick brain. × 1,600.

FIG. 12. SF antigen in the wall of a large artery in a 7-day old chick embryo. The endothelium (E) is not stained while bright fluorescence is seen in the more peripheral layers of the vessel. Lumen (L). × 800.

## Discussion

These and previous studies have established that the chick SF antigen is present (*a*) in fibroblasts in loose connective and primitive mesenchymal tissue, (*b*) in limiting tissue membranes throughout the organism, and (*c*) in soluble form in circulation (serum and plasma). The data also verify that SF antigen is cell-type specific. Despite the lack of staining of SF antigen in vast areas of the brain, fluorescence was demonstrable in some cells of chick brain cell cultures (unpublished observations). These positive cells may be specialized glia cells, since, recent results from our laboratory (unpublished) show that cultured human glia cell lines (11, 12) contain SF antigen. The parenchymal cells in the different organs and tissues showed no SF antigen in immunofluorescent staining.

In connective tissue in vivo and fibroblast cultures in vitro SF antigen had a fibrillar appearance. A similar distribution was recently described for both actin

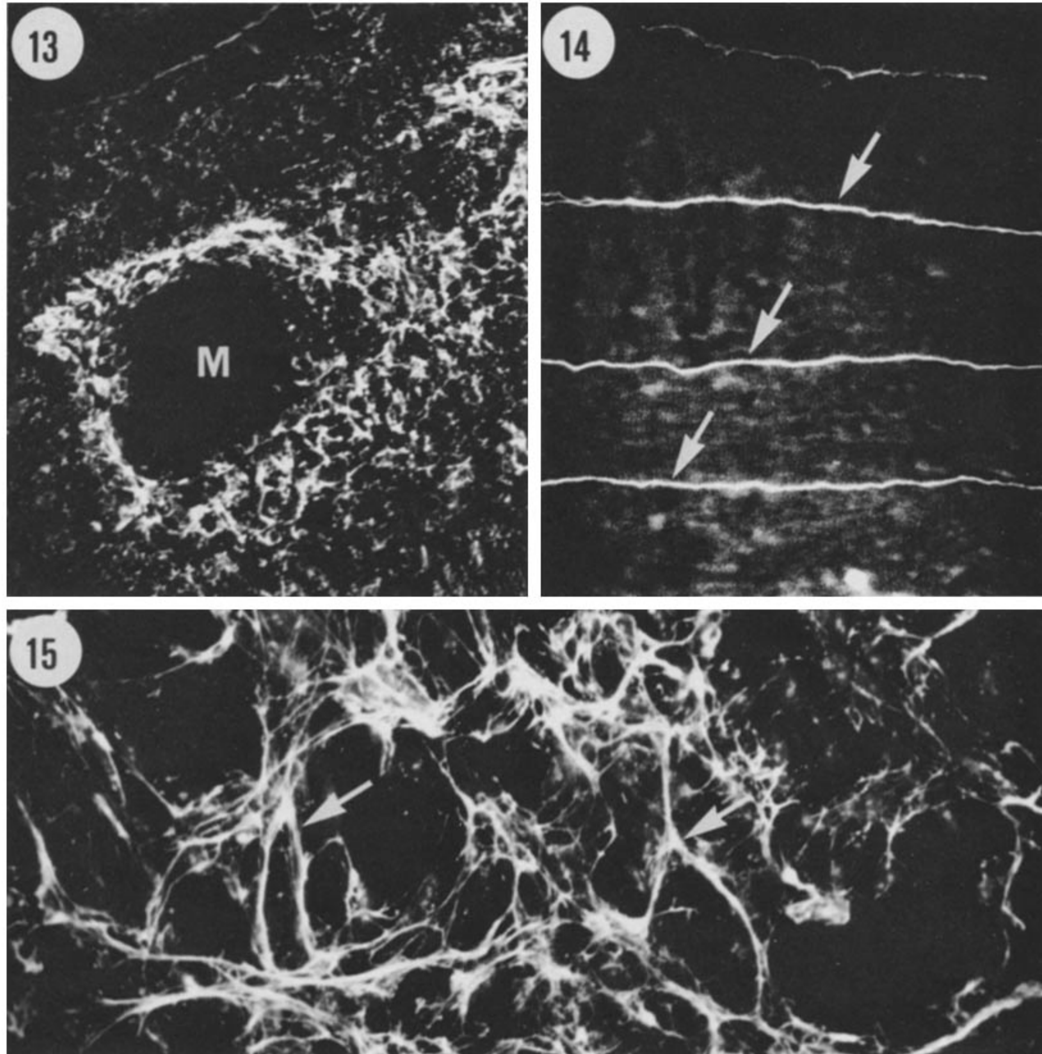


FIG. 13. SF antigen in the loose connective tissue surrounding a myotome (M) of a 7-day old chick embryo. Note network of fibrillar material. × 400.

FIG. 14. SF antigen in sarcolemmal sheaths (arrows) around bundles of striated muscle fibers in adult chick femoral muscle. × 400.

FIG. 15. SF antigen in confluent in vitro culture of embryonic chick fibroblasts. A tendency of fine fluorescent fibrils to form thicker branching bundles can be seen (arrows). × 1,000.

and myosin molecules in cultured fibroblasts (10, 13). SF antigen, however, seems not to be related to these molecules as indicated by its absence in parenchymal muscle cells containing actomyosin filaments. The possibility that SF antigen molecules in fibroblast surface may have an association with actin is supported by indirect evidence as reported and discussed elsewhere (2, 3).

SF antigen in cultured fibroblasts is a major surface component (about 0.5% of total cell protein) and as shown here is widely distributed in vivo throughout the connective tissue. SF antigen appears to be one of several fibroblast-derived

molecules (collagen and glycosaminoglycans) that form the intercellular matrix. Our recent work has shown that human plasma SF antigen has an affinity to fibrin and fibrinogen (14) and is identical to what has been previously called "cold insoluble globulin." The significance of the SF antigen- fibrinogen interaction is not known.

Expression of SF antigen seems to be a property of primitive mesenchymal cells acquired early in ontogenesis. Its expression appeared to be terminated during their differentiation in parenchymal direction. Cells of the nephrogenic mesenchyme of young embryos contained readily demonstrable fibers of SF antigen. Epithelial kidney tubules derived from the mesenchymal cells (15) lacked the antigen. This is in keeping with previous observations (9) indicating that tubulogenesis involves early loss of connective tissue antigen(s) present in nephrogenic mesenchymal cells. Parenchymal cells of striated and smooth muscle, cartilage, and bone, also derived from the mesenchyme, similarly lacked the antigen.

These and previous data indicate that the expression of SF antigen correlates with the morphological differentiated state of normal fibroblast. SF antigen is lost both in differentiation of primitive mesenchymal cells into more specialized cells, as demonstrated in this study, as well as in trypsinization (4), during the mitotic state[3] and in malignant transformation (reference 4 and footnote 2).

## Summary

Fibroblast surface (SF) antigen is present in fibrillar surface structures of cultured normal fibroblasts, shed to the extracellular medium, and is also found in circulation (serum and plasma). Malignant fibroblasts (transformed by viruses) do not express SF antigen on the cell surface.

In this study the in vivo differentiation and distribution of SF antigen has been investigated in the developing chick embryo using cryostat sections and immunofluorescence. The major findings were: (*a*) SF antigen was detectable in the loose connective tissue of very early (2-to 3-day old) embryos. (*b*) Condensation of SF antigen was seen in various boundary membranes such as the glomerular and tubular basement membranes of the kidney, the boundary membranes of the notochord, yolk sac, and vitelline membranes and liver sinusoids. (*c*) SF antigen was found to be cell-type specific. It was seen as a fibrillar network in the loose connective tissue of different organs but not in the parenchymal cells. It was not found in muscle cells at any stage of development. (*d*) The antigen was present in the undifferentiated mesenchymal cells of the kidney, but not found after their development into epithelial cells of the secretory tubules. (*e*) Both in vivo and in fibroblast cultures SF antigen was distributed as a fibrillar network. These data indicate that SF antigen is a "differentiation antigen" restricted to certain cells of mesenchymal origin and character, and that it accumulates in the connective tissue during embryogenesis.

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[3] Vaheri, A., et al. Manuscript in preparation.

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