

ABSTRACT

Linwood McAuley Sawyer. A FINE STRUCTURAL ANALYSIS OF 8-DAY CHICKEN COMB AND COMB-FEATHER CHIMERAS. (Under the direction of Dr. Irvin E. Lawrence, Jr.) Department of Biology, August, 1973.

Epithelial-mesenchymal interactions in 8-day chick skin were studied in chimeras prepared for electron microscopy. Chimeric grafts were formed by enzymatic separation and recombination of comb epidermis on feather dermis and feather epidermis on comb dermis. The chimeras were grown on the chorioallantoic membrane for 24, 48, and 72 hours.

Enzymatic separation of the tissues removed the basal lamina. The dermal fibroblasts did not retain their characteristic epidermally oriented pattern. Microfilament bundles in the fibroblasts were affected, and collagen was disorganized. After recombination the basal lamina was gradually reformed, dermal cells resumed their normal oriented pattern and anchor filaments were also modified. Since acid mucopolysaccharides have been implicated in epithelial-mesenchymal interactions, ruthenium red was used to demonstrate their presence. Acid mucopolysaccharides were observed in the basal lamina of 8-day comb epidermis, but were not apparent in the basal lamina of 8-day feather epidermis. These morphological findings provide further evidence for an inductive role for epidermis at 8 days. This inductive activity may be expressed through differences in the matrix material.

A FINE STRUCTURAL ANALYSIS
OF 8-DAY CHICKEN COMB
AND COMB-FEATHER CHIMERAS

A Thesis

Presented to

the faculty of the Department of Biology
East Carolina University

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts in Biology

by

Linwood McAuley Sawyer

August 1973

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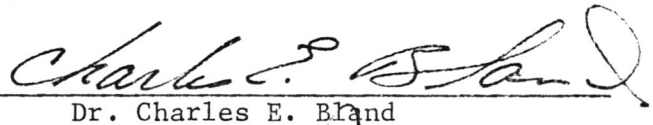
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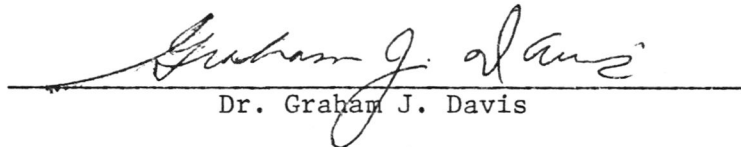
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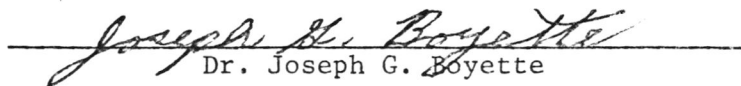
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DEDICATION

This thesis is dedicated to my wife, Jean, who has been most understanding during its completion.

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INTRODUCTION

The results of recombination grafts of chicken comb and feather primordia indicate that the epidermis may have an inductive role by the eighth day (Lawrence, 1971). If day eight is a critical period in development of chicken skin, fine structural changes occurring when 8-day (stage 34) epidermis is in contact with 8-day dermis might reveal morphological evidence for an information communication network between the two interacting tissues. Studying changes in such cellular components as microfilaments, microtubules, junctional complexes, coated vesicles, oriented organelles; and such extracellular materials as collagen, mucopolysaccharides, and anchoring filaments may provide insight on how information is transmitted between the two developing tissues.

It has been reported that interaction between two different tissues may lead to the formation of a matrix with elements not present in either of the original matrices (Grobstein, 1954). If comb epidermis does have an inductive capacity at 8 days, interaction with a new matrix may be expressed in new forms of cellular architecture. The purpose of this study is to provide morphological evidence for an inductive role of epidermis at eight days by observing fine structural changes in eight day chimeric grafts.

THE REVIEW OF LITERATURE

Comb Development: The comb, which is a featherless type skin, first appears after about seven days of development (stage 30) as an ectodermal elevation which results from rapid proliferation of the underlying mesenchyme. The proliferation of mesenchyme results in localized condensations of cells forming a compact ridge. Thus a primary source of comb tissue appears to be localized mitotic activity rather than cell migration from other areas (Lawrence, 1963). During the formation of the comb primordium, the ectoderm thickens to form a two layered epidermis consisting of a periderm and a germinative basal layer. At eight days of development (stage 34) the comb primordium has a network of fibers extending from the base of the comb elevation to the periphery. Later these fibers condense along the vertical axis of the comb and form the core. Transverse fibers develop during this period, forming a loose "net-like" system of fibers extending outward between the central core and the epidermis. After 12 days of development (stage 38) the dermis becomes trilayered (Lawrence, 1963).

Feather Development: The organogenesis of feather is similar to the formation of comb in many respects. The feather primordium (placode) first appears on the dorsal surface at 6.5 to 7 days of development (stage 30) as localized thickenings (Kischer, 1968). The localized thickenings appear when the loose aggregates of mesenchyme condense. Controlled mitosis and cellular rearrangement seem to be responsible for the formation of the dermal condensation during this period (Rawls, 1963; Wessells, 1965).

The basal layer of the epidermis forms thickenings (stage 28) or "spurs" that extend down into the mesenchyme (Wessells, 1965; Kischer, 1968). Fiber-like processes (anchoring bundles) extend from these areas of the basal epidermis deep into the dermis. Some of these anchoring filament bundles may terminate in the area just above the neural tube. Anchoring fibers occur in the skin during the period of placode formation (stages 29-34) and 24 hours later when the elevation of the epidermis occurs (stage of elongation) they are no longer apparent in the same region (Kallman et al., 1967).

Recombination Experiments: Wessells (1965) points out that "recombination experiments can only ask whether the mesoderm or epidermis in combination with some other reactant has the capacity to form feather germs. It cannot define the stage of inductive activity due to the fact that following recombination there is often major rearrangements of cells and tissues before feather germs are again established." It should be noted that recombination experiments can give some indication of the stage at which interaction between two tissues occur.

The results of recombination grafts of comb and feather primordia indicate that the epidermis may have an inductive role between seven and eight days (Lawrence, 1971). Comb dermis of 5 to 13 days can induce comb in tarsometatarsal epidermis of 8 to 13 days and mid-dorsal epidermis of 5 to 8.5 days. Lawrence (1963) found that mid-dorsal epidermis (7.5 days) with formed feather placodes in contact with 4 to 7.5 day comb dermis does not form feathers. Comb dermis does not erase feather induced in 8 to 8.5 day mid-dorsal epidermis and scale induced in 8 to 13 day tarsometatarsal epidermis. Mid-dorsal dermis of 5 to 8.5 days shows an

inability to erase comb induced in 8 to 10 day and all 13 day comb epidermis. It was concluded that the epidermal potential does change and that the comb epidermis may have an inductive role by eight days (Lawrence, 1971). Sengel (1958) reports that the dermis induces transformation of the ectoderm into epidermis and determines the polarity of the epidermis (fixed by 6 days). The transformation of the ectoderm results in the formation of the epidermal placode and determines the regional character of epidermal differentiation. In the feather the epidermis orients feather germs and induces differentiation and growth of the germ (from 6 day). At 8 days the epidermis induces dermal cells to form the axis of the feather germ and to furnish an epidermal sheath which is capable of inducing the dermis.

Wessells (1965) reports that control cultures of whole skin placed in a protein free defined medium develops feathers only if the explant came from stage 28+ chick. The only morphological difference found between stage 27 skin which never forms feathers in vitro and stage 29 skin which always forms feathers is the presence of a condensed dermis. Wessells suggests that cultures of recombined skin indicate that both the epidermis and dermis of the dermal condensation stage have an inductive capacity in feather. Rawls (1963) finds that 8 to 8.5 day mid-dorsal epidermis in contact with 13 to 15 day scale dermis develops feather unlike 5 to 7.5 day epidermis. This may indicate a change in the competence of the epidermis at 8 to 8.5 days. Rawls (1963) concludes that the formation of aberrant feather is the only indication of an effect of the strong dermal stimulus. Grafts of epidermis of 8 to 13 days show some epithelial specificity. Grafts produced with skin younger than

eight days develop according to the regional specificity of the dermis. Grafts produced with epidermis of 8 days and older develop according to the specificity of the epidermis. This is suggestive of an inductive role for bird epidermis (Lawrence, 1971).

Intercellular Matrix: If the epidermis does have an inductive role at eight days it seems that the basal lamina might very well have an active role in the expression of new characteristics. Numerous studies suggest that the extracellular matrix (glycocalyx) located at the interface between the embryonic epithelium and mesenchyme may play an active role in morphogenesis (Bernfield and Banerjee, 1972). The matrix material is composed of the basal lamina, collagen, glycoprotein, neutral mucopolysaccharides, and acid mucopolysaccharides. These components are thought to be of epidermal origin or produced as a result of epidermal action on the dermis (Fawcett, 1966; Briggaman et al., 1971).

Grobstein (1961) suggests that large "molecular mediators" may play an important role in induction. These materials are normally closely associated with cell surfaces but are able to exist away from the cell surfaces for limited distances. The concept of "molecular mediators" is in agreement with the thesis that the intercellular matrix might be directly involved in interactions between two tissue interfaces. It has been suggested that the mesenchyme supplies the precursors and the epithelium supplies polymerizing and organizing factors. Therefore, the differentiation of a structure might result from interaction of intercellular matrices of the two interacting tissues. Thus induction would not be the result of direct transfer of substances from one interacting tissue into the other, but would be dependent on the

interaction of surface associated materials which would affect the involved cells. New properties of the basal lamina may arise through controlled polymerization of precursors on one side by the other side. Alternatively new properties of the basal lamina may arise from a combination of precursors from both sides to produce new macromolecules (Grobstein, 1961).

Grobstein (1954) emphasizes the importance of the intercellular matrix by pointing out that it is a "labile intercellular continuum" which can undergo local changes in permeability and other properties. It has a high degree of specificity, is closely responsive to the genotype and is able to condense to higher levels of order thus providing boundaries and interfaces. Grobstein (1954) also suggests that when matrices of two different tissues are compatible, adhesion might occur, thus producing a matrix with new properties which could alter the properties of the interacting tissues. Interaction at the molecular level might lead to the formation of new elements not present in either of the original matrices.

Thomson (1964) has demonstrated the presence of acid mucopolysaccharides during the formation of scales in chick skin. The acid mucopolysaccharides were localized in the dermis and at the basement membrane during the formation of the fibrillar network. She suggests that acid mucopolysaccharides play a structural role.

Sengel (1962) found that mucopolysaccharides are particularly abundant in the dermis before feather formation. When the primordium appears, the concentration of mucopolysaccharides increases in the lenticular mesenchymal component of the primordium. Later the

mucopolysaccharides are concentrated at the base of the primordium and disappear from the apex. Observations of the development of skin in the scaleless mutant suggest that the dermal-epidermal junction has altered properties since mutant epidermis can be separated from the dermis between 10 and 15 days without EDTA treatment. Scaleless shank skin appears to be unable to participate in the formation of a normal basal lamina complex. This may result in the failure of the scale placode formation which in normal skin occurs between stages 35 and 36. Recombinations between epithelial and mesenchymal components of scaleless and normal skin suggest that the defect is in the epithelial component (Goetinck and Abbott, 1963; Sawyer et al., 1972). Since recombinations of normal and scaleless epidermal and dermal components have shown that the defect is in the epidermis, it seems reasonable to conclude that scale placode formation is controlled by the epithelium rather than the mesenchyme (Sawyer et al., 1972).

Bernfield and Banerjee (1972) report that the disruption of the basal lamina in the embryonic salivary gland results in the loss of morphology and cellular orientation and that morphogenesis is not initiated until the basal lamina has reformed. By the use of autoradiographic techniques, newly synthesized acid mucopolysaccharides have been shown to be localized in the areas of morphogenetic activity (Bernfield and Wessells, 1970). Wessells (1970) finds that the greatest amount of collagen is localized in "morphogenetically quiescent" areas. This would agree with the idea that collagen maintains morphology (stabilizing tendency) by providing support to the intercellular matrix in the form of tridimensional networks (Dische, 1970).

Flint (1972) has suggested that there is an inverse relationship between concentrations of mucopolysaccharides and the amount of collagen found in rabbit tendo-achilles undergoing repair. In the initial stages of repair there is an increase of unsulphated mucopolysaccharides such as hyaluronic acid. Later during repair of the divided tendon, non-sulphated mucopolysaccharide is replaced by sulphated material which appears to be associated with the reaggregation of collagen fibers. As the reaggregation of collagen occurs in a later phase of the repair period, the amount of mucopolysaccharides decreases. These observations agree with the finding that hyaluronic acid will disperse collagen which is normally strongly bound by chondroitin or dermatin sulphate (Flint, 1971).

Bernfield (1970) suggests that the mesenchyme is the major source of epithelial collagen and that the epithelium influences the amount of collagen in the mesenchyme. There is a possibility that extracellular materials produced by the epithelium play a major role in induction (Hay, 1964). In the development of feather, dermal cells become aligned along lattice-like tracts of collagen (Stuart and Moscona, 1967). It has been suggested that the formation of the basement membrane is the result of the transfer of soluble tropocollagen from the mesenchyme where it is synthesized, to the epithelial surface where it is polymerized (Kallman and Grobstein, 1965). This is in agreement with the idea that a variation in the composition of the mucopolysaccharide fraction and the degree of sulfates in different tissues could act as morphogenetic agents by influencing the distribution and organization of collagen fibrils (Meyer, 1960). Polyanions such as glycoproteins and acid

mucopolysaccharides found in the ground substance may be involved in the formation of abnormal collagen fibrils (Dische, 1970; Silberberg, 1970).

The interaction properties of tropocollagen are altered by treatment with proteolytic enzymes such as pepsin. Such treatment liberates the terminal covalently bound peptides and the linkage between alpha chains. The alpha chains are covalently linked to form beta chains. This suggests that it is the protease labile portion of the tropocollagen molecule that participates in the polymerization of collagen, rather than the non-labile portion of the molecule (Rubin et al., 1963). Collagen fibrils grow in diameter by apposition of new tropocollagen molecules (Dische, 1970). The short alpha chain may play a role in the inductive interactions during epithelial formation (Dische, 1970).

Acid mucopolysaccharides occur as large polymers and are covalently linked to protein. The polyanionic properties are due to sulfate ester and carboxylic acid groups (Bernfield and Banerjee, 1972). Pease (1971) has suggested that the interactions between carbohydrates and collagen at different levels of organization involve different bonding mechanisms and carbohydrate molecules of different sizes. The presence of divalent cations may provide intermolecular cross-linkages and thus force a gel formation. A monovalent cationic environment would result in sols. Such a system would allow the association and aggregation as well as disaggregation of collagen fibers.

MATERIALS AND METHODS

Experiments were performed with White Leghorn eggs purchased from Castlebury Poultry Farm in Apex, N. C. Eggs were held at 17°C prior to incubation in a forced air incubator with a controlled humidity and temperature of approximately 37.5°C. Donor eggs were incubated a period of 7 to 8 days for stages 33 and 34 embryos (Hamburger and Hamilton, 1951). Host eggs were incubated for a period of 9 to 11 days and prepared for chorioallantoic grafting (Lawrence, 1963).

The entire comb primordium and feather tracts from the mid-dorsal area were removed intact and cut to an approximate block size of 1 X 1.5 mm. The tissue blocks were cut with iridectomy scissors and transferred with a Spemann pipette to sterile watch glasses containing a 1% trypsin (Difco 1:250) in Tyrode solution. The tissues were incubated in trypsin for 1 to 1.5 hours at 4°C.

After trypsinization, the tissues were pipetted into a cold solution of 1:1 egg albumen in chick Ringers with 0.05 mg/ml of streptomycin sulfate and 100 units/ml of penicillin (Rawls, 1963). Chimeras were assembled from the isolated comb and feather regions. Using watch maker forceps and glass needles the epidermis from both isolates was removed and placed on the opposite dermis to allow physical contact (Fig. 1).

The chimeras were removed from the medium with a small spatula and watch maker forceps, and placed on the chorioallantoic membrane (CAM). Excess fluid was removed with a Spemann pipette to insure good contact with the membrane. A round 22 mm glass coverslip was

placed over the hole in the host egg and sealed with paraffin.

After 24, 48 and 72 hours of development on the CAM, the respective grafts were removed with iridectomy scissors and a Spemann pipette. The chimeras were fixed in cold 1.2% glutaraldehyde in Millonig's buffer at pH 7.6 for 30 to 60 minutes and washed in two changes of buffer for one hour. Tissues were post fixed in 2% osmium in Millonig's buffer at pH 7.8 for 20 minutes, dehydrated and embedded in Araldite 6005. A second series of grafts were stained en block with ruthenium red (RR) utilizing Luft's (1971) procedure. Tissues were fixed for one hour in a solution containing equal parts of 3.6% glutaraldehyde, 0.2 M cacodylate buffer, pH 7.4, and RR stock solution containing 10 mg/ml in water. Tissues were washed in three changes of 0.15 M cacodylate buffer for ten minutes. Tissues were post fixed for 3 hours at room temperature in a solution containing equal parts of 4% osmium in distilled water, 0.2 M cacodylate buffer, pH 7.4, and RR stock solution 10 mg/ml in water. The tissues were rinsed in buffer, dehydrated and embedded in Araldite 6005. Sections were cut with a Reichart OMU-2 ultra-microtome and a diamond knife. Sections were stained with uranyl acetate and Reynolds lead citrate (1963) and examined on an Hitachi HS-8 electron microscope.

RESULTS

Normal Comb

The chicken comb is an integumentary derivative composed of an epidermis and a dermis. The epidermis of 8-day comb consists of a thin layered periderm, usually one to two cell layers thick, and a basal cell layer of low columnar cells (Fig. 2). Microvilli and marginal folds occur along the free surface of the periderm (Fig. 3). On the other surface of the periderm tight loops are formed by interdigitation of processes (Figs. 2, 4). In the basal layer, the low columnar cells have large elongate nuclei (almost ovoid in appearance) perpendicular to the basal lamina (Fig. 2). Throughout the epidermis coated vesicles are present and are conspicuous in areas adjacent to the basal lamina (Fig. 5). Ribosomes are numerous in the epidermis and the endoplasmic reticulum is fine and not swollen. There are electron dense mitochondria in both layers of the epidermis. The basal lamina is trilaminar, having a fuzzy appearance along the dermal interface (Figs. 5, 6).

A subepidermal space separates the epidermis from the underlying dermis. This space contains a network of fine dermal cell processes which extend toward the epidermis, bending at right angles to that tissue and then running parallel to the epidermal surface. From the basal lamina connective tissue fibers originate and extend inward (Figs. 6, 7). Anchoring filaments often occur between dermal cell processes (Fig. 8). Deeper in the dermis elongated dermal fibroblasts extend toward the epithelium in a perpendicular arrangement. It is

the fine processes of these cells that reach the basal lamina and assume the parallel orientation. These cells contain large bundles of microfilaments which run parallel to this long axis (Fig. 9). Collagen bundles form parallel to the long axis of these fibroblasts and appear in vertical, horizontal, and oblique planes.

Normal Feather

The papillary area of feather skin is similar in fine structure to comb. It differs from comb, however, in the organization of collagen and some unidentified filaments that may be the anchoring filaments described by Kallman (1965) and Kischer (1968). The collagen bundles are more irregular. The anchoring filaments have a dark central core surrounded by an electron lucent matrix (Fig. 10). A detailed description of feather development is given by Kischer (1968) and Matulionis (1970).

Separated Tissue

The epidermal cells of separated tissue maintained close contact by means of cell interdigitations and desmosomes (Fig. 11). Its fine structure was much the same as that found in normal tissue. A few aberrant cilia and centrioles were present in both the periderm and basal layer. The Golgi was present (Fig. 11). Extensive cytoplasmic projections protruded from the basal layer of the epidermis, however, and the basal lamina was absent (Fig. 12).

The dermis of separated tissue had cells that appeared to have rounded-up and their numerous cytoplasmic projections withdrawn (Fig. 13). The microfilaments in these cells appeared as conspicuous aggregates (Fig. 14). Peculiarly oriented ribosomes were observed in several

cases (Fig. 15). This type of pattern is known to occur in chicken embryos when incubation is ceased and the eggs are allowed to cool slowly. Watson (1970) suggests that this type of aggregation indicates that protein synthesis has temporarily stopped. Collagen appeared disorganized in the separated comb dermis. Anchoring filaments in the separated feather dermis were still present and remained unchanged in appearance.

Recombined Tissues

Tissues that had undergone tryptic separation and then were recombined for a period of 24 hours showed a distinct change from tissues that had only been separated with trypsin. The extensive cytoplasmic projections noted above were no longer present. A basal lamina had reformed but was discontinuous and did not yet have the trilaminar appearance of normal tissue (Fig. 16). The desmosomes found in the periderm of feather epidermis were enlarged (Fig. 17). Anchoring filaments were also present in feather tissue.

CE/FD 24 Hours: The endoplasmic reticulum of comb epidermis and feather dermis grafts (CE/FD) after 24 hours on the CAM was swollen and had many associated ribosomes (Fig. 18). These chimeras had many polyribosomes and a few aberrant cilia with a 9 + 0 arrangement (Fig. 19). The mitochondria of the periderm were electron dense while those of the basal epithelium were enlarged and electron lucent (Figs. 18, 20). The basal lamina was fine, discontinuous, and contained amorphous material (Fig. 20). Where large space occurred between the dermis and epidermis there was an accumulation of amorphous material which coated many dermal cell processes (Fig. 21). Anchoring bundles

which resemble the type found in normal feather were frequently associated with the basal lamina and dermal cells (Figs. 22, 23). The Golgi was present in both epidermis and dermis.

Throughout the cytoplasm of the dermis there were numerous enlarged mitochondria. There were areas of dense collagen accumulation and some dermal cells showed extensive bundles of microfilaments (Fig. 24).

FE/CD 24 Hours: Feather epidermis of FE/CD grafts after 24 hours on the CAM had large vesicles near the free surface which may be remnants of mitochondria (Fig. 25) (Porter and Bonneville, 1968). The epidermis appeared to be similar to that of comb epidermis. Enlarged desmosomes with an extensive network of tonofilaments were present in the periderm (Fig. 26).

The rough endoplasmic reticulum of the comb dermis was swollen and less tortuous than that found in feather dermis of 24 hour chimeras. The mitochondria tended to be enlarged and collagen occurred in a few areas. The anchoring filaments found in the comb dermis were atypical in appearance and more closely resembled the anchoring filaments found in feather (Fig. 27). The basal lamina was discontinuous, and numerous free erythrocytes and phagocytes were present, demonstrating that the irritating effects of tryptic action were still present after 24 hours (Figs. 15, 28, 29).

48 Hour Chimeras: After 48 hours of development, the chimeras showed fewer signs of trauma. The subepidermal space had decreased and only occasional free erythrocytes and phagocytes were seen. Numerous ribosomes were found in the basal region of the epidermis and in the dermis. The basal lamina was fine and continuous (Fig. 30).

The rough endoplasmic reticulum was not as dilated as that found in 24 hour chimeras.

The dermis contained numerous cytoplasmic projections, and fibroblasts appeared to have elongated somewhat. The amount of amorphous material coating feather dermal cells had decreased (Fig. 31). Collagen appeared to be localized in specific areas of the dermis. Anchoring filaments occurred frequently in the dermis and along the basal lamina (Fig. 32).

72 Hour Chimeras: After 72 hours on the CAM, the chimeras were similar in appearance to the 48 hour chimeras. The mitochondria were more uniform in size. The basal lamina was continuous and had a trilaminar appearance (Fig. 33). The Golgi was more extensive in both epidermis and dermis (Fig. 34). Microfilaments resembled those found in normal tissue.

Ruthenium Red

The reaction with RR imparts a density on cellular surfaces that is very specific and easily distinguished from the reaction obtained with lead citrate and uranyl acetate (Luft, 1971). Utilization of RR demonstrated differences in the composition of the basal lamina of comb and feather tissue. In feather tissue the basal lamina was unstained and the dermis contained very little RR positive material (Fig. 35). Comb tissue gave a very intense reaction at the basal lamina with the dermis also showing a positive staining reaction (Fig. 36). After tissues had undergone tryptic separation, there was no RR positive material along the basal lamina (Fig. 37). Deeper in the

separated dermis some RR positive material was associated with the cells (Fig. 38).

Tissues which had been separated with trypsin and recombined demonstrated the same staining characteristics found in normal tissue. When feather was treated in this manner the epidermis had a negative reaction (Fig. 39).

Analysis of chimeric grafts gave results that were consistent with the staining reaction found in normal tissue. FE/CD chimeras had a staining reaction similar to the recombined feather tissue (Fig. 40). The basal lamina was not positive and very little RR positive material was associated with the dermis. CE/FD chimeras usually demonstrated a RR positive basal lamina and positive material was usually associated with dermal cells (Fig. 41).

Normal Feather: After treatment with RR, feather gave a negative reaction. The basal lamina and coated vesicles along its surface did not show the typical electron density attributed to the RR technique (Fig. 42). Dermal cells, anchoring filaments, and collagen fibrils were devoid of RR positive material (Fig. 42).

Normal Comb: Comb tissue gave an intense reaction when it was treated with RR. A slight reaction could be observed along the tight cell interdigitations found in the periderm, although on occasions this region was not stained (Fig. 43). Along the basal lamina there was a positive reaction and collagen fibrils could be found associated with this region (Fig. 44). RR positive material also coated dermal cell surfaces and collagen fibrils. Coated vesicles associated with the epidermis and dermis were stained and RR positive material could

be found along the shafts of cilia (Figs. 45, 46). Inner leaflets of the plasma membrane demonstrated a positive staining reaction (Fig. 47).

Separated Tissue: The thick area of the basal lamina stained in the normal tissue was no longer stained in the separated tissue (Fig. 48). The staining reaction of the basal cell surface more closely resembled the free surface of the periderm. Coated vesicles that occurred along the basal lamina demonstrated a positive reaction (Fig. 48).

Recombined Tissue: The material in the intercellular space stained positive for RR in regions where the basal lamina was positive. The positive RR reaction was found in this series more frequently than was found in feather tissue. Areas where the basal lamina was stained coated vesicles were also stained. There was a dense accumulation of collagen in this area which also demonstrated a positive reaction (Fig. 49). The majority of the basal lamina was RR negative and in these negative regions the coated vesicles did not show the positive reaction (Figs. 50, 51).

Recombined comb tissue was similar to normal comb tissue. The dermal cells were coated with a RR positive material and collagen was embedded in the material.

Chimeras: The basal lamina of 24 hour CE/FD chimeric grafts gave a positive RR reaction (Fig. 52). The dermal cells, anchoring bundles and coated vesicles demonstrated a positive reaction (Fig. 53). In some cases it appeared that the recesses of collagen fibrils were penetrated by the stain (Fig. 54).

The results after 48 and 72 hours on the CAM were similar. The basal lamina was continuous and collagen was associated with it (Fig. 55).

Coated vesicles in the epidermis and dermis were RR positive. The collagen showed a periodicity of binding with RR positive deposits (Fig. 56).

After 24 hours on the CAM, FE/CD grafts resembled the recombined feather series. The basal lamina was negative and occasionally positive material was seen in the dermis. After 48 and 72 hours on the CAM the basal lamina was continuous. The basal lamina demonstrated a negative staining reaction and the dermis was also negative (Fig. 57). In areas where the basal lamina was positive the dermis and collagen fibers below it demonstrated a positive reaction (Fig. 58).

DISCUSSION

Several epithelial-mesenchymal interactions are considered to be developmentally significant. Most early studies tried to determine at what time in the developmental sequence, important information was transferred between the two interacting tissues. New techniques and recent advances in molecular biology have made it possible to investigate more critically some of the mechanisms that may be involved in tissue interactions. Oriented organelles, such as filopodia, microtubules, and microfilaments; junctional complexes; coated vesicles; anchoring filaments; collagen; and acid mucopolysaccharides (MPS), as revealed by electron microscopy, are some of the fine structural details that have been cited as being involved in epithelial-mesenchymal interactions.

The mechanical properties of a tissue are influenced by the composition and physiochemical state of the matrix material (Myers et al., 1973). Luft (1971) has found that RR precipitates a large variety of polyanions by ionic interaction. He reports that RR fails to precipitate neutral polysaccharides and suggests that the density associated with cell coats is probably due to the presence of acid MPS. Myers et al. (1973) suggested that the dense globules of RR positive material bound to collagen and cell surfaces represent linear aggregations of acid MPS. RR then is considered an ultrastructural indicator of acid MPS. Many of the sites of RR localization in Luft's study were the same areas in which substantial mechanical forces were known to exist.

The epidermally oriented dermal filopodia with microfilament bundles may serve as retractable probes in an information communication network. Since the contractility of microfilaments is dependent on the availability of calcium ions and may be regulated by surface associated MPS, the concentration of MPS on the filopodia would implicate these processes as active probes (Gingell, 1970; Bernfield et al., 1972). The regular pattern of coated vesicles may be another means of cellular communication, since the MPS that lines these vesicles may have properties suitable for selective binding of particular classes of ions or molecules (Bennett, 1963). Anchoring filaments are also thought to play an important role in cellular communication. The occurrence of these filaments during early feather placode formation is a possible indication of some type of "initiatory activity" on the lower side of the epidermis (Wessells, 1965). Kallman et al. (1967) found that these filaments were present during the time of placode formation and were not apparent in the same region 24 hours later. Although the filaments found in the normal feather and all chimeric grafts done in this study differ from the anchoring filaments found in the earlier stages of feather placode formation they could be the same or related structures. They may represent shortened or dissolving anchoring filaments (Wessells, 1965). The filaments found in the chimeras resemble those of normal 8 day feather tissue, even when derived from comb dermis. Anchoring filaments in normal day 8 comb tissue had a uniform electron density.

Collagen is implicated in cellular communication (Dische, 1970). Collagen maintains morphology by providing support to the intercellular matrix in the form of tridimensional networks (Dische, 1970).

The disorganized appearance of collagen represents a change due to the removal of surface associated MPS (Bernfield and Wessells, 1970). Dische (1970) points out that polyanionic substances like MPS can regulate the distribution and diameter of collagen fibrils. This is in agreement with the idea that a variation in the composition of the MPS fraction and the degree of sulfates in different tissues could influence the organization of collagen (Dische, 1970; Meyer, 1960).

Enzymatic treatment at 4°C digests the basal lamina (Rawl, 1960; Hay and Dodson, 1973; Briggaman et al., 1971). The cytoplasmic projections which protrude from the epidermis after treatment with trypsin result from the removal of the basal lamina (Hay and Dodson, 1973). Huet and Herzburg (1973) suggest that the cytoplasmic projections found in the basal epidermis after enzymatic separation indicate a stress at the level of the cell coat. This is in agreement with the idea that the extracellular MPS coat on the surface of cells is of "mechanical importance". This layer combined with the inner cytoplasmic leaflet could provide the tensile strength which is not accounted for by the continuous lipid bilayer model of the plasma membrane (Luft, 1971). Alternately this type of phenomenon could represent a response to an unfavorable environment. There is evidence that MPS may regulate the in vitro phenomenon of contact inhibition (Pessac and Defendi, 1972).

Following recombination there is often a major rearrangement of cells and tissues before morphology of the developing structure is again established (Sengel, 1958). It has been shown that disruption of the basal lamina in embryonic salivary glands results in a loss of

morphology and that acid MPS-protein complexes within the basal lamina are required for maintenance of morphology and continuation of morphogenesis (Bernfield et al., 1972). Several studies indicate that MPS may play an important role in the development of various structures. Sengel et al. (1962) found differences in the distribution of acid MPS during feather development, which may be related to different cell orientations observed in such areas (Wessells, 1965). Thomson (1964) reported that acid MPS was localized in the dermis and basal lamina during the formation of scales in chick skin and suggested that they may play a structural role. Experiments with the skin from the scaleless mutant indicate that the failure to form scale placodes may be due to the failure of the epidermis to form a normal basal lamina (Goetinck and Abbott, 1963; Sawyer et al., 1972).

In surveying the results of the RR series it is apparent that there are differences in the basal lamina of the comb and feather tissue. The differences in the staining reaction can most likely be attributed to the presence of acid MPS. Variation in the composition of the intercellular matrix may be responsible for the initiation of morphogenetic patterns which would result in new phenotypic expression. The chimeric grafts indicate that the inductive activity of the epidermis at this stage may be expressed through differences in the basal lamina. Grafts of comb epidermis and feather dermis routinely show an accumulation of acid MPS in the basal lamina and in the dermis. This is in agreement with Sawyer's (1972) conclusion for the scale placode in that formation of the primordium is controlled by the epidermis rather than the dermis. The results of the RR series also indicate that the

basal lamina is a product of the epidermis, since it is completely removed by the separation technique and reforms with the staining properties of its respective epidermis. This finding would agree with the theory that the epidermis may have an inductive role during this stage.

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FIGURES

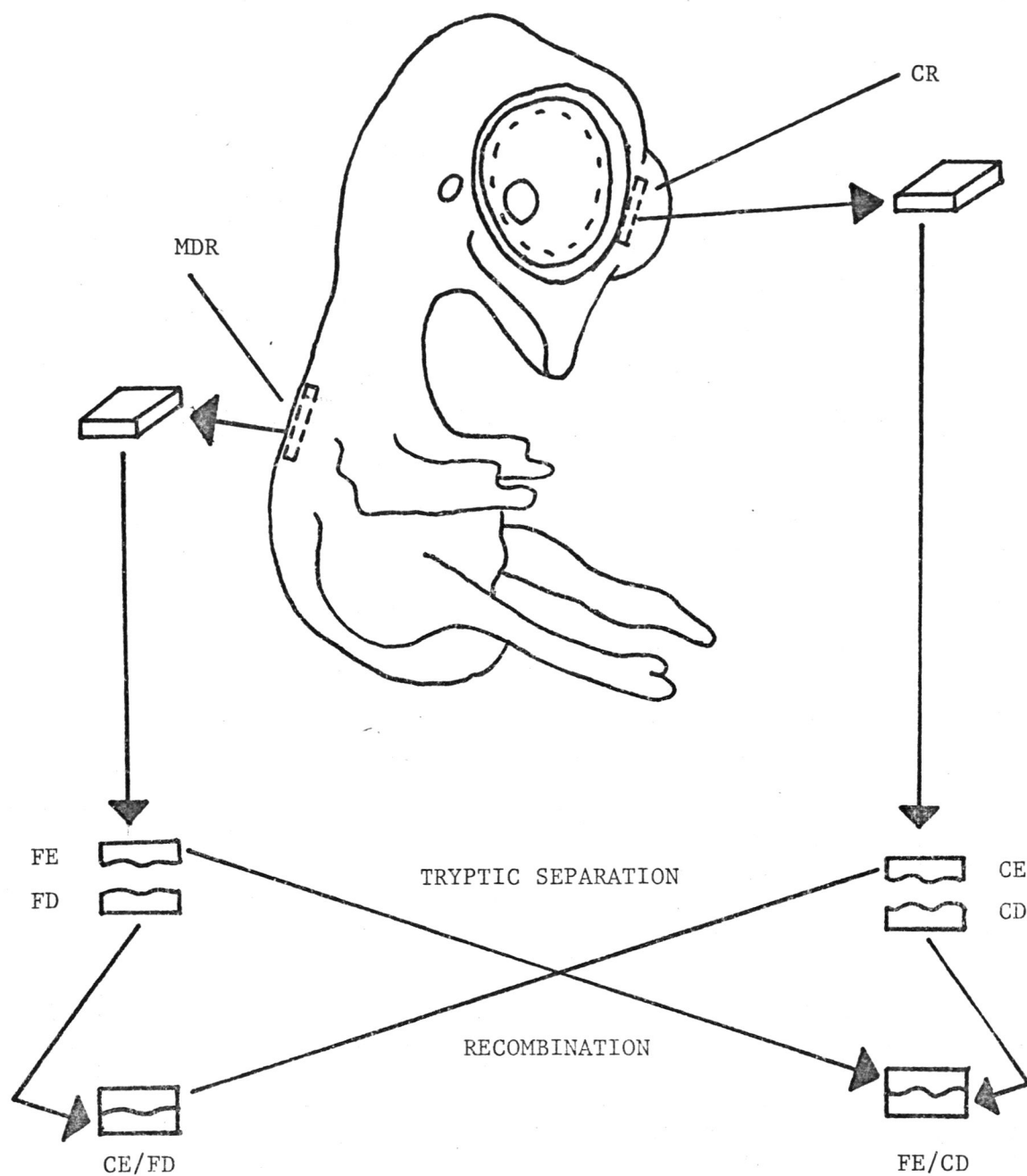


Figure 1. Procedure for preparation of recombination grafts. Comb region (CR), mid-dorsal region (MDR), comb epidermis (CE), comb dermis (CD), feather epidermis (FE), and feather dermis (FD).

Figure 2. Comb epidermis showing thin layered periderm (PE). Basal layer (BE) containing large nuclei (N). 8,400 X.

Figure 3. Marginal folds (MV) along the free surface of the periderm of the comb. 22,000 X.

Figure 4. Cell interdigitation (Id) in comb epidermis. 22,000 X.

Figure 5. Coated vesicles (CV) in the basal epidermis and along the basal lamina (BL). 32,000 X.

Figure 6. Basal lamina (BL) of comb epidermis. Dermal cell processes (CP) extending toward the basal lamina. 22,000 X.

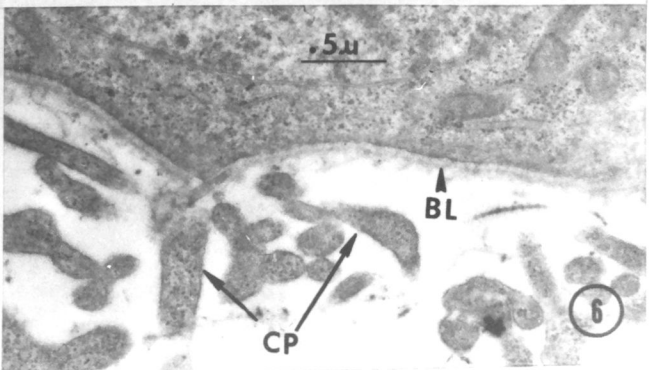
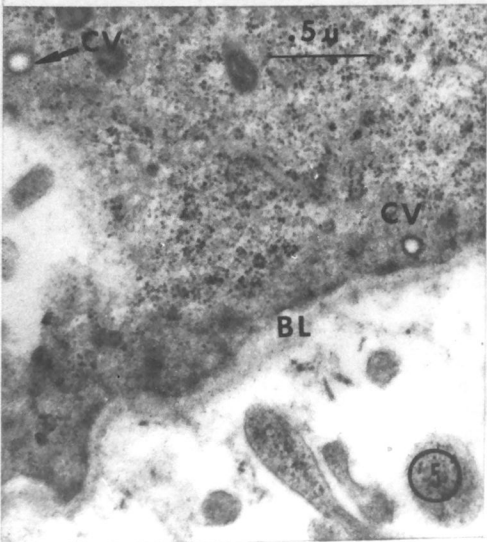
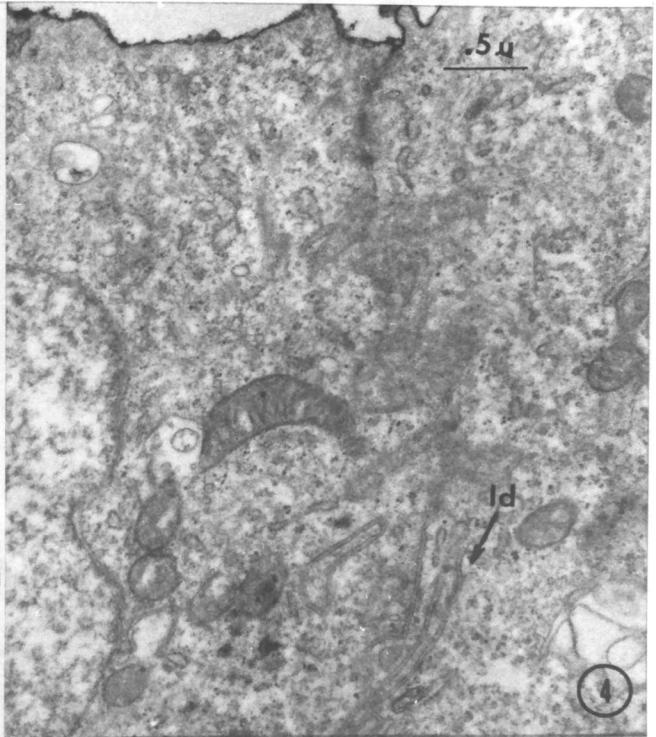
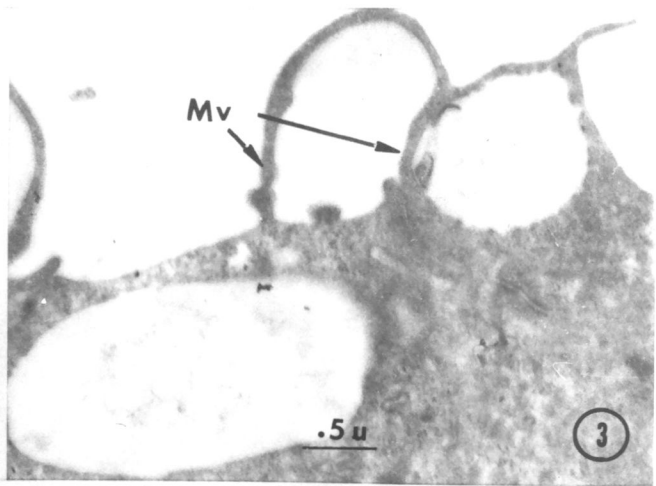
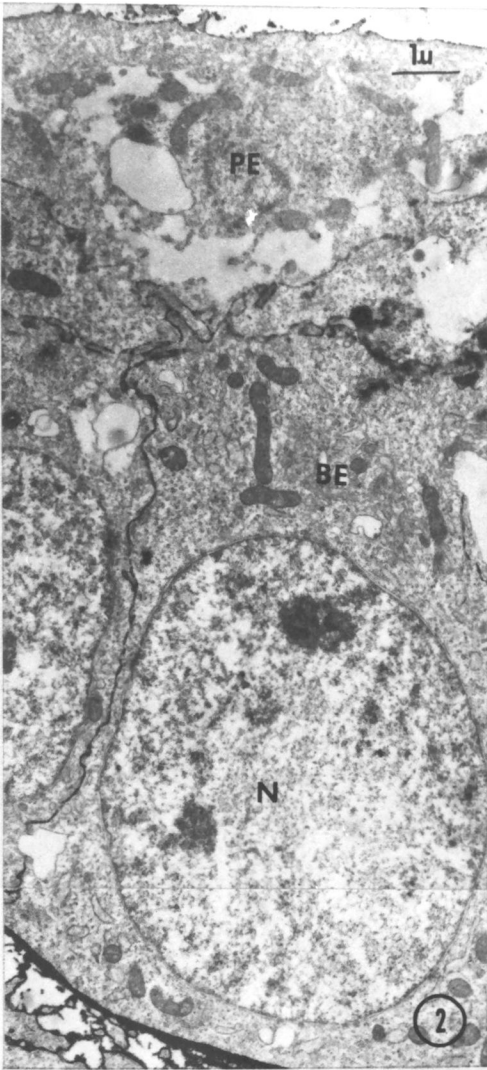


Figure 7. Dermal cell processes (CP) extending toward the basal lamina in comb tissue. 8,400 X.

Figure 8. Anchoring filaments (AF) in comb dermis. 90,000 X.

Figure 9. Dermal fibroblasts in comb dermis containing large bundles of microfilaments (MF). 22,000 X.

Figure 10. Anchoring filaments (AF) of normal feather dermis. 32,000 X.

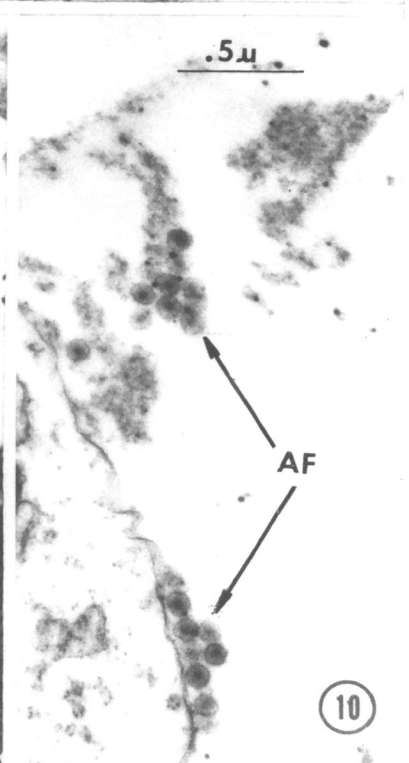
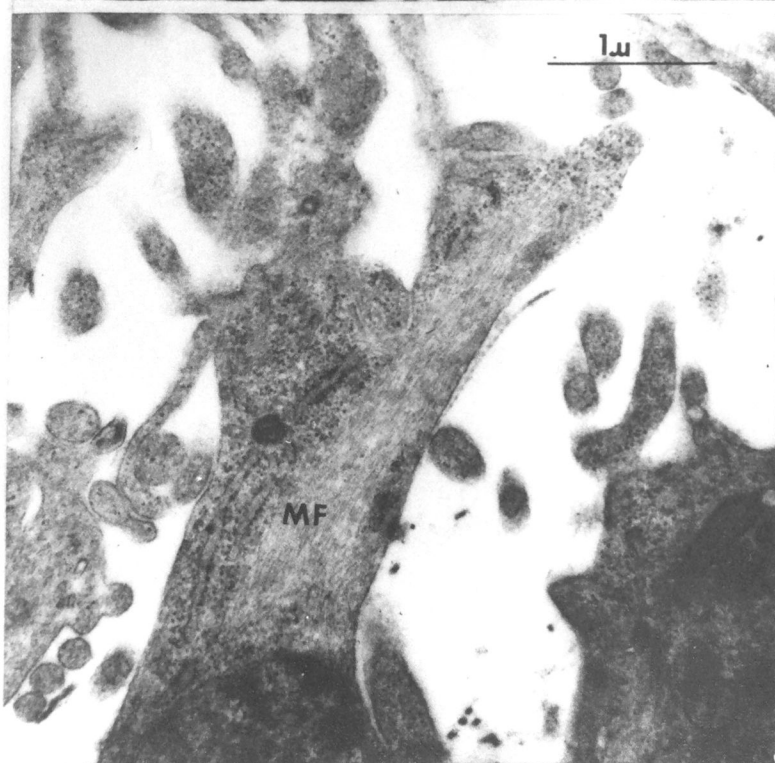
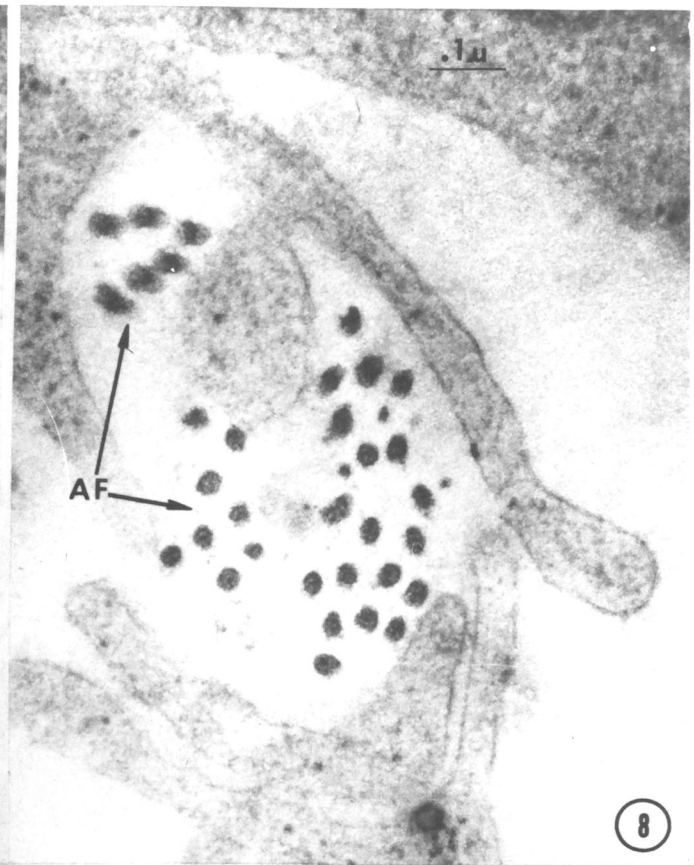
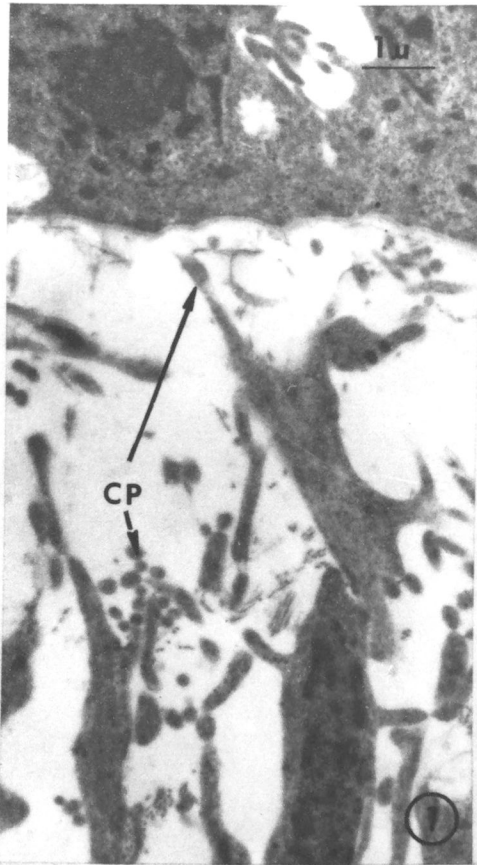


Figure 11. Separated comb epidermis showing cell interdigitations (Id), desmosomes (D), and Golgi (G). 19,200 X.

Figure 12. Separated comb epidermis showing the cytoplasmic projections found in the basal layer. 12,000 X.

Figure 13. Separated comb dermis showing withdrawn cytoplasmic projections (CP). 8,400 X.

Figure 14. Separated comb dermis showing aggregates of microfilaments (MF). 19,200 X.

Figure 15. Separated comb dermis showing oriented ribosomes (R). 19,200 X.

Figure 16. Recombined feather tissue showing the reformed basal lamina (BL). 8,400 X.

Figure 17. Recombined feather epidermis with enlarged desmosomes (D). 22,000 X.

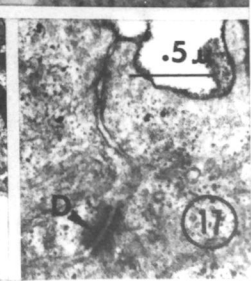
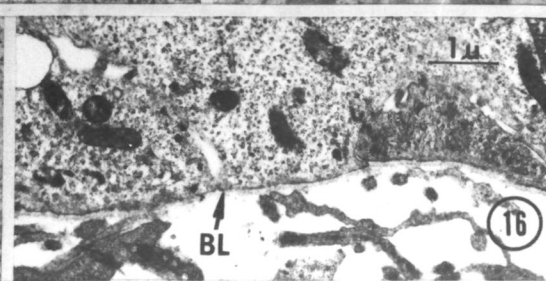
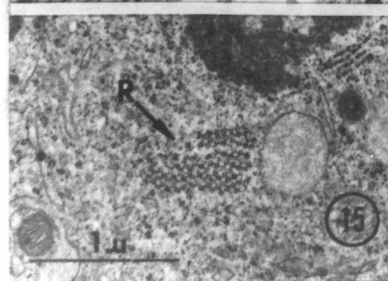
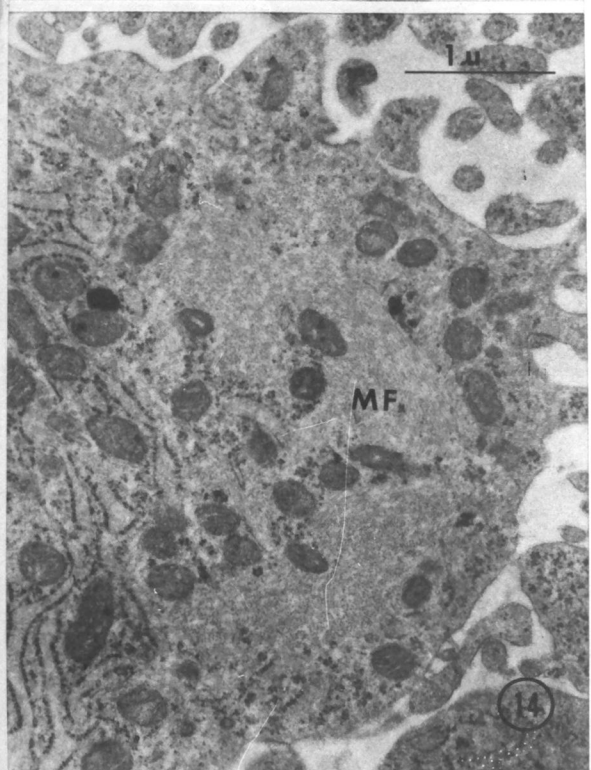
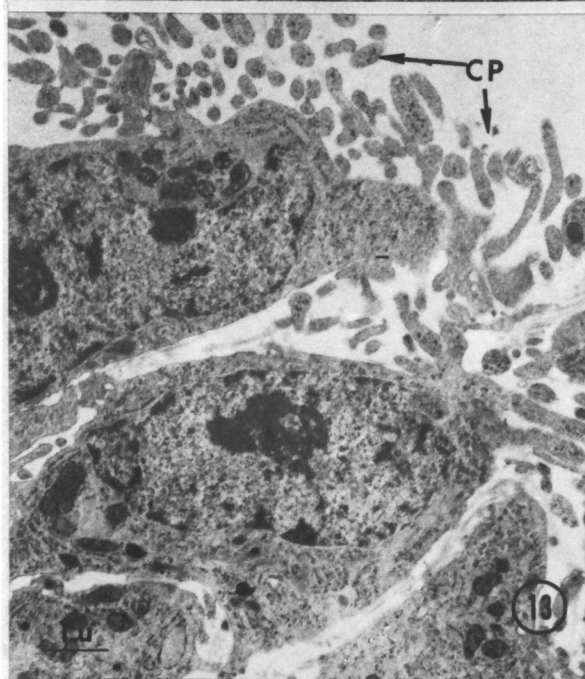
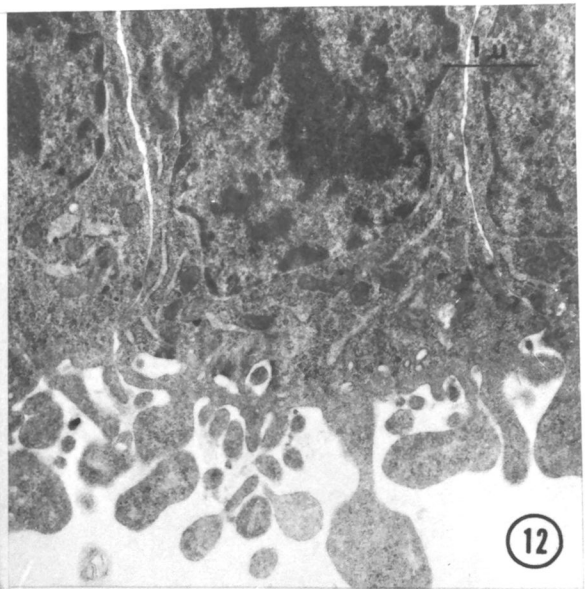
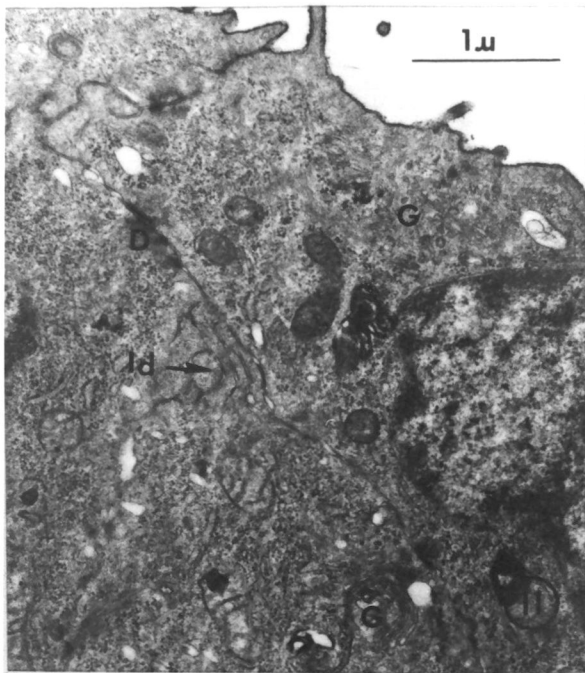


Figure 18. CE/FD chimera showing small electron dense mitochondria (M). 8,400 X.

Figure 19. CE/FD chimera showing aberrant cilium (C) with 9 + 0 arrangement. 36,000 X.

Figure 20. CE/FD chimera showing enlarged mitochondria (M) and basal lamina containing amorphous material. 8,400 X.

Figure 21. CE/FD chimera showing amorphous material (arrow) associated with dermal cells. 8,400 X.

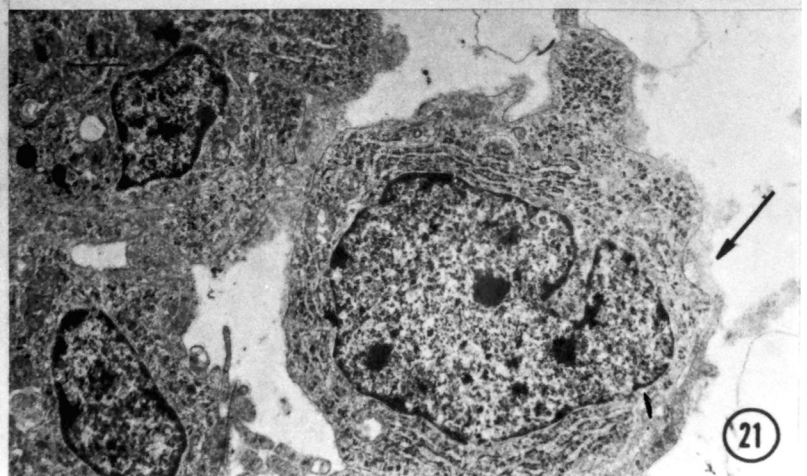
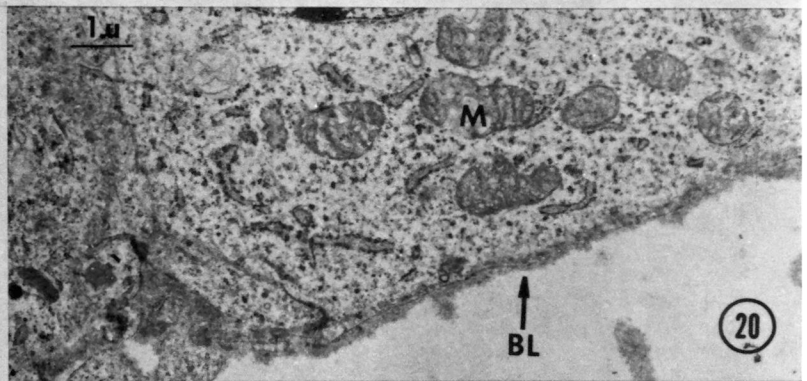
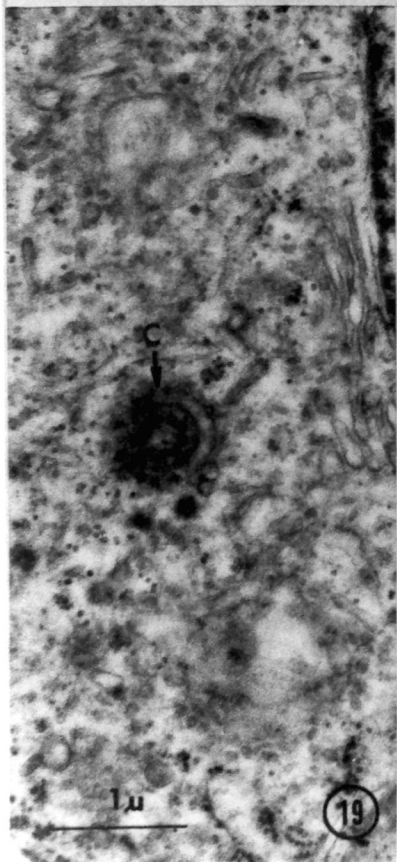
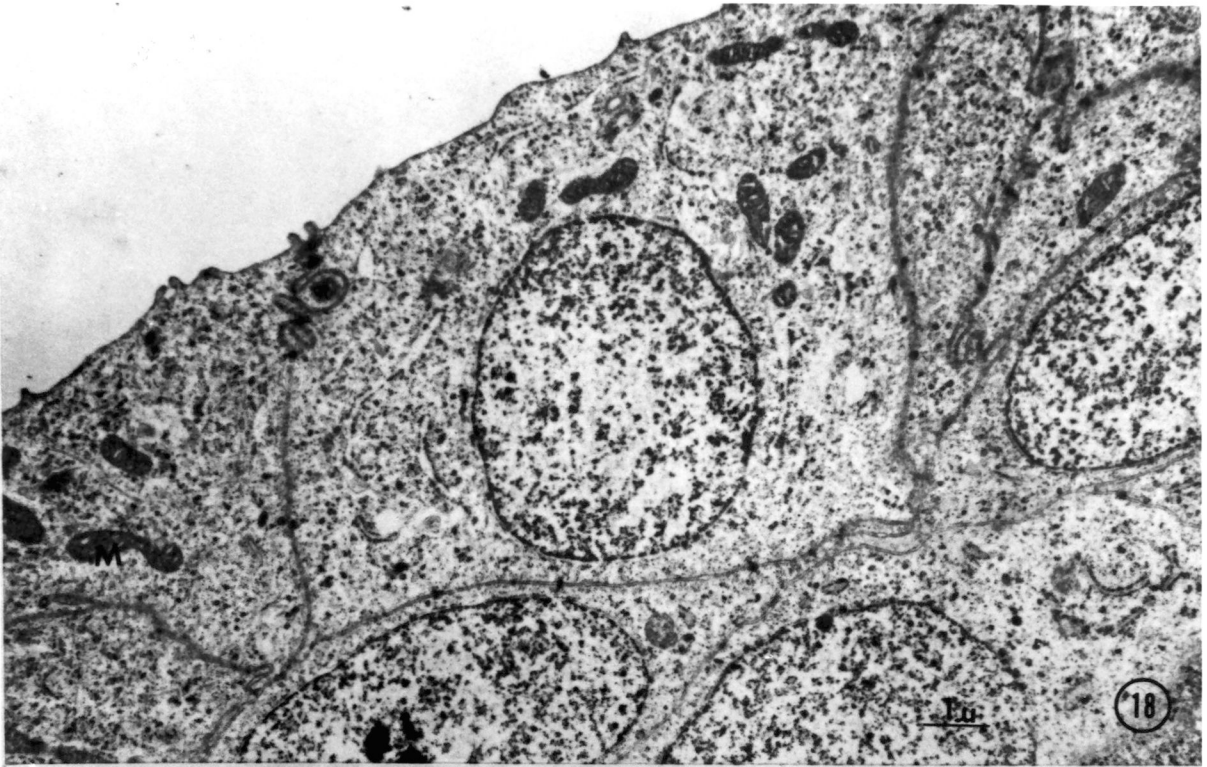


Figure 22. CE/FD chimera showing anchoring filaments (AF) in longitudinal section along the basal lamina. 15,800 X.

Figure 23. CE/FD chimera showing anchoring filaments (AF) in longitudinal and cross sections in the dermis. 36,000 X.

Figure 24. Dermis of CE/FD chimera showing microfilaments (MF), enlarged mitochondria (M), and collagen (Co). 10,500 X.

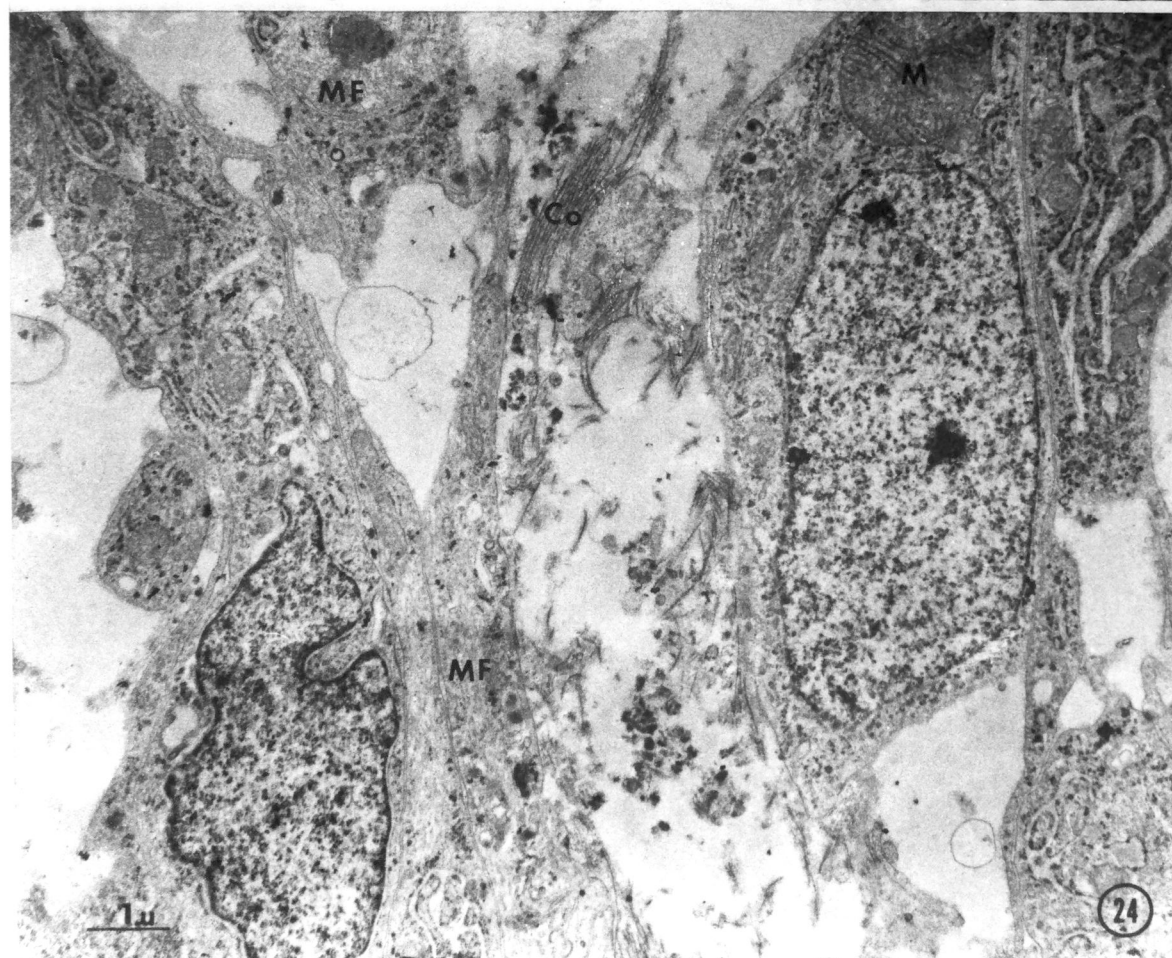
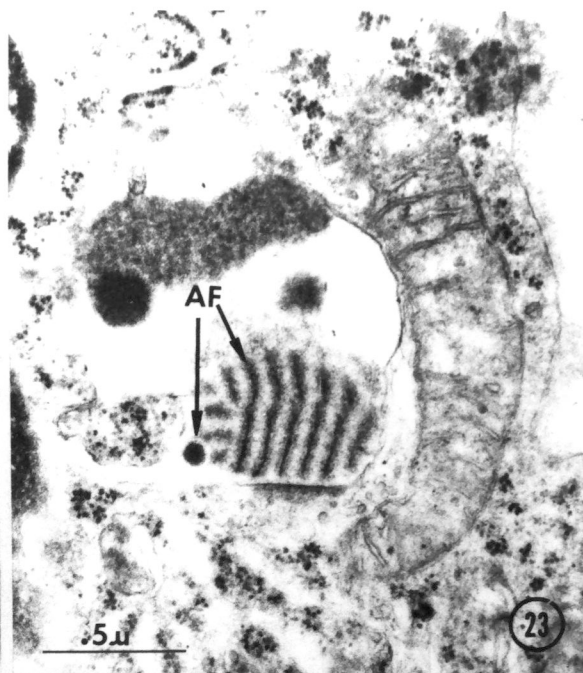
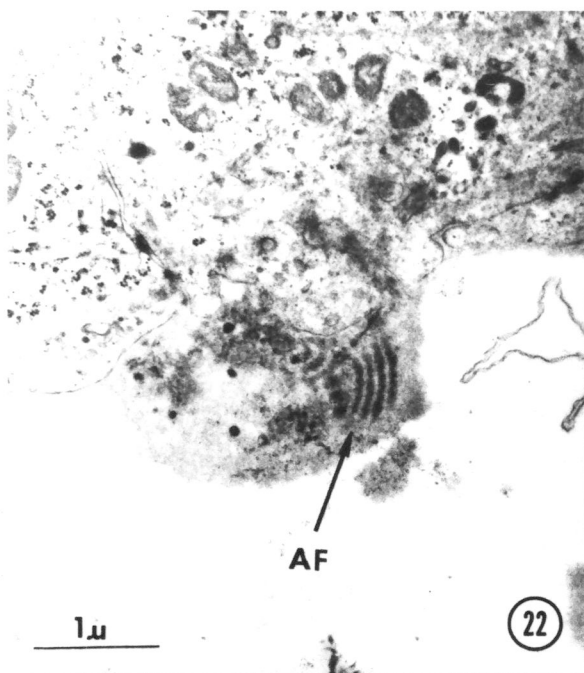


Figure 25. FE/CD chimera showing large vesicles (V) in the periderm. 66,000 X.

Figure 26. FE/CD chimera showing enlarged desmosomes (D) in the periderm. 22,000 X.

Figure 27. FE/CD chimera showing anchoring filaments (AF) in the dermis. 48,000 X.

Figure 28. FE/CD chimera showing free erythrocytes in the dermis. 8,400 X.

Figure 29. FE/CD chimera showing phagocytes in the dermis. 8,400 X.

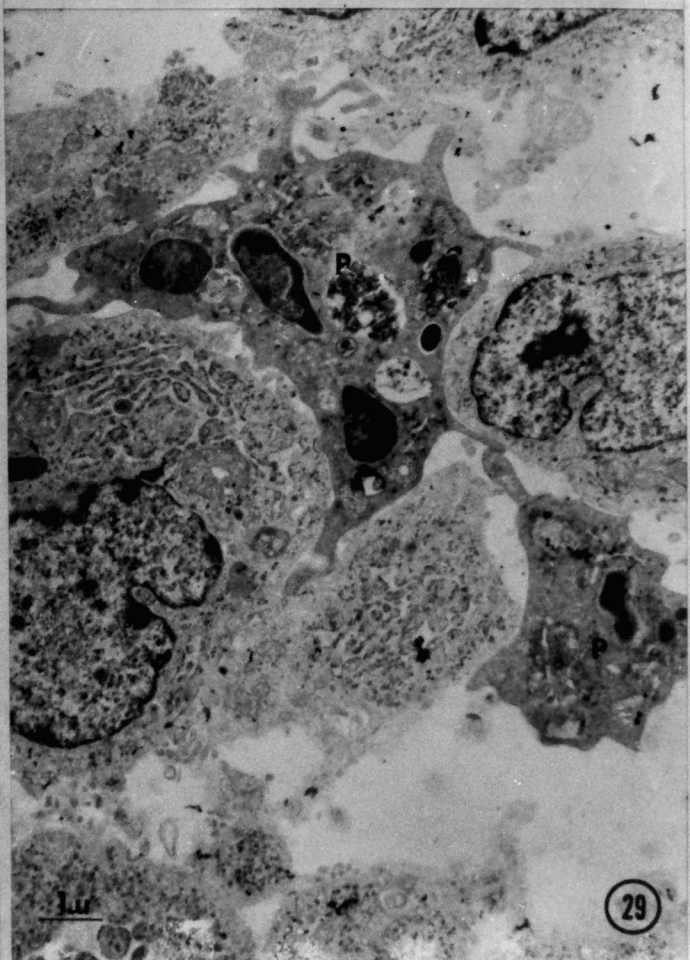
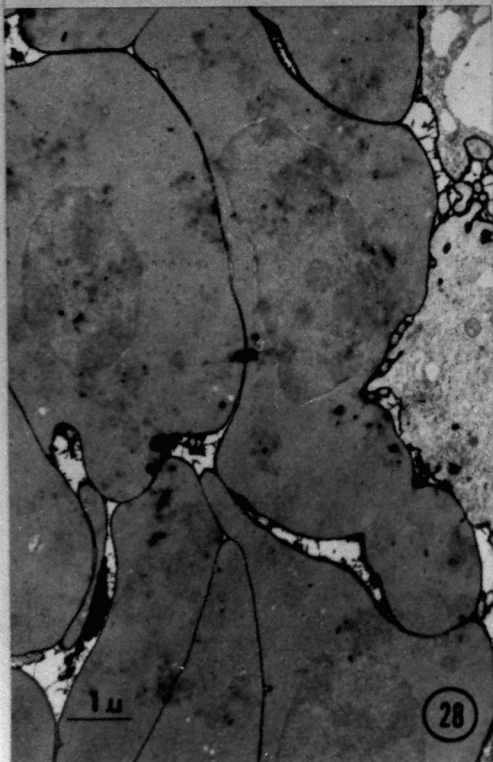
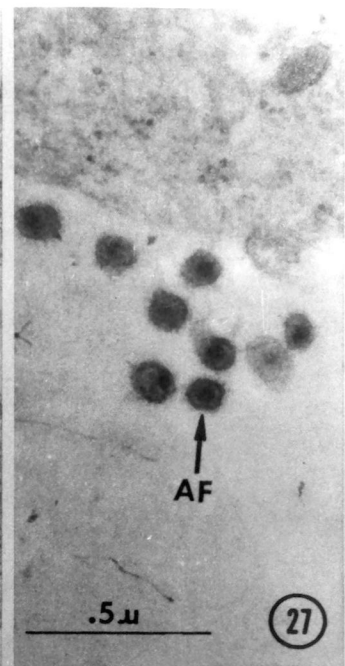
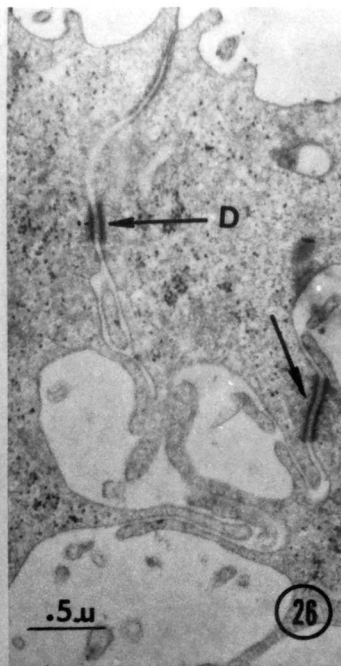
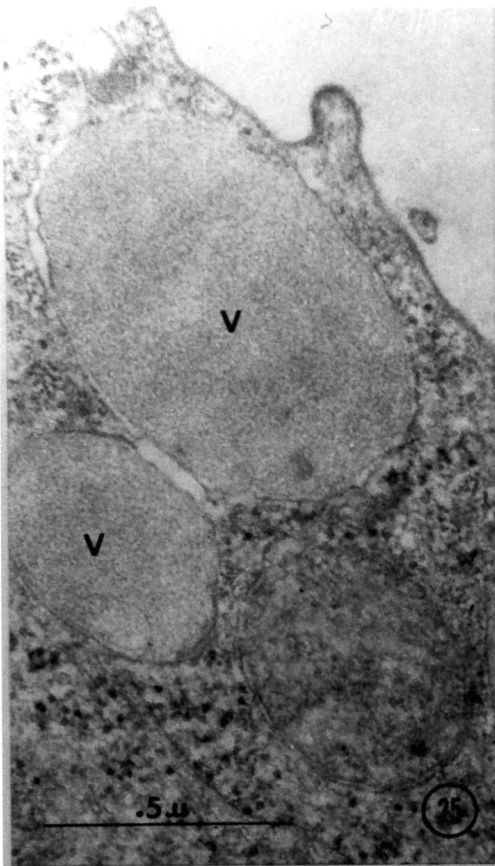


Figure 30. CE/FD chimera after 48 hours on the CAM showing the basal lamina (BL). 36,000 X.

Figure 31. CE/FD chimera after 48 hours on the CAM showing the subepidermal space. 8,400 X.

Figure 32. FE/CD chimera after 48 hours on the CAM showing collagen and anchoring filaments (AF) in the subepidermal space. 40,000 X.

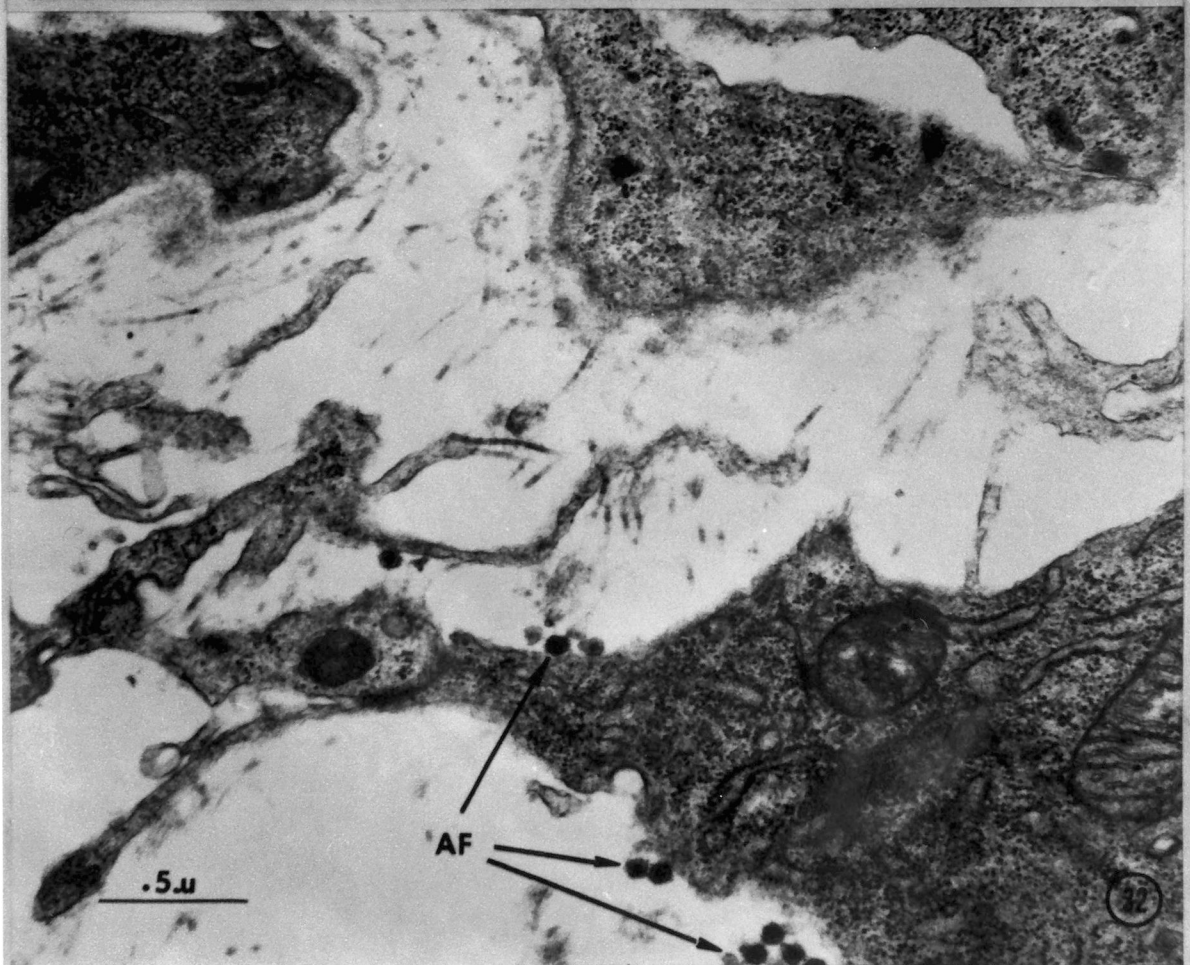
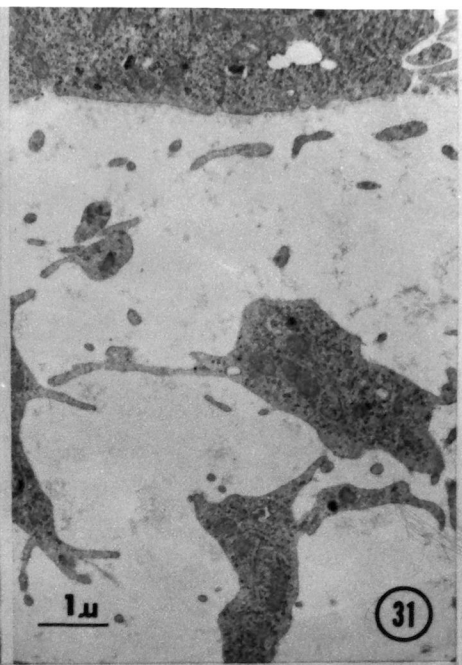
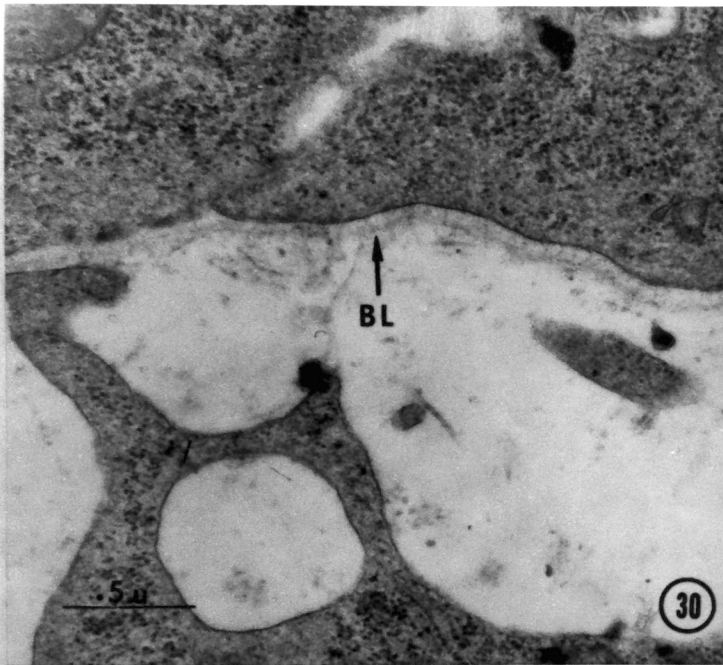


Figure 33. FE/CD chimera after 72 hours on the CAM showing mitochondria (M) in the basal epidermis, basal lamina (BL), and dermal cells. 10,500 X.

Figure 34. FE/CD chimera after 72 hours on the CAM showing extensive Golgi complex (G). 40,000 X.



Figure 35. Light micrograph of normal feather tissue stained with RR showing a negative reaction along the basal lamina (arrow). 406 X.

Figure 36. Light micrograph of normal comb tissue stained with RR showing a positive reaction along the basal lamina (arrow). 406 X.

Figure 37. Light micrograph of separated comb epidermis stained with RR showing a negative reaction along the basal lamina. 260 X.

Figure 38. Light micrograph of separated comb dermis stained with RR showing a negative reaction along the free surface of the dermis. 260 X.

Figure 39. Light micrograph of recombined feather tissue stained with RR showing a negative reaction along the basal lamina (arrow). 260 X.

Figure 40. Light micrograph of FE/CD chimera stained with RR showing a negative reaction along the basal lamina (arrow). 650 X.

Figure 41. Light micrograph of CE/FD chimera stained with RR showing a positive reaction along the basal lamina (arrow). 260 X.

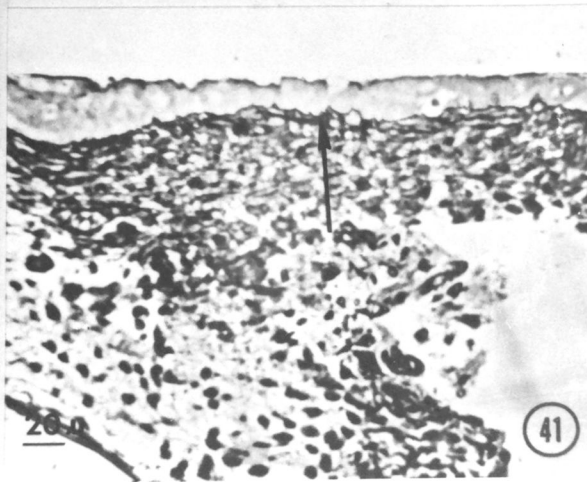
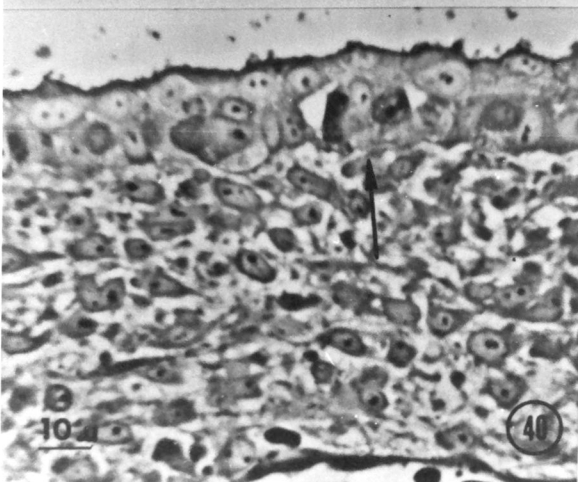
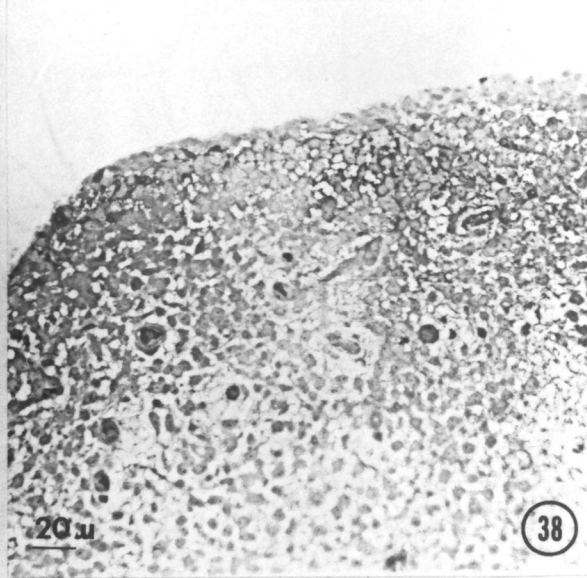
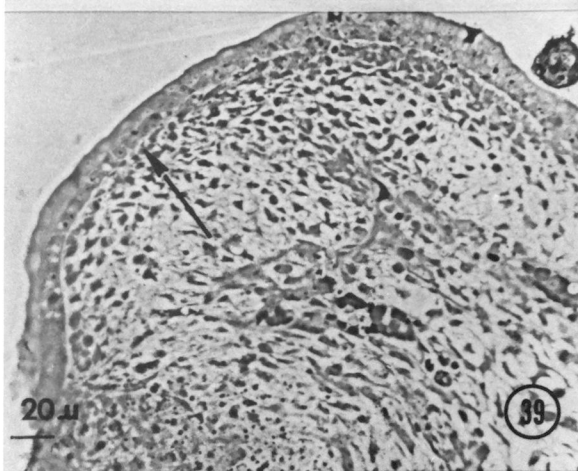
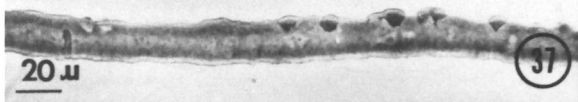
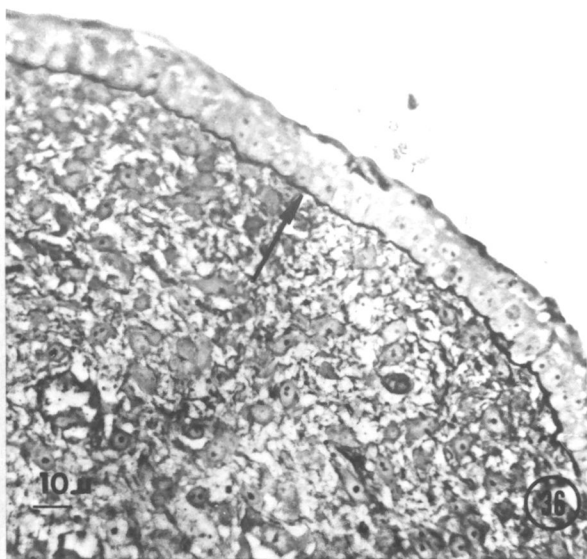
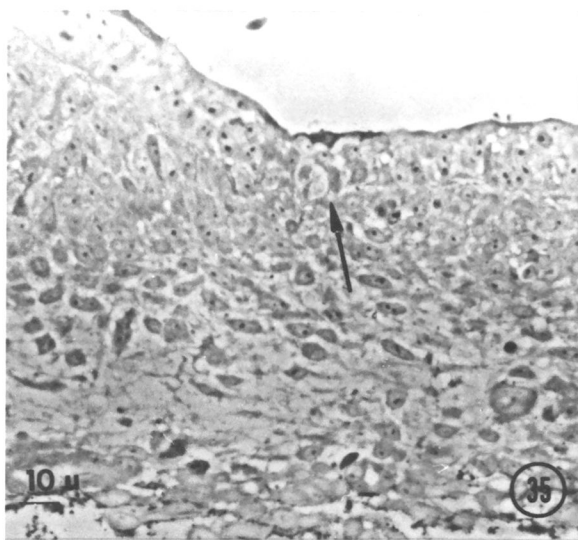


Figure 42. Normal feather tissue stained with RR showing a negative reaction along the basal lamina (BL) and with the coated vesicles (CV). Note Golgi (G) in dermal cells. 8,400 X.

Figure 43. Normal comb tissue stained with RR showing a positive reaction along cell interdigitations (arrows), the basal lamina (BL), and dermal cells. 8,400 X.

Figure 44. Normal comb tissue stained with RR showing a positive reaction along the basal lamina (BL) and on the dermal cells. Note collagen (Co) associated with the basal lamina. 22,000 X.

Figure 45. Normal comb tissue stained with RR showing coated vesicles (CV) with a RR positive reaction. 36,000 X.

Figure 46. Normal comb tissue stained with RR showing a positive reaction along the shafts of a dermal cilium. 36,000 X.

Figure 47. Normal comb tissue stained with RR showing a positive reaction along the inner leaflet (IL) of the plasma membrane. 72,000 X.

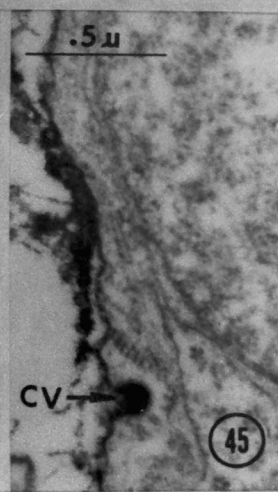
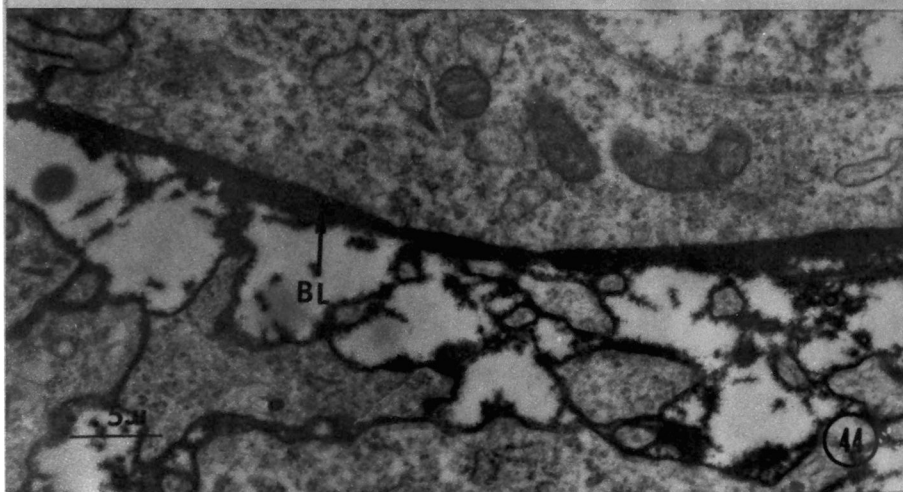
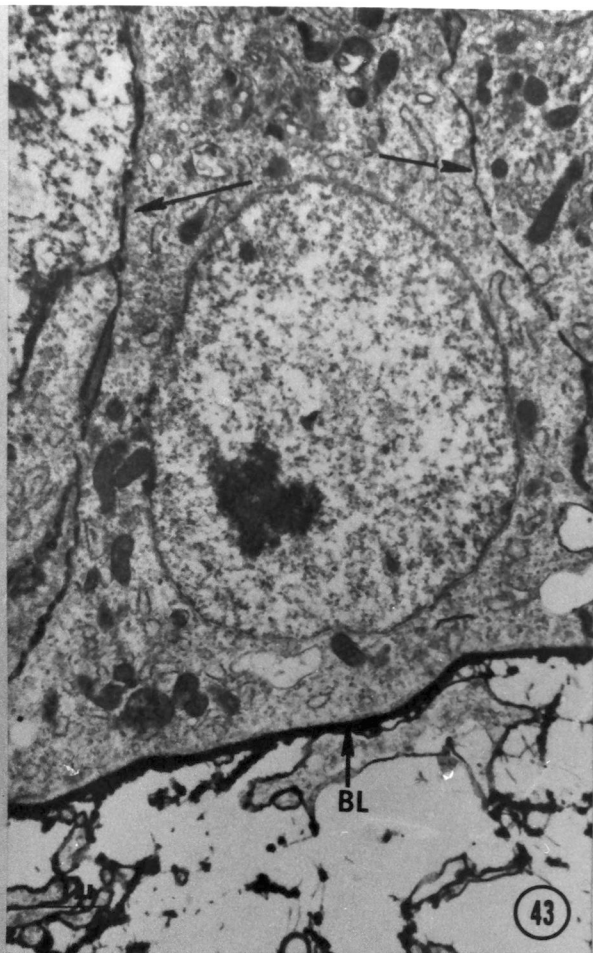
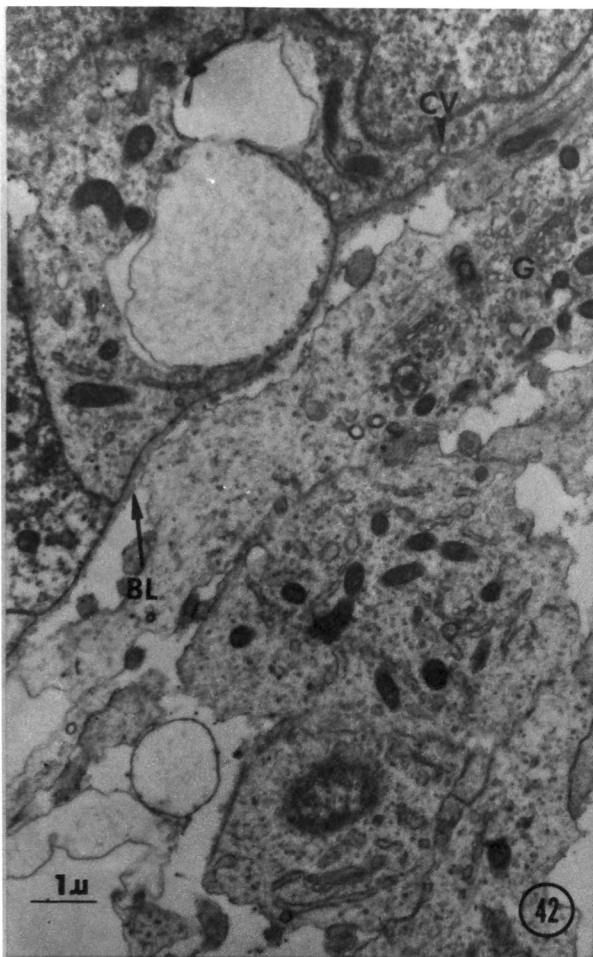


Figure 48. Separated comb epidermis stained with RR showing the basal epidermis and coated vesicles (CV). 36,000 X.

Figure 49. Recombined feather tissue stained with RR showing a positive reaction along the basal lamina and in the dermis. 8,400 X.

Figure 50. Recombined feather tissue stained with RR showing a negative reaction along the basal lamina (BL) and in the dermis. 8,400 X.

Figure 51. Recombined feather tissue stained with RR showing coated vesicles (CV) with a negative reaction. 36,000 X.

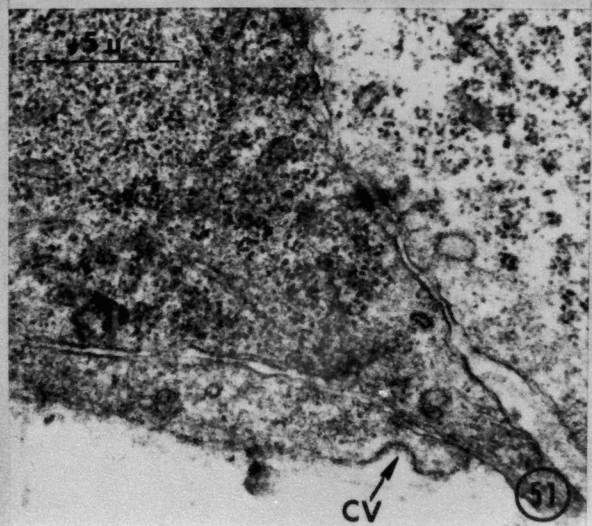
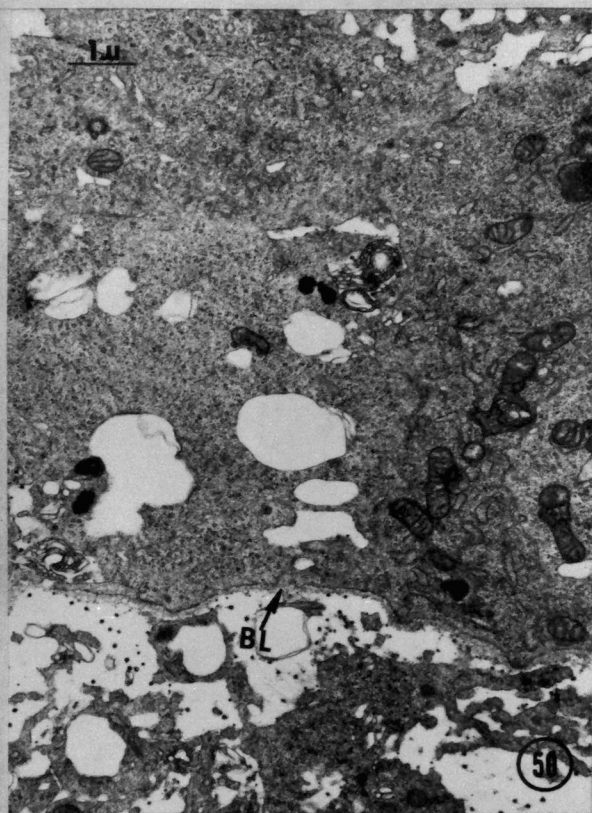
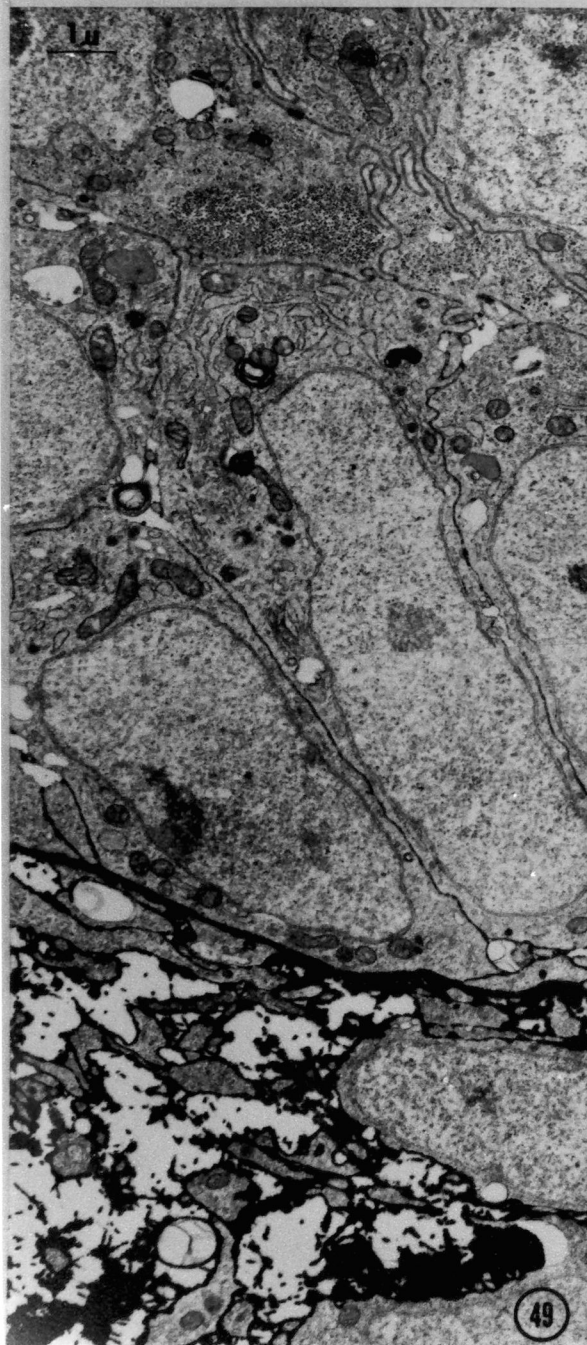
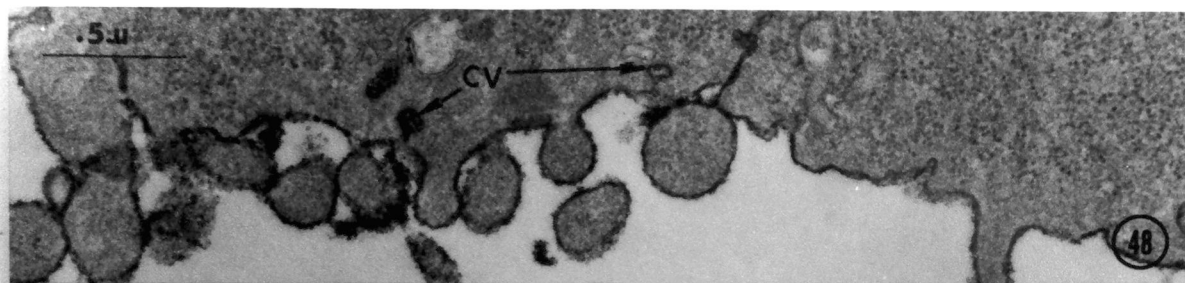


Figure 52. CE/FD chimera stained with RR showing a positive reaction along the basal lamina (BL) and on dermal cells. 10,500 X.

Figure 53. CE/FD chimera stained with RR showing positive material associated with anchoring filaments (AF) in the dermis. 36,000 X.

Figure 54. CE/FD chimera stained with RR showing a positive reaction in the recesses of collagen fibrils (Co). 36,000 X.

Figure 55. CE/FD chimera after 48 hours on the CAM stained with RR showing a positive reaction along the basal lamina (BL). Note collagen fibrils (Co) associated with the basal lamina. 12,000 X.

Figure 56. CE/FD chimera after 48 hours on the CAM stained with RR showing collagen (Co) with a periodicity of binding with RR positive deposits. 36,000 X.

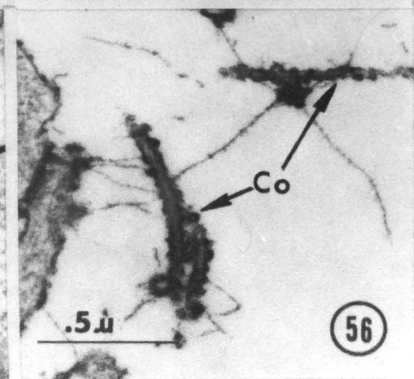
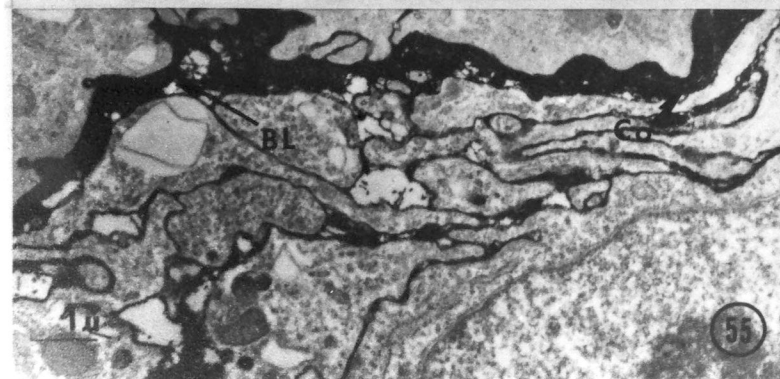
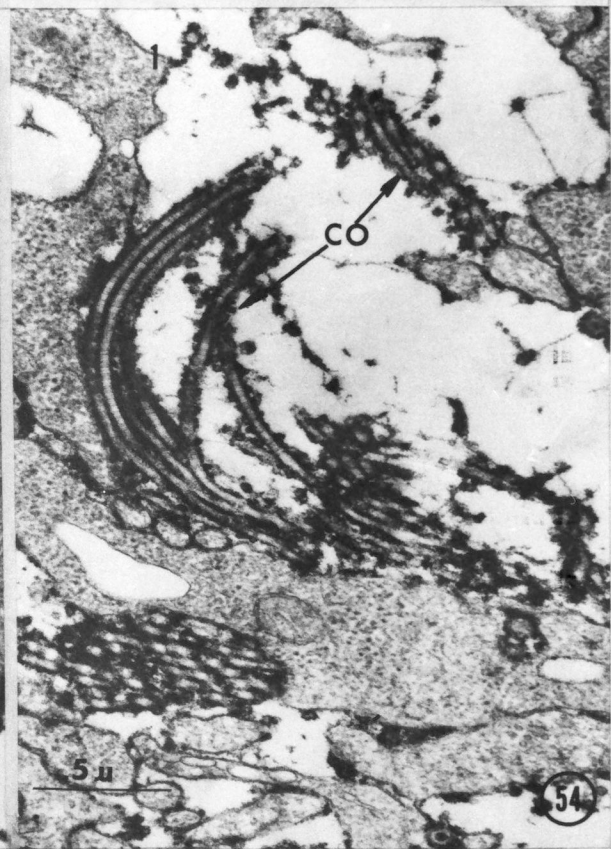
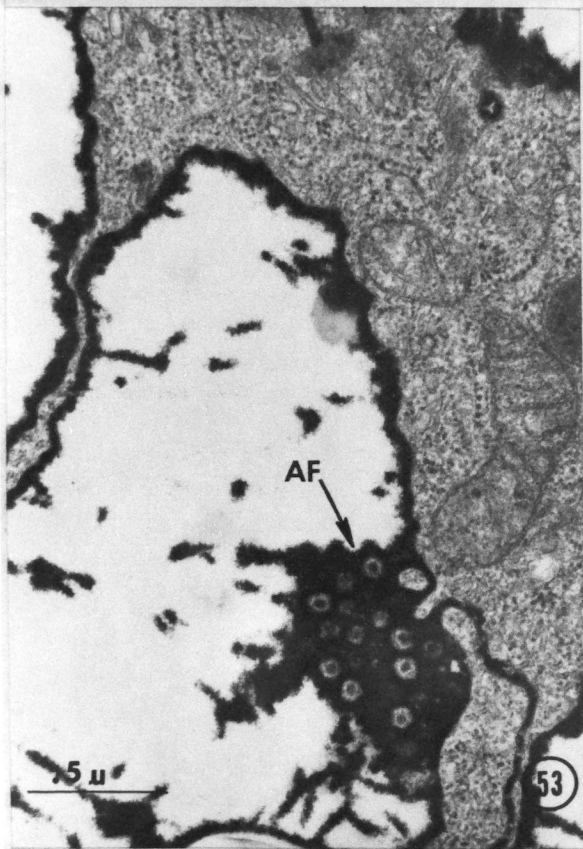
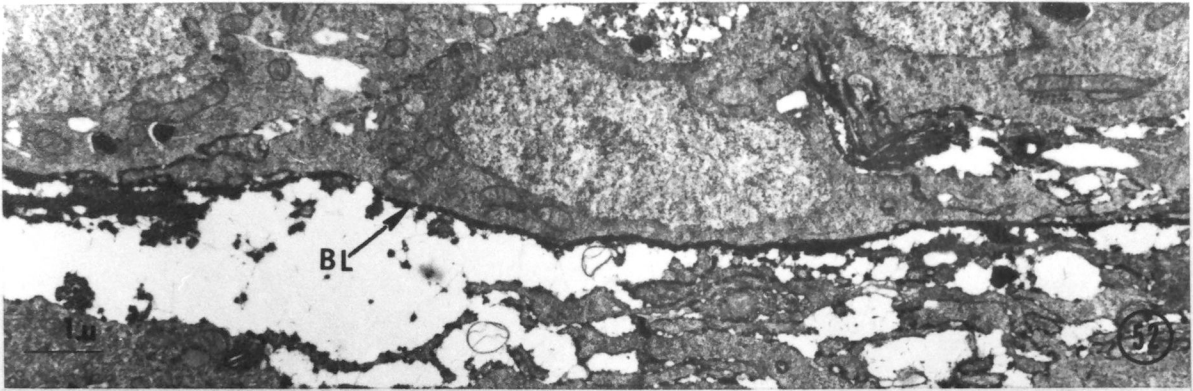


Figure 57. FE/CD chimera after 48 hours on the CAM stained with RR showing a negative reaction along the basal lamina (BL) and on dermal cells. 19,750 X.

Figure 58. FE/CD chimera after 48 hours on the CAM stained with RR showing a positive reaction along the basal lamina (BL) and on dermal cells. 10,500 X.

