Fine structure of the developing down feather

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KISCHER, Clayton Ward, 1930—
FINE STRUCTURE OF THE DEVELOPING DOWN FEATHER.

Iowa State University of Science and Technology
Ph.D., 1962
Zoology

University Microfilms, Inc., Ann Arbor, Michigan
FINE STRUCTURE OF THE DEVELOPING DOWN FEATHER

by

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A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Embryology

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1962
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INTRODUCTION

The development of any organ requires the mobilization of cells in a specific locale, their interaction to set off a train of chemical events leading to differentiation of new structure, and the continuous supply of substances from the environment for the maintenance of the organized cellular population. An understanding of these processes requires observations on the movements and orientation of the cells involved, the identification and sequence of appearance of chemical substances peculiar to them, and studies of the nature of the interaction. Of particular interest in connection with the chemical and physical relationships between the component cells, is the nature of the cellular boundary between two interacting tissues such as the mesoderm and epidermis of the developing down feather. This organ has well-known chemical constituents localized at rather specific sites which make it of particular interest and value for studies of fine structure through electron microscopy.

The down feather of the chick embryo arises as an outgrowth of the skin. It is composed of an outer covering of epidermis and an inner pulp of mesenchyme. It forms by a localized accumulation of mesenchymal cells which induce an elevation of the skin. The first primordia (on the back) appear between 6½ and 7 days (Stage 30, Hamburger and Hamilton, 1951). Growth of the feather continues at a rapid pace. During development there is a gradient of morphogenesis occurring within the feather. Differentiation of the tip precedes that of the base. The outer layer of epidermal cells differentiates into an encircling sheath, the inner layer into 10-15 longitudinal barb ridges. Thus, in cross section the feather
epidermis appears scalloped along its inner margin. By the end of the 14th day, it has attained its definitive form of a long taper or cone.

Koning and Hamilton (1954) investigated the normal histochemistry of the down feather and found two chemical constituents appearing in abundance, ribonucleic acid (RNA) and alkaline phosphatase. The former is highly concentrated in the epidermis, especially near the dermo-epidermal boundary. The latter is concentrated in the pulp. Each shows a gradient pattern of appearance and disappearance from the 7th to 14th days of development. The above authors concluded that the coincident appearance of RNA and alkaline phosphatase in the early- and mid-developmental stages was in harmony with the proposal of Danielli (1953, p. 72) that alkaline phosphatase functions as a phosphokinase, and therefore as a transport enzyme. It could supply the epidermis with phosphate complexes which might pass across the basement membrane and support rapid synthesis of RNA. This, in turn, would support the synthesis of feather protein (keratin).

In separate investigations, Hamilton and Koning (1956), Fabiny (1959), Gibley and Hamilton (1959), and Kischcr and Hamilton (1960) found that when there was interference with either the phosphatase or RNA, morphogenesis was curtailed. The abnormal picture as well as the normal one confirmed the importance of the basal region of the feather as the center of morphogenetic events. When feathers were treated with a known phosphatase inhibitor, the basal regions of the epidermis showed a diminished RNA concentration (Hamilton and Koning, 1956; Fabiny, 1959).
Hamilton and Kischer (1961) showed that the basophilia in this region was localized in rods and spheres of varying size and dimension which proved to be mitochondria. They concluded that ribonucleoprotein is a component of mitochondrial activity, although not essential for the structural integrity of the mitochondria.

The most active period of growth and differentiation in the down feather occurs on the 11th day at the time when barb ridges are developing. This coincides with the stage of maximal concentrations of RNA and alkaline phosphatase.

The present investigation was undertaken to examine in greater detail, through the use of electron microscopy, the basal epidermal area during this active period (the 11th day). Special attention was given to the sites where alkaline phosphatase and ribonucleic acid are localized and to the dermo-epidermal boundary across which all transfer must take place. For comparison, the fine structure of the same region was examined in younger stages, older stages, and inter-follicular skin.
REVIEW OF LITERATURE

Search of the literature revealed a paucity of work done on the sub-microscopic structure of the embryonic down feather. Mercer (1958) showed three photomicrographs of feather cells, including the dermo-epidermal boundary. However, he did not identify the type of feather or stage of development. Porter (1954a) used epidermis of 12- and 14-day-old chick embryos, but did not say if this skin contained feathers.

Several electron microscopic studies have been made of skin and hair from various vertebrate sources. In his review on the structure and function of skin, Montagna (1956, p. 19) described human embryonic epidermis as two-layered, each layer being one cell in thickness. The outer layer, the periderm, was flattened and the inner layer cuboidal. As development ensued, the inner or basal cells became columnar. He stated that as the epidermis became stratified the lower surface of the basal cells became serrated, and cytoplasmic processes imposed upon the upper layer of the dermis.

Selby (1955) studied human embryonic skin, adult rat skin, and adult human skin. She described the epidermis as separated from a "dermal membrane" by a distance of about 300 Å. This membrane continued without interruption across the space between the bases of adjacent epidermal cells. In addition to mitochondria, endoplasmic reticulum, and submicroscopic particles in the epidermal cell cytoplasm, she also described occasional filaments of less than 100 Å in diameter in the basal portions of the cytoplasm. In all skin types studied, the space between the basal cell membrane and the submicroscopic membrane limiting connective tissue
from the epidermal cells was approximately 300 Å.

Menefee (1957) investigated the epidermis of embryonic mice during differentiation. At a very young stage (15 days) these embryos had a stratified epidermis in which the outer layer lacked keratinization. The basal cells were more elongate than the spinosal cells, which were more spherical. The basement membrane consisted of a homogeneous layer which followed the contour of the basal cells closely and was separated from the latter by a zone of lesser electron density than itself. Tonofilaments of 40 Å in diameter were in close association with mitochondria, sometimes in direct contact. A paucity of Golgi material was noted in this and in older stages. The cytoplasm of cells in the stratum corneum was filled with granules of 40 Å in diameter.

In an early study of epidermis of larval forms of Amblystoma, Porter (1954b) described the basement membrane as consisting of three parts: 1) a layer of granules subjacent to the basal plasma membrane, 2) a layer of unorganized material under this, blending into 3) a fabric of fine fibrils of 130 Å in diameter and appearing striated at 350-400 Å intervals.

Kemp (1959) reported on the development of basement lamellae in larval anuran skin. He showed the development of "bobbins", patches of electron dense material, and microfibrils, at the periphery of basal epidermal cells, as differentiation of the epidermis progressed. He stated that the increase of these structures was indicative of active intracytoplasmic synthesis. He also suggested that, in the early stages, cisternae of endoplasmic reticulum were formed by a fusion of many cyto-
plasmic vesicles.

In a study of the development of hair, Pinkus (1958) reported that there was an initial, rapid, mitotic division of the basal layer which projected into the dermis, so that the piliary complex comprised ectodermal and mesodermal components which developed as a result of mutual influences. Mercer (1958) showed that in hair, skin, and feather follicles, the dermo-epidermal boundary consisted of a single continuous membrane, 200-300 Å thick, separated from plasma membranes of the epidermal cells by a lighter zone. He considered this zone to be a cementing layer. He characterized the cells of the basal layer as columnar in the more keratinized tissues. These cells also contained an abundance of dense particles of ribonucleoprotein (RNP), numerous mitochondria, and granular vesicles, but no organized endoplasmic reticulum. He proposed that these features are characteristic of cells with a great ability for protein synthesis. He claimed that epidermal cells which retained their products within them, characteristically had poorly developed endoplasmic reticulum but large numbers of free particles of RNP.
MATERIALS AND METHODS

White Leghorn chick embryos of Stages 29-40 (7-14 days of incubation) were used. The shell was removed directly above the embryo, and the chorion and amnion were torn away to expose the surface of the body. Feathers were removed from back skin in the area between the brachial and lumbo-sacral levels, using iridectomy scissors under a dissecting microscope. In some cases, principally at younger stages, pieces of skin containing several feather germs were removed from the same area. The feathers and skin pieces were then fixed for 30 minutes in isotonic 1 percent OsO₄, buffered to a pH of 8.1, consisting of the following components:

10.00 parts 2 percent OsO₄
5.00 parts veronal acetate
2.50 parts 0.16 percent CaCl₂
0.37 parts 20 percent NaCl
2.20 parts 0.1N HCl.

This mixture contained a 0.97 percent normal saline equivalent for chick tissue. Some tissues were fixed at room temperature, others at 0-4°C. Dehydration was accomplished by passing through two changes of 50 percent ethyl alcohol for 5 minutes each (to prevent excessive "blackening reaction", von Hahn, 1961), 50, 75, and 95 percent ethyl alcohol for 10 minutes each, and two changes of 10 minutes each in 100 percent alcohol. The material was then soaked in three changes (15 minutes each) of

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methacrylate monomer with 1 percent benzoyl peroxide added as catalyst. The monomer was made by mixing 7 parts n-butyl methacrylate with 3 parts ethyl methacrylate, and filtering through anhydrous Na₂SO₄ prior to use. Individual feathers and tissue pieces were then placed in size 0 or size 2 gelatin capsules containing pre-polymerized methacrylate, and let stand at room temperature for 5-15 hours. For final polymerization, the capsules were placed in a 60°C oven for 16 hours.

The specimens were sectioned at 25 μμ on a Porter-Blum microtome and placed on copper Athene grids with a 400 mesh. The grids had previously been coated with a thin methacrylate membrane to support the tissue. The sections were stained with 1 percent potassium permanganate (Lawn, 1960) or with monobasic lead acetate (Dalton and Zeigel, 1960). They were then covered with another methacrylate membrane after the method of Roth (1961).

The sections were examined in an RCA EMU 3F electron microscope, operated at 50 kv with a 30 μμ objective aperture. Photographs were taken at magnifications ranging from 3,000 to 13,000 and enlarged photographically.

Several modifications of the fixation, dehydration, and embedding procedures were tried, but without improvement in the results obtained by the processes described above. These included variations in the length of time for fixation, from 5 minutes to 2 hours, and temperatures of 0-4°C and ca. 25°C. Five different fixatives were used: 1) the isotonic mixture previously described, 2) the isotonic mixture, but with 1.3 parts of 0.2N HCl and 1 part of 5 percent methyl cellulose², 3) Dalton's Chrome-

Osmium (1955), 4) Palade's (1954), and 5) Caulfield's (1957). The pH of the fixatives was varied from 7.2 to 8.4.

The graded series of alcohols was extended from 20 percent to 100 percent. In another test, the alcohols were made isotonic up to and including the 70 percent dilution. Some tissues were passed directly to 100 percent alcohol from the fixative. Others were washed for 5-10 minutes between fixation and dehydration in distilled water, de-ionized water, or veronal buffer adjusted to a pH of 8.1. Dehydration was usually accomplished at room temperature, but in one trial was continued to the 95 percent step at 0-4°C.

Embedding methods included n-butyl methacrylate alone, mixtures of 6:4, 7:3, 8:2, and 9:1 n-butyl methacrylate to ethyl methacrylate, pre-polymerization of the monomer to thick, medium, and thin consistencies, and final polymerization by 15-watt, germicidal, ultra-violet lamps.

In addition, some feathers were processed after Luft's epoxy resin method (1961) and a Vestopal W procedure (Zelickson and Hartmann, 1960).
RESULTS

Epidermis of the 11-Day Feather

The periderm is the outer single layer of cells lying atop an epidermis of three to four cells in thickness. It is horizontally oriented and connections between cells are highly interdigitated (Fig. 1). Desmosomes are present. The free surface shows numerous small surface blebs. Mitochondria are variable in size, but not abundant. Endoplasmic reticulum appears in the shape of lamellae with attached granules. It is not well-developed. Agranular vesicles are also noted, and some of the smaller ones reveal an electron-dense interior. Tonofilaments are observed, and some give the suggestion of contact with mitochondria (Fig. 2).

The middle cells (Figs. 2 and 3) are more spherical or columnar in shape. Connections between the cells are not complex. Intercellular spaces are of the order of 10-15 μ. Mitochondria are not abundant. They are usually found in the basal portions of the cells (Fig. 3). Free granules are present in the cytoplasm which measure 10-20 μ, with the majority about 14 μ in diameter. Endoplasmic reticulum appears poorly developed in the mid-epidermal region. Mitoses are encountered in this area almost as frequently as in the basal zone (Fig. 3). Fig. 9 shows a centriole in cross section from the central cell in Fig. 3. The cell in the upper left of Fig. 3 shows the typical annuli of the nuclear envelope.

The basal cells are roughly columnar, with nuclei centrally located
so as to leave free cytoplasm at the apices and bases. Fig. 4 illustrates a portion of the basal zone of a barb ridge. Interspersed in this area are "puff-clouds" of electron-dense material, some isolated and some in close association with the mitochondria. On closer examination (Fig. 5) the cloudy areas are seen to consist of dark granules approximately 14 μ in diameter, not unlike the granules in the background material. They are set within an amorphous matrix appearing more electron-dense than the surrounding hyaloplasm. Associated with these areas, are occasional small vesicles. Lamellae of the endoplasmic reticulum appear increased over those in more superficial epidermal cells (Fig. 14). The cloudy areas sometimes appear to extend into the lighter zone beneath the epidermal cell membrane (Fig. 6). The mitochondria appear to be concentrated in the basal cytoplasm (Figs. 4 and 13) and although usually small and sub-spherical in the barb ridge area, may be tortuous or vermiform in other areas (Fig. 14). Their average diameter is about 0.5 μ. Some measure up to 2 μ in length. Tonofilaments are identified in the basal-most cytoplasm and microfibrils are observed on the dermal side (Figs. 13 and 15). Insofar as the resolution will demonstrate, the tonofilaments appear similar to those in Fig. 2. The microfibrils suggest periodicities of approximately 60 μ; occasionally they are seen to blend with the granular layer beneath the basal cell membrane. Infrequently, spherical structures are observed with a single outer membrane and an electron-dense center which suggests internal structure (Fig. 11).

When two epidermal cells contact each other at the dermo-epidermal boundary, their edges curve inward to form a channel, wider than the
normal intercellular space, leading upwards into the epidermis (Figs. 11, 12 and 14). Lodged within these channels, in some cases, are discrete membrane-bound structures with a granular center (Fig. 12). Close observation of the plasma membranes indicates they are of a double nature (Fig. 11). Whole portions of cells, not sectioned through the nucleus are observed in the basal area (Figs. 7 and 13). These are not seen as frequently in the upper layers of the epidermis. The plasma membrane next to the dermo-epidermal boundary shows small invaginations or pockets (Figs. 10 and 20) which occasionally appear to house sections of other cells (Fig. 10).

Structure of Epidermis Between Feathers

The interfollicular epidermis of 10- and 11-day feathers has two layers of cells. Again, the outermost layer is horizontally oriented (Fig. 16). The cytoplasm lacks the abundance of free granules found in the cells of the feather. Vacuoles and small agranular vesicles are present, as they are in cells of the feather. The cells appear devoid of the "puff-clouds" and there is very little endoplasmic reticulum. The epidermis shows rather large intercellular gaps which are thought not to be artifacts, but which could have been enlarged by the processing of the tissue.

Comparison of 11-Day Feather Epidermis with Other Stages

In the 9-day feather, epidermal cells are seen with increasing numbers of mitochondria and lamellae of endoplasmic reticulum (Fig. 17).
Golgi-like material is identifiable. The mitochondria appear to be in close proximity with the nucleus in some cells, but this was not a consistent finding. Generally, they are confined to the basal portions of the cells. They appear varied in size and shape and display the usual double membrane. The cristae are well-developed and sometimes extend across the complete diameter (Fig. 18). Discrete granules of the same dimension (14 nm in diameter) previously described as free in the cytoplasm and in the "puff-clouds" are found within the matrix of the mitochondria. This has been a consistent finding in all areas of the feather studied. Fig. 8 shows a series of irregularly-shaped structures (angulates) bounded by incomplete membranes, and with no definite internal structure.

The outermost epidermal cells in the 14-day feather show degenerative features with fragmentation of cellular parts (Fig. 19). Tonofilaments are present. The mitochondria are still intact, but are reduced in size (compare Figs. 17 and 19). The underlying epidermal cells still retain their internal structural integrity (Figs. 19 and 23). In the basal cells the mitochondria are similar to those of the earlier stages (Fig. 23). There appears to be a large increase in agranular vesicles. The hyaloplasm contains an abundance of free granules of the type previously described (Fig. 22).

The Dermo-Epidermal Boundary

The epidermis is separated from the dermis by a space of about 50 μm (Fig. 20). Approximately 35 μm of this distance is a lighter zone, sub-
jacent to the epidermal plasma membrane. These were consistent findings in all of the samples studied. The lighter zone appears to have within it particles of a granular nature, but no fibers. Beneath this area is a granular layer of approximately 15 μ width which appears to be composed, at the most, of two rows of granules (Fig. 20). This layer is continuous and shows no breaks or pores. It follows closely the contour of the epidermis (Fig. 25). Microfibrils are more conspicuous next to or in contact with the granular layer in the 11-day feather.

The Dermis

There was not extensive contact between the mesenchymal cells and the granular layer of the dermo-epidermal boundary in any of the stages of the feather which were studied (Figs. 21, 22, and 25), or in the interfollicular skin. Instead, there were short areas of contact between the dermal cells and granular layer, with frequent interruption by vesicles. Undoubtedly, these represent the intercellular spaces of the mesenchyme, accentuated somewhat by artifacts of preparation. The spaces are larger in earlier stages (Fig. 21) than in later ones (Figs. 23 and 25), which may be a reflection of lesser density of cells and a more delicate mesenchymal network in the former. Fig. 21 of a 9-day feather shows isolated portions of cells in the spaces which are probably sections through stellate processes of the mesenchymal cells.

The dermal cells, although smaller and irregular in shape, are well supplied with the usual intracellular organelles and structures. There is variation in size and shape among the mitochondria, and the cristae are
well-developed. Endoplasmic reticulum is present in the form of lamellae and vesicles. Numerous clusters of agranular, electron-dense vesicles, resembling Golgi material, are seen (Figs. 24, 25 and 26). Such clusters appeared to be more frequent in the mesenchymal cells than in the epidermal cells. There is a suggestion that the cells bordering the epidermis, or those in close proximity to it, reveal more fine structural features than the deeper-lying pulp cells (compare Fig. 25 with Figs. 26 and 27). In addition, extracellular microfibrils were observed (Figs. 26 and 27). Their periodicities could not be measured accurately due to inadequate resolution. However, they appear to be similar to those described in close proximity to the granular layer of the dermo-epidermal boundary.
DISCUSSION

The Epidermis and the Dermis

The differences between the epidermal cells, with respect to the ultrastructural components, in the various stages of the feather, reflect a gradual transformation of an undifferentiated group of cells into a definitive organized structure. Growth and differentiation must be assessed in terms of differences in the gross features as well as the fine structure of the component cells.

The most obvious change is the increase in the number of cells. In the early stages and in primordial skin intercellular gaps are common. As growth of the organ proceeds, mitoses and cell number increase, and the gaps are reduced. Birbeck and Mercer (1957) found large intercellular gaps between the cells of the matrix in the bulb of the human hair follicle. They suggested that the gaps indicated flexibility and lack of cellular adhesiveness. It would appear that these gaps are real; however, the possibility of their being accentuated due to artifacts exists.

The cells of the early feather are of the low cuboidal type. Later, as development progresses, they become more columnar. The nuclei are generally seen in a central position, so that most of the cytoplasm is located in basal and apical regions of the cell. There is some evidence that the basal cells become somewhat pyramidal during the most active stages of growth and differentiation (Figs. 7 and 13). The isolated portions of cells observed and the cross sections of slender processes seen wedged between intercellular spaces (Fig. 12) lend some support,
also, to the idea that they possess pseudopodia-like processes. Mercer (1961) described cross sections of double-membraned structures formed by tongues of one cell pushing into the neighboring cell. These observations lend support to the idea that the basal cells have extensive surface area contact with neighboring cells and with the dermo-epidermal boundary.

The change in shape of the epidermal cells from a cuboidal type to a columnar type may account for the positioning of the mitochondria. One may claim that this is a logical point of view. However, whether the mitochondria arrive at the basal and apical portions of the cell as a result of the change in shape or whether they position themselves prior to this change for some other reason, is not known.

It has been known for some time that the intense basophilia of the basal epidermis is RNA, which is considered necessary for the synthesis of keratin in the feather (Koning and Hamilton, 1954; Hamilton and Koning, 1956; Koning, 1957). Hamilton and Kischer (1961) identified the basophilia as localized in the mitochondria. In addition to the conspicuous basal border, there was a less apparent, discontinuous stratum of basophilia corresponding to the mitochondria occupying apical positions. Novikoff (1956) isolated mitochondria virtually free of RNA; whereas, Birbeck and Reid (1956) found the RNA in mitochondria to be 3 percent of the total protein content. Rendi (1959) reported a fraction of rat liver mitochondria to contain an RNA content of about 15 percent, and that this RNA was the site for amino acid incorporation into mitochondrial proteins. Intact mitochondria showed no sensitivity to ribonuclease. However, Kalf,
Bates, and Simpson (1959) found that mitochondria isolated from calf heart acquired sensitivity to ribonuclease after disruption by sonic oscillation.

In all stages of the feather studied, except the 14-day, the 10-20 μ granules appear in approximately the same concentration in all cells of the epidermis. Palade (1955) described cytoplasmic particles of 100-150 Å in diameter as being abundant in many cell types. Cells which were differentiated, he suggested, showed a preferential attachment of the granules to the membranes of the endoplasmic reticulum. Embryonic cells demonstrated the highest number of granules and the fewest associations with the endoplasmic reticulum. In such cells, therefore, the majority of the granules were found free in the cytoplasm. He described them as appearing in different forms, such as rosettes, loops, spirals, and rows. He tentatively concluded that there was a high correlation between the granules and the basophilia associated with RNA. In a later investigation, Palade and Siekevitz (1956) proved the direct association of the small dense granules with ribonucleic acid. The present findings are in accordance with the above. The free cytoplasmic granules are numerous and dispersed throughout the cytoplasm in all cells. They show the various types of clusters, rosettes, spirals, and rows. Only in the 14-day feather do they reveal a different appearance. There the granules show a more even dispersement throughout the cytoplasm and fail to demonstrate the patterns seen in the earlier stages (Fig. 22). This change in the granules may be correlated with the greater keratinization in the older feather. Koning and Hamilton (1954) and Koning (1957) have shown that basophilia due to RNA diminishes as protein increases.
The endoplasmic reticulum is poorly developed throughout all stages. This is consistent with the observation that cells which tend to retain their proteins (keratinizing cells), as opposed to secretory cells, will show very little endoplasmic reticulum, but an abundance of free granules, presumably RNP (Mercer, 1958, p. 94; 1961, pp. 112 and 115). The endoplasmic reticulum appears to be somewhat increased in the dermal cells over that of the epidermal cells. Whether this is indicative of differentiation of the mesenchyme, is not known.

One of the more striking differences between epidermal and dermal cells was the increased number of Golgi membranes and vesicles in the latter (Figs. 24, 25 and 26). The functions of the Golgi substance are thought to involve storage and possibly modification of some substances, particularly lipids. Synthesis of secretory products may occur in the Golgi apparatus, too, but less is known of this (Dalton, 1961). The possibility exists that alkaline phosphatase is sequestered by the Golgi material, but it would be difficult to attribute the histochemical demonstration of the enzyme in the feather to the Golgi substance alone because it is far more pervasive.

No attempt to localize the enzyme ultra-structurally was made in the present investigation, and no evidence is presented for postulated sites of its activity. This should be done in future researches. However, if alkaline phosphatase is synthesized or stored in the Golgi material there is the possibility it could diffuse to other parts of the cell, such as membranes, mitochondria, and the nucleolus, where it would participate in metabolic reactions or transfers.
The tonofilaments described in Figs. 2 and 9 probably represent precursors of keratin. Mercer (1958) describes the formation of tonofibrils (tonofilaments)\(^3\) in mammalian epidermis, and states that they are precursors of keratin.

It should be pointed out that not all of the fine structural components of the epidermal and dermal cells can be identified. Vesicles and vacuoles of varying size and shape, aside from those described above, remain of unknown origin and function. Of necessity, only the more readily identifiable structures are discussed in the present study.

One of the most interesting observations has been that of the "puff-clouds". They were seen only in the barb ridge area of the 11-day feather. If the granules within them are indeed of the same type as the free cytoplasmic granules, then it could be assumed they contribute to the intense basophilia of the basal zone. Hunt (1961) described similar structures in studies of the eye during induction. He concluded that the granules were embedded within or adsorbed to a mucopolysaccharide matrix. The clouds were found in the interepithelial space between optic vesicle and lens ectoderm. He offered this finding as support for the view of McKeehan (1956) that RNA was transferred from optic vesicle to the lens ectoderm during induction. Figs. 6 and 7 indicate that the clouds may extend into the lighter zone beneath the epidermal cell membrane. However, at no time, were the "puff-clouds" observed in the vesicular space of the mesenchyme. It would not be expected that the feather show identity in its fine structure with the eye, even though both have

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\(^3\)Tonofilament is a term used to denote a subunit of a structure which histologists refer to as a tonofibril. The latter term is usually employed in light microscopy studies.
inductive processes, since in the latter, basement membranes of two epithelia oppose each other, whereas in the former, dermal cells may contact the granular layer of the dermo-epidermal boundary. It is possible that these clouds represent a transfer of RNA from the dermis. It is also possible that they represent a transfer of phosphates complexed with alkaline phosphatase. To lend some support to this idea, Montagna (1956, p. 215) has demonstrated that alkaline phosphatase in skin and hair follicles is associated with mucopolysaccharides. They are found together in the dermal papilla of the growing hair follicle, but not in the resting follicle. The dermal cells, especially those next to the epidermis, do show abundant cytoplasmic granules. It is possible that on contact with the granular layer, the mesenchymal cells give up particles, which diffuse through the latter, and are then adsorbed onto a mucopolysaccharide matrix in the lighter zone. It is evident from the present findings that granules are present in this zone (Fig. 20). The "puff-clouds" might then be formed and moved into the epidermal cells by a "matrix interaction" proposed by Grobstein (1954) or through adsorption by an exudate on the surface of the cell (Weiss, 1958).

Nature of the Basement Membrane

Basement membranes are present where connective tissue comes into contact with epithelium. The light microscopists have not used the term consistently because their presence depended on stainability which could be enhanced by a plentiful deposit of collagen fibers underlying the epidermis. Pease (1958) described the basement membrane as "the
a lamella that is electron-dense after osmium tetroxide fixation. He described the space between this and the plasma membrane of the adjacent cell surface as a cement layer. He stated that the distance between cell membranes and basement membranes was 150 Å, and preferred not to refer to the latter as belonging to the dermis. Selby (1955) described the basement membrane as belonging to the dermis and introduced the term "dermal membrane". She measured its distance from the epidermal cell membrane as 300 Å. Salpeter and Singer (1960) made a plea for universal terminology with respect to the basement membrane. They introduced the term "adepidermal membrane". The present author prefers to reserve judgement as to a proper name for the basement membrane and refers to it, in this study, as the "granular layer". Measurements of the distance from the granular layer to the epidermal cell membrane in this investigation are closer to those of Selby. Consistently, in all stages of the feather, this distance was approximately 35 μm. The granular layer measured about 15 μm in thickness.

Unlike developing skin, the epidermis of the feather is not underlain with collagen fibers. Some collagen precursors are probably formed in the early stages (Figs. 13 and 15), but they are conspicuously absent in the later stages (Fig. 22). Since the pulp eventually shrinks away, there would be no means of continuing the production of collagen. One function of the basement membrane is claimed to be that of mechanical attachment of the epidermis to the underlying connective tissue (Pease, 1958). Its origin has been said to be from interaction between an ectodermal exudate forming the intercellular cement and a more diffusible
component emanating from the mesodermal cells (Mercer, 1961, p. 8). Of concern in the present study is its relationship with possible transfer of products from dermis to epidermis during growth and differentiation of the feather. The chemical nature of the basement membrane is not definitely known, but it is thought to be rich in polysaccharides (Pease, 1958). Some water-soluble products of small size might diffuse through this membrane. If the particles are of larger size they might be adsorbed onto the layer and then passed through to the epidermis by diffusion. It is of interest that no electron-dense particles which might represent materials being transported from dermis to epidermis were found in the spaces below the granular layer. Therefore, it may be tentatively concluded that contact with the granular layer is necessary for transfer of materials between the two germ layers. This would be in accordance with the view of McLoughlin (1961) that contact and proximity between mesenchyme and embryonic epidermis are necessary for the normal differentiation of the latter.

It was thought in the early observations that the vesicular spaces beneath the epidermis could be a result of artifacts. However, all the modifications of tissue processing failed to remove or reduce these spaces. It is possible that they were accentuated by the stresses imposed during the processing of the tissue. It was observed that the plasma membranes of the mesenchymal cells were more frequently damaged than those of the epidermal cells. This condition seemed not to be as prevalent in the older stages. It is suggested therefore, that the protein content of the dermal cell membranes changes, as the feather differentiates, rendering them less susceptible to fixation damage.
SUMMARY

1. Down feathers from the back skin of the 11-day chick embryo were examined under the electron microscope, giving special attention to the barb ridge area and the dermo-epidermal boundary. Other stages and interfollicular skin were also examined for comparison.

2. It is tentatively concluded that the basophilia of previous histochemical studies resides, in part, in the free cytoplasmic granules of 10-20 mμ in diameter, as well as in the mitochondria. Granules of a similar size are found in the matrix of the mitochondria.

3. Structures referred to as "puff-clouds" are seen in the basal cytoplasm of the innermost epidermal cells of the barb ridge area. They are composed of granules measuring approximately 14 mμ in diameter and lie within an amorphous matrix, more electron-dense than the surrounding hyaloplasm. Some are isolated while others are in close association with mitochondria. Some also appear in the dermo-epidermal boundary lying within the light zone between the epidermal cell membrane and the granular layer on the dermal side.

4. The basement membrane is identified as a "granular layer" 15 mμ in width, separated from the basal cell membranes by a space of about 35 mμ. It shows no breaks or pores, and closely follows the contour of the basal cell membranes throughout its length.

5. The mesenchymal cells appear to have more Golgi material and less mitochondria than the epidermal cells. Otherwise, there are no striking differences between the two in their sub-cellular components.

6. The vesicular spaces beneath the granular layer are real and probably
represent intercellular spaces between mesenchymal processes.

7. The 14-day down feather, which is known to have nearly completed its growth, shows dissolution or degeneration of its cellular organoids in its outer, more keratinized layers. There is also less fixation damage to the dermal cells, probably as a result of greater content of protein.
LITERATURE CITED


ACKNOWLEDGEMENTS

This investigation was supported in part by a research grant, RG-3813(C7), from the Division of Research Grants and Fellowships, National Institutes of Health, U.S. Public Health Service, and by the Industrial Science Research Institute of Iowa State University of Science and Technology.

The author wishes to thank Dr. Howard L. Hamilton for suggesting the problem and for assistance and encouragement during the course of this investigation. A special note of appreciation is extended to Dr. L. Evans Roth, Department of Biophysics and Biochemistry, Iowa State University, whose direction and guidance sustained the author's efforts for competence in the techniques and their application in this study.
APPENDIX

Explanation of Plates

Figs. 1-27 are electron micrographs of portions of the down feather and interfollicular skin removed from the back of chick embryos from 9-14 days of incubation. Unless otherwise specified in the explanation of figures, the material was fixed in isotonic 1 percent osmium tetroxide for 30 minutes, embedded in methacrylate monomer consisting of 7 parts N-butyl methacrylate and 3 parts ethyl methacrylate, sectioned on a Porter-Blum microtome at 25 μm and stained with 1 percent potassium permanganate. All material was examined in an RCA EMU 3F electron microscope. Each figure is oriented on the plate so that the outer portion of the skin is towards the top of the photo. Labels used in the figures are listed below.

A, angulates
B, surface blebs
C, puff-clouds
D, desmosomes
E, plasma membrane
F, microfibrils
G, free cytoplasmic granules
H, intercellular channel
I, invaginations of plasma membrane
L, light zone of dermo-epidermal boundary
M, mitochondria
N, intercellular boundaries
O, Golgi material
P, periderm cell
R, granular layer of dermo-epidermal boundary
S, spindle fiber
T, tonofilaments
U, isolated section of cell
V, vesicles
X, dermo-epidermal boundary
Y, intercellular gaps
AN, annuli of nuclear envelope
CE, centriole
CG, dark granules in puff-clouds
CP, cell process
CR, cristae of mitochondria
DN, degenerate epidermal cell nucleus
DV, vesicles with electron-dense interior
EL, lamellae of endoplasmic reticulum
EN, epidermal cell nucleus
EV, vesicles of endoplasmic reticulum
GM, granules in mitochondria
MC, mesenchymal cell
SS, unidentified spherical structures
VA, vacuole
VM, vesicular space of mesenchyme
Fig. 1. 11 days. A cell from the periderm (P) is seen with surface blebs (B), and highly-interdigitated intercellular boundaries (N). Vesicles (V) are identified, some with an electron-dense interior (DV). Lamellae of endoplasmic reticulum (ER) are scarce. Desmosomes (D) are observed. Mitochondria (M) are varied in size. Magnification: X 19,500

Fig. 2. 11 days. A cell from the periderm (P) is seen in the upper portion of the figure. Tonofilaments (T) are observed which suggest close proximity to a mitochondrion (M). The lower cells from the middle layer of the epidermis reveal many free cytoplasmic granules (G). The intercellular boundaries (N) are not as remarkable as those in the periderm. Magnification: X 18,000
Fig. 3. 11 days. The middle layer of the epidermis. The cells are generally columnar and the mitochondria (M) are usually located in the basal and apical portions of the cell. Vesicles of endoplasmic reticulum (EV) are noted. In the cell in the upper left of the figure may be seen annuli (AN) of the nuclear envelope. A cell in mitosis is seen in the center of the figure. Magnification: X 19,500

Fig. 4. 11 days. The basal zone of a barb ridge area of the epidermis is shown. Puff-clouds (C) appear isolated and in close proximity to the mitochondria (M). The dermo-epidermal boundary (X) is shown in the lower portion of the figure. Magnification: X 9,000

Fig. 5. 11 days. A portion of the basal cytoplasm from Fig. 4. The puff-clouds (C) contain dark granules (CG) set within an amorphous matrix. Magnification: X 28,000
Fig. 6. 11 days. A portion of the basal zone of the epidermis in a barb ridge showing the puff-clouds (C) extending into the light zone (L) of the dermo-epidermal boundary. Magnification: X 33,000

Fig. 7. 11 days. The basal zone shows an isolated section of a cell (U) apparently belonging to a cell whose major portion is located either in front of or in back of the section shown. Magnification: X 18,000

Fig. 8. 9 days. Angulates (A) are seen in the basal zone of the epidermis which are indistinctly bound by a membrane and show no evidence of internal structure. Magnification: X 20,000

Fig. 9. 11 days. A centriole (CE) and spindle fibers (S) from the central cell in Fig. 3. Magnification: X 55,000

Fig. 10. 11 days. An invaginated portion of the plasma membrane (I) of a basal epidermal cell. Within the invagination, a cell process (CP) may be seen. Magnification: X 27,500

Fig. 11. 11 days. The basal zone showing an intercellular channel (H) formed by the meeting of two basal cells. Unidentified spherical structures (SS) are seen bounded by a membrane and suggesting internal structure. The plasma membrane (E) appears double. Lead acetate stain. Magnification: 27,500

Fig. 12. 11 days. The basal zone showing cell processes (CP) in the intercellular channel (H). Lead acetate stain. Magnification: X 27,500
Fig. 13. 11 days. The isolated section of the basal cell (U) contains tonofilaments (T) near the dermo-epidermal boundary (X). Microfibrils (F) occur on the dermal side, sometimes impinging upon the granular layer of the dermo-epidermal boundary (R). Mitochondria (M) are abundant. Magnification: X 23,500

Fig. 14. 11 days. A portion of the basal zone showing relatively extensive lamellae of the endoplasmic reticulum (EL) with attached granules. A tortuous mitochondrion (M) is noted. An intercellular channel (H) appears in the middle of the figure. Lead acetate stain. Magnification: X 27,500
Fig. 15. 11 days. Microfibrils (F) appear underneath the granular layer of the dermo-epidermal boundary (R). Magnification: X 27,500

Fig. 16. Interfollicular skin. A transverse section through the entire epidermis. The cells of the periderm (P) are oriented in a longitudinal fashion. Large intercellular gaps (Y) are common. Vacuoles (VA) are observed. Magnification: X 12,000
Fig. 17. 9 days. The epidermis shows numerous mitochondria (M) particularly in the basal cells. Lamellae of endoplasmic reticulum (EL) are scattered. Golgi material (G) is seen in a middle cell. The dermo-epidermal boundary (X) is located in the lower portion of the figure. Magnification: X 9,000

Fig. 18. 9 days. Mitochondria (M) from the basal area of Fig. 17. The cristae (CR) are well-developed. Granules within the matrix of the mitochondria (GM) appear similar to those in the cytoplasm (G). Magnification: X 47,000
Fig. 19. 14 days. The upper portion of the epidermis showing a degenerate nucleus (DN) and cytoplasm. Bands of tonofilaments (T) are scattered. The mitochondria (M) are small, elongated, and appear degenerate. The underlying epidermal cell nucleus (EN) does not show signs of degeneration. Fixation in isotonic osmium with added sucrose. Magnification: X 11,000

Fig. 20. 11 days. A portion of the basal zone showing an invagination of the plasma membrane (I). The light zone of the dermo-epidermal boundary (L) contains granules. The granular layer (R) appears to be composed of not more than two rows of granules. Magnification: X 50,000

Fig. 21. 9 days. The mesenchyme (VM) contains cell processes (CP) reflecting the stellate nature of mesenchymal cells. Fixation in isotonic osmium with methyl cellulose. Magnification: X 12,000
Fig. 22. 14 days. The basal zone of the epidermis is in the upper portion of the figure and the mesenchyme in the lower portion. Numerous vesicles (V) are noted. The vesicular space of the mesenchyme (VM) is reduced. Cytoplasmic granules (G) are abundant. Fixation in isotonic osmium with added sucrose. Magnification: X 28,500

Fig. 23. 14 days. Mitochondria (M) in the basal portion of the epidermal cell. Fixation in isotonic osmium with added sucrose. Magnification: X 12,000

Fig. 24. 11 days. A portion of a basal epidermal cell in the upper portion of the figure, and a portion of a mesenchymal cell in the lower portion. Golgi material (G) is abundant, and lamellae of endoplasmic reticulum (EL) are present. Magnification: X 26,500
Fig. 25. 11 days. A montage showing portions of the basal epidermis and the underlying mesenchyme. The granular layer of the dermo-epidermal boundary (R) is seen to follow the epidermal cell membranes closely. Golgi material (G) is frequently seen in the mesenchyme. The stellate nature of the mesenchymal cells is particularly well demonstrated by the presence of many cell processes (CP) in the vesicular space of the mesenchyme (VM). Magnification: X 3500
Fig. 26. 11 days. Mesenchymal cells showing Golgi material (G) and microfibrils (F). Magnification: X 14,000

Fig. 27. 11 days. Mesenchymal cells and microfibrils (F).
Magnification: X 14,000