CELL DIFFERENTIATION IN THE LOWER OUTER SHEATH OF THE ROMNEY WOOL FOLLICLE: A COMPANION CELL LAYER

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[Manuscript received January 27, 1971]

Abstract

Morphological evidence is presented showing that, in the Romney wool follicle, the layer of cells in the outer root sheath lying next to Henle's layer differentiates in the bulb as a separate and distinct layer from other outer root sheath cells. The term "companion cell layer" is suggested for this layer. Its possible role in the movement of the inner root sheath toward the skin surface is discussed.

I. Introduction

The presence of morphologically distinct cells in the lower outer root sheath (ORS), lying next to Henle's layer, has been reported in the follicles of man (Pinkus 1927; Montagna 1962), the Australian opossum (Gibbs 1938), sheep (Auber 1952), mouse and guinea pig (Straile 1962, 1965), and the rat (Rogers 1964). In general these cells differ from other ORS cells in their flattened form and their attachment to keratinized Henle's layer cells. Rogers (1964) has shown that they form a membrane complex with keratinized Henle's cells which, unlike that between keratinized inner root sheath (IRS) cells, has no central dense component. This complex has no parallel where ORS cells appose.

Evidence for the role of these cells in the ORS is limited but it is believed that they are involved in the movement of the IRS toward the skin surface. Pinkus (1927) believed that the cells were "pulled along" by the upward movement of the fibre–IRS complex toward the skin surface. On the basis of morphological evidence, Rogers (1964) hypothesized that these cells moved upward with Henle's layer. However, the picture is confused by other evidence suggesting that there is a general movement of ORS cells upwards (Straile 1962, 1965; Epstein and Maibach 1969).

This paper presents morphological evidence showing that, in the wool follicle, the ORS cells lying next to Henle's layer differentiate as a layer separate and distinct from other ORS cells. Because of their close association with Henle's cells, the term "companion" is proposed for these cells to distinguish them from other ORS cells.

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II. Materials and Methods

The lower halves of wool follicles in anagen VI (Chase, Rauch, and Smith 1951) were dissected from midside biopsies of 18-months-old Romney wethers. The follicles were fixed in Karnovsky's (1965) fixative, pH 7·4, for 4 hr at 4°C. They were then washed in three changes of 0·1M cacodylate buffer, pH 7·4, with 7·5% sucrose, post-fixed in 1% OsO_4 in 0·1M cacodylate buffer, pH 7·4, at 4°C, dehydrated through a graded series of ethanols, and embedded in Epon. Sections were cut with glass knives on an LKB ultramicrotome and stained with uranyl acetate and lead citrate (Venable and Coggeshall 1965) for examination in a Philips EM300 electron microscope operating at 60 kV.

III. Results

(a) *Comparative Morphology of Companion Cells*

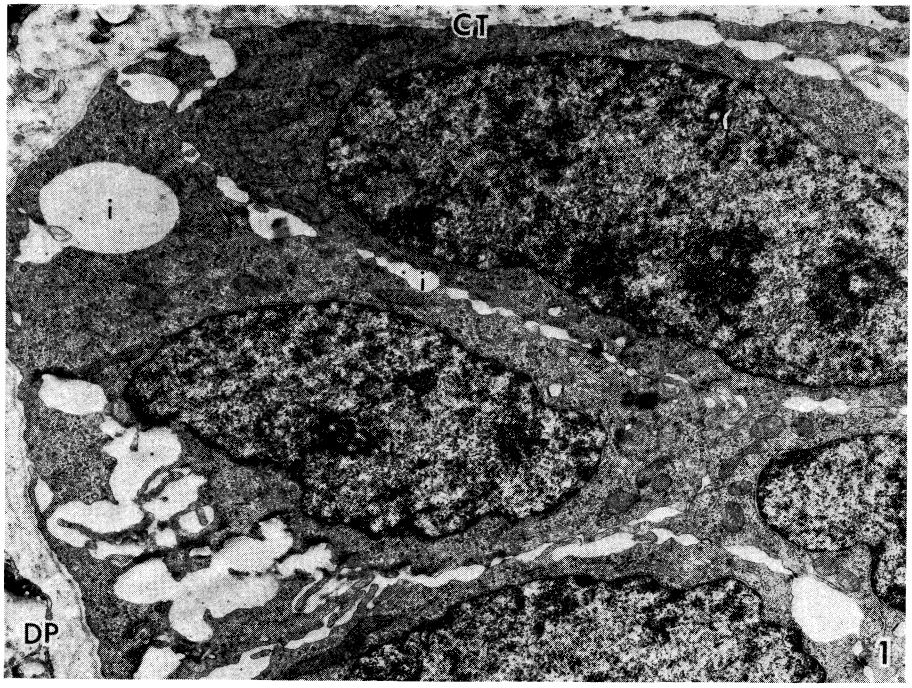
Examination of the presumptive and differentiated ORS cells of the lower follicle from the base of the dermal papilla to the level where the cortex keratinizes revealed the development of two layers each containing cells of a distinctive type. Figures 1–5 show the salient features observed during the development of these two cell types.

In the bulb, the cells near the base of the dermal papilla form no obvious layers; they have shapes ranging from columnar to ovoid and are often found to be dividing (Fig. 1). At 4–5 cells from the base of the dermal papilla, the cells adjacent to the connective tissue sheath show changes that distinguish them from the other cells in this region of the bulb (Fig. 2). The outermost cells form a layer comprising the presumptive outer root sheath (pre-ORS) cells. These cells have become flattened and elongated. The cell surfaces attached to the basement membrane of the connective tissue sheath show few infoldings whereas at the junctions of apposing pre-ORS cells there are many intercellular gaps and infoldings of the cell surfaces so that the cells appear to be only loosely attached to each other. During this phase there is, in some follicles, evidence of condensation of the cytoplasmic and nuclear contents.

In the same region, the cells adjacent to the pre-ORS cells also start to elongate and flatten, but generally at a slower rate so that the cells of this layer reach a comparable stage of elongation further from the base of the dermal papilla. The elongation and flattening of these cells marks the first detectable stage of differentiation of the companion cell layer. At this stage, there is no evidence of trichohyalin in the presumptive Henle's layer, which lies next to the companion cell layer, although the pre-Henle's cells are often distinguishable by their shape.

Companion cells at this stage have characteristics which distinguish them from the pre-ORS cells. These relate mainly to the attachment of companion cells with neighbouring cells. The pre-ORS–companion cells appear to be only loosely attached

Fig. 2.—Mitotic zone cells about six cells from dermal papilla base. The outer two layers of cells have elongated and flattened with the outermost layer, the pre-ORS cells (*pOR*) being more elongated and showing condensation of nuclear and cytoplasmic contents. Companion cells (*CC*) are slightly condensed while pre-Henle's cells (*pH*) are not as elongated and do not contain trichohyalin. Intercellular gaps (*i*) are common between pre-ORS cells and companion cells and adjacent pre-ORS cells. Desmosomes (arrows) are more common between companion cells and Henle's cells. *CT*, connective tissue sheath. ×9400.



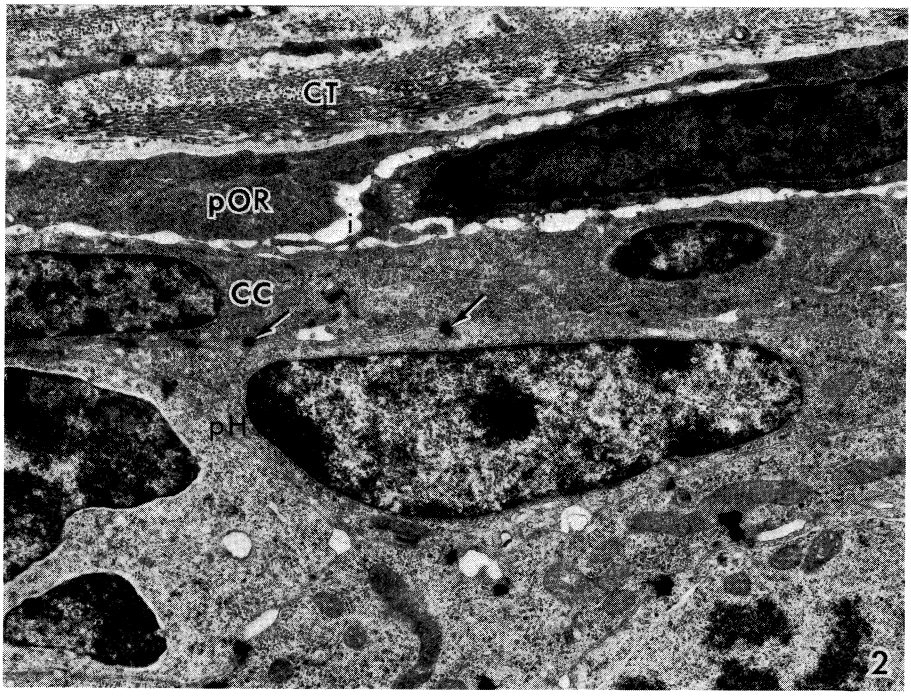
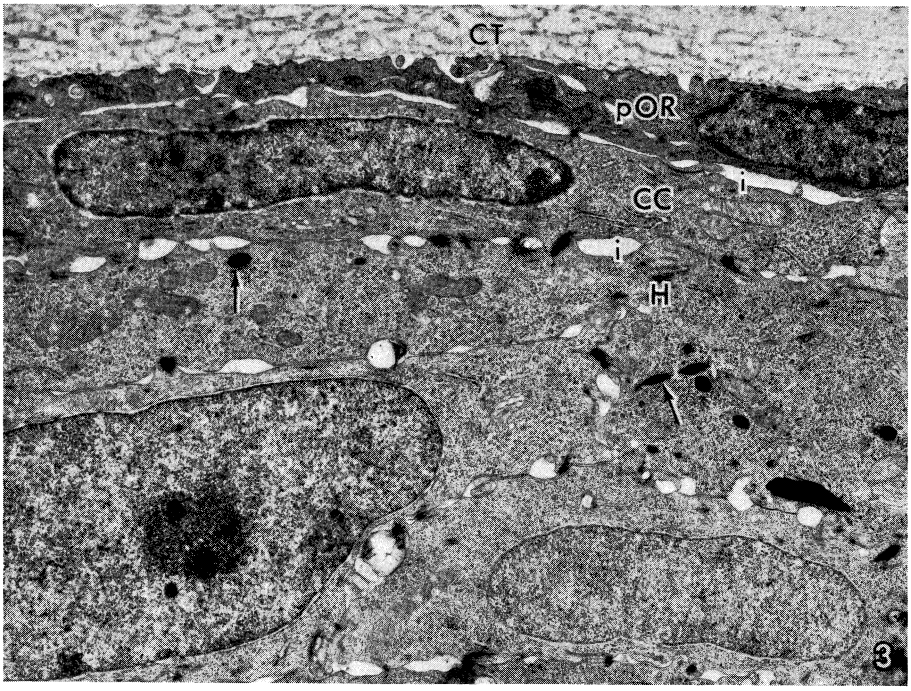


Fig. 1.—Mitotic zone cells at the base of the dermal papilla (*DP*). These cells are similar in appearance. *CT*, connective tissue sheath; *i*, intercellular gap. ×8700.



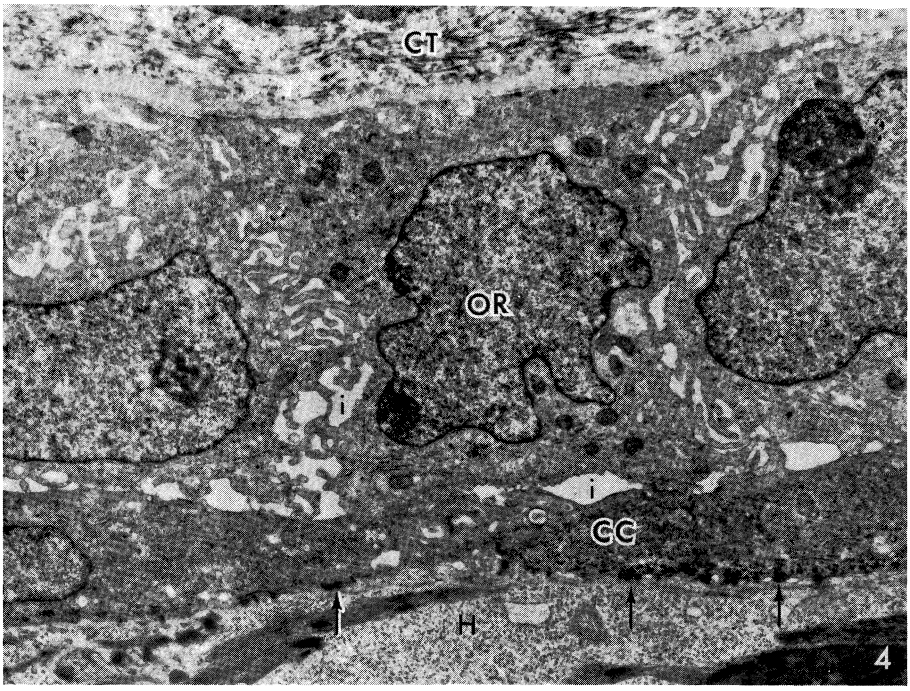


Fig. 3.—Bulb cells at the differentiation level of Henle's layer (*H*). The two outermost layers are still elongated and flattened with pre-ORS cells (*pOR*) showing some signs of condensation. Intercellular gaps (*i*) are common between companion cells (*CC*) and pre-ORS cells but rarer between companion cells and Henle's cells. Arrows point to trichohyalin in Henle's cells. *CT*, connective tissue sheath. ×9100.

as long narrow intercellular spaces occur frequently between these cell types. Cell surfaces show frequent invaginations while specialized membrane differentiations such as desmosomes are infrequent. In contrast, the surfaces of apposing companion cells are relatively flat with few intercellular gaps between membranes which are otherwise the usual distance of 15 nm apart. Desmosomes are more common. Companion cell–pre-Henle's cell surfaces have a similar appearance. Numbers of intercellular gaps are seen but the membranes for the greater part of their length are the usual distance apart with desmosomes and other membrane differentiations occurring relatively frequently. In some follicles, the contents of the cytoplasm and nucleus of companion cells also undergo condensation, again usually reaching a comparable stage further from the dermal papilla than pre-ORS cells (Fig. 2).

Definite identification of Henle's cells can be made with the appearance of trichohyalin in their cytoplasm. At this stage, the two cell layers comprising the companion cell layer and the ORS cell layer are readily apparent alongside Henle's layer (Fig. 3). The differences (described earlier) between the cell types are still apparent but intercellular gaps between companion cells and Henle's cells are rare. Elongation and flattening of both companion and ORS cells and condensation of nuclear and cytoplasmic contents usually reach their ultimate limits at this stage. Divisions in companion cells have not yet been observed in this region or above it.

As differentiation proceeds, the differences between companion cells and ORS cells become quite marked. At the next stage, where the ORS and companion cells are approximately level with the top of the dermal papilla, the ORS cells start assuming the form typical of ORS cells in the proliferation zone (Straile 1962). Here, the infolding of cell surfaces of apposed ORS cells and the intercellular gaps between ORS and companion cells are clearly visible (Fig. 4). In contrast, the surface of these cells in contact with the basement membrane still shows few infoldings. Also, where the cell contents of companion cells and ORS cells had been condensed at an earlier stage, as noted above, more typical densities are apparent in this region. The most important difference is, however, that the ORS cells are no longer elongated or flattened but block shaped while companion cells still retain their elongated or flattened form. The other characteristics of companion cells are also retained except that small infoldings of the companion cell surface into Henle's cells are sometimes apparent.

When Henle's layer keratinizes, several other differences become visible. ORS cells at this level no longer show marked convolutions between their apposing surfaces although there are many intercellular gaps. Furthermore, the ORS is generally multilayered and the cells usually have large quantities of glycogen visible in the cytoplasm. In comparison, the single-layered companion cells retain their elongated and flattened form and show little change in their contacts with ORS cells while little or no glycogen is visible in their cytoplasm (Fig. 5). The infoldings of companion cells into Henle's cells are preserved as such when Henle's layer hardens.

Fig. 4.—Outermost cells above bulb of the follicle. The two outermost layers of cells have markedly different appearances. The ORS cells (*OR*) are now block shaped with many infoldings of the cell surface. Many intercellular gaps (*i*) occur between the elongated and flattened companion cells (*CC*) and ORS cells but are rare and small between companion cells and Henle's cells (*H*). Desmosomes (arrows) are common at these surfaces but not between companion cells and ORS cells. *CT*, connective tissue sheath. ×8200.

These morphological characteristics are retained throughout the remainder of the lower third of the follicle, i.e. up to the level where the cortex keratinizes.

(*b*) *Morphology of Companion Cells*

Although companion cells are basically similar to other wool follicle cells, there are some aspects of their morphology worthy of comment. Firstly, cytoplasmic microtubules are most frequently oriented circumferentially to the axis of the follicle (Fig. 6). Secondly, a fibrous material is laid down in the cytoplasm close to the membrane. This material is of sufficient density to exclude ribosomes and other cytoplasmic organelles from contact with the cell membrane (Figs. 6 and 7). It first appears during the early differentiation of the companion cell layer (Fig. 7). In most cases, the fibrous substance develops as a layer on the Henle's side of the companion cell, but eventually extends around the circumference of the cell (Figs. 6, 7, and 8). There may be two types of material synthesized or one may be an aggregate of the other, as high and low electron-dense* material can be detected in this layer. The material of low electron density is apparently synthesized first and seems to be similar in appearance to the material seen near the surfaces of ORS cells apposing the basement membrane (Fig. 7). The material of high electron density is usually first apparent in companion cells in the region level with the top of the dermal papilla, where it often occurs on the inner edge of the low electron-dense material (Fig. 6). It is fibrous in appearance and, unlike most of the low electron-dense material, accumulates in the cytoplasm as differentiation proceeds (Figs. 8 and 9). At the cell margins it is usually oriented at right angles to the cytoplasm. A similar material is seen in ORS cells (Fig. 9) but it usually appears first in companion cells.

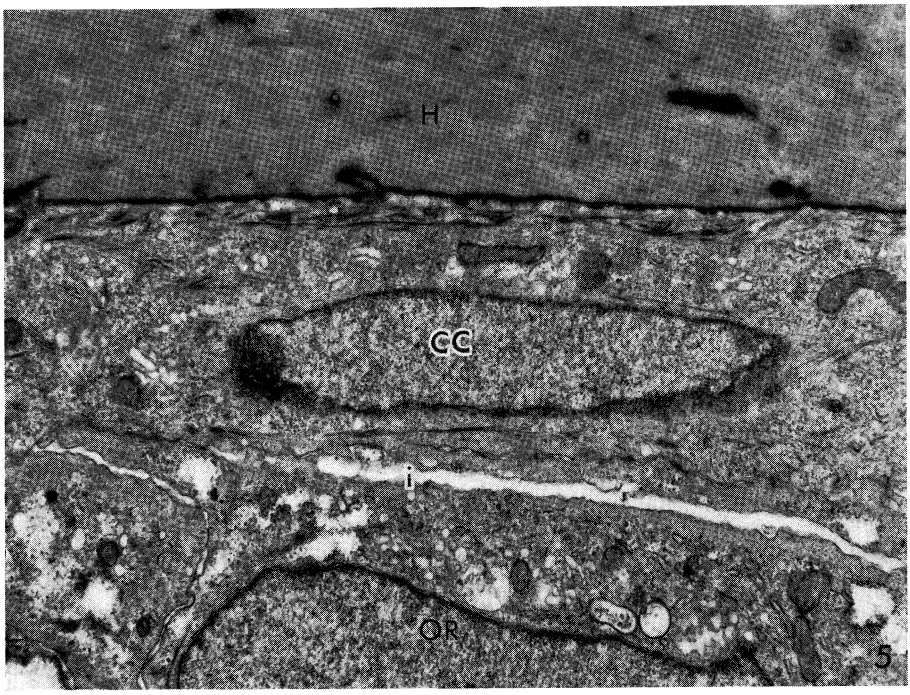
As a result of keratinization (hardening) of Henle's cells, minor changes occur in the membranes between apposing companion and Henle's cells. Firstly, the apposing membranes of the two cell types are straighter than usual except where occasional infoldings of companion cells into Henle's cells occur (Fig. 10). This is the result of the form taken by the Henle's keratin, which fills the cells up to or very close to the cell membrane. Secondly, where desmosomes occur in the membranes of these apposed cell types, cytoplasmic plaques are apparent on the companion cell side but not on the Henle cell side (Fig. 10).

IV. Discussion

The evidence presented in this paper shows that in the ORS the elongated and flattened cells found next to Henle's layer (Pinkus 1927; Gibbs 1938; Auber 1952; Montagna 1962; Straile 1962, 1965; Rogers 1964) originate in the bulb of the wool follicle as a cell type which is distinct from other cell types in the ORS. The location in the bulb and early differentiation of this cell type suggests that it arises from cells

Fig. 6.—Companion cell (*CC*) above bulb. This cell has separated from the ORS during processing and shows the presence of low electron-dense fibrils (*f*) along most of the cell margins. Electron-dense fibrils (*F*) are also present along the margin of the cell apposed to Henle's layer (*H*). Arrows point to microtubule cross-sections. ×34,600.

* Electron density is analogous to optical density in the corresponding context.



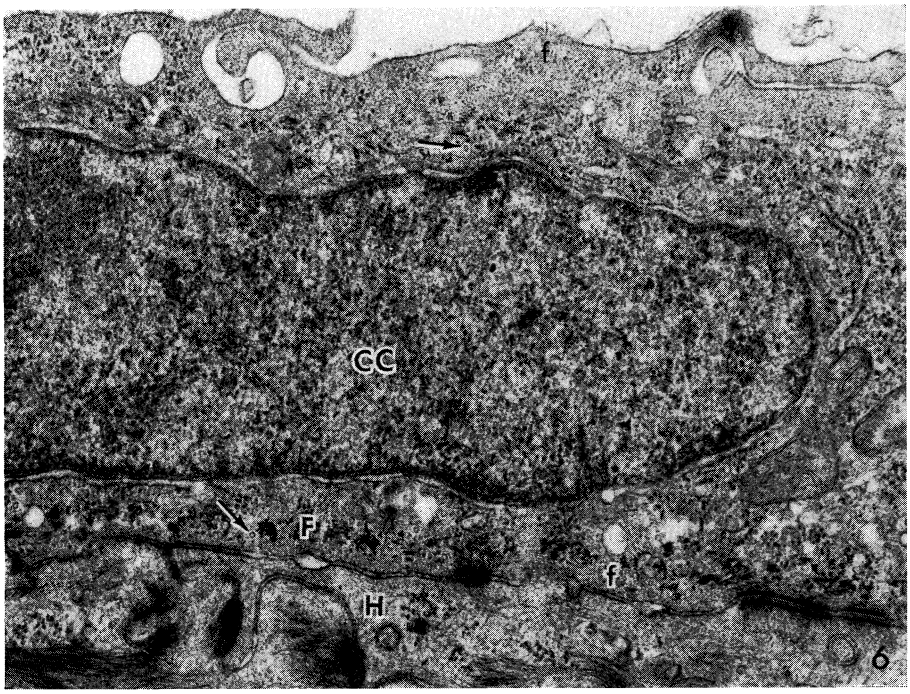


Fig. 5.—ORS zone of proliferation. Companion cells (*CC*) are still elongated and flattened with no intercellular gaps apparent between them and the keratinized (hardened) Henle's cells (*H*). Intercellular gaps (*i*) are present between companion cells and ORS cells (*OR*). ×9600.

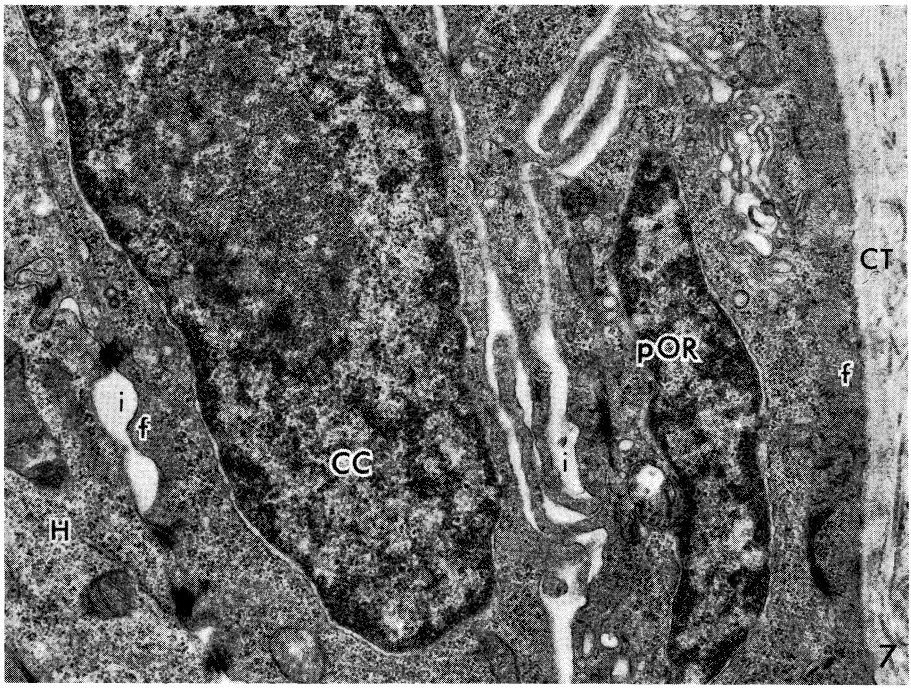
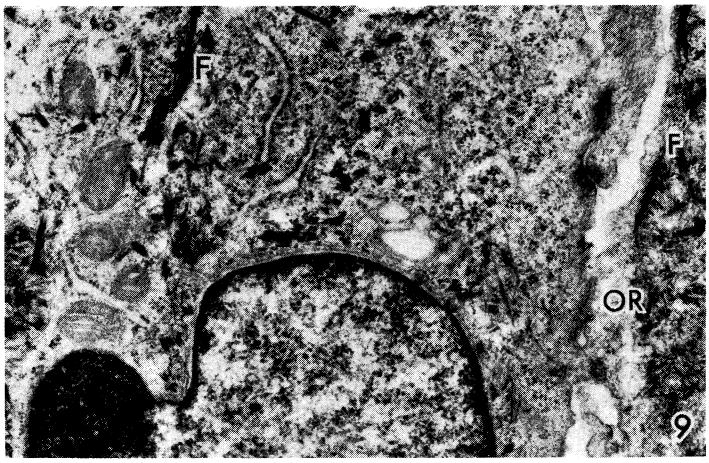


Fig. 7.—Companion cell (*CC*) in lower bulb. Low electron-dense fibrils (*f*) are present in some regions of the cell margin of the companion cell and also near the surfaces of the pre-ORS cell (*pOR*) apposed to the connective tissue sheath (*CT*). *H*, Henle's cell; *i*, intercellular gap. ×20,200.

Fig. 8.—Companion cell (*CC*) in ORS zone of proliferation (*OR*). Electron-dense fibrils (*F*) occur in the cytoplasm and around the cell margins where they are oriented at right angles to the cell membrane. *H*, Henle's cell. ×31,800.

produced near the base of the dermal papilla. This is in agreement with several authors' (Pinkus 1927; Auber 1952; Epstein and Maibach 1969) findings that the different cell types in the follicle originate from dividing cells located on or near particular regions of the dermal papilla surface.



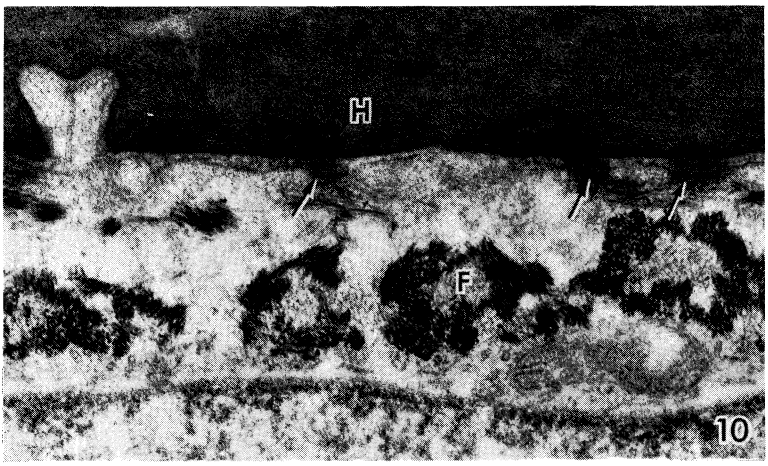


Fig. 9.—Companion cell above follicle bulb. Electron-dense fibrils (*F*) in the companion cell appear similar in appearance to those in ORS cytoplasm (*OR*). ×21,000.

Fig. 10.—Companion cell margin apposed to keratinized (hardened) Henle's cell (*H*). An infolding of the companion cell into the Henle's cell has been retained after keratinization of the Henle's cell. The protein of the Henle's cell extends close to the cell membrane so that the desmosomes (arrows) appear to lack cytoplasmic plaques. *F*, electron-dense fibrils. ×43,700.

Apart from their origin, other differences between companion cells and ORS cells in the lower third of the follicle, as observed in this and other studies, would include the following characteristics. Companion cells are elongated and flattened in shape, are tightly apposed to keratinized Henle's cells but not to ORS cells, do not accumulate large quantities of glycogen in contrast to other ORS cells, and are the

first of the cell types to accumulate a fibrillar keratin-like material. These differences mainly support Rogers' (1964) hypothesis that companion cells move towards the skin surface as part of the fibre–IRS complex. In particular, the close apposition of the membranes of companion cells and Henle's cells including the infolding of companion cells into keratinized Henle's cells and frequent occurrence of the junctional complexes—the desmosomes—suggest that relative movement between these two types would involve considerable and continual reorganization of their surfaces. In contrast, the flattened and elongated shapes of companion cells and the intercellular gaps and relatively few desmosomes where companion cells and ORS cells are apposed point to easier reorganization of the cell surfaces if companion cells move relative to ORS cells. Furthermore, the fibrillar material synthesized near the cell membrane and in the cytoplasm may help to retain the form of companion cells during such movements.

Although the morphological evidence suggests that companion cells move with keratinized Henle's cells, this study indicates further that, in the wool follicle at least, this may occur from the early stages of differentiation of these cell types. In other words, a companion cell may be a true companion to a Henle's cell. Evidence to support this possibility is limited, however. Epstein and Maibach (1969) found that cells labelled with tritiated thymidine and closely associated with IRS cells moved from the bulb into the ORS. However, they could not determine whether these cells represented a separate and distinct layer which moved up with the IRS. The present findings would indicate that there are likely to be two layers of cells closely apposed to the IRS cells which would further complicate Epstein and Maibach's interpretation of their data. However, in the bulb, they did find that IRS cells migrated upwards faster than precortical cells and companion cells may be important in this movement. These authors and Straile (1962, 1965) observed mitoses in the zone of proliferation of the lower ORS, indicating that ORS cells migrate upwards with the IRS. The evidence presented here, although not comprehensive, would indicate that companion cells do not contribute to the mitoses in this zone of the ORS as dividing companion cells were not observed above the level where trichohyalin is first observed in Henle's cells. Obviously, evidence for movement of companion cells relative to Henle's cells and/or other ORS cells awaits further experimentation.

Finally, in view of their close association with Henle's layer, it is tempting to suggest that companion cells may have a role in the differentiation and breakdown of these cells.

V. Acknowledgments

The technical assistance of Miss H. Gilmore and Mr. R. W. Thomson is gratefully acknowledged.

VI. References

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