THE DISTRIBUTION OF COATED VESICLES IN KERATINIZING CELLS OF THE WOOL FOLLICLE

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Abstract

The presence and distribution of coated vesicles in the cells of the lower third of the Romney wool follicle are described, with special reference to cortical cells. Coated vesicles were typically found free in the cytoplasm and associated with plasma membranes and Golgi complexes. They were most prevalent in the bulb and in the elongation and lower keratogenous zones of the cortex. However, the numbers of coated vesicles decreased markedly in the remainder of the keratogenous zone, especially of those associated with plasma membranes. A similar decrease in the numbers and sizes of Golgi complexes in this zone was also noted. The possible role of coated vesicles in the lower wool follicle is discussed.

I. Introduction

Coated vesicles have been reported in various tissues. These vesicles have been divided into two populations on the basis of mean diameter in the tissues studied by Friend and Farquhar (1967), Garant and Nalbandian (1968), Bonneville, Weinstock, and Wilgram (1968), and Arstila *et al.* (1971). Furthermore, the coated vesicles of smaller diameter have been shown to have multiple functions. In general, depending on the tissue studied, coated vesicles are involved in the transport of material from the Golgi complex to various organelles or from the plasma membrane surface to various organelles in the cytoplasm or both processes (Palay 1963; Bruni and Porter 1965; Friend and Farquhar 1967; Kanaseki and Kadota 1969; Cornell 1970; Arstila *et al.* 1971).

In this study the occurrence of coated vesicles in the lower region of the wool follicle is described. Their distribution is examined with special reference to the cortex as a first stage in determining their role.

II. Materials and Methods

Follicles were dissected from biopsies taken from the midside of Romney wethers and fixed in Karnovsky's cacodylate-buffered (pH 7·4) glutaraldehyde–formaldehyde fixative for 4 hr at 4°C. After post-fixation in 1% OsO_4 in the same buffer, the follicles were dehydrated in a graded series of ethanols and embedded in Epon. Silver to gold longitudinal sections of the lower halves of the wool follicles were stained with uranyl and lead salts and examined in a Philips EM300 electron microscope operating at 60 kV.

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Ten follicles were used for the quantitation of the numbers of coated vesicles present in cortex cells. In order to follow changes in vesicle numbers during differentiation of the cortex the follicle was divided into four zones as shown in Figure 1:

Zone A is the base area of the follicle up to a line drawn through the top of the dermal papilla.

Zone B is the area between zone A and the level where Henle's layer hardens. It includes mainly the elongation zone.

Zone C is the area between zone B and the level where the cell membrane of cortex cuticle cells apposed to inner root sheath cuticle cells has a continuous layer of cortex cuticle keratin associated with it. This zone finishes approximately one-third of the way up the keratogenous zone.

Zone D is the area from zone C to the level where the remaining inner root sheath cell lines harden. This zone includes the remainder of the keratogenous zone.

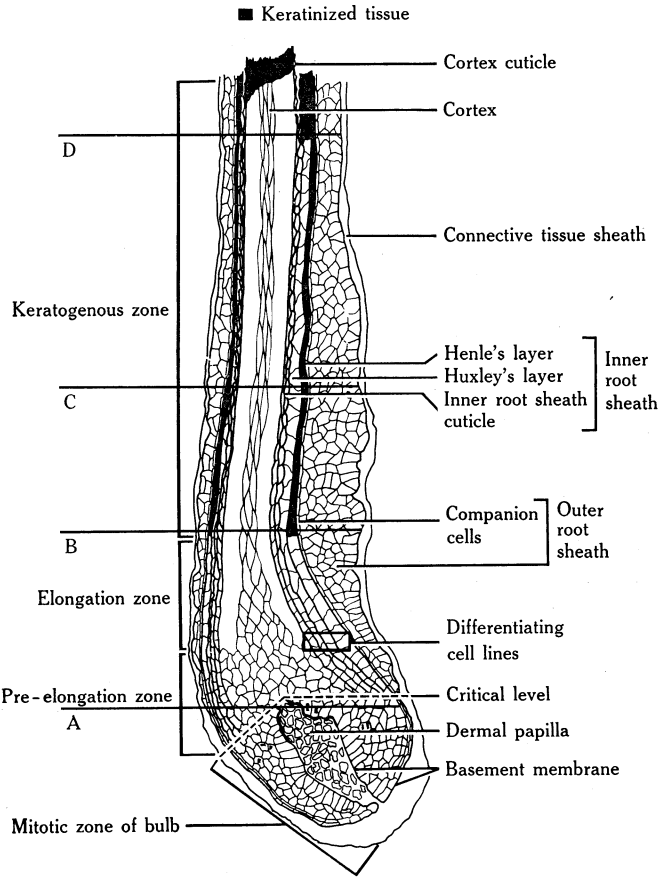
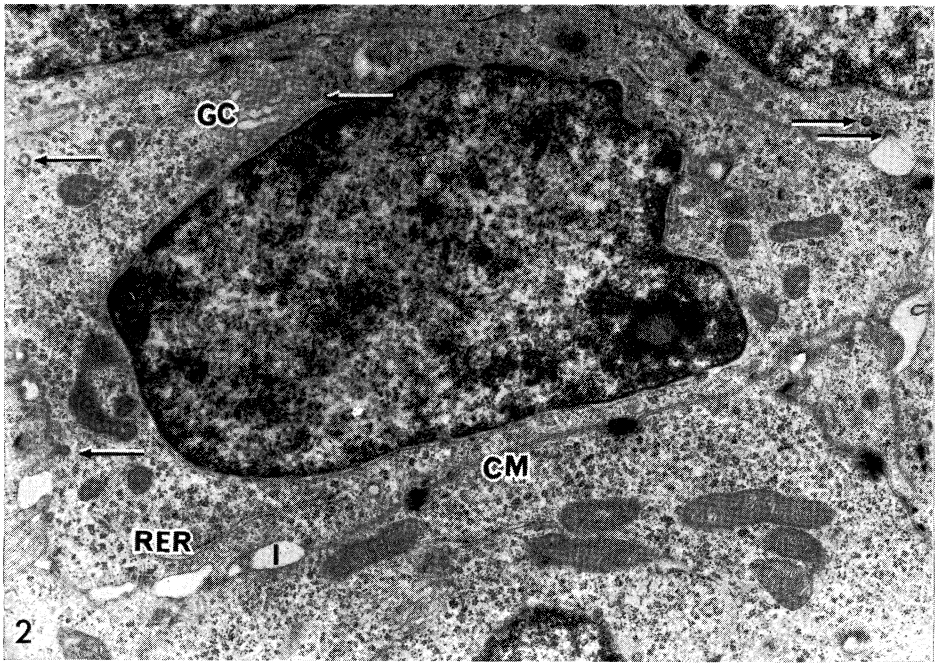


Fig. 1.—Diagrammatic representation of lower wool follicle. Letters refer to the zones used in this study (see Section II for details). Cortex cell outlines are only partly included.

The numbers of coated vesicles were counted in 10 longitudinal cell sections per zone for each follicle. Thus the total number of cell sections examined for coated vesicles in each zone was 100. Although it was not possible to ensure that each follicle was cut precisely longitudinally, attempts were made to ensure that variations were kept to a minimum. Furthermore, coated



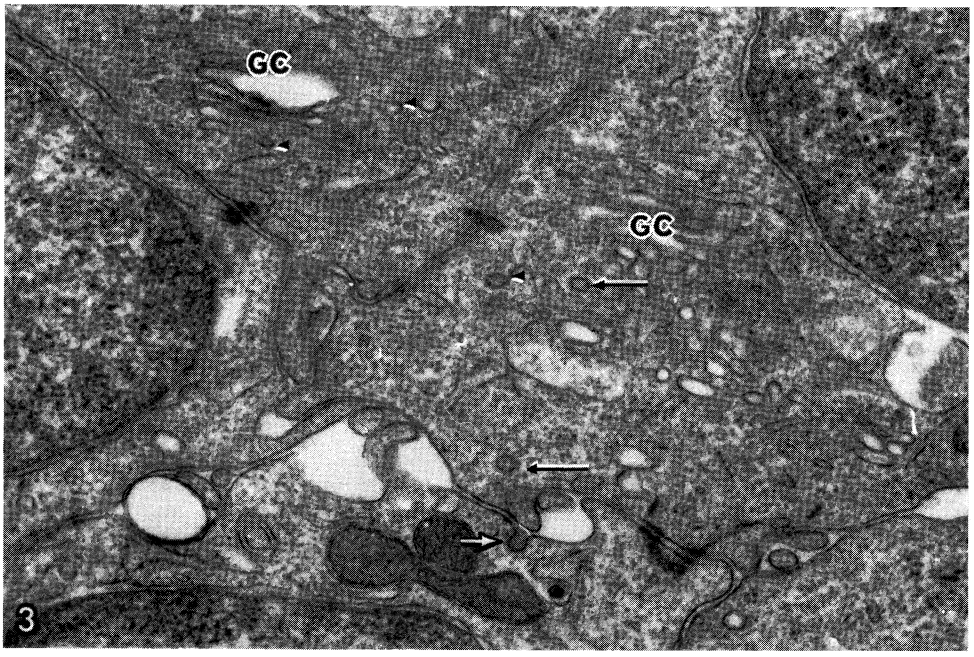
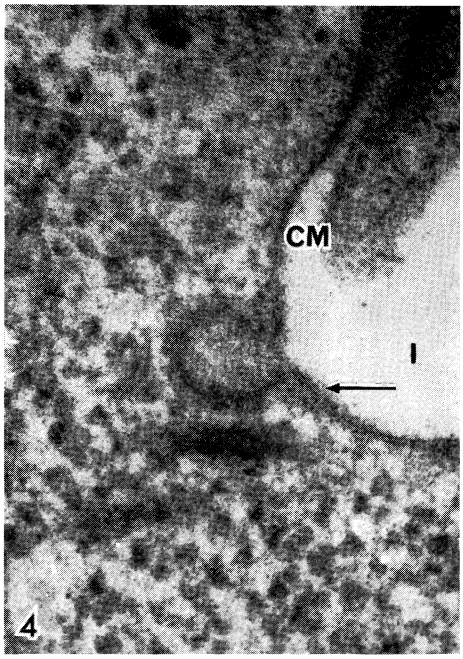
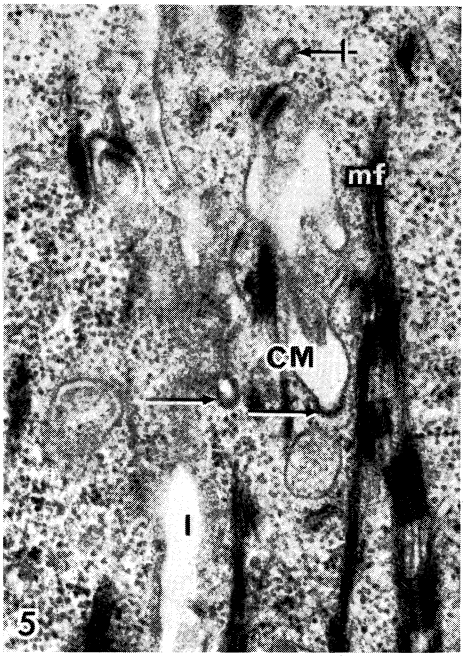
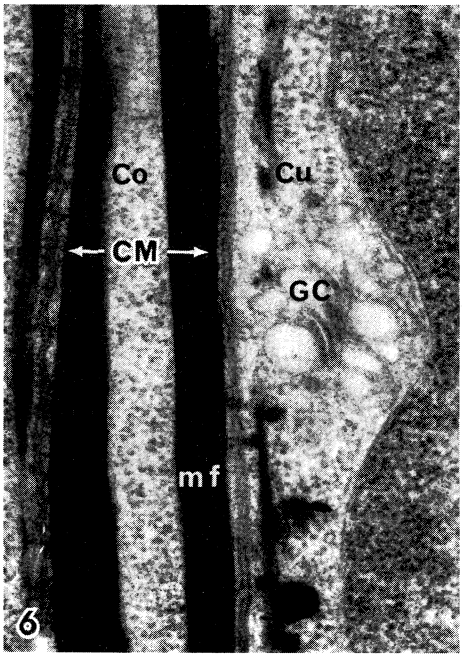


Fig. 2.—Zone A cell. Coated vesicles (arrows) are seen associated with the Golgi complex (*GC*) and cell membrane (*CM*) and free in the cytoplasm. Small amounts of rough endoplasmic reticulum (*RER*) are present. *I*, intercellular gap. ×12,000.

Fig. 3.—Zone A cortex cells. Two Golgi complexes (*GC*) are shown. Coated protrusions are visible at the ends of cisternae and some sacs in the *GC* (arrowheads). Free coated vesicles are also present (black on white arrows). A coated invagination of the plasma membrane at a region where the membranes are closely apposed is also shown (white on black arrow). ×25,000.







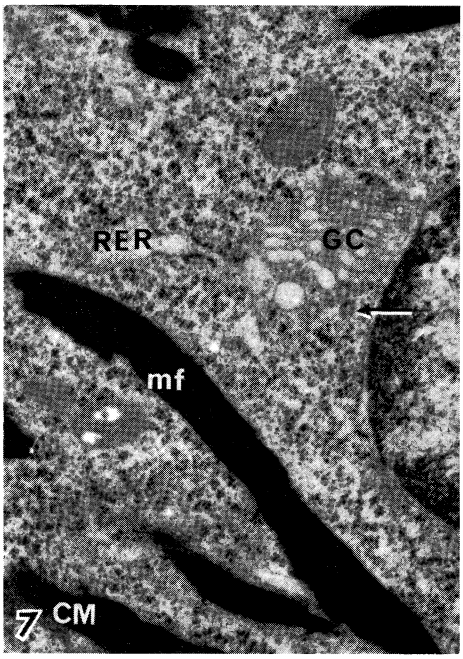


Fig. 4.—Coated invagination of cell plasma membrane (*CM*). The membrane of the invagination appears to be continuous with that of the plasma membrane. The contents of the coated invagination are of similar electron density to the material coating the plasma membrane (arrow). *I*, intercellular gap. ×113,000.

Fig. 5.—Zone B cortex cells. Coated invaginations of the cell plasma membranes (*CM*) are apparent at an intercellular gap (*I*) and where the cell plasma membrane is closely apposed (lower arrows). A free coated vesicle is present near a membrane (upper crossed arrow). *mf*, forming macrofibril. ×36,000.

vesicles were only counted in the largest of the cell longitudinal sections present in any one follicle section. This was done in order to restrict coated vesicle counts to cells which had been cut longitudinally through the middle. Visual assessments of the numbers of coated vesicles and Golgi complexes in the other cells in the lower follicle were also made.

Measurements of the diameter of 34 coated vesicles were made with an ocular micrometer ($\times 7$) graduated to 0·1 mm.

III. Results

In zone A, coated vesicles were present in all cell lines of the wool follicle. In particular, they were found free in the cytoplasm and associated with the Golgi complex, plasma membrane (Fig. 2), and occasionally with endoplasmic reticulum and vacuoles.

The Golgi complex in zone A cells consists of relatively few cisternae but many interconnected sacs. Usually associated with these complexes were free, single membrane-bounded, coated vesicles among the smooth vesicles also present (Fig. 3). In addition, blebs or protrusions of Golgi cisternal or sac membranes occurred which were coated on their cytoplasmic side. It seems likely that these protrusions either pinch off to give the free coated vesicles or represent coated vesicles joining into the cisternae and sacs of the Golgi complex (Fig. 3). The electron density of the material in these coated protrusions is similar to that in free coated vesicles and to the coating on plasma membranes (Fig. 4).

Table 1

Distribution of coated vesicles in the cortex of the wool follicle

Values given are mean numbers per longitudinal cell section

Zone	Coated vesicles			Golgi complexes	
	Free, in cytoplasm	Associated with plasma membrane	Free, near Golgi complex	Number	Coated protrusions
A	2·65±0·091	1·44±0·047	1·24±0·004	0·97±0·030	1·43±0·049
B	3·49±0·120	1·98±0·065	1·19±0·093	1·96±0·071	1·35±0·047
C	2·82±0·099	1·36±0·044	1·46±0·080	1·98±0·064	1·39±0·047
D	0·83±0·037	0·063±0·032	0·34±0·010	0·83±0·028	0·30±0·010

Free coated vesicles were found in all parts of the cytoplasm (Fig. 2). Their mean diameter was 70±14 nm with a range of 50–100 nm.

Coated invaginations of the cell plasma membrane were also seen (Figs. 2, 3, 4, and 5). The appearance of these suggested that either free coated vesicles were fusing with the cell plasma membrane or that they were forming at the cell surface. These coated invaginations occurred both at intercellular gaps and in regions where the plasma membranes of two cells were closely apposed (Figs. 3, 4, and 5).

Fig. 6.—Upper zone C cortex (*Co*) and cortex cuticle (*Cu*) cells. The membranes (*CM*) of these cells are closely apposed with no intercellular gaps present. The Golgi complex (*GC*) in the cuticle cell appears vesiculated and lacking in coated vesicles. *mf*, forming macrofibril. ×38,000.

Fig. 7.—Zone C cortex cell. The Golgi complex (*GC*) is smaller than those normally found in zone A cells (cf. Fig. 3) and has one coated vesicle (arrow) associated with it. The rough endoplasmic reticulum (*RER*) is swollen and contains some material of low electron density. *CM*, cell plasma membrane; *mf*, forming macrofibril. ×23,000.

Table 1 shows the distribution of coated vesicles in the cortex of the wool follicle. The most obvious feature is that while coated vesicles occur in all locations in zones A, B, and C (Figs. 3, 4, and 5), they decrease markedly in number in zone D (Fig. 6) especially those associated with the plasma membrane. This trend parallels the decrease in intercellular gaps in zones A, B, and C (Figs. 2 and 5). These gaps disappear by upper zone C and zone D so that only the normal space between closely apposed plasma membranes is evident (Fig. 6).

The numbers of free coated vesicles and coated membrane invaginations (coated vesicles associated with plasma membranes) increase from zone A to reach a maximum in zone B, but decrease in zone C despite the continuing increase in cell size. Conversely, the maximum numbers of free coated vesicles near the Golgi complexes occur in zone C cells.

The number of Golgi complexes increases from zone A to zone B, paralleling the increase in cell size. By zone D the number and size of Golgi complexes have decreased (cf. Figs. 3 and 7) and by upper zone D, prior to keratinization, it is very difficult to define any complexes at all.

Visual assessment of the numbers of coated entities in other keratinizing and hardening cell lines showed similar trends to those seen in cortical cells. Coated vesicles, coated Golgi protrusions, and coated plasma membrane invaginations were prevalent during the early stages of differentiation of these cells but as the cells became more closely apposed to neighbouring cells and reached their final sizes the number of coated vesicles decreased rapidly until coated entities were usually absent before keratinization or hardening took place.

An unusual case occurred in some follicles at the apposing surfaces of inner root sheath cuticle and Huxley's cells. Here, the surfaces usually became closely apposed by the top of zone B. In some follicles, however, these surfaces separated again to give long intercellular gaps (Fig. 8). These intercellular gaps remained evident in zone C and often in lower zone D. Coated invaginations of the cell plasma membranes were often seen in these zones (Fig. 9). Increasing cell adhesion occurred in zone D and, a few cells before the hardening of these two cell layers, intercellular gaps and coated vesicles disappeared (Fig. 10). Although the means by which this separation occurred was not apparent, it is noteworthy that it occurred in the region where the cuticle cells of the inner root sheath and cortex were undergoing considerable changes in shape. Where separation of these two cell layers did not occur, coated vesicles were rarer in zones C and D and were usually associated with Golgi complexes.

Within companion cells and outer root sheath cells, a similar distribution of coated vesicles to that in keratinizing and hardening cells was found. However, the distribution of coated vesicles did not follow the same changes as keratinizing or hardening cells in zones A to D, and coated vesicles and Golgi complexes were still

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Fig. 9.—Zone C inner root sheath cuticle (*IRSC*) and Huxley's layer (*Hu*) cells. Coated vesicles (arrows) are associated with the plasma membranes at intercellular gaps (*I*). *D*, desmosome; *t*, fibrous trichohyalin. ×37,000.

Fig. 10.—Upper zone D. Intercellular gaps are no longer apparent between the plasma membranes (arrows) of inner root sheath cuticle (*IRSC*) and Huxley's (*Hu*) cells. *He*, Henle's layer; *t*, trichohyalin. ×11,500.

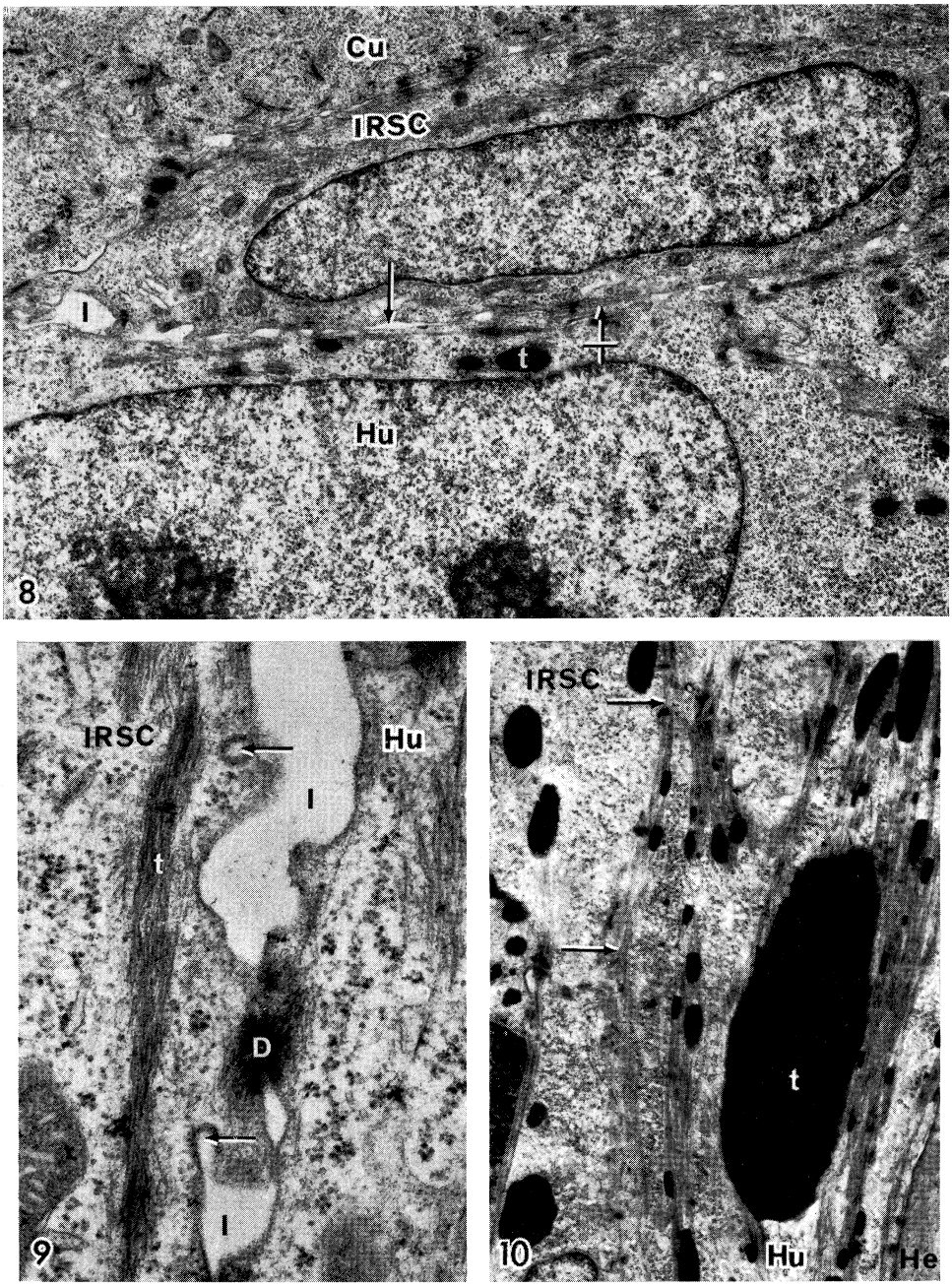
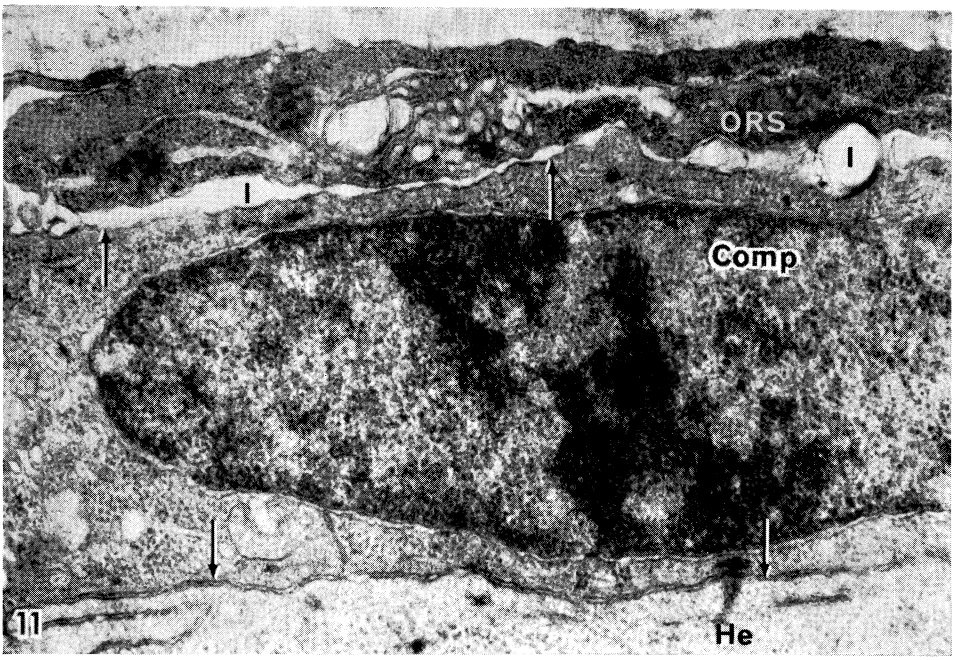


Fig. 8.—Zone B. The close attachment of Huxley (*Hu*) and inner root sheath cuticle (*IRSC*) cell surfaces that normally occurs by this zone is shown at the crossed arrow. The reappearance of intercellular gaps (*I*) that occurs in some follicles and is maintained in the remainder of zones B and C is also shown (arrow). *Cu*, cortex cuticle; *t*, trichohyalin. ×11,000.



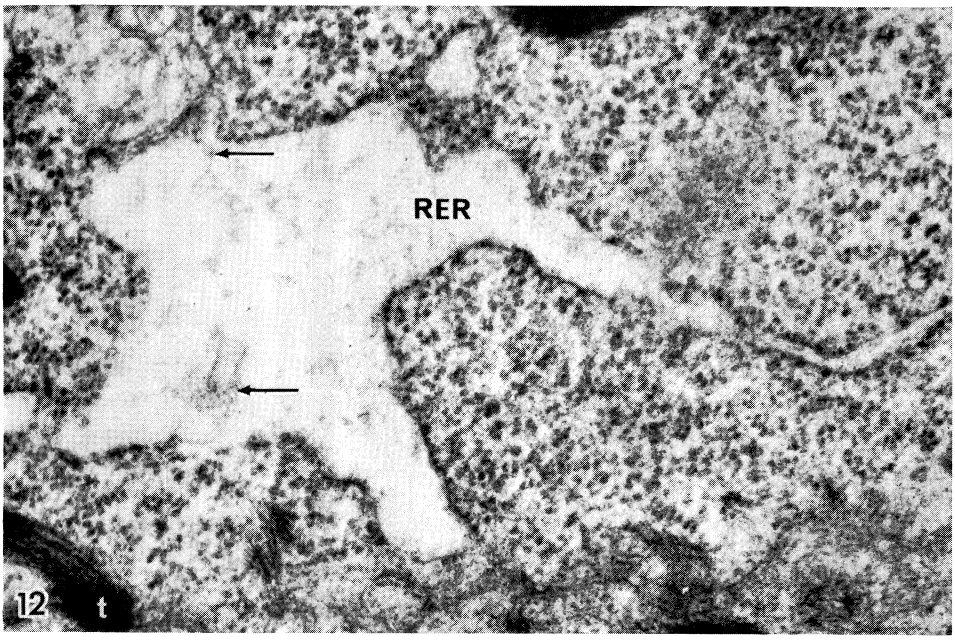


Fig. 11.—Zone B companion cell. The plasma membranes (arrows) are closely apposed between the Henle's (*He*) cell and companion (*Comp*) cell whereas extensive intercellular gaps (*I*) occur between the outer root sheath (*ORS*) cell and companion cell. ×33,000.

Fig. 12.—Zone B Henle cell. The rough endoplasmic reticulum (*RER*) has become very swollen. Small amounts of a slightly electron-dense material are present (arrows). *t*, trichohyalin. ×60,000.

present in zone D cells. Despite this, the intercellular gaps found between companion cells and Henle's cells at the base of the follicle decreased as differentiation proceeded until the plasma membranes of these two cell lines were closely apposed whereas the intercellular gaps between companion cells and outer root sheath cells remained (Fig. 11).

During this study, it was noted that the decrease in size and numbers of Golgi complexes in keratinizing and hardening cells was often associated with increasing vesiculation of both Golgi complexes and rough endoplasmic reticulum before their final breakdown prior to keratinization or hardening (Fig. 7).

This was most marked in Henle's cells in the later stages of differentiation where a major part of the membranous system appears to form large vacuolar-like swellings of rough endoplasmic reticulum (Fig. 12). The slightly electron-dense material which is often found within these swellings may represent a method of storing material (enzymes?) involved in the transition of trichohyalin to the hardened material found in inner root sheath cells.

IV. Discussion

The coated protrusions and free vesicles of the Golgi complex described in this study parallel findings in other tissues (Bruni and Porter 1965; Friend and Farquhar 1967; Bonneville, Weinstock, and Wilgram 1968; Weinstock and Wilgram 1970; Arstila *et al.* 1971). Similarly, coated invaginations of the plasma membrane have been described in the tissues studied by Palay (1963), Bruni and Porter (1965), Friend and Farquhar (1967), Cornell (1970), and Arstila *et al.* (1971).

The mean diameter of free wool follicle coated vesicles corresponds to that of the small population of coated vesicles reported in the tissues studied by Friend and Farquhar (1967), Garant and Nalbandian (1968), and Arstila *et al.* (1971).

In other tissues, these small coated vesicles have been reported to have several functions as follows: to carry material from the Golgi complex to the cell plasma membrane in the rat *vas deferens* (Friend and Farquhar 1967); to carry hydrolases from the Golgi complex to multivesicular bodies in rat liver and *vas deferens* and in human epidermis (Bruni and Porter 1965; Friend and Farquhar 1967; Bonneville, Weinstock, and Wilgram 1968); and to carry material from the cell surface into the cytoplasm in the rat cerebellum (Palay 1963), rat liver (Bruni and Porter 1965), guinea pig liver and brain (Kanaseki and Kadota 1969), mouse embryo cell strains (Cornell 1970), and Hela cells (Arstila *et al.* 1971).

The distribution of coated vesicles in the wool follicle, while not providing direct evidence of their function, nevertheless indicates likely roles. In general, the association of coated vesicles with Golgi complexes and plasma membranes suggests that they are involved in the transport of material from the Golgi complex to the cell plasma membranes or *vice versa*. The decrease in coated vesicle numbers in zone D associated with completion of cell adhesion indicates that the former pathway may be the more likely. There are several studies in other tissues to support this pathway as evidence for the synthesis of cell coat material in the Golgi complex and its transport in Golgi-derived organelles to the cell surface has been reported by Neutra and Leblond (1966*a*, 1966*b*), Rambourg, Hernandez, and Leblond (1969), Fleischer, Fleischer, and Ozawa (1969), Wise and Flickinger (1970), Bennett (1970), Bennett and Leblond (1970).

However, only Friend and Farquhar (1967) have presented evidence of coated vesicles following this pathway. If coated vesicles are the transport organelles of intercellular material in the wool follicle, then this material is likely to be the polysaccharide-containing cell coat material found on cells in other tissues (Pease 1966; Rambourg 1967; Rambourg and Leblond 1967; Mercer, Jahn, and Maibach 1968) as well as the wool follicle (Orwin 1970).

There are no reports of coated vesicles transporting material in other tissues from the cell surface direct to the Golgi complex. Also, there is no evidence yet that wool follicle cells in the region studied have an absorptive function involving coated vesicles, as described for rat cerebellum (Palay 1963), rat liver cells (Bruni and Porter 1965), mouse embryo cell strains (Cornell 1970), and Hela cells (Arstila *et al.* 1971). Furthermore, coated invaginations of the plasma membranes were found in zones A to C in regions of close membrane apposition as well as at intercellular gaps. If coated vesicles are involved in the uptake of extracellular material there seems to be no *a priori* reason why this should not occur in zone D where all the plasma membranes are closely apposed.

This study also presents some indication that the presence of coated vesicles may depend on Golgi complexes. Although the Golgi complexes decrease in size as cortex differentiation proceeds (Forslind and Swanbeck 1966), their numbers increase in zones B and C. This is paralleled by greater numbers of coated vesicles. Similarly, when the numbers of Golgi complexes decrease in zone D, the numbers of coated vesicles also decrease.

However, there are sufficient anomalies to the hypothesized role of coated vesicles presented here to suggest that coated vesicles in the wool follicle may have multiple functions. Although rarely observed, coated blebs were found in the membranes of vacuoles and rough endoplasmic reticulum. Furthermore, in companion cells in zone A (Orwin 1971), coated vesicles were observed associated with the plasma membranes apposing outer root sheath cells and Henle's cells. Yet as differentiation proceeded in zone B, the plasma membranes of companion and Henle's layer cells became closely apposed while those of the companion and outer root sheath cells did not.

Finally, the presence of coated vesicles in zone D associated with the Golgi complex and free in the cytoplasm but not associated with the plasma membrane may also indicate a population of vesicles with a different function. Obviously, this study provides, at best, a working hypothesis for more direct studies on the function of coated vesicles in wool follicle cells.

V. Acknowledgment

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