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Fine structure of bulbar end of human foetal hair follicle at stage of differentiation of inner root sheath

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INTRODUCTION

The fine structure of the human foetal hair follicle from pregerm to early bulbous-peg stages of development has been described in previous papers (Breathnach & Smith, 1968; Robins & Breathnach, 1969). These studies provide an essential background for examining further differentiation of the various components of the developing pilo-sebaceous unit, and for an analysis of the onset and progress of keratinization or hardening within it. Presumptive evidence of early keratinization was found only within the intra-epidermal portion of the hair-tract at the early bulbous-peg stage (Robins & Breathnach, 1969), and it might seem logical, in extending observations to a later stage as at present, that primary attention should be directed towards further developments in this region. However, the fate of the hair-tract is so bound up with the later extension of the tip of the emerging hair anlage along it, that it is desirable first to consider changes taking place at the lower end of the follicle, which gives origin to the hair and its inner root sheath. The present report therefore, deals with further differentiation of the hair-bulb and hair-cone and their development to a stage when a definitive inner root sheath with clear-cut Henle and Huxley layers can be recognized.

To date, light microscopic studies of human foetal hair follicles through the stages described here have shown little detail (Pinkus, 1958). Valuable comparisons, however, can be made between the present observations and accounts of the ultrastructure of adult human anagen (Birbeck & Mercer, 1957; Puccinelli, Caputo & Ceccarelli, 1967) and rodent (Parakkal & Matoltsy, 1964; Roth & Helwig, 1964; Roth & Clark, 1964; Kint, 1967) follicles. These studies have shown that the final step in the maturation of inner root sheath cells is abrupt, so that adjacent cells of widely different appearance are encountered. Examination of graded foetal material might be expected to throw further light on this transition, and on related problems such as the origin of trichohyalin, and its alleged conversion into the filaments of the hardened cell.

MATERIALS AND METHODS

Observations were made on 20 human foetuses, of C.R. length 130 mm and upwards, obtained at hysterotomy. Skin was taken from upper lip, eyebrow, scalp, back, arm and leg regions, and fixed either in 2% buffered osmium tetroxide for 2 h, or in 2% buffered glutaraldehyde for 0·5 h, followed by postfixation in osmium tetroxide for another 2 h. After dehydration in 70, 90% and absolute alcohols, tissue

blocks were embedded in Araldite, and thin sections mounted on large-hole carbon-coated grids were stained with lead hydroxide and examined in a Siemens Elmiskop I electron microscope. Some blocks were bulk-stained with alcoholic PTA during dehydration

To obtain true longitudinal sections of hair follicles, blocks were roughly orientated when first removed from the foetus, and final adjustments in cutting orientation were made by studying 1 μm thick toluidine blue stained sections of the Araldite blocks by light microscopy. Hair follicles at various stages of development were present in the different localities and foetuses examined, and primary follicles of younger foetuses and secondary follicles of older ones were found suitable for observation of the stages under consideration. The extent of envelopment of the dermal papilla by the hair-bulb was used as a guide in estimating the general degree of development of individual follicles, particularly when making comparisons.

OBSERVATIONS

The term 'keratinization' can have several different meanings depending upon the sense in which it is used, i.e. whether physical, chemical or morphological. In this report it is used mainly in the last sense to cover the whole sequence of structural changes involved in the transformation of relatively undifferentiated cells present at the early bulbous-peg stage (Robins & Breathnach, 1969) into elements with homogeneous, predominantly filamentous cytoplasm, and which, by comparison with the mature follicle, can be regarded as almost completely or fully keratinized. The final stages in the process will be referred to as 'hardening'.

(1) *Hair-bulb at onset of keratinization*

Fig. 1 presents a central longitudinal section through one half of the bulb of a follicle, within which, at the immediately higher level of the hair-cone (Fig. 4), small granules of trichohyalin can be detected. The elongated bulbar cells adjacent to the papilla (*DP*) may be designated matrix cells (*M*), and their cytoplasmic features are essentially similar to those of the basal cells of the earlier bulbous-peg as previously described (Robins & Breathnach, 1969). In the upper left-hand area of the field, i.e. above a line drawn transversely at the level of the apex of the papilla (*A*), more rounded presumptive hair-cone cells (*PHC*) can be seen, and at the periphery, cells of the presumptive outer root sheath (*ORS*).

Presumptive hair-cone cells are more rounded than matrix cells, and spaces of varying size occur between them (Fig. 2). Desmosomes are present along the plasma membrane, and tonofilaments are associated with them. Elsewhere in the cytoplasm,

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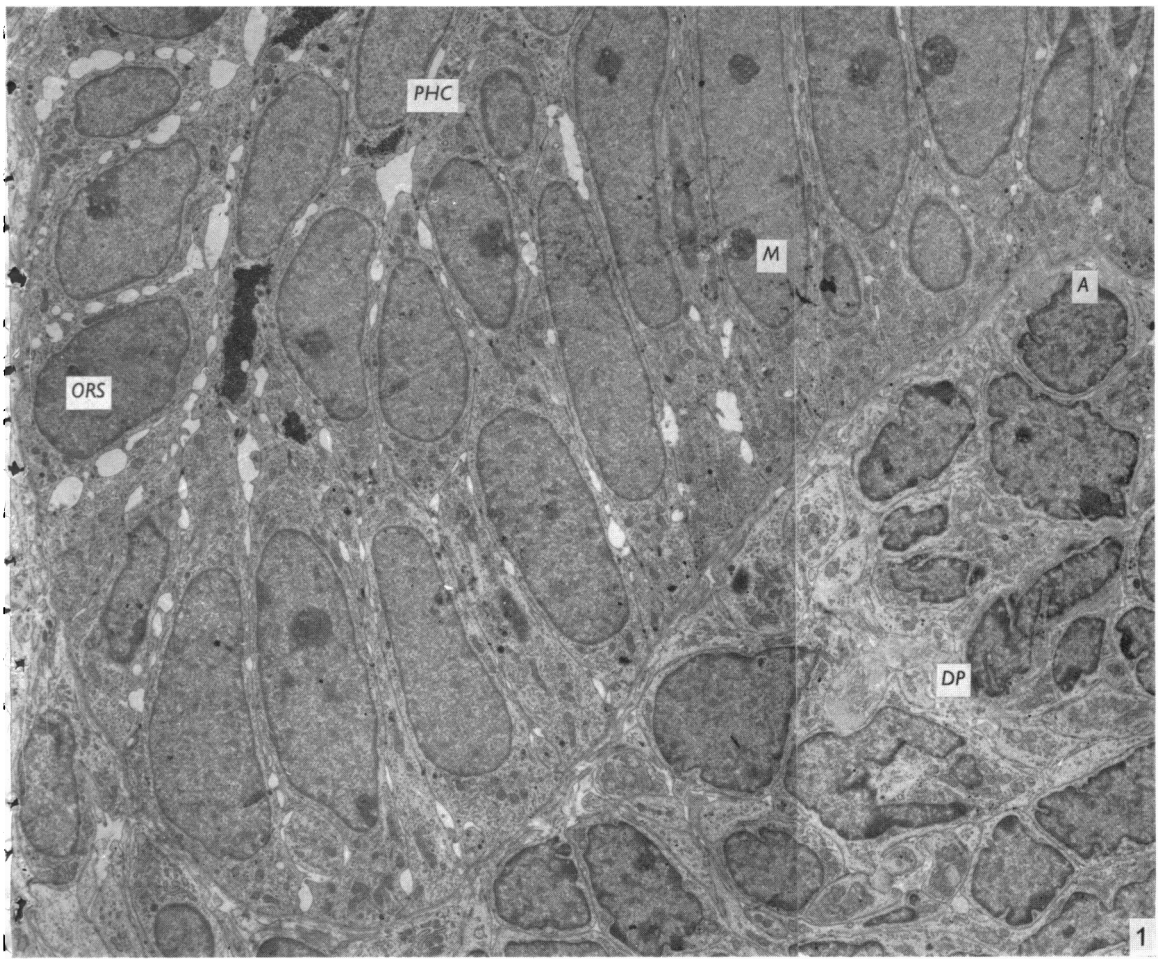
Fig. 1. Montage of central longitudinal section through one half of bulb of follicle within which production of trichohyalin granules has commenced at a higher level (Fig. 4). *DP*, dermal papilla; *A*, apex of papilla; *M*, matrix cells; *ORS*, outer root sheath; *PHC*, presumptive hair-cone cells. ×4500.

Fig. 2. Presumptive hair-cone cells (*PHC*) and outer root sheath cells (*ORS*) showing general features and disposition. ×12250.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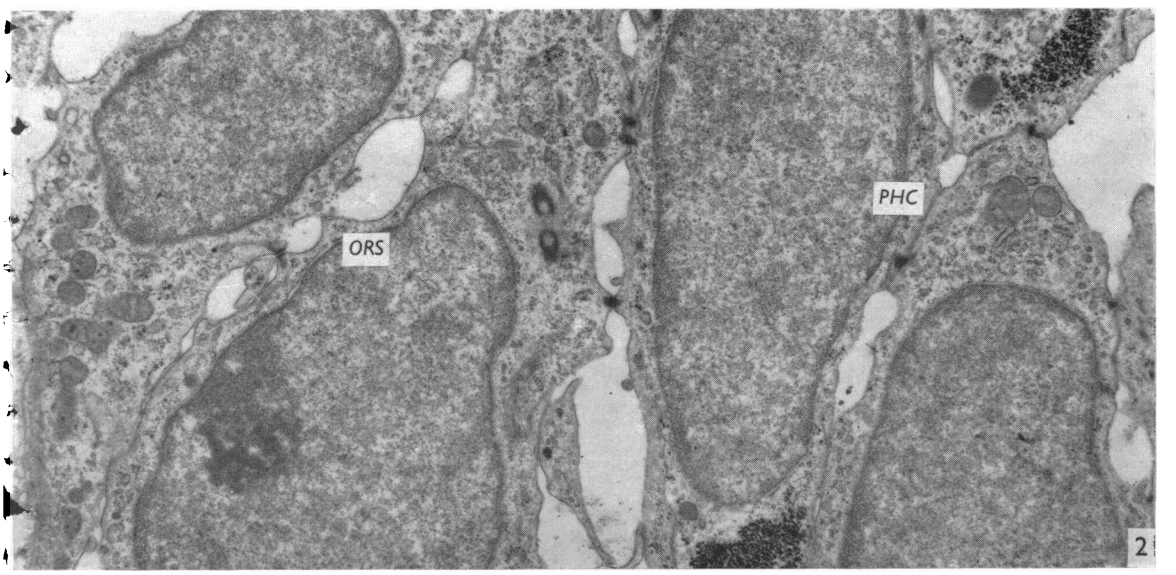
1


ORS
PHC
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filaments are very sparsely distributed—being limited to one or two fine bundles in any one section. Golgi membranes, centrioles, mitochondria, isolated glycogen deposits and numerous free ribosomes are present. Presumptive outer root sheath cells are not significantly different in respect of the above features.

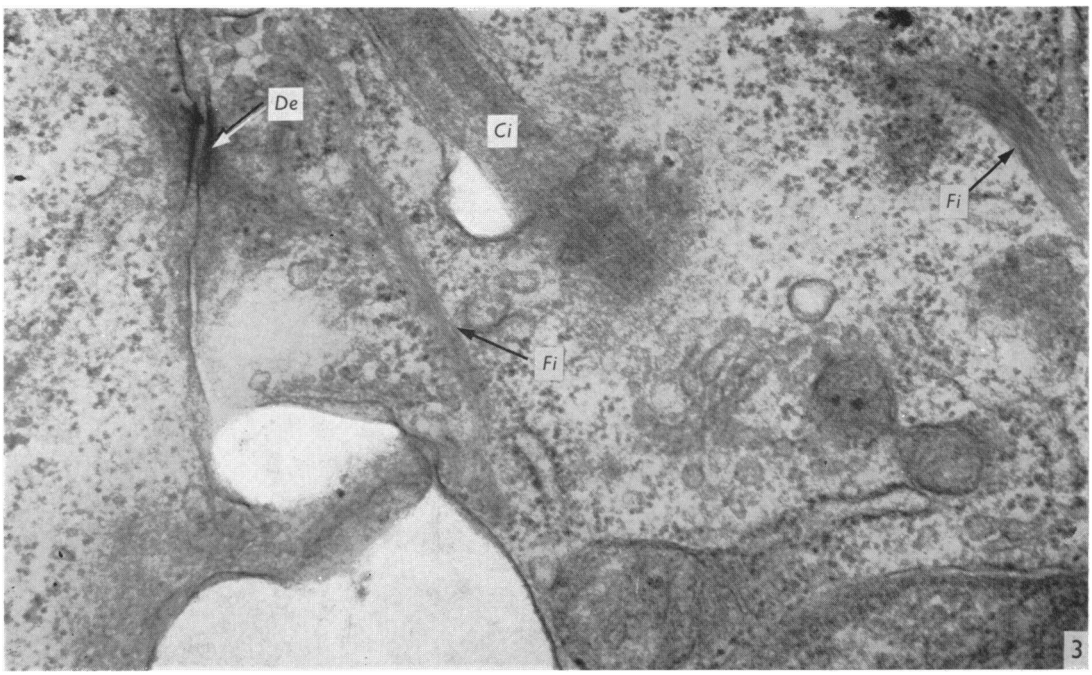

Fig. 3. Cytoplasm of hair-cone cell of earlier bulbous-peg stage, i.e. before appearance of trichohyalin granules. In addition to common organelles, filaments (*Fi*) are present in the cytoplasm. *De*, desmosome; *Ci*, cilium. × 50000.

(2) *Appearance of keratohyalin in hair-cone*

Immediately above the presumptive cone cells is the hair-cone itself, centrally placed in the follicle and surrounded by cells of the outer root sheath (see Robins & Breathnach, 1969). At the early bulbous-peg stage immediately preceding that under consideration here, hair-cone cells present the appearance illustrated in Fig. 3. Desmosomes and occasional cilia are associated with the plasma membranes and intercellular spaces are prominent. The cytoplasm contains the common organelles

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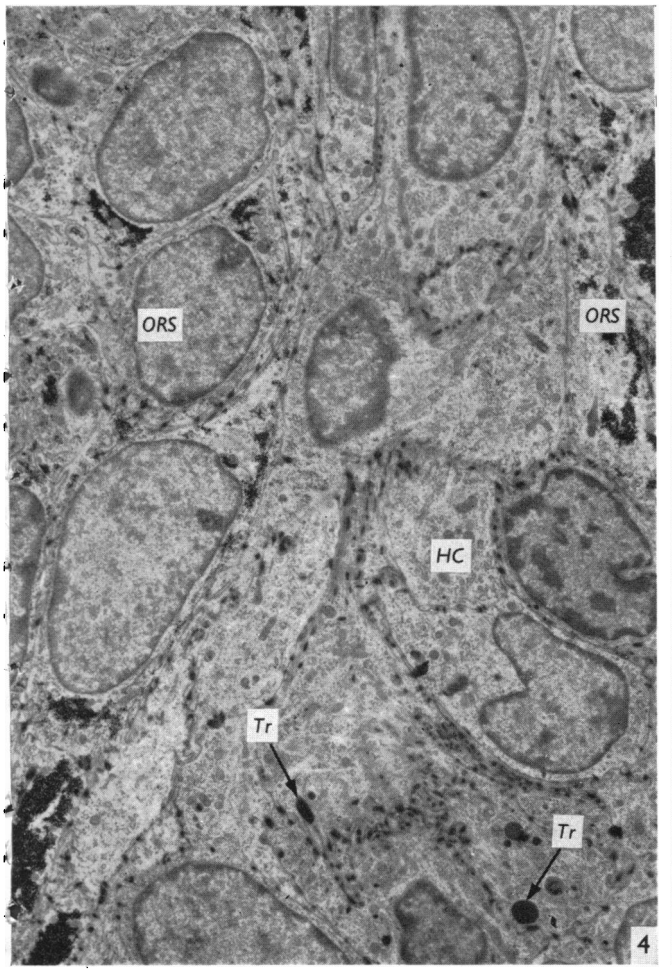
Fig. 4. Central longitudinal section of hair-cone (*HC*) of follicle illustrated in Fig. 1. *Tr*, trichohyalin granules in basal cells of cone; *ORS*, outer root sheath. × 5700.

Fig. 5. Trichohyalin granules and filaments in cytoplasm of hair-cone cell. Filaments can be traced for some distance into the upper two granules. The lower granule exhibits typical 'speckled' appearance. × 73500.

Fig. 6. Cytoplasm of hair-cone cell showing filaments and associated electron-dense trichohyalin granules. Bulbar end of follicle towards the right. × 40300.


ORS
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4


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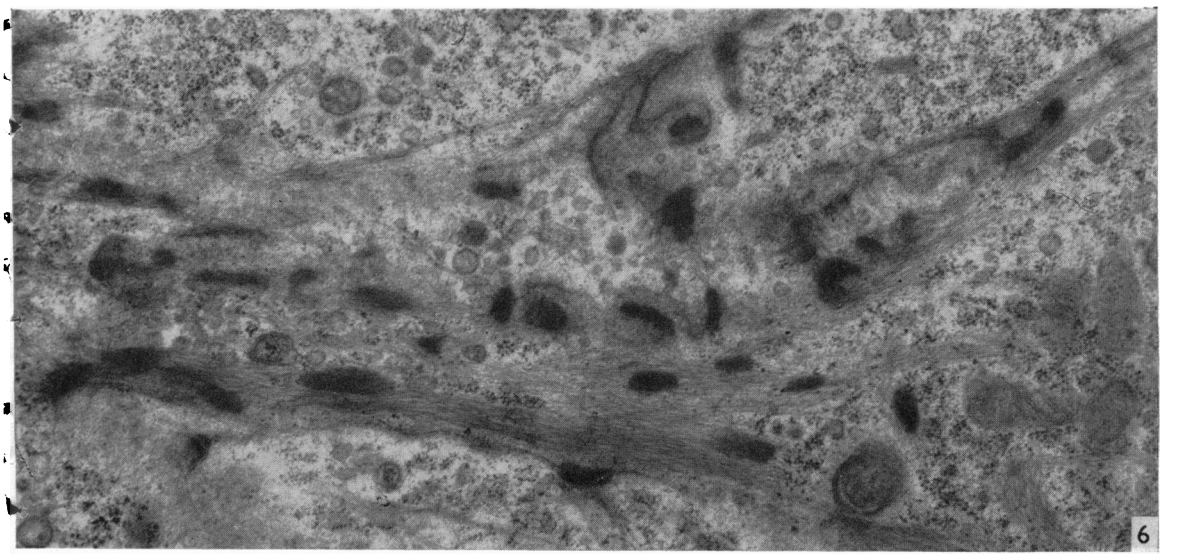
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and scattered small bundles of filaments. The latter are not very prominent, but it may be stressed that they are present within hair-cone cells before any evidence of trichohyalin can be detected.

Hair-cone cells at an immediately later stage of development (judged by extent of envelopment of the dermal papilla by the bulb; see Fig. 1) than those in Fig. 3, are presented in Fig. 4. The cells are now more compactly arranged, intercellular spaces being virtually absent and desmosomes more numerous. Cytoplasmic contents of the cells are similar to those of earlier hair-cone cells, and of presumptive hair-cone cells of the bulb of the same follicle (Figs. 1, 2), except that filamentous bundles are more numerous, and small electron-dense areas, morphologically identical with trichohyalin granules of more advanced follicles, are associated with them (Fig. 6). At this stage, trichohyalin-containing cells are located exclusively above the level of the apex of the papilla, but as this latter becomes more extensively enveloped (Fig. 9) they extend downwards to a region which corresponds to the 'critical level' (Montagna, 1962) of the bulb of the mature follicle.

Trichohyalin granules (Figs. 5, 6) are invariably associated from first appearance with filaments which appear to run into them. Filaments are clearly visible at the periphery of the granules, but not at the centre. It is not possible from purely morphological observations to make any definite statement concerning the origin of trichohyalin. We could find no evidence from examining these early trichohyalin granules, or smaller granules within non-hardened inner root sheath cells at a later stage of development (Fig. 13) that the increase in electron density whereby they become manifest is due to the deposition of an added amorphous component along, or between the filaments. At high magnification (Figs. 5, 14, 15) the granules present a finely granular 'speckled' appearance which we do not feel it is justifiable to interpret as being due to unaltered filaments embedded in an amorphous matrix. We feel rather that the pattern presented is more consistent with a suggestion that trichohyalin granules are regions where some chemical or molecular reorganization of filamentous substance occurs. However this may be, it is clear that in ontogeny, filaments antedate trichohyalin granules in the cells of the hair-cone.

(3) *Further differentiation of hair-cone*

At a slightly later stage of development than that illustrated in Figs. 1 and 4, the base of the hair-cone just above the apex of the dermal papilla is composed of elongated cells with closely apposed parallel plasma membranes (Fig. 7). The more centrally placed cells contain small elliptical trichohyalin granules, while those im-

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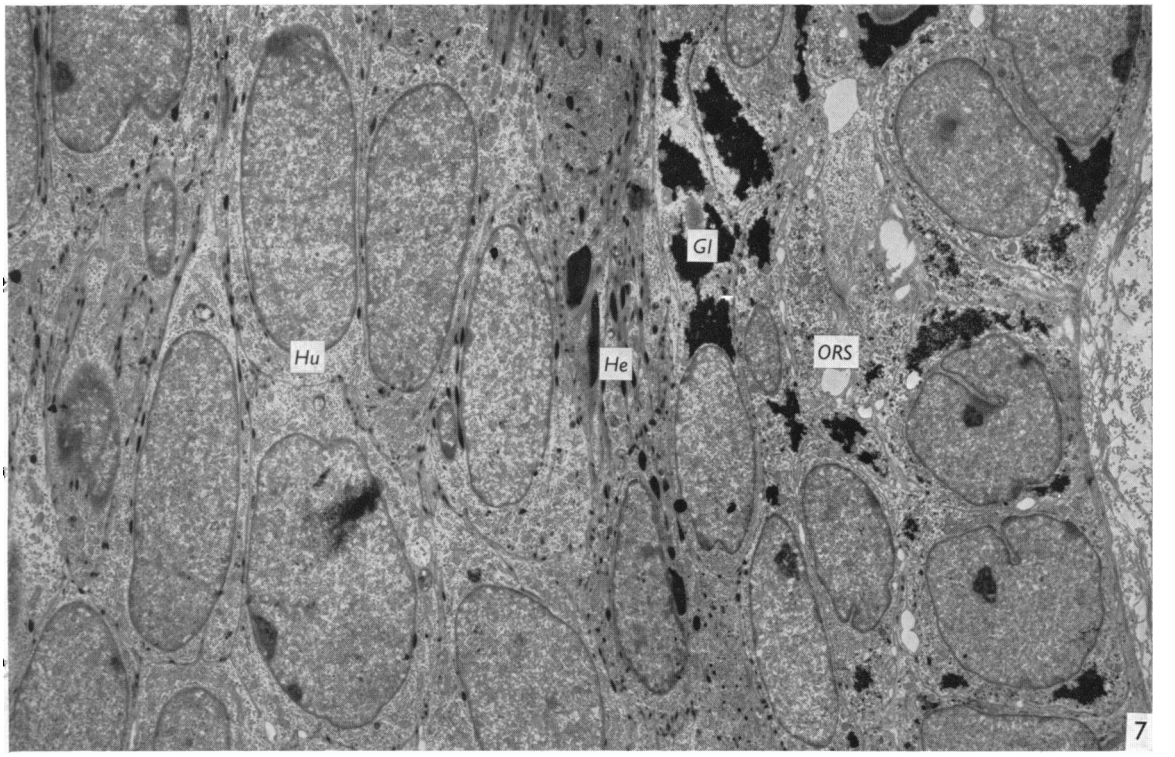
Fig. 7. Central longitudinal section through basal part of hair-cone and outer root sheath (*ORS*) of a follicle at a more advanced stage of development than that in Fig. 4. The outermost cells of the hair-cone containing large trichohyalin granules form the Henle (*He*) layer of the inner root sheath, and the more centrally lying cells with smaller granules, constitute the Huxley (*Hu*) layer. *Gl*, glycogen in cells of outer root sheath. × 3200.

Fig. 8. Transverse section of apical region of hair-cone before hardening has commenced. The central core of cells containing trichohyalin granules (*Tr*) forms the hair-cone (*HC*) of inner root sheath cells, and is surrounded by glycogen-containing cells of the outer root sheath (*ORS*). *Ar*, electron dense and vacuolated areas indicative of autophagic activity. × 8000.



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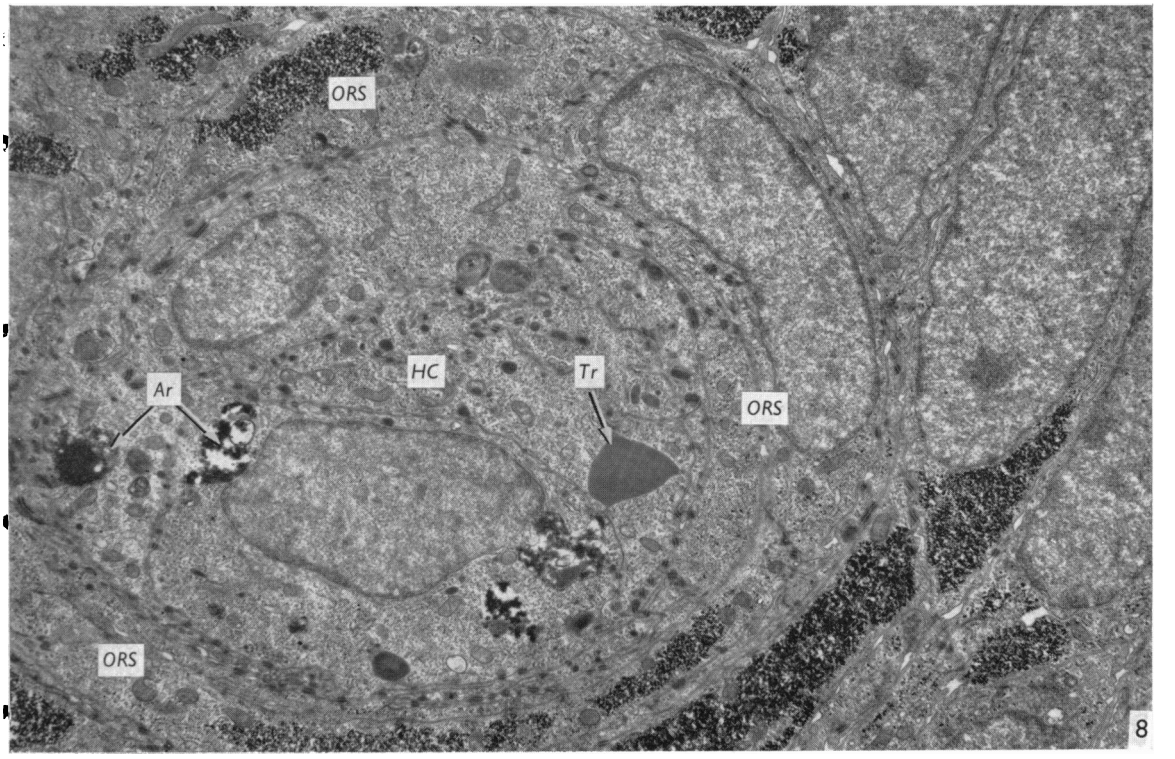

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mediately adjacent to the outer root sheath contain much larger ones and more prominent filamentous bundles. These outermost trichohyalin-containing cells can now be identified as forming the Henle layer of the inner-root sheath, and those more centrally placed, the Huxley layer. Of the future keratinized components of the follicle, they are the first to be differentiated, and the hair-cone at the stage illustrated contains no identifiable cuticular or cortical elements.

Transverse sections through the apical region of the hair-cone at this or a slightly earlier stage (Fig. 8) reveal a concentric core of trichohyalin containing Henle and Huxley layer cells, within the outer root sheath. It is evident that the advancing tip of the cone is formed entirely of these inner root sheath elements. In addition to trichohyalin granules, cells in this situation may also contain partially membrane-limited deposits of varying density, to which further reference will be made later.

(4) *Transformation or 'hardening' of hair-cone cells*

Shortly after the establishment of the hair-cone of inner-root sheath cells, a process of maturation or 'hardening' commences at the tip of the cone, and extends downwards. The changes involved, which are identical in the two layers, occur earlier in the Henle layer, and the advancing tip of the cone eventually consists of a cap of hardened Henle layer cells overlying a core of Huxley layer cells (Fig. 10). Just prior to hardening, the inner root sheath cells contain an abundance of filamentous bundles orientated mainly along the length of the follicle, and many trichohyalin granules of varying size and shape (Fig. 11). As at earlier stages, the trichohyalin granules are associated with filaments (Figs. 12, 13) which in some instances (Fig. 13) appear to run right through them. Desmosomes are very numerous along the plasma membrane of cells at this stage.

The next observable stage in differentiation of inner root sheath cells (hardening) is illustrated in Fig. 16. Here, a lower cell with characters described above, is in contact with an immediately adjacent one of entirely different appearance. The plasma membrane of the latter is thickened, and the cytoplasm contains numerous filamentous bundles orientated along the long axis of the cell. The filaments are less compactly arranged than in cells at an earlier stage of differentiation, and interspersed between the bundles are areas of cytoplasm with islands of clumped ribosomes, mitochondrial remnants and occasional vesicular structures. Typical electron-dense deposits of trichohyalin are absent from cells of this type, but ill-defined more translucent amorphous deposits are present. The final condition reached by inner root sheath cells up to the stage under present consideration is illustrated in Fig. 17. The cytoplasm, apart

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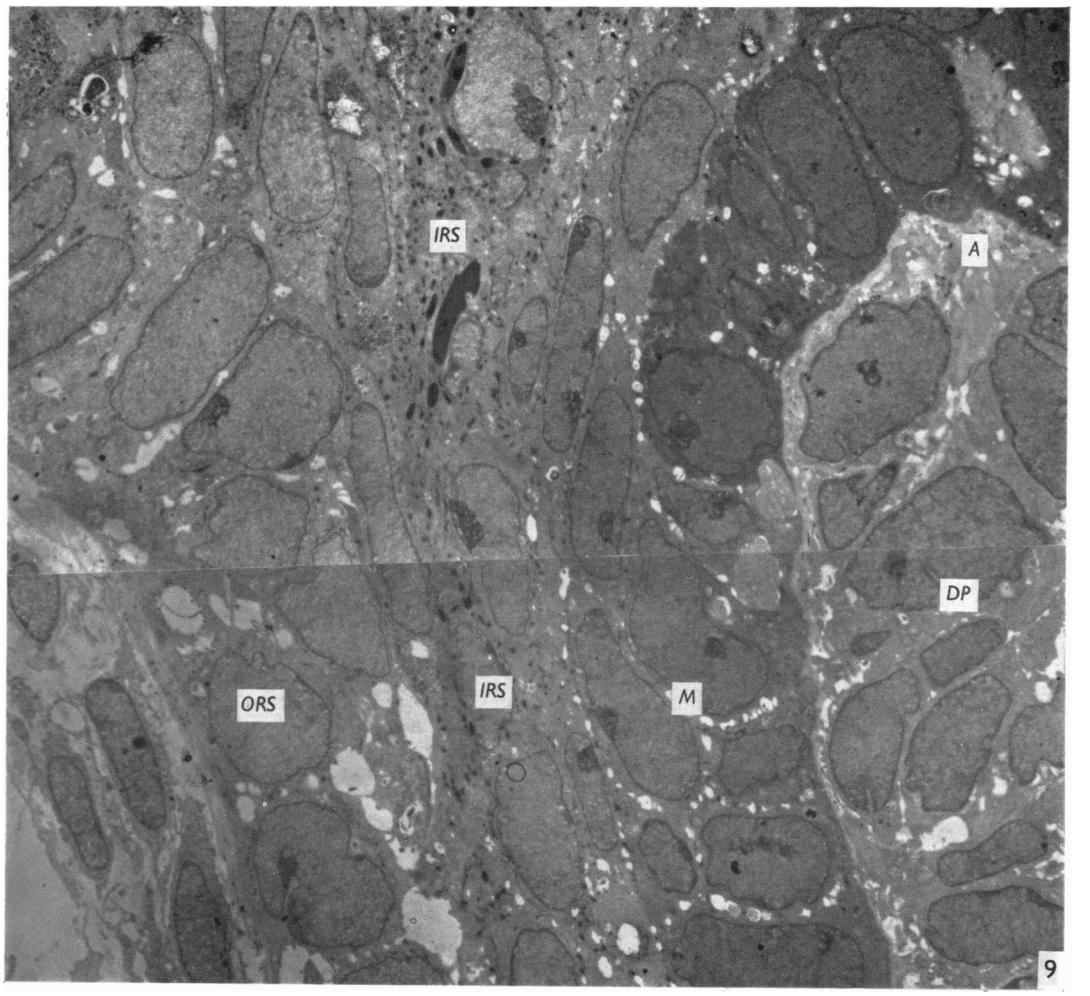
Fig. 9. Longitudinal section through bulbar region of follicle in which envelopment of the dermal papilla (*DP*) has progressed considerably further than the follicle in Fig. 1. Trichohyalin-containing presumptive inner root sheath cells (*IRS*) are present in the bulb well below the level of the apex (*A*) of the papilla. *M*, matrix cells; *ORS*, outer root sheath. × 3400.

Fig. 10. Longitudinal section of hair-cone in which hardening of inner root sheath cells has occurred. The narrow advancing tip of the cone towards the left, is formed of hardened Henle (*He*) layer cells, while more basally (towards the right), the cone consists of an outer layer of hardened Henle cells and a central core of unhardened Huxley (*Hu*) layer cells containing large trichohyalin granules. *ORS*, outer root sheath. × 3400.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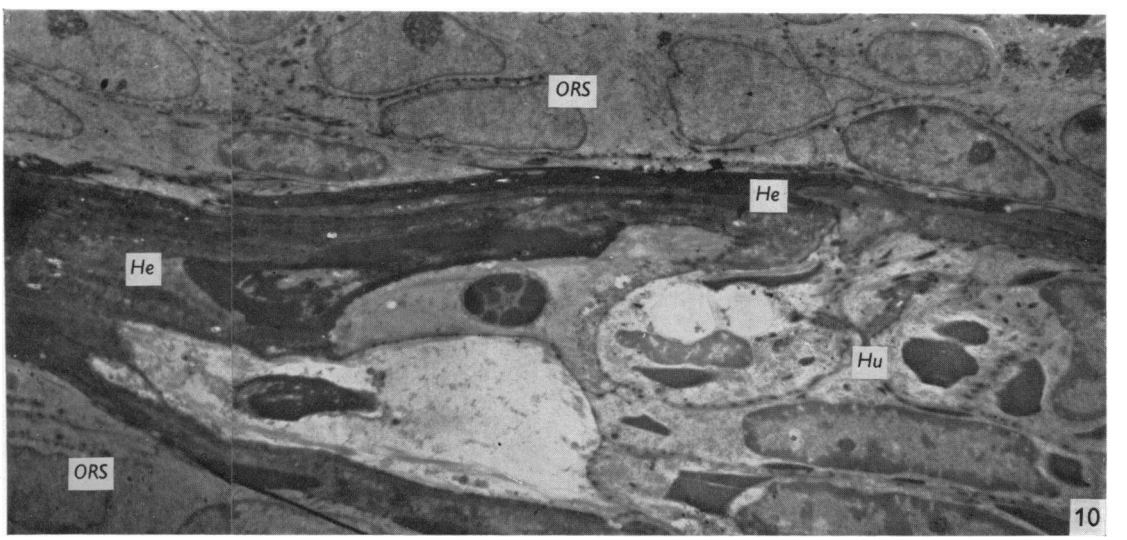
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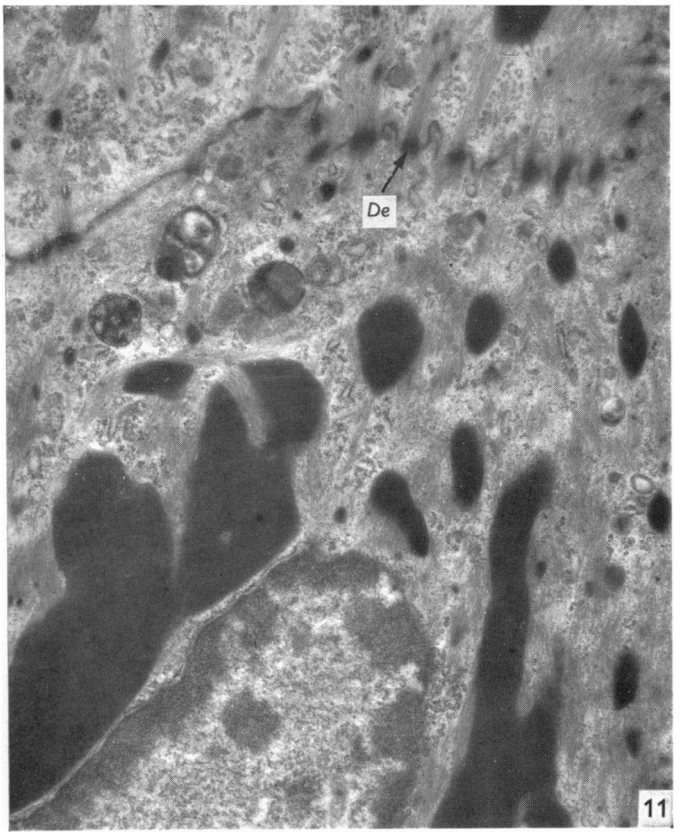
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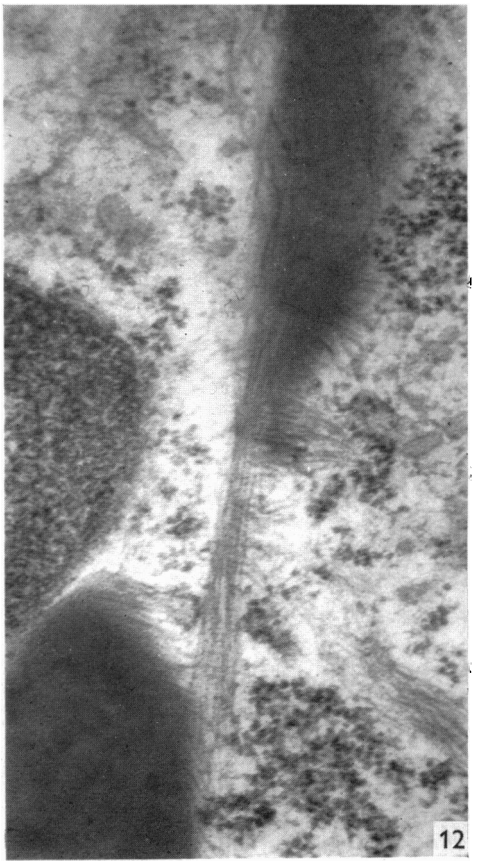
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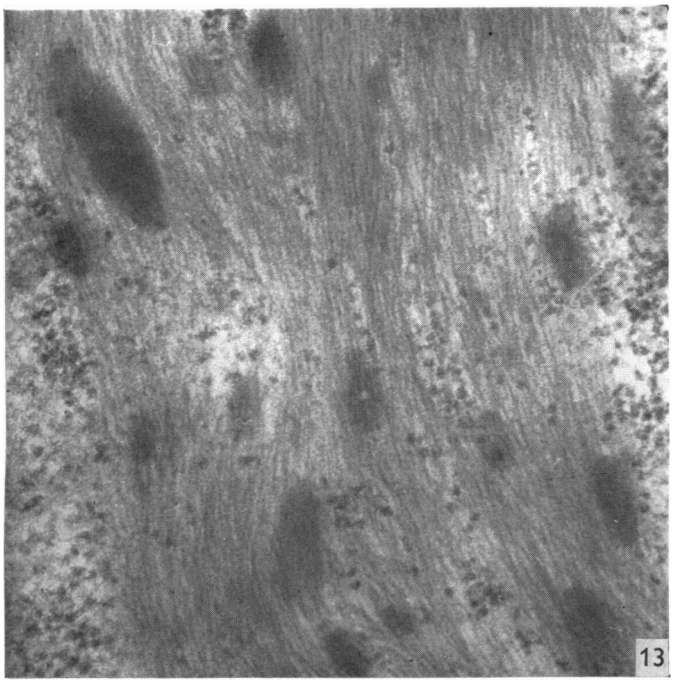
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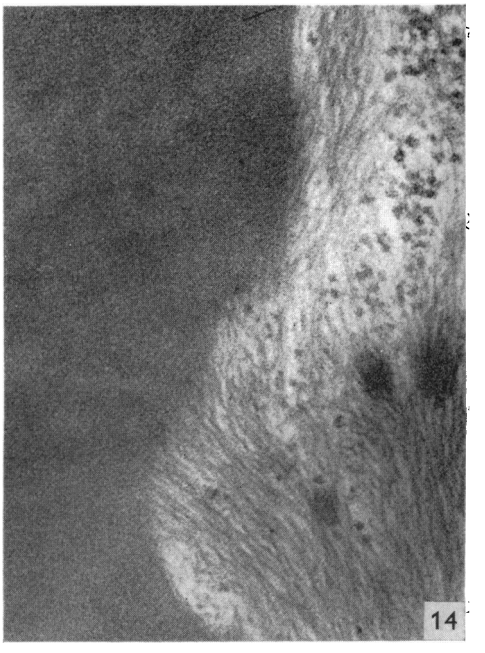
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from occasional islands of ribosomes is completely occupied by closely packed filaments, and no interfilamentous matrix is detectable. The nucleus lacks a membrane, and the chromatin is clumped to form granular patches of different densities. Observations at later foetal stages reveal that apart from disappearance of the nucleus and some increase in electron density of the filamentous cytoplasm, inner root sheath cells do not differentiate significantly beyond the condition illustrated here (Fig. 17).

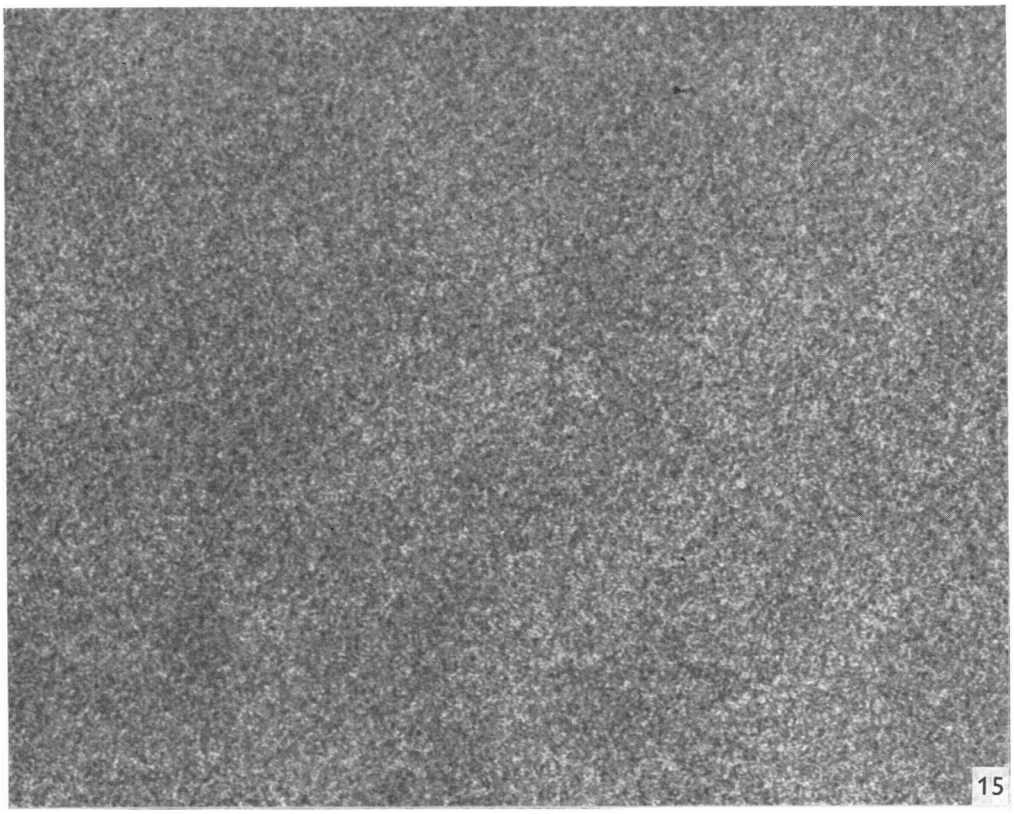
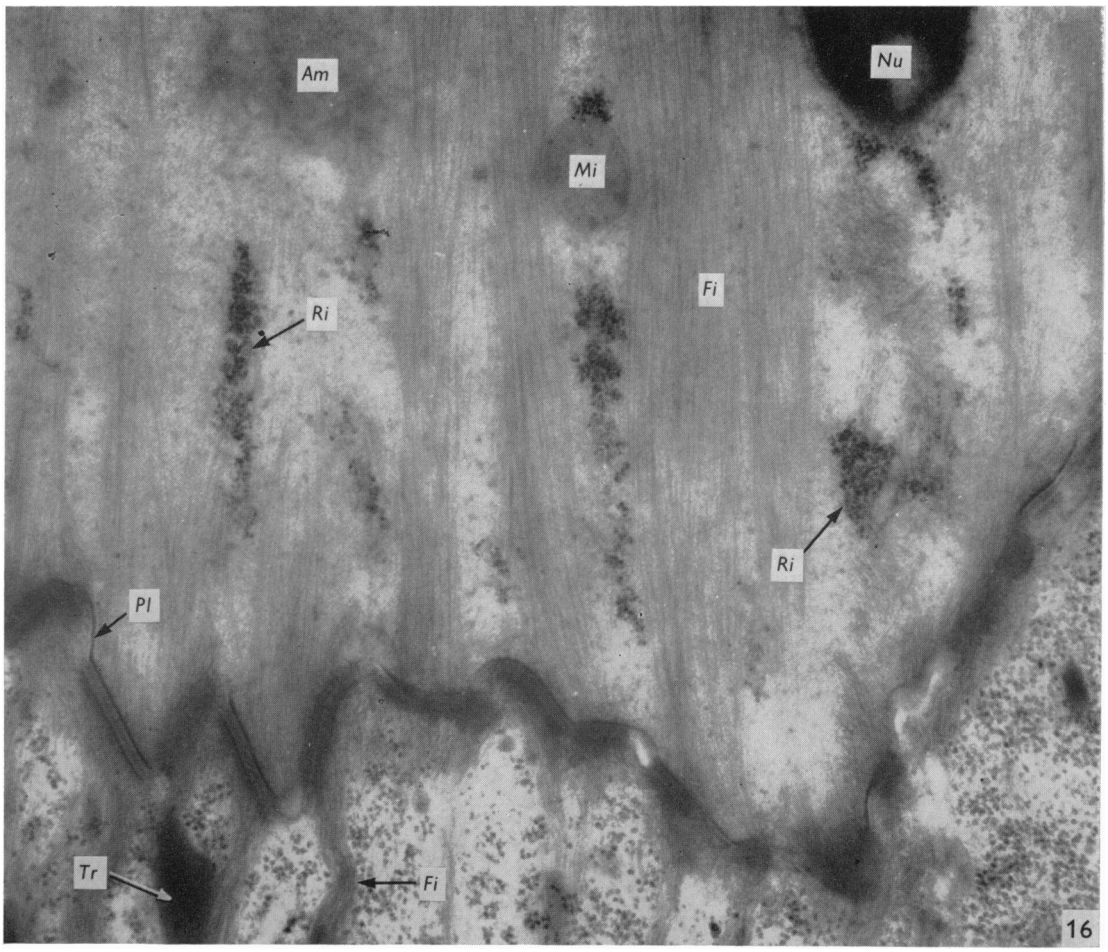


Fig. 15. Portion of trichohyalin granule at high magnification. A 'speckled' pattern of sorts is evident, but this cannot be interpreted as being due to unaltered filaments set in an amorphous matrix. ×280000.

Fig. 11. Inner root sheath cells immediately prior to onset of hardening. Numerous trichohyalin granules of varying size are present in the cytoplasm, associated with filamentous bundles orientated along the length of the follicle. Note numerous desmosomes (*De*) associated with plasma membrane. ×14000.

Figs. 12–14. Trichohyalin granules and filaments from inner root sheath cells immediately prior to hardening. Magnifications, Fig. 12, ×61000; Fig. 13, ×65000; Fig. 14, ×80000.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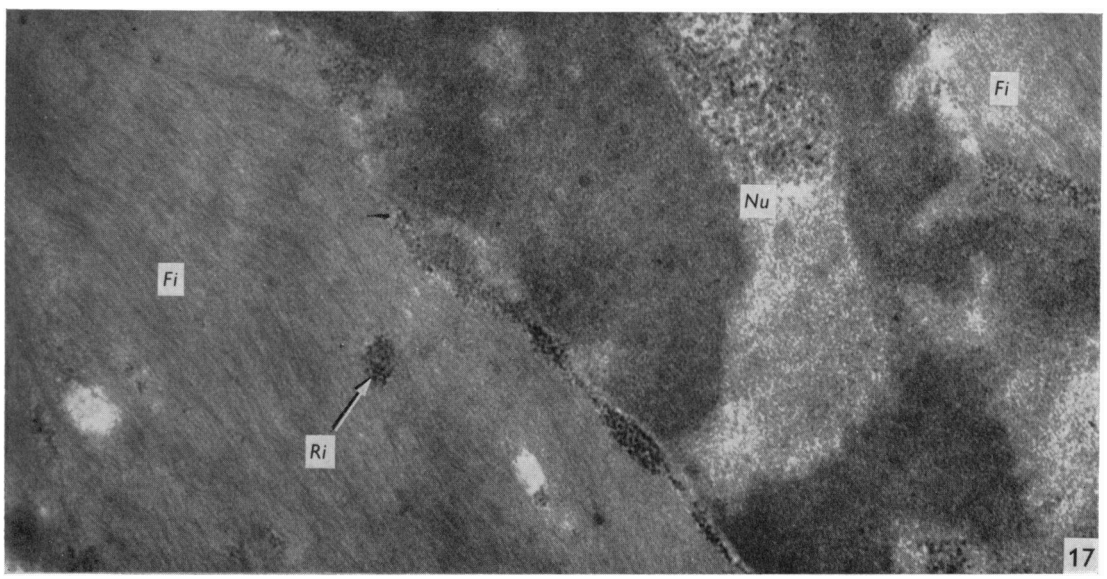
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17

Degenerating elements in hair-cone

At the earlier bulbous-peg stage (Robins & Breathnach, 1969) individual cells of the hair-cone exhibited degenerative features characteristic of morphogenetic cell death (Saunders & Fallon, 1966). Similar elements were encountered in the present material. One such is seen in Fig. 18. The plasma membrane is poorly defined, and patchy, but desmosomal contacts with adjacent cells are still evident. The cytoplasm is densely granular, lacking the common organelles, and several empty vesicles are present, as well as trichohyalin deposits unassociated with filaments. The nucleus exhibits characteristic loss of membrane and commencing segregation of chromatic and achromatic areas within it. Fig. 19 illustrates an irregular electron dense area surrounded by inner root sheath cells of normal appearance. No limiting membrane can be defined, but the presence of desmosomes along the periphery clearly indicates that this is a remnant of a cell. The appearance of these dying cells is sufficiently characteristic to allow them to be distinguishable from cells undergoing hardening (cf. Figs. 16, 17). Membrane-limited bodies of varying size and of irregular density (Figs. 8, 20) seen in some cells resemble cytolysosomes, and are indicative of impending autolysis.

DISCUSSION

This report extends previous observations on the lower (bulbar) end of the human foetal hair follicle (Breathnach & Smith, 1968; Robins & Breathnach, 1969) to a stage when the first keratinized or hardened elements become differentiated. These are the Henle and Huxley layers of the inner root sheath, which reach an advanced stage of maturation before any definitive cuticular or cortical elements can be recognized. The advancing end of the hair-cone is made up exclusively of inner root sheath cells, and the tip consists of a cap of hardened cells of the Henle layer.

In recent years a number of authors have attempted analysis at the ultrastructural level of the process of maturation and hardening of inner root sheath cells of postnatal human and rodent follicles. All have drawn attention to the rapid build up of filaments and trichohyalin granules within the cells as they ascend from the matrix of the bulb, and to the abruptness of their final transformation. There is considerable disagreement among authors, however, as to the origin, nature and fate of trichohyalin, and as to the source of the filaments which pack the cytoplasm of the hardened cell. Birbeck & Mercer (1957) who concluded that trichohyalin antedates filaments in differentiation, suggested that the filaments are formed directly from trichohyalin, that this process is already occurring within the non-hardened cells, and that its sudden stepping up at the level of hardening accounts for the final complete

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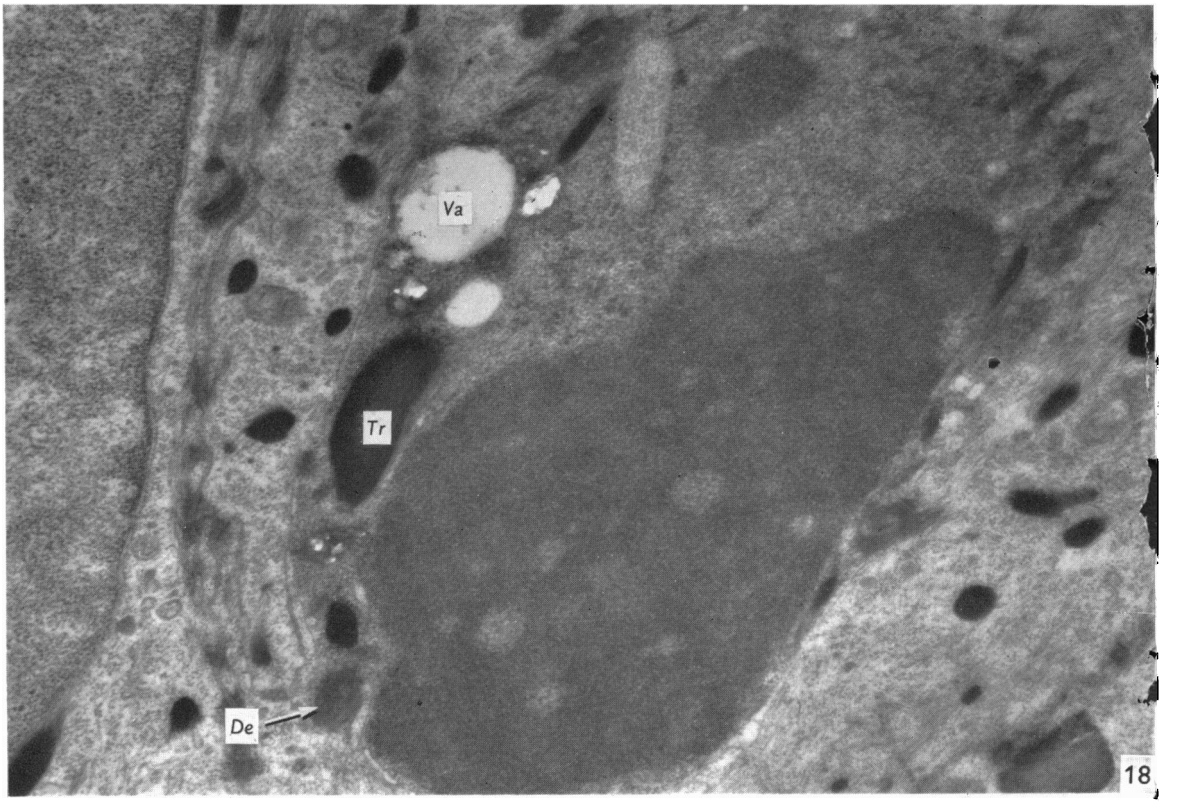
Fig. 16. Hardening of inner root sheath cell. The lower of the two cells illustrated is a mature non-hardened cell with features similar to cells in Fig. 11. *Tr*, trichohyalin granule; *Fi*, filaments. In the upper cell, the process of hardening is well advanced. *Fi*, filaments; *Am*, amorphous deposit; *Mi*, mitochondrion; *Ri*, islands of ribosomes in areas of cytoplasm free of filaments; *Nu*, nucleus; *Pl*, thickened plasma membrane. ×40000.

Fig. 17. Cell of inner root sheath that is almost fully hardened. *Fi*, closely packed cytoplasmic filaments; *Nu*, nucleus exhibiting granular areas of different density; *Ri*, ribosomes. ×42000.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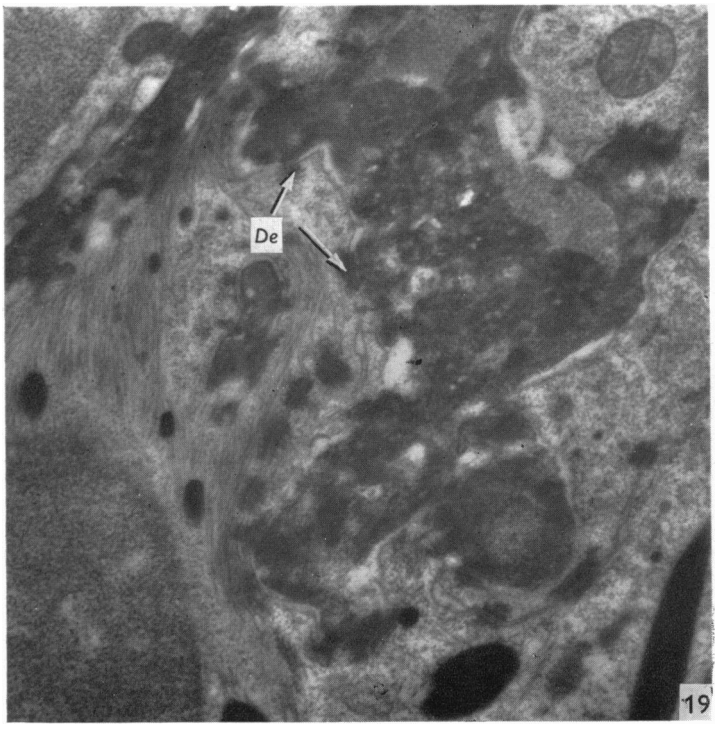
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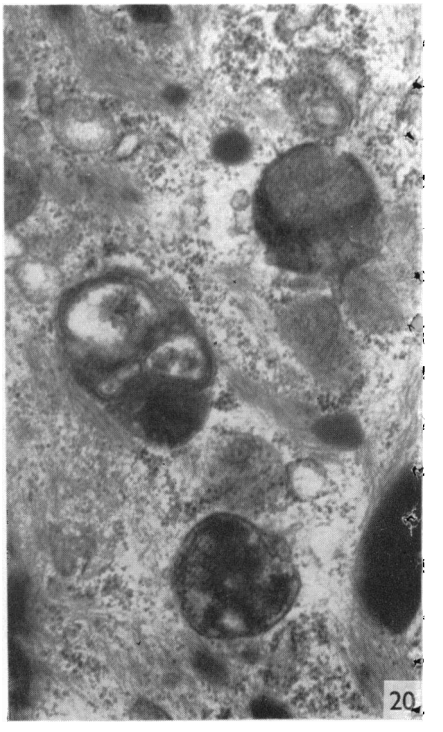
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disappearance of trichohyalin. Later workers (Charles, 1959; Parakkal & Matoltsy, 1964; Roth & Helwig, 1964; Roth & Clark, 1964; Kint, 1967) however, established that filaments are present before the appearance of trichohyalin granules, and therefore questioned the view that the latter serve as precursor material. Parakkal & Matoltsy (1964) and Roth (1967) suggested that trichohyalin is formed by deposition of an amorphous substance at local sites around the filaments, and that the granules therefore are constituted of two components, but neither they, nor the other authors mentioned, were able to draw any firm conclusions concerning their ultimate fate or possible role in the final process of hardening. It is clear from an examination of the literature that this latter process in turn requires further elucidation, and controversial matters, such as whether or not an interfilamentous matrix is present in the fully hardened cell, remain to be clarified.

It was hoped that examination of the present graded foetal material spanning the ontogenetic evolution of the processes under consideration, might allow them to be studied, so to speak, in slower motion than in the fully developed follicle, with resulting clarification of some of the problems presented. In the event, this hope was not realized to any extent. The successive morphological stages of maturation of inner root sheath cells as they ascend from the matrix of the postnatal follicle were mirrored in temporal sequence in the present material, but the transition between one stage and another in time was equally abrupt. Thus, for example, while we were in a position to examine follicles at a stage before the cells of the hair-cone contain any trace of trichohyalin (Fig. 3), the granules were already present within cells of follicles at an immediately later stage of general development (Fig. 4). The present observations, therefore, provide no precise information as to the source of trichohyalin, or its manner of formation. That the increase in density whereby trichohyalin granules become manifest occurs originally in association with pre-existing filaments is, however, clear and this supports the view that trichohyalin *per se* is not the precursor of the filaments of the non-hardened cell. We could find no definite evidence that the increase in density indicative of trichohyalin formation is due to deposition of an added substance upon, or among the filaments (Parakkal & Matoltsy, 1964), and while a pattern of sorts could be discerned within the granules (Figs. 5, 14, 15) it could not be interpreted as representing unaltered filaments set in an amorphous matrix. Our impression is rather that the pattern indicates some fundamental reorganization of filamentous protein, and that the filaments bound up in trichohyalin granules differ in some way from those lying free in the cytoplasm. Preferential extraction of the granules similar to that carried out by Fukuyama, Buxman & Epstein (1968) on keratohyalin granules of interfollicular epidermis might throw

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Fig. 18. Cell in hair-cone exhibiting features characteristic of morphogenetic cell death. Nuclear and plasma membranes are indistinct, and the dense granular cytoplasm lacks filaments or the common organelles. Note trichohyalin granules (*Tr*) and vacuoles (*Va*). *De*, desmosome. × 26000.

Fig. 19. Irregular electron-dense element in hair-cone. Desmosomes (*De*) indicate that this is a degenerate remnant of a cell. × 30000.

Fig. 20. Membrane-limited bodies resembling cytolysosomes in cytoplasm of hair-cone cell. × 33000.

further light on this problem. We would conclude that further primarily morphological studies are unlikely to solve the questions under consideration, and until the chemical nature of trichohyalin is established, it is not easy to suggest other lines of approach.

SUMMARY

1. Previous observations on development and differentiation of the bulbar end of the human foetal hair follicle have been extended to a stage when the first keratinized or hardened elements have appeared.

2. The first layers to become differentiated within the hair-cone are the Henle and Huxley layers of the inner root sheath, which reach an advanced stage of maturation before presumptive cuticular or cortical elements can be recognized. The advancing tip of the hair-cone is made up exclusively of inner root sheath cells.

3. Filaments antedate trichohyalin granules in ontogeny, so the latter are unlikely to serve as precursor material for the filaments of non-hardened inner root sheath cells. No evidence was obtained to support the view that trichohyalin granules consist of unaltered filaments in an amorphous matrix. Micrographs suggest rather that the electron-dense appearance of the granules is due to some fundamental reorganization of filamentous protein without any added material.

4. The present observations on maturing and hardened inner root sheath cells are discussed in relation to previous studies on postnatal human and rodent follicles. Examination of graded foetal material throws little light on the final stages of the hardening process which occurs as abruptly as it does in fully developed hair.

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