

SHORT REPORTS

Apoptosis as the Mechanism of the Involution of Hair Follicles in Catagen Transformation

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Abstract. Electron microscopy was performed on mouse skin to study the mechanism of the spontaneous involution of hair follicles during catagen. The reduction in follicle size appears to result from cell deletion by apoptosis, a distinct type of cell death with importance in tissue kinetics. These ultrastructural changes have been misinterpreted in the past as autophagic vacuole formation because of the prominent phagocytosis of the apoptotic fragments by adjacent, surviving cells of the hair follicle.

Key words: Apoptosis; Cell death; Catagen; Hair cycle

The mechanism responsible for the involution of the hair follicle during catagen has never been satisfactorily explained. Textbooks of dermatopathology dismiss the process briefly as a collapse (6), regression (8), disintegration (11) or involution (1) of the lower follicle. Montagna and Parakkal, in their comprehensive review of the hair cycle, attributed the reduction in follicle size to the formation of autophagic vacuoles within the cells of the lower follicle (9). In the same year, Garrett & Hashimoto suggested that phagocytosis of necrotic cells by other epithelial cells in the outer root sheath played a role in the continuous reshaping of the outer root sheath (4).

The electron micrographs in these two papers (4, 9) depict the process of apoptosis, the morphologically unique form of cell death which is the mechanism of cell deletion in a wide range of circumstances (15). To confirm that apoptosis is responsible for catagen involution we have undertaken an ultrastructural study of spontaneous catagen in mice.

MATERIAL AND METHODS

Newborn mice from a local "Hall" strain were obtained, and pairs sacrificed on days 15 to 18 inclusively. A previ-

ous pilot study had shown that the initial anagen phase lasted for approximately 16 days. Representative samples were taken from both ventral and dorsal regions of each animal. Part of each specimen was fixed in 3% glutaraldehyde, while the remainder was formalin-fixed for later paraffin embedding. The tissue for electron microscopy was post-fixed in 1% osmium and later embedded in Araldite. Sections were stained with uranyl acetate and lead citrate prior to examination in an AEI Corinth 500 electron microscope.

RESULTS

Light microscopy showed a marked reduction in hair follicle size in mice sacrificed at 17 days of age compared with matched areas of mice killed a day earlier. Those follicles still undergoing involution on day 17 showed scattered, shrunken epithelial cells in the outer root sheath, some barely visible in the light microscope. Nuclear remnants were present in some fragments, aiding recognition of the

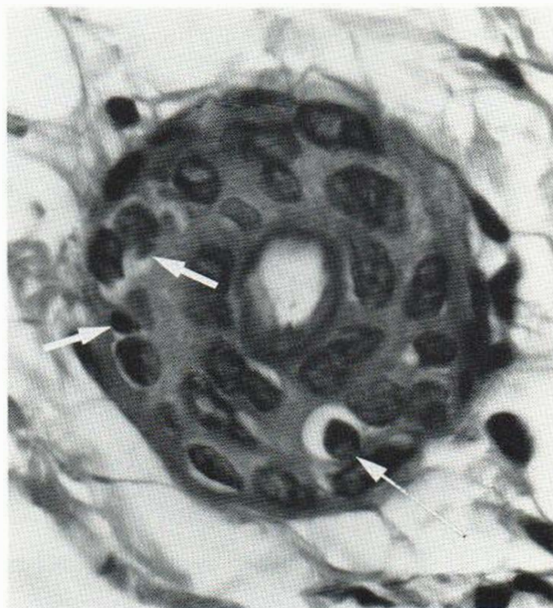


Fig. 1. Transverse section of early catagen follicle in the mouse. Note the shrunken epithelial cells with pyknotic nuclei (arrows) characteristic of apoptosis (H&E, $\times 1235$).



Fig. 2. Lower end of a catagen follicle. There are numerous apoptotic bodies at various stages of intracytoplasmic digestion. A few apoptotic bodies (arrows) have not yet been ingested. $\times 3535$.

apoptotic process (Fig. 1). Only an occasional cell could be seen undergoing apoptosis in the hair follicles of mice killed at 16 days of age, while this process was absent altogether in those animals sac-

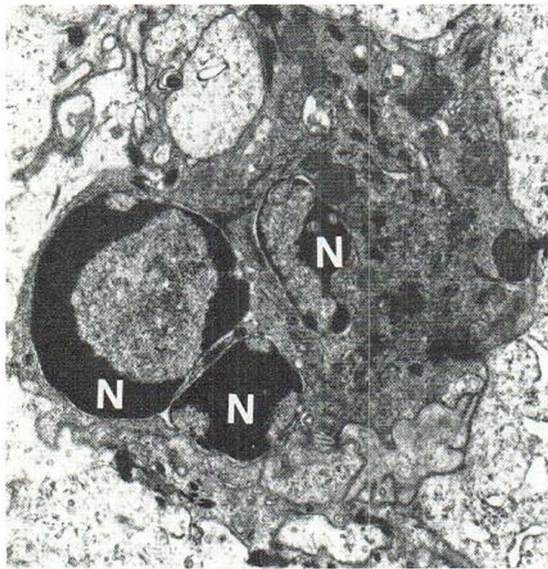


Fig. 3. Early apoptosis in a cell of the outer root sheath. Note the marked condensation of the cell, with well-preserved organelles and fragmentation of the nucleus (N). $\times 7500$.

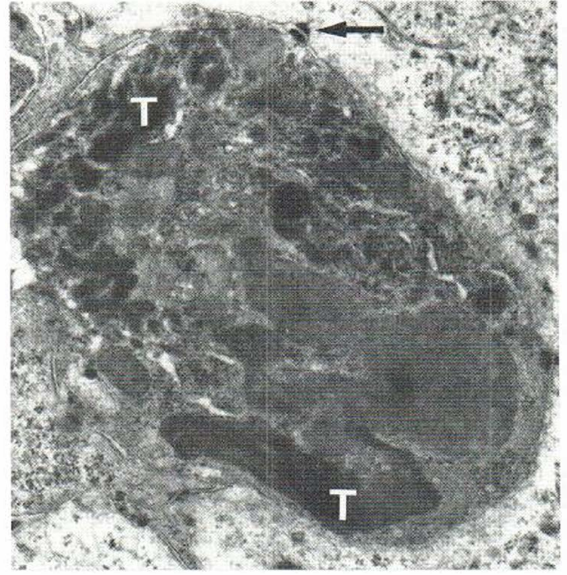


Fig. 4. Early apoptosis with condensation and some redistribution of the tonofilaments. (T). An intact desmosome is present (arrow). This cell is shown in Fig. 2. $\times 18000$.

rificed a day earlier. Mitotic activity appeared to cease in most follicles by day 16.

Ultrastructural examination confirmed the presence of extensive apoptosis involving the epithelial cells in the outer root sheath of hair follicles undergoing catagen involution (Fig. 2). Most of the apoptotic fragments were in the cytoplasm of the surviving epithelial cells of the hair follicle.

Only after prolonged examination were the early stages of apoptosis seen, with cells showing marked condensation (Fig. 3). Condensed cells, containing abundant tonofilaments, were sometimes seen (Fig. 4) and, in some of these, intracytoplasmic desmosomes were present. Condensation of the dying cell was often accompanied by the development of areas of narrowing (Fig. 5) leading to the rapid division of the cell into smaller fragments which were then engulfed by adjacent viable epithelial cells. Organelles were well-preserved in the early stages of apoptosis but following intracytoplasmic ingestion the apoptotic bodies were progressively degraded. The presence of characteristic nuclear remnants in some of the ingested fragments allowed them to be distinguished from autophagic vacuoles (Figs. 6 and 7).

In those follicles in which catagen was well advanced, some condensation of collagen was present

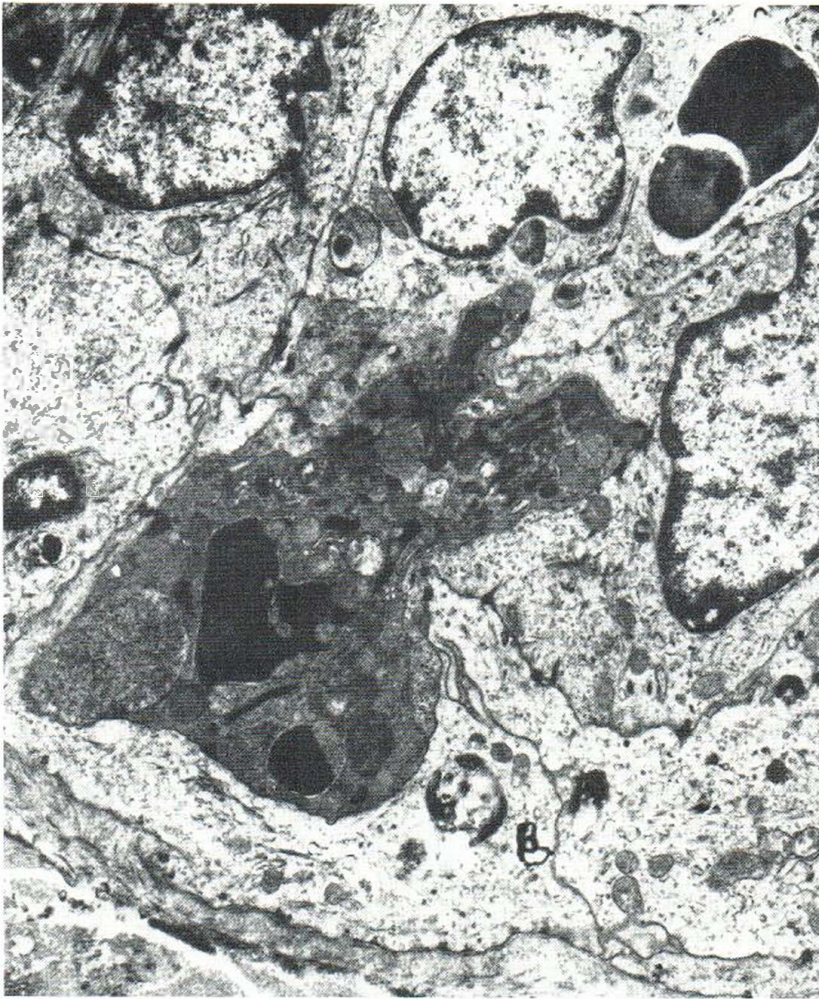


Fig. 5. Lower end of a catagen follicle. A condensed apoptotic cell is present. Smaller fragments are about to be "pinched" or "budded" off the upper part of the cell. $\times 7500$.

around the hair follicle. A few macrophages containing apoptotic fragments at all stages of digestion, together with a small amount of intracytoplasmic collagen were noted at an even later stage of catagen involution.

DISCUSSION

Apoptosis, involving as it does a rapid condensation of the cell with budding into smaller fragments which are later phagocytosed by adjacent epithelial cells or macrophages, is the method of cell deletion in the elimination of phylogenetic vestiges during embryonic development and the steady-state kinetics of normal adult tissues (15). One advantage of apoptosis over the other form of cell death, coagulative necrosis, is that apoptosis does not itself evoke

an inflammatory response (15). This is clearly important in embryogenesis or when cyclical phenomena such as catagen are involved.

In a previous review (15), we discussed some of the modifications to the basic process of apoptosis that occur in different circumstances. In the apoptosis of epidermal cells in lichen planus (15), graft-versus-host reaction (2) and regressing plane warts (14), filament-rich fragments remain which are usually resistant to phagocytosis by adjacent epithelial cells or macrophages. These filamentous bodies (5) are extruded into the dermis where they become coated with immunoglobulin to form colloid bodies. In basal cell carcinomas, where spontaneous apoptosis appears to account for the paradoxically slow growth of tumours possessing numerous mitotic figures, phagocytosis of apoptotic remnants by



Fig. 6. Ingested apoptotic bodies composed largely of nuclear remnants (arrow). The cell also contains smaller bodies at an advanced stage of degradation. $\times 5\,320$.

adjacent tumour cells is quite prominent (15). In embryogenesis, apoptotic cells are usually quickly engulfed by adjacent epithelial cells, and macrophages play little part in the removal of these

fragments (3). Our studies confirm that this situation also occurs in catagen follicles, the only phagocytosis by macrophages involving the last remaining cells of the lower follicle, towards the end of catagen.

The presence of these partly-degraded apoptotic fragments within the phagolysosomes of the viable cells of the catagen follicle has led to confusion with autophagic vacuole formation (9, 13). This latter process involves the isolation of cytoplasmic organelles within membrane-bounded vacuoles and their later destruction by lysosomes discharged into the autophagic vacuole. Their distinction from apoptotic bodies may be very difficult unless recognizable nuclear remnants, or organelles foreign to the ingesting cell, are present within the phagocytosed apoptotic fragments. However, this distinction is crucial to the correct understanding of the kinetics of catagen involution. With the development of the concept of apoptosis, the role of autophagy in tissue involution needs to be re-evaluated. Garrett & Hashimoto (4) demonstrated heterophagocytosis by outer root sheath cells in mouse vibrissae, but they did not relate these findings to the involutional changes of the catagen follicle. Our Figs. 6 and 7 clearly demonstrate the presence of heterophagocytosis by showing intracytoplasmic bodies containing nuclear material.

The close contact between adjacent cells of the hair follicle undoubtedly contributes to the limited



Fig. 7. Lower end of a catagen follicle with partly digested intracytoplasmic bodies. A nuclear fragment is clearly visible in one of these apoptotic bodies (arrow). $\times 4\,990$.

budding of the dying cells which is a feature of the early stages of apoptosis in other situations (15). Phase-contrast microscopy has shown that the cellular condensation and budding phases of apoptosis take only a few minutes (15). This, combined with the avidity with which viable cells engulf the apoptotic fragments, accounts for the difficulty in finding the early stages of the process by electron microscopy.

This has undoubtedly contributed to its confusion with autophagic vacuole formation as the later stages of intracytoplasmic digestion are the most prominent feature. It was only after prolonged searching, aided by our knowledge of apoptosis in other circumstances, that we found early apoptosis in lichen planus (15) and in this study of catagen follicles.

Since Olson & Everett (10) first suggested that apoptosis might be involved in the involution of hair follicles during catagen, we have examined hair follicles for its presence in biopsies from patients with alopecia. We have found that apoptosis is a useful marker of catagen in follicles of human scalps. Interestingly, apoptosis has been demonstrated ultrastructurally in alopecia areata, although it was not recognized as such (12).

It remains to be determined what is responsible for inhibiting mitosis and initiating apoptosis in catagen transformation under various circumstances. In spontaneous catagen, some genetically programmed phenomenon is probably involved, but studies of molting in animals (7) would seem to indicate that numerous other factors, including climate, nutrition and light also have some influence on this process. Further work is needed to clarify these aspects.

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Formation of Glutathionedopa in Albino Rats after DOPA Injection

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Abstract. 5-S-Glutathionedopa was demonstrated in a homogenate of the choroid and the retinal pigment