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ELECTRON MICROSCOPY OF EMBRYONIC HUMAN EPIDERMIS AT SEVEN AND TEN WEEKS

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The onset of the keratinization process in human embryonic epidermis has not been previously documented, and the appearance of desmosomes and tonofilaments has been described in no younger than the twelve week human embryo. In the 12 week and the 14 week human embryo a few desmosomes with short tufts of tonofilaments which spread only a short distance into the cytoplasm have been described, although no evidence of keratinization such as formation of a keratin layer or keratohyalin granules has been observed (1, 4).

We are here reporting the differentiation of desmosomes and tonofilaments in 7 and 10 week human embryonal epidermis which, at this stage, still consists of one to three cell layers.

Materials and Methods

Two surgically aborted human Japanese embryos of 7 and 10 weeks menstrual age were used for this study. Skin specimens obtained from the scalp, face, trunk, forearm, gluteal region, and ankle were fixed for 120 minutes at 4°C in 6.25 % glutaraldehyde containing 0.33 M sucrose, buffered at pH 7.2 with 0.072 M cacodylate. After the specimens were fixed, they were washed twice for 5 minutes in cold 0.33 M sucrose. They were post-fixed for 60 minutes at 4°C in 1 % osmium tetroxide buffered to pH 7.2 with Veronal acetate buffer. After fixation, the specimens were dehydrated in a series of increasing concentrations of ethanol and embedded in Epon 812. Serial sections were cut with a Porter-Blum microtome or an LKB Ultratome, mounted on Formvar-coated copper specimen grids and stained with 5 % aqueous uranyl acetate and Reynold's lead citrate. Specimens were examined with an RCA EMU-3F or EMU-3G microscope.

Results and Discussion

In the scalp of our seven week embryo the epidermis consists of a superficial layer of flattened cells or periderm, and one germinative layer of cuboidal cells having large nuclei (Fig. 1). The embryonic keratinocytes composing this germinative layer are of two types, moderately electron dense dark cells and light cells having a distinctly clearer cytoplasm. The dark keratinocytes contain many glycogen granules, moderately well developed rough-surfaced endoplasmic

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reticulum, mitochondria, and some Golgi vesicles (Fig. 2). The light keratinocytes generally contain only a few scattered glycogen granules, mitochondria, Golgi vesicles, and immature endoplasmic reticulum. These keratinocytes join together very closely without desmosomes or macula adherens in most areas and form close junction or fascia occludens (3) (Fig. 2). However, at this initial stage of epidermis development it is already observed that the specialized cell-to-cell attachments appear between dark keratinocytes or between the two types, and that the attachments also join these cells to the periderm. These attachments have so far revealed no distinct intercellular contact layer and therefore appear to be zonulae adherentes (3, 4) or simple desmosomes by Rupec's definition (5). These desmosomes lack the well organized attached tonofilaments seen in adult epidermis but possess fuzzy moderately electron dense narrow areas (Fig. 2, 3). The dark cells often contain less electron dense cytoplasmic areas where many glycogen granules but few organelles are seen (Fig. 2). Small villi are distinctly visible on the outer surface of the periderm (Fig. 2).

In the ten week old human embryonal epidermis the desmosome-tonofilament complex is more highly developed than in the seven week embryo and shows a greater number of distinctly prominent electron dense complexes although the close junctions, fasciae occludentes can be seen in many areas (Fig. 4). Furthermore, maculae adherentes, desmosomes having a distinct intercellular contact layer, can be observed and very long loosely organized fine fibrils, which seem to be immature cytoplasmic tonofilaments, have been found within the cytoplasm (Fig. 5). In this embryonic epidermis, keratinocytes often contain few organelles but already possess a number of zonulae adherentes and nucleus (Fig. 6). These keratinocytes are often packed with glycogen-like particles (Fig. 7).

The formation of neither a keratin layer nor keratohyalin granules has been found in both 7 and 10 weeks embryos. The dermis consists of scattered immature mesenchymal cells in which mitosis is occasionally seen. The basement membrane is already distinctly formed and the accumulation of short immature collagen fibers in close association with the membrane can be observed (Fig. 7).

The appearance of simple desmosomes with immature tonofilament-like structures precedes the formation of keratin and keratohyalin granules and probably of cytoplasmic tonofilaments. Embryonic keratinocytes which possess only a few organelles in their cytoplasm, have been observed to have already formed these simple desmosomes (Fig. 6, 7). The formation of such immature desmosome-tonofilament complexes in the early embryonic human epidermis seems to have a primary function in maintaining the integrity of the developing epidermis rather than in the synthesis of keratin.

SUMMARY

1. In the seven week embryo the "dark" and "light" keratinocytes of the one to two layer epidermis are joined to each other and to the periderm by simple desmosomes or zonulae adherentes without well differentiated tonofilaments. These cells are also attached to the already formed distinct basement membrane by immature half-desmosomes.

2. In the ten week embryonic skin, in addition to a more developed desmosome-tonofilament complex (macula adherens), cytoplasmic tonofilaments are recognizable.

3. In both seven and ten week embryonic skin, no keratinization process such as formation of keratin layer or of keratohyalin granules is seen in spite of the presence of zonula adherens or macula adherens.

4. In contrast to adult human epidermis, the embryonic epidermis often forms an obliterated intercellular space, the fascia occludens, resulting in a continuous belt-like structure encircling the cell.

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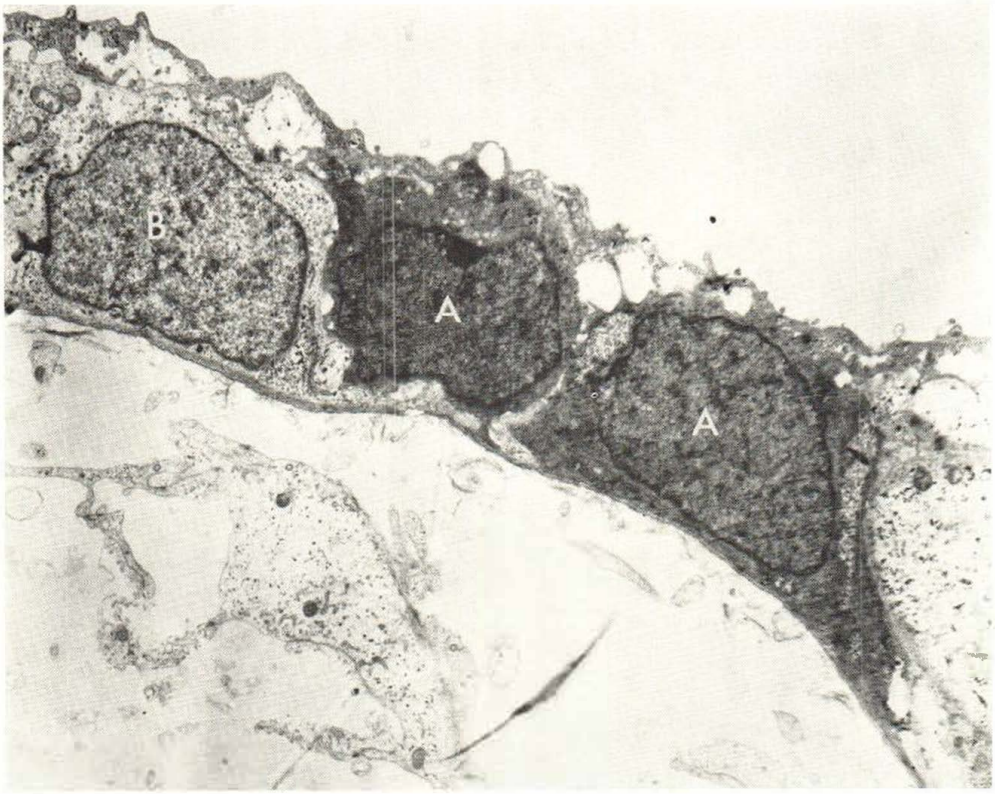

Fig. 1. Two layer epidermis of seven week embryo (scalp), showing "dark" (A) and "light" (B) keratinocytes in the germinative layer. Uranyl acetate and Reynold's lead citrate stain. ×6470.

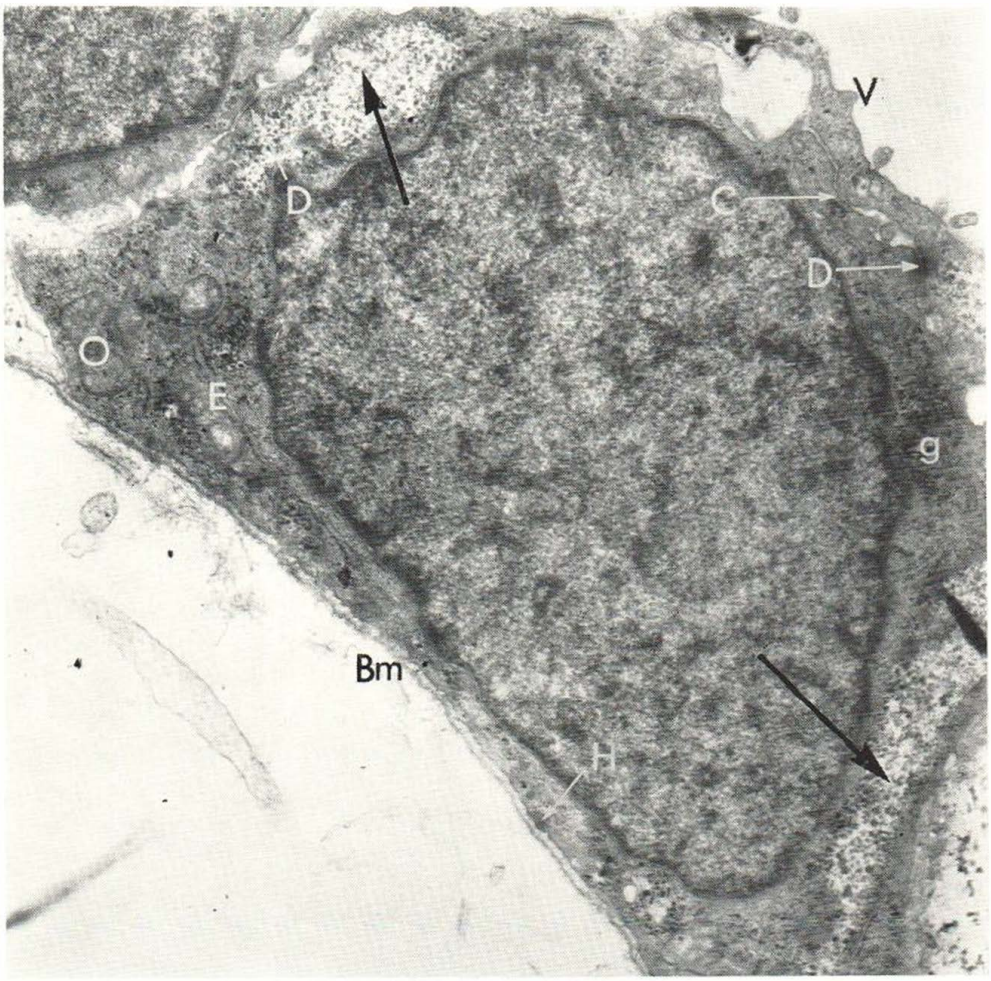

Fig. 2. "Dark" cell of Fig. 1 with light area (arrow) containing numerous glycogen granules. These embryonic keratinocytes are joined very closely without macula adherens or desmosomes and form close junction (C) in many areas. The remainder of the cytoplasm is rich in organelles. D: simple desmosome, V: small villi, Bm: basement membrane, H: half desmosome, E: rough surfaced endoplasmic reticulum, g: glycogen granules, O: mitochondrion. Uranyl acetate and Reynold's lead citrate stain. ×6600.

Fig. 3. Simple desmosome (D) between periderm (top) and germinative keratinocyte of the scalp of a seven week embryo, showing immature tonofilaments and less electron dense intercellular space of 240 Å. N: nucleus, G: Golgi apparatus, E: ergastoplasm. Uranyl acetate and Reynold's lead citrate stain. ×55,200.

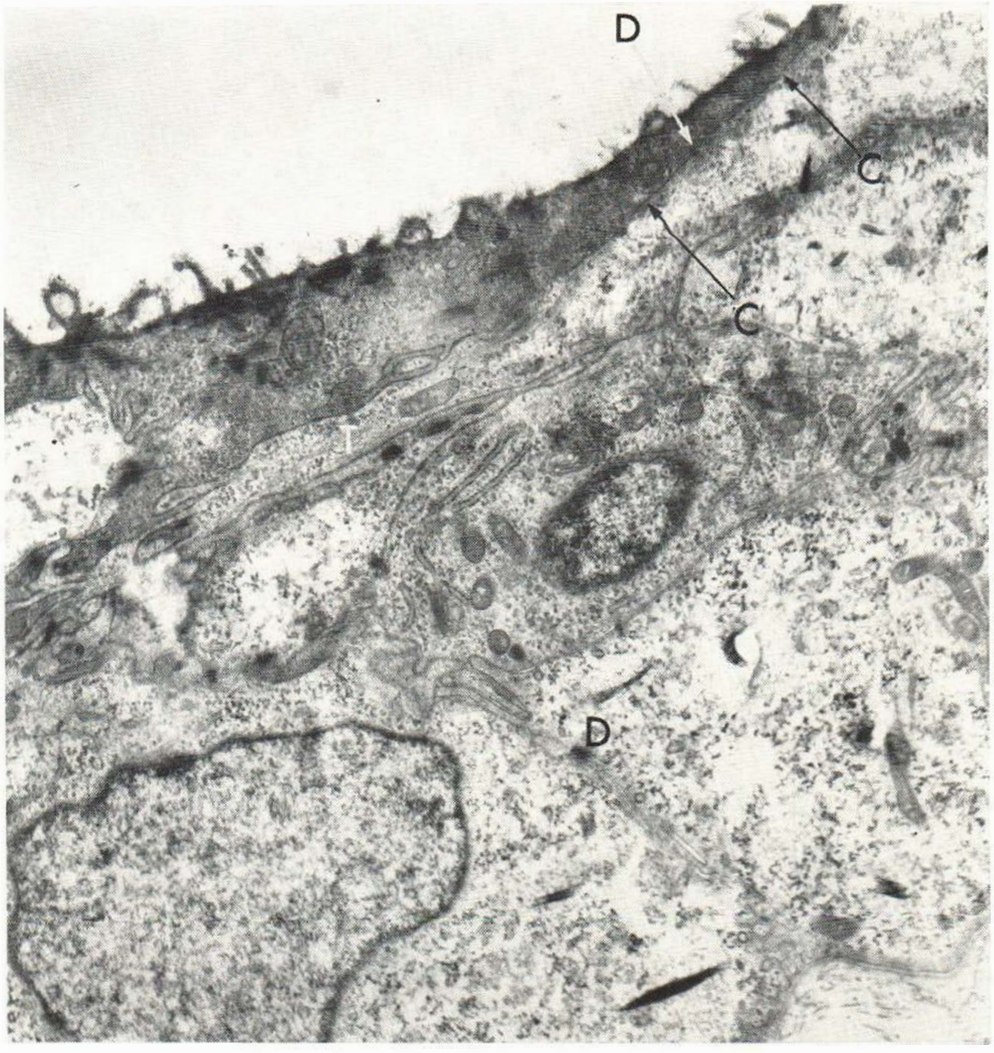

Fig. 4. Two to three layer epidermis of the scalp of a ten week embryo, showing moderately well devolped desmosomes (D) with fine cytoplasmic filaments although close junctions (C) are present in many areas. Long, loosely organized, fine fibrils (T) which seem to be immature cytoplasmic tonofilaments. More and longer peridermal villi are seen. Uranyl acetate and Reynold's lead citrate stain. ×27,300.

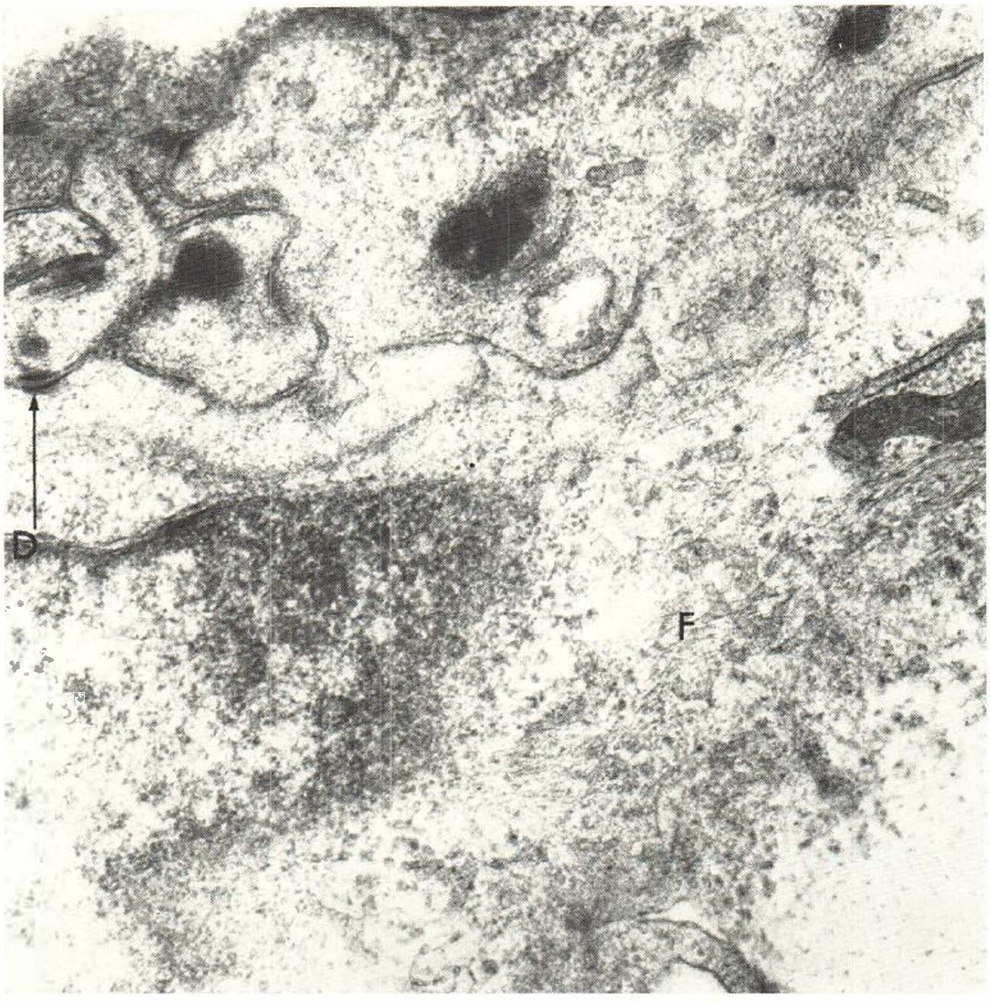

Fig. 5. Long, loosely organized fibrils (F) in the cytoplasm of keratinocyte of scalp of ten week embryo. An intercellular contact layer of the desmosome (D) is visible. Uranyl acetate and Reynold's lead citrate stain. ×27,900.

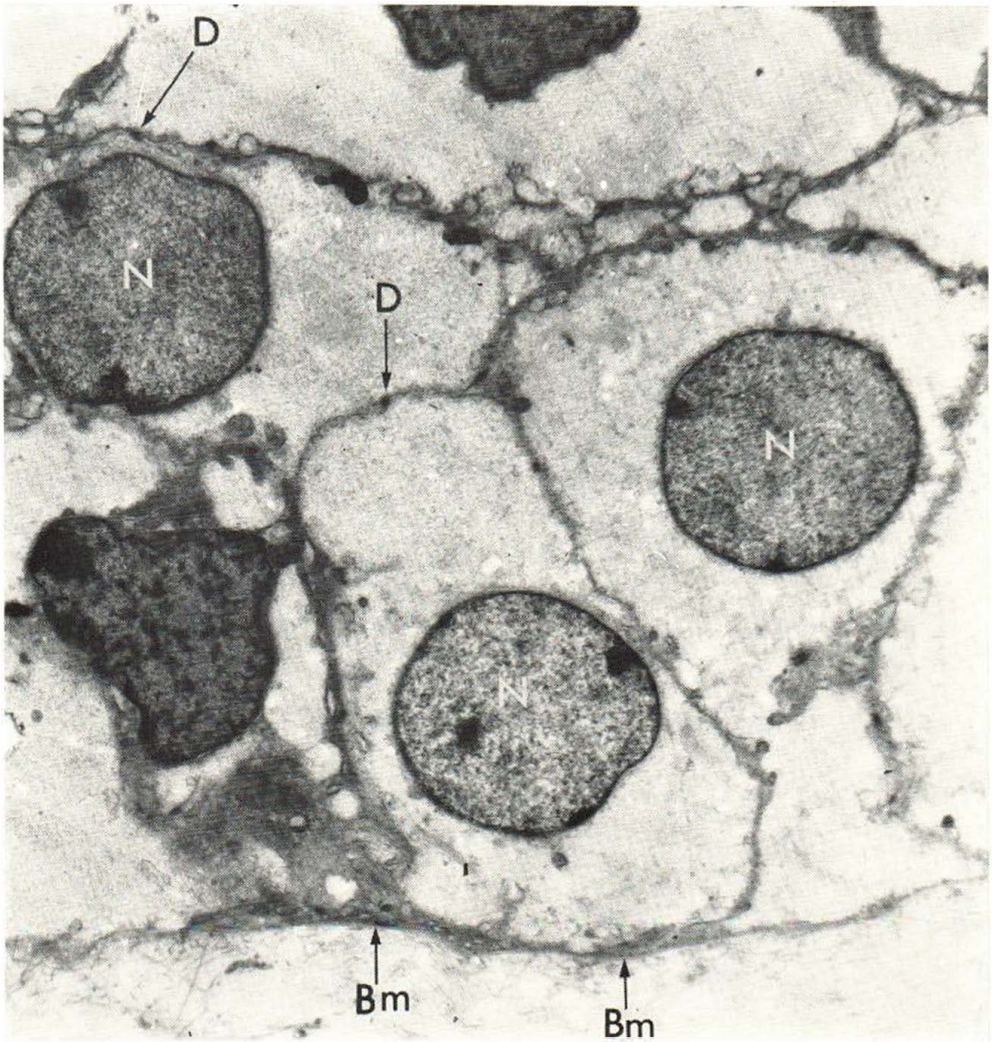

Fig. 6. Ten week human embryonic epidermis of trunk showing keratinocytes (N) which contain few organelles, but which have already formed definite simple desmosomes, zonulae adherentes (D). Bm: basement membrane. Uranyl acetate and Reynold's lead citrate stain. ×2800.

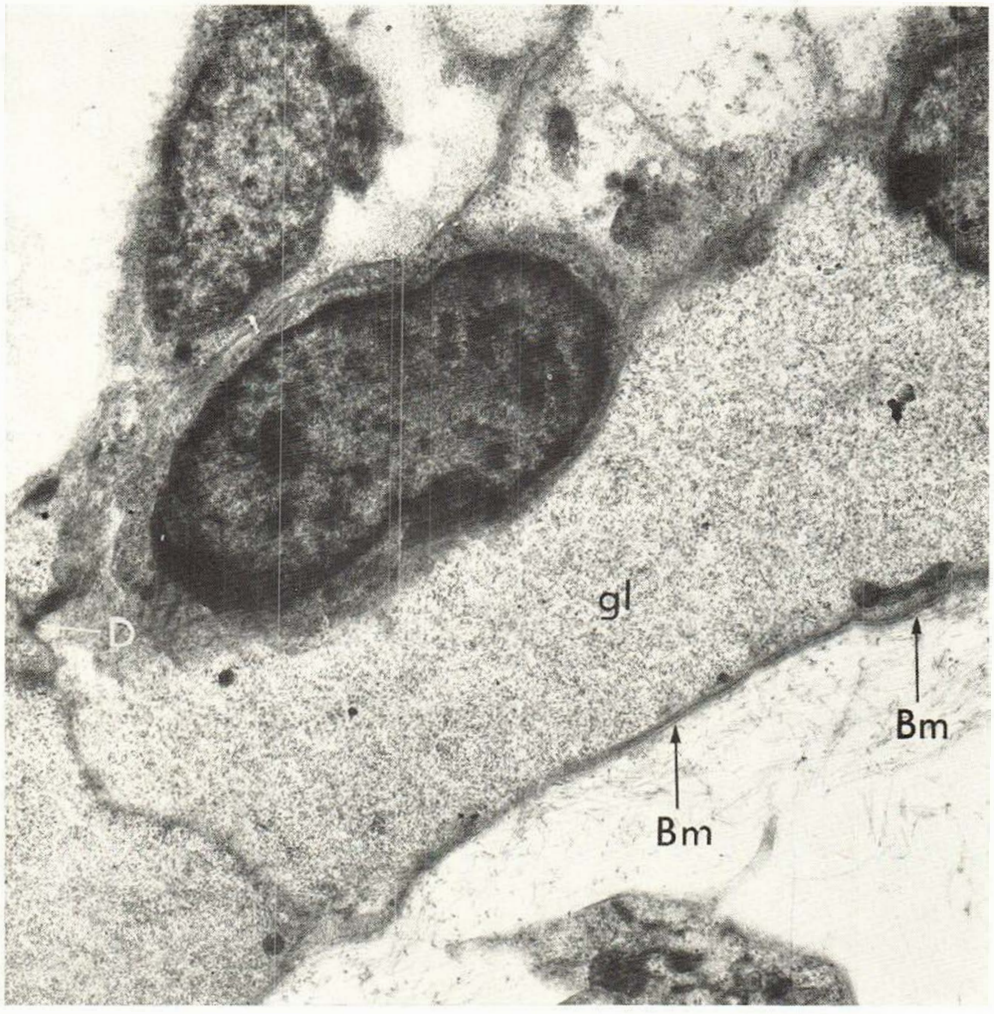

Fig. 7. Ten week human embryonic keratinocytes of scalp containing very few organelles but abundant glycogen-like granules (gl). D: simple desmosome, Bm: basement membrane. Uranyl acetate and Reynold's lead citrate stain. ×9540.