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EFFECT OF RETINOIDS ON THE MORPHOLOGY OF EPIDERMAL DIFFERENTIATION IN VITRO

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Keratinocytes grown in submerged tissue culture progress from a single cell layer to more than ten layers while going through a series of alterations similar to differentiation in the intact epidermis. Many of the cytological features of the different epidermal cell types are retained in these stratifying cultures. Vitamin A has an important influence on epithelial differentiation. The amount of Vitamin A and its analogs (retinoids) available to keratinocytes will determine the degree of keratinization achieved during differentiation. Vitamin A deficiency can result in hyperkeratinization and squamous metaplasia. Excess of Vitamin A can result in mucuse metaplasia. The aim of this investigation is to study the effect of Vitamin A on the morphology of differentiating keratinocytes in vitro.

Keratinocytes from neonatal rat epidermis are grown in tissue culture and allowed to differentiate in medium containing different amounts of Vitamin A as follows: a)normal medium, MEM + 10% fetal calf serum (FCS); b) high retinoid, MEM + 10% FCS + 4-12 ug/ml retinoic acid or retinol + 0.02% DMSO; c) low retinoid, MEM + 10% FCS from which retinoids are removed with other lipids by ethanol-acetone extraction, followed by ethylether wash.

Differentiating keratinocytes in normal medium (Fig. 1) are characterized by cells in the basal layer containing mitochondria, lysosomes, tonofilaments, desmosomes, numerous free ribosomes and a few cisternae of rough endoplasmic reticulum. The Golgi apparatus is well developed. Cells in the upper layers are more flat and nearly devoid of organelles; they have pycnotic nuclei, cornified envelopes, filaments, desmosomes and occasional round dark bodies resembling keratohyalin granules.

Keratinocytes differentiating in low retinoid medium form flat layers. There are more layers of cells with cornified envelopes than in cultures grown in high retinoid or normal medium. Numerous desmosomes and filaments are present (Figs. 1 and 2).

Differentiating cultures in high retinoid medium stratify into 4-5 layers. Cells in the basal layer have interdigitating elongated microvilli, some of them enveloping other cells. At the upper surface, the cells are rounded, oddly shaped and detach easily into the medium. Very few desmosomes and filaments are observed. Cells at all layers contain numerous vacuoles of different sizes and shapes. Cells with an apparently cornified envelope are rounded and have ruffled membranes (Figs. 4 and 5).

Cultures grown in high retinoid medium appear to lose the ability to stratify and keratinize. At the same time, they develop many vacuoles that may contain products other than keratins. On the other hand, cultures grown in low retinoid medium have more components that are typical for keratinization as compared to those grown in normal or high retinoid medium. These structural components indicate a high degree of keratinization.

Key Words: Vitamin A, keratinocytes, tissue culture, differentiation, ultrastructure.

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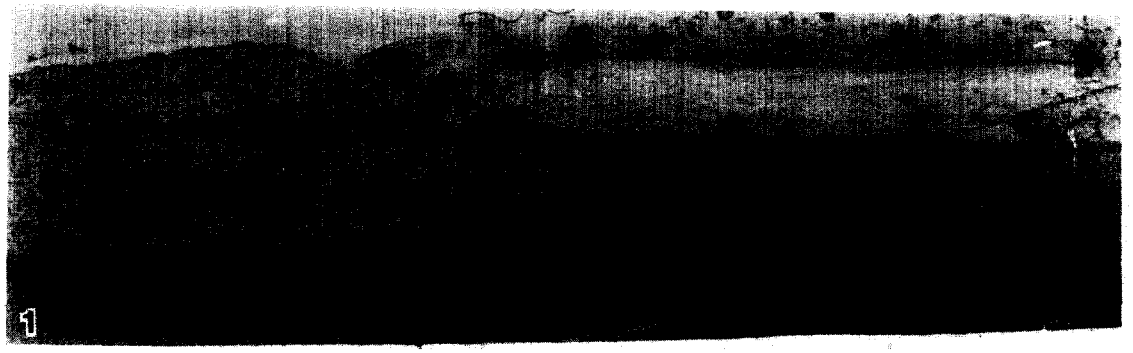

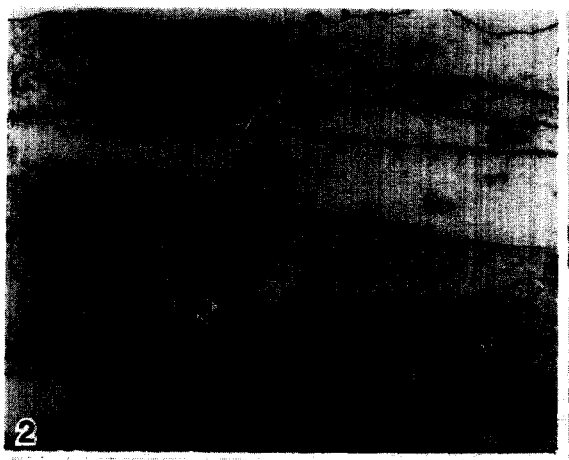

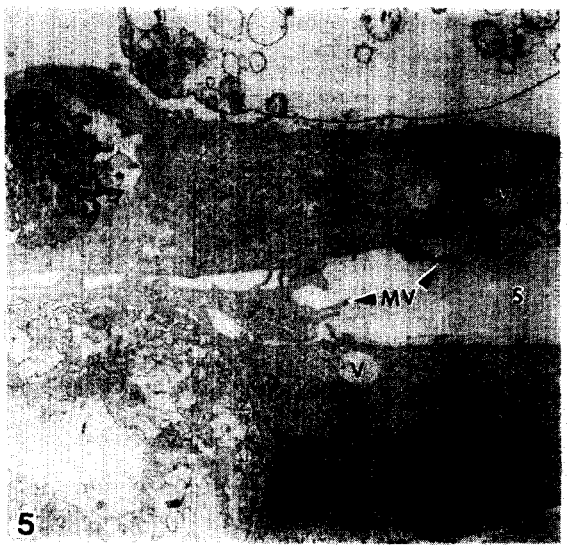

Fig. 1. Normal culture, 6 days old, showing four layers, nucleus (N).
Fig. 2. Low retinoid culture, 6 days old, showing seven layers with numerous tonofilaments (T) and desmosomes (D).
Fig. 3. Low retinoid culture, 10 days old, showing cornified envelopes (CE), nucleus (N), tonofilaments (T), and basal cell (B).
Fig. 4. High retionoid (retinol) culture, 18 days old, illustrating single cell layer with numerous microvilli (MV).
Fig. 5. High retinoid (retinoic acid) culture, 18 days old, with wide intracellular spaces (S), elongated microvilli (MV), nucleus (N), vacuolated structures (V) and reduced numbers of desmosomes.