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Vitamin A and β-Carotene Concentrations at Different Depths of the Epidermis: A Preliminary Study in the Cow Snout

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ABSTRACT

Vitamin A (retinol) is an anti-keratinizing agent essential for normal epithelial differentiation. In order to examine the epidermal distribution of vitamin A and provitamin A (ß-carotene), we took advantage of the extraordinarily thick snout epidermis of the cow which can be cut horizontally into at least 6 layers, representing keratinocytes at different stages of maturation. Extracts of saponified samples were analyzed for retinol and ß-carotene by reversed phase high-performance liquid chromatography. The highest retinol concentration (0.8 μg/g protein; n=3) was recorded closest to the dermis; progressively decreasing amounts of retinol were found in the upper parts of epidermis. Maximum values of ß-carotene (1.0 μg/g; n=7) were found in the lower parts of epidermis; substantially lower levels were seen at the dermal transition zone and in the upper parts of epidermis. The results suggest that the endogenous concentration of vitamin A in snout epidermis is inversely related to the degree of cellular differentiation.

INTRODUCTION

Epidermal keratinocytes, while undergoing terminal differentiation, continuously move outwards from the basal layer to the skin surface .The mechanisms controlling these events are still not clear. Vitamin A is a potent inhibitor of keratinization (4) and, in experimental animals, deficiency of the vitamin causes epithelial hyperkeratosis (13). It may thus be proposed that normal keratinocytes become progressively deficient in vitamin A as they move towards the surface, thereby facilitating their transformation into corneocytes. This 'programmed vitamin A-deficiency' could operate through a restricted supply of vitamin A to the upper parts of epidermis or through an inherent decline in the retinoid-responsiveness of keratinocytes as they move away from the basal layer. Whereas the former mechanism does not seem to have been investigated, the latter is supported by the work of Green and Watt (5), who showed that cultured keratinocytes from various sources (conjunctiva, esophagus, vagina and epidermis) differ in their responsiveness to retinoids in vitro, with epidermal keratinocytes being most resistant to the anti-keratinizing action of the compounds.

In vivo, vitamin A is transported to the target cells by serum retinol-binding protein (RBP) which delivers the vitamin by binding to cell-surface receptors (9,10). Subsequently, retinol enters

a series of metabolic steps leading to the formation of e.g. retinyl esters, retinaldehyde and retinoic acid. Whether ß-carotene (provitamin A) contributes to the supply of retinol to the epidermis is uncertain. However, an additional function of the carotenoid pigments in epidermis may be related to their capacity to quench free radicals.

In previous analyses of epidermal vitamin A and carotenoids, we used specimens of whole epidermis, i.e. mixtures of cells at different stages of keratinization (12). In the present study, we have focused on the distribution of retinol and ß-carotene in cow snout epidermis which can be easily cut into 5 or 6 horizontal layers of sufficient s size to permit chemical characterization (7).

MATERIAL AND METHODS

Cow snouts were removed immediately after slaughter and were kept cool in the dark until cut into cubes (20x20x5 mm) within 30 min. The samples were then firmly squeezed between two glass plates and frozen by flushing with liquid CO_2. Slices of 0.1 mm thickness were produced with the sample mounted horizontally in a hand driven cryostat (Leitz Wetzler, FRG). A punch biopsy from the center of each slice was preserved for routine histology. The rest of the slices were kept frozen (-70°C) until hydrolyzed in ethanolic KOH (80°C; 20 min) and extracted with n-hexane as previously described for human skin (11). The evaporated extracts were redissolved in methanol and analyzed for retinol by high-performance liquid chromatography (HPLC) as described elsewhere (11). A separate series of samples were analyzed for carotenoids by a modification of the HPLC procedure of Driskell et al (3). Thus, the sample extract (prepared as before) was dissolved in 100 µl of acetonitrile and applied to a Zorbax ODS column (4.6 x 150 mm) coupled to a dual pump system (Altex 110, Altex Scientific Inc., Berkeley, Ca.) initially delivering acetonitrile:dichloromethane: methanol (83:2:15) at a flow rate of 1.5 ml/min for 6 min. The composition of the mobile phase was subsequently shifted (concave gradient) to 70:18:12, reaching a plateau at 12 min. The eluate was monitored by UV-absorption at 436 nm (Waters 440, Waters Assoc. Inc, Milford, Ma.). Standard curves (peak height vs. mass) were derived by injecting variable amounts of a solution containing eight different authentic caroteinoids. In a typical experiment, ß-carotene eluted at 27 min. The ß-carotene and retinol concentrations were related to the protein content of the sample as determined by the biuret assay (12).

RESULTS

Histological preparations of the tissue sections produced by cutting a cow snout specimen parallel to the surface are shown in Fig. 1. As indicated, the first section comprises the stratum corneum and the upper portion of stratum granulosum. The second and third sections comprise the upper and mid portions of the stratum spinosum; sweat ducts penetrating the epidermis can be seen. The three last sections contain the basal layers and variable amounts of papillary dermis.

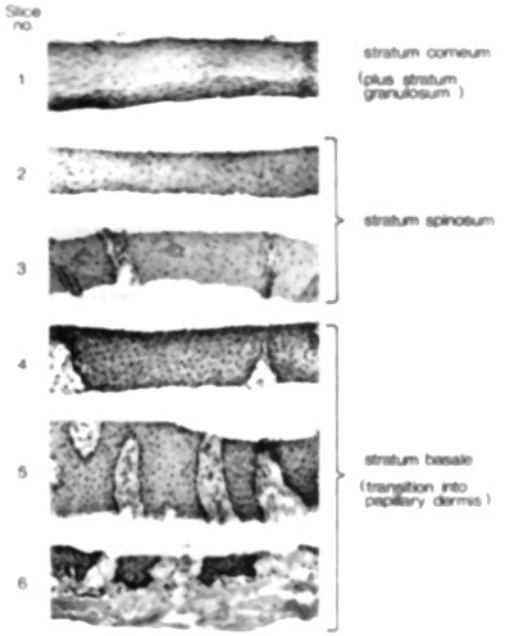

Figure 1. Sections of epidermis stained with hematoxylin and eosin. Six epidermal slices of 0.1 mm thickness taken through the epidermis from the stratum corneum (1) to the papillary dermis (6).

Fig.2 shows the mean retinol and ß-carotene concentrations in the sections produced from 10 samples of epidermis. The highest retinol concentration is seen at the dermal transition zone (section no. 6) and the lowest concentration is in the upper part of stratum spinosum (section no.2). Occasionally, 3-dehydroretinol (vitamin A_2) was detected in the samples (data not shown), but not to the same extent as in human epidermis (12). The concentration profile of ß-carotene is different from that of retinol; high values are seen in mid to lower parts of epidermis and low values are seen both in the stratum corneum and in the dermal transition zone. Small amounts (<0.1 µg/g protein) of lutein were also present in the samples. This carotenoid appeared to be evenly distributed in the epidermis (data not shown).

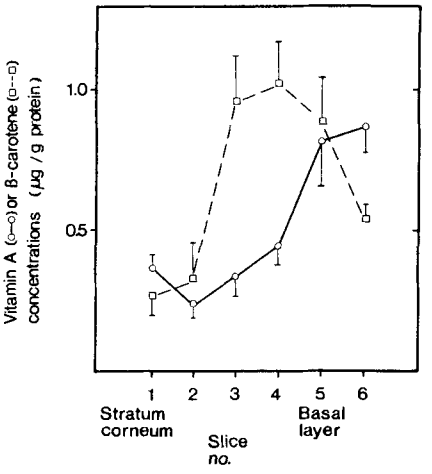

Figure 2. Changes in retinol and ß-carotene contents of epidermis with depth in the epidermis. Mean (±SE) values for three (retinol) and seven (ß-carotene) samples, respectively. Factors to convert the values to nmol/g: x3.497 (vitamin A) and x1.863 (ß-carotene).

DISCUSSION

The low retinol concentration found in the upper layers of epidermis (saponified samples) is consistent with the proposed 'programmed vitamin A-deficiency' in the keratinizing zone. However, it should be emphasized that the number of samples investigated was small and that it is not known whether the observed retinol concentration is sufficiently low to precipitate terminal differentiation of keratinocytes. Also, the vitamin A status of a tissue depends not only on its total content of retinol; a number of other factors, including variable formation of inactive retinyl esters or highly active retinoic acid (not detected by our assay) may markedly influence the expression of retinoid activity.

The origin of the retinol gradient in snout epidermis should be sought among one or several of the following explanations: (a) restricted passage of serum retinol-RBP to the outer parts of epidermis (1), (b) decreased number of RBP receptors on differentiated keratinocytes (8), (c) enhanced metabolic degradation of retinol in upper epidermis, and (d) destruction of retinol by solar radiation penetrating the superficial layers of the integument (2). The possibility of a decreased uptake of retinol in the upper epidermis is supported by a recent in vitro study on cow snout. Autoradiography showed that the uptake of tritiated retinol from RBP is highest in the basal layers of epidermis (Törmä, Gillberg & Vahlquist, to be published).

Although one should excercise care when extrapolating results from cow snout to other types of epithelia, there are indications that a similar concentration gradient of vitamin A exists in human epidermis. For example, the stratum corneum of the human foot sole contains much less vitamin A than the underlying epidermis (12). Unfortunately, the thickness of human epidermis (about 0.1 mm) precludes sectioning of the samples by the technique used for snout epidermis. Clearly, further studies on the occurrence, metabolism and function of vitamin A in different types of epithelia are required before the observed vitamin A gradient in epidermis can be implicated in the process of keratinization.

It is noteworthy that the distributions of retinol and ß-carotene in epidermis differ. Using less sophisticated methods, we (12) and others (6) reported previously that human epidermis has a higher affinity for carotenoids than dermis, but it is not known to which epidermal component(s) the compounds bind. As yet, there is no proof that epidermal ß-carotene is converted to retinol but other processes, such as quenching of free radicals, may be equally important functions of the carotenoids in the epidermis. Tentatively, the accumulation of carotenoids in the lower parts of epidermis could help to protect the proliferating basal cells from solar damage.

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