THE EFFECTS OF EPIDERMAL GROWTH FACTOR ON NEWBORN MICE EPIDERMIS

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SUMMARY : In this study, the effects of epidermal growth factor EGF on newborn mice epidermis have been examined. EGF is an active agent on mesothelium and epithelium originated cells with its own receptor in various tissues. In this study, 10 of the 22 newborn mice were given EGF subcutaneously at the dose of 10 µg/kg/day for ten days after birth. As the control group, for the same period saline was given at the same dose to 12 newborn mice. After 10 days all the mice were decapitated and tissue samples from skin were examined by electron microscopy. In the EGF injected group, epidermis was composed of basal, intermediate and superficial cells. In the superficial cells, keratohyalen granules were observed clearly. Keratinization initially appeared on the surface. Desmosomes were present among these epidermal cells. Basement membrane within the two layers was completely developed. In the control group, the epidermal layers were not developed and the granules present in this area were thought to be the precursors of keratohyalen granules. The development of basement membrane was incomplete.

***Key Words** : Epidermal Growth Factor, Skin, Epidermis, Electron Microscopy, Ultrastructure.*

INTRODUCTION

Epidermal growth factor (EGF) was first isolated from a male mouse submaxiller (submandibular) gland by Dr.Stanley Cohen in 1962. Since it caused a thickening in epidermis by increasing keratinization, it is called the epidermal growth factor (2, 3).

EGF is the most widely examined polypeptid that causes growth. Besides causing normal growth, EGF also causes cancer development in skin carcinoma, pancreatic carcinoma, gastric carcinoma, mammary tumors and thus, it has become the subject of numerous studies (4, 6, 7, 9).

EGF has also been reported to promote epidermal growth and inhibit hair follicle development and hair growth (5).

In this study, the effects of epidermal growth factor on newborn mice epidermis which is mitogenic on mesothelium and epithelium orginated cells-with its own receptor in numerous tissues, were examined.

MATERIALS AND METHODS

In this study 10 of the 22 newborn mice were given EGF subcutaneously at the dose of 10 µgr/kg. day for ten days after birth. As the control group, for the same period, saline was given at the same dose to 12 newborn mice. After 10 days all the mice were decapitated and tissue samples from skin were examined by electron microscopy.

Tissues were fixated in phosphate - buffered 2.5 % gluteraldehyde at 0-4° C for 2h. They were then postfixated in 1 % OsO_4 and dehydrated in a series of graded alcohols. After passage through propylene oxide, the specimens were embedded in Araldyt CY212, DDSA, BDMA. Ultrathin sections were cut perpendicular to the outer surface of epidermis and stained with uranyl acatate lead citrate to be examined with a Carl Zeiss EM 952 electron microscope.

RESULTS

In the control group, epidermal layers were not developed and granules present in this area were thought to be the precursors of keratohyalin granules (Fig 1). Development of the basement membrane was also incomplete (Fig 2).

In the EGF applied group epidermis was composed of basal, intermediate and superficial cell layers (Fig 3, 4). In the superficial cells, keratohyalen granules were observed clearly. Keratinization was found to have begun on the surface (Fig 5). Desmosomes among these epidermal layers were also found to be completely developed (Fig 6, 7).

DISCUSSION

In the control group, epidermis had not developed completely. The cells on the basal lamina were flat. The cells which were placed on the uppermost flat cells had a keratohyalen granulary structure which suggested that they were primordial struc-

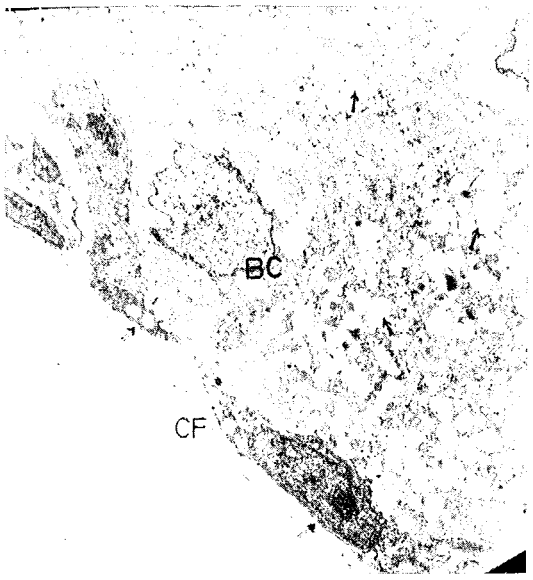

Fig - 1 : Representation from control group demonstrating flat basal cells (BC), on the basement membrane (double arrows) and unmature collagen fibers (CF). Granules of keratohylalen (arrows) lying on the basal cells are also observed x 8500.

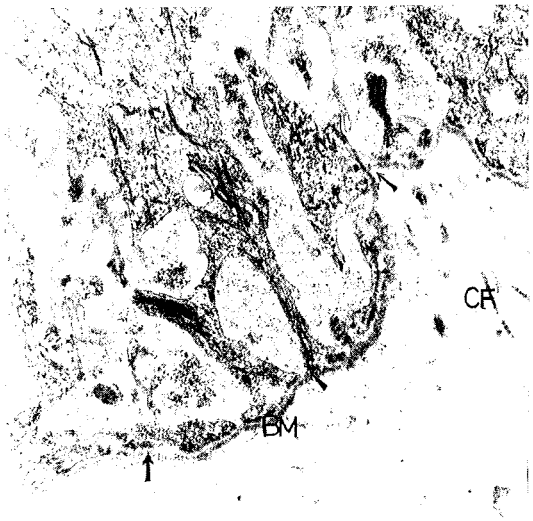

Fig - 2 : In the control group, unmature and discontinuous basement membrane (BM) is seen (arrows). Few collagen fibers are found in the dermis x 47.500.

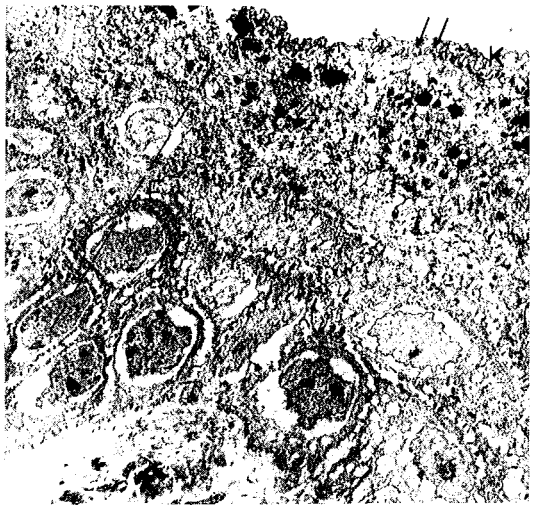

Fig - 3 : EGF injected group : Mature epidermis with all layers (E). Thickened cell membrane (arrows) and keratinization (K) on the flattened cells of the uppermost layerare observed x 9000.

Fig - 4 : EGF injected group : Mature epidermis with all layers. Basal cells form a cuboidal layer on the basement membrane (BC); while on the suprabasement and upper spinosum layers, there are flattened cells (FC). Str. corneum (SC), keratohyalin granule (KG), x 8500.

Fig - 5 : This picture shows that Str. corneum has been fully matured. Str. corneum (SC), keratinized layer (KL), basal cell (BC) x 9000.

Fig - 6 : This picture shows desmosomes (D) lying between basal cells in the EGF injected group on high magnification x 140.000.

tures of the keratohyalen granules.

It was also observed by higher magnification that the basement membrane of the control group had not developed sufficiently and had lost its continuity step by step.

A few of the collagen fibers in the connective tissue of dermis underneath the basement membrane had started to grow in the control group.

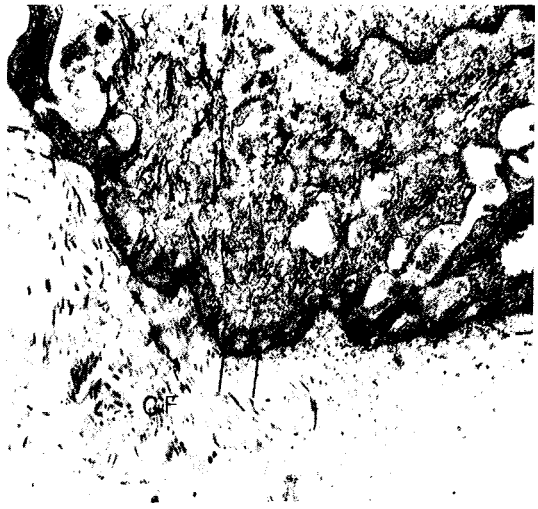

Fig - 7 : Completely matured and continuous basement membrane (arrows), together with the collagen fibers in dermis (CF) x 47.500.

In explants cultured with epidermal growth factor (EGF), hidrocortison (HC) and delipidized fetal calf serum (dFCS) tonofilament bundles and electron dense masses were found to be poorly developed in the superficial flattened cells forming the membrane thickening (1).

In our study, the cell membranes of the superficial layer had a significant thickening. In the granular layers, keratohyalen granules appeared as amorphous eletron dense material; sometimes in contact with the tonofibrils (8). We also demonstrated a large number of keratohyalen granules in str granulosum cells of the epidermis in the EGF administrated group. Desmosomes and hemidesmosomes were still found abundantly in the basal cells (1). The higheat magnification possible by the electron microscope revealed a substantial number of desmosomes between the basal cells in the EGF injected group.

In sections of the keratinizing epidermis from 13 day-old chick embryos, the basement membrane was intact, and appeared as a continuous structure running along its basal surface. The lamina densa and rara were also clearly observed (1).

During the duration of the study, only two hair follicles which were not completely developed were observed and the microscopic image of one of them was photographed. According to Seguin in the ultrastructure of the neoepidermis (20 days) tissue was composed of keratocyts, cuboidal in the basal and suprabasal layers while flattened in the upper spinous and granular layers (8).

Although all the epidermal layers had developed; cuboidal basal cells of the 10 day mouse epidermis were more prominent. In the 13 day old chick embryos, basement membrane became discontinuous with many gaps and was sometimes found to be even detached from the epidermal basal layer protruding into the underlying dermis to from folds or wrinkles. Occasionally, basal cell processes appeared in the dermal region and extended beyond the basement membrane (1). We did not observe a discontinuity with in the basement membrane in EGF injected newborns. However, it was clearly observed that the epidermal basal cells invaded the dermis and the basement membrane adapted to this situation as well. The st. corneum was clearly individualized (1).

As a conclusion, in our study, EGF caused a substantial development in the str. corneum and in addition, a significant increase was observed in the thickness of the keratinized layer.

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