0022-202X/84/8202-0165$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 82:165–169, 1984

Vol. 82, No. 2
Printed in U.S.A.

Visualization of Epidermal Growth Factor Receptors in Human Epidermis*

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The localization of epidermal growth factor (EGF) receptors in normal human epidermis was examined with two independent experimental methods. The distribution of EGF receptor sites was studied using light microscopic autoradiography with [^{125}I]EGF and direct immunocytochemical techniques with EGF receptor antibodies and protein A–colloidal gold complexes. Direct visualization by autoradiography indicated that the concentration of EGF receptors was greatest in the lower epidermal layers. Ultrastructural morphometric analysis of protein A–gold complexes showed that EGF receptors were primarily associated with the plasma membranes although intranuclear and cytoplasmic localization was also evident. This postembedment immunolocalization method also confirmed the relative differences in the number of EGF receptors found in individual epidermal layers (basalis > spinosum > granulosum > corneum layers). This inverse relationship between numbers of EGF receptors and the degree of epidermal differentiation and/or keratinization may suggest a physiologic role for EGF in these processes in human epidermis.

The polypeptide hormone epidermal growth factor (EGF) is a potent mitogen for a variety of mammalian epithelial tissues and cells [see reviews 1,2]. Evidence indicates that the initial interaction of EGF with target cells occurs on specific membrane receptors [3,4]. Investigations with ferritin and fluorescein-labeled EGF indicate that receptor-ligand complexes are diffusely distributed and laterally mobile in the membrane [5–7]. Subsequent to binding, EGF receptor-hormone complexes form clusters, are internalized, and become incorporated into lysosomes. Elucidation of this sequence following binding of EGF has been facilitated by using cultured A-431 cells, a human epidermoid cancer cell line with large numbers of EGF receptors [8,9]. Much less is known concerning in vivo EGF receptor interactions. When EGF was discovered, it was so named for its ability to increase growth and keratinization of skin epithelium [10]. Binding studies indicate that EGF receptors are found in neonatal and fetal mouse skin [11] and cultured keratinocytes [12]. In vitro, morphologic differentiation and keratin production appear to parallel detectable decreases in binding of [^{125}I]EGF [12,13].

In the present studies, the localization of EGF receptors in normal human skin was investigated by two differing methods. Light microscopic autoradiographic techniques were used to localize receptor sites with covalently coupled [^{125}I]EGF. Alternatively, immunoelectron microscopic techniques with a monospecific antibody against the EGF receptor were developed to localize antigenic sites in aldehyde-fixed and Epon-embedded tissues.

MATERIALS AND METHODS

Reagents

EGF was purchased from Collaborative Research (Waltham, Massachusetts and ^{125}I was obtained from New England Nuclear (Boston, Massachusetts). NTB-2 emulsion for autoradiography was obtained from Eastman Kodak (Rochester, New York). Protein A and polyethylene glycol (M_r 20,000) were obtained from Sigma Chemical Co. (St. Louis, Missouri) Colloidal gold solutions were purchased from Polysciences, Inc. (Warrington, Pennsylvania). Dulbecco's modified Eagle's medium was obtained from Grand Island Biological Co., 10% calf serum from Flow Laboratories, and gentamycin from Microbiological Associates. Affi-Gel was obtained from BioRad. The A-431 human epitheloid carcinoma cells were provided by Dr. J. DeLarco, National Institutes of Health. All other reagents and chemicals were purchased from common suppliers.

Antibody Preparation

The A-431 cells were grown to confluency in 100-mm Falcon dishes containing Dulbecco's modified Eagle's medium supplemented with 10% calf serum and gentamycin. Polyclonal antibody to the EGF receptor was prepared as described by Stoscheck and Carpenter. [14]. Briefly, EGF receptor from A-431 cells was purified by affinity chromatography as previously described [15]. The purified receptor, which migrates as one Commassie-stained band on sodium dodecyl sulfate gel electrophoresis, was injected into rabbits to produce immune sera.

Autoradiography

Normal human skin specimens were obtained from patients undergoing elective surgery. Specimens were processed either for light microscopy (LM) or transmission electron microscopy (TEM). Previous binding experiments with cell membranes served as the guideline for the [^{125}I]EGF incubation conditions. Modifications were adapted from the methods of Gordon et al [7] and O'Keefe et al [11]. EGF was iodinated by the method of Carpenter and Cohen [16]. Samples destined for autoradiographic studies were cut into small pieces and incubated for 90 min at room temperature with shaking in 0.5 ml Hanks' balanced salt solution containing 1 mg/ml bovine serum albumin, 20 mM HEPES pH 7.4, and [^{125}I]EGF (final concentration approximately 10 ng/ml). The skin samples were washed repeatedly for 45 min in the incubation medium devoid of EGF or, alternatively, samples were washed in the incubation medium containing excess unlabeled EGF (2 μg). After 24 h of fixation in 10% neutral buffered formalin, the skin was routinely processed for LM and embedded in paraffin. Sections (7 μm) were cut onto subbed glass slides, deparaffinized, and dipped into a 1:1 mixture of NTB-2 emulsion and distilled water. The emulsion was dried and,

Manuscript received July 7, 1983; accepted for publication September 7, 1983.

This work was supported by the University Research Council and the American Association of Plastic Surgeons (LBN), GM 27153 and EYO221 (JAM), CA-24071 and BC-294A (American Cancer Society) (GC), 1R01-AM26518 and V. A. funds (LEK). Dr. Stoscheck was a research fellow, NIH training grant HD-07043; Dr. Carpenter is an Established Investigator of the American Heart Association.

A preliminary report of this work was presented at the national meeting of The Society for Investigative Dermatology, May 1982.

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Abbreviations:
EGF: epidermal growth factor(s)
LM: light microscopy
pA-gold: protein A-colloidal gold complex(es)
s.: stratum
TEM: transmission electron microscopy

following exposure at 4°C for 4–8 weeks, developed for 2 min in Kodak D19 developer at 10°C. Sections were lightly stained with hematoxylin and eosin and photographs taken on a Leitz Ortholux microscope equipped with epi-illumination. Photomicrographs were taken using standard bright-field illumination as well as dark-field illumination to accentuate the silver grains on the tissue sections.

Preparation of Skin for TEM

Other specimens were processed for TEM by fixation at 4°C for 24 h in 4% gluteraldehyde buffered with 0.2 M phosphate at pH 7.2. Following rinsing in 0.3 M sucrose containing 0.2 M phosphate buffer, tissues were postfixed in 1% osmium tetroxide buffered with 0.2 M phosphate for 2 h. After rinsing with distilled water, tissues were dehydrated in graded concentrations of ethanol and propylene oxide and embedded in Medcast (Ted Pella, Inc.). For orientation, semithin sections were cut and stained with toluidine blue. Ultrathin sections (400–600 Å) were cut on a diamond knife with an LKB ultramicrotome and floated onto nickel grids. Sections were examined and photographed with an Hitachi 600 transmission electron microscope.

Preparation of Protein A–Colloidal Gold Complexes (pA–gold)

Protein A (pA) was complexed to colloidal gold by minor modifications of the methods described by Roth, Bendayan, and Orci [17]. The optimum concentration of protein A was determined by adding serial dilutions of pA to a constant volume of colloidal gold. After 5 min, 0.5 ml of 10% NaCl was added and flocculation judged visually. A color change from red to blue-violet indicated flocculation and therefore an excess of pA. This routine testing to check the optimum pA concentration was necessary because there was great variability in concentration of colloidal gold solutions. For the working stock solution of colloidal gold, the pH was adjusted to 6.9 with 0.2 M K_2CO_2. After adding excess pA to the gold solution, the pA-gold solution was centrifuged at 100,000 *g* for 1 h at 4°C. The supernatant which contained uncomplexed pA was discarded and the pA-gold precipitate resuspended in 5 ml of phosphate-buffered saline (pH 7.4) with 0.2 mg/ml polyethylene glycol as a stabilizer. To verify that pA was complexed to colloidal gold particles, negative staining of the precipitate placed on Formvar-coated grids was done using uranyl acetate. A clear halo around colloidal gold particles was noted by the negative staining technique whenever pA was complexed in sufficient amounts. The pA-gold solutions were stored at 4°C for a maximum of 14 days.

Immunocytochemical Staining

Human skin was labeled after ultrathin sectioning of Epon-embedded specimens by the following procedures:

1. Ultrathin, unstained sections on nickel grids were floated on drops of experimental antisera (diluted 1:4) for 60 min at 24°C.
2. Grids with attached sections were washed by holding them with forceps and agitating through a series of beakers containing distilled water.
3. Grids containing treated sections were incubated on drops of pA-gold for 60 min at 24°C.
4. The rinsing procedure described in step 3 was repeated.
5. For early testing, uranyl acetate was used for staining, but lead citrate counterstaining was later added because it did not interfere with labeling and counting of gold particles.

Immunocytochemical Controls

To determine specificity of the method, the following controls were performed:

1. Incubation of sections with pA-gold complex alone for 60 min.
2. Incubation of sections with experimental sera or control sera followed by non-pA-complexed colloidal gold.
3. Incubations of sections with rabbit preimmune serum instead of experimental antiserum before adding the pA-gold.

RESULTS

Identification of EGF Receptor Sites by Autoradiography

The EGF receptor distribution in representative autoradiographs of epidermis from normal human skin incubated with [^{125}I]EGF is shown by silver grain deposition in Fig 1*B,C*. The highest density of silver grains representing specific EGF receptors was localized in the stratum (s.) basalis and, to some extent, s. spinosum. A sharply decreased number of silver grains over the region of the s. granulosum and s. corneum was apparent. There was no detectable difference in the upper portion of the epidermis between experimental and control autoradiographs. The specificity of the binding of [^{125}I]EGF was demonstrated by the ability of excess unlabeled EGF (2

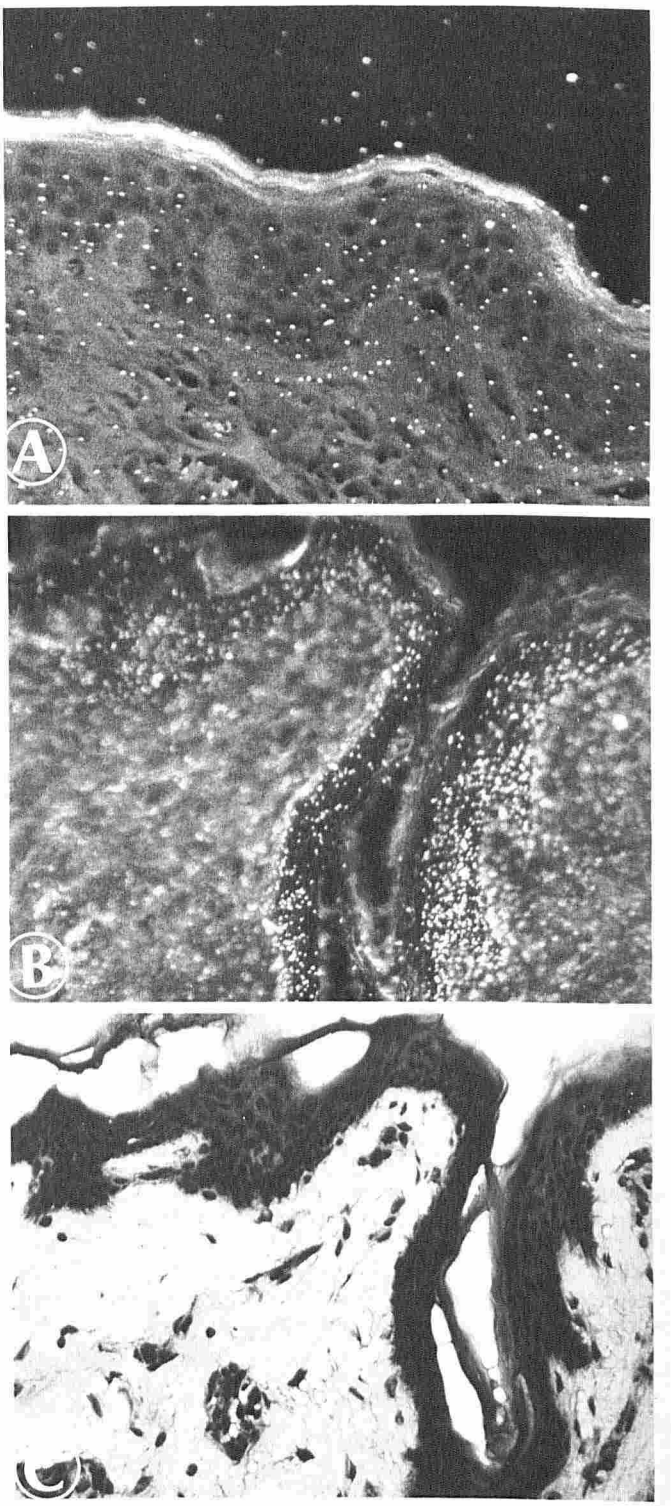

FIG 1. [^{125}I]EGF labeling of normal human skin. Small samples of normal human skin incubated in media containing 2 μg of unlabeled EGF (*A*) and media containing approximately 10 ng/ml [^{125}I]EGF (*B and C*). Tissues were fused, processed for autoradiography, stored for 10 weeks, developed, and stained with hematoxylin and eosin. *A and B* were photographed under dark-field illumination; *C* was photographed under standard bright-field illumination. *A*, × 640; *B*, × 525; *C*, × 525.

μg) to displace the bound [^{125}I]EGF (Fig 1*A*) or to prevent [^{125}I] EGF binding (data not shown).

Morphometric Analysis of EGF Receptor Sites

Colloidal gold provides an electron-dense particle which can be visualized by TEM [17–23]. We used a "sandwich" technique with a monospecific anti-EGF receptor followed by pA-gold to label EGF receptors in epidermal layers. With this immunocytochemical procedure, the keratinocyte plasma membrane was labeled with gold particles (Fig 2). Gold particles were also found dispersed within the cytoplasmic and nuclear compartments. An occasional gold particle was observed over the extracellular space without apparent proximity to any cell surface. Morphometric analysis of the colloidal gold labeling procedure was deemed necessary because the labeling patterns were very complex and impossible to judge by visual examination.

Skin samples were obtained from several body regions in patients ranging in age from 15–85 years. A large number (300) of randomly selected micrographs from the skin were enlarged to 33,500×. Counting and measuring determinations were greatly facilitated by the use of a computerized morphometric system (E. Leitz Co.). On each micrograph the percentages of label per micron of plasmalemma and per square micron of cytoplasm, nucleus, and extracellular space were assessed. In all cases, 50 micrographs or more were analyzed from each epidermal layer. The actual numbers varied because the total sum of the length of the plasmalemma was held approximately equal. Adjustments were also necessary because s. basalis cells generally have more cell processes and therefore more plasma membrane than s. corneum cells, making it necessary to count more micrographs from s. corneum to avoid bias in the measurements.

For plasma membrane labeling, only those particles were counted which appeared to be associated with the membrane. This counting method posed some problems, however, because the proximity of particles to the membrane was variable, especially in regions where the membrane passed obliquely through the plane of section [24]. Therefore, by counting only particles that appeared to be associated with the membrane, our counting procedure may underestimate the number of membrane-bound EGF receptors, due to plane of section counting errors.

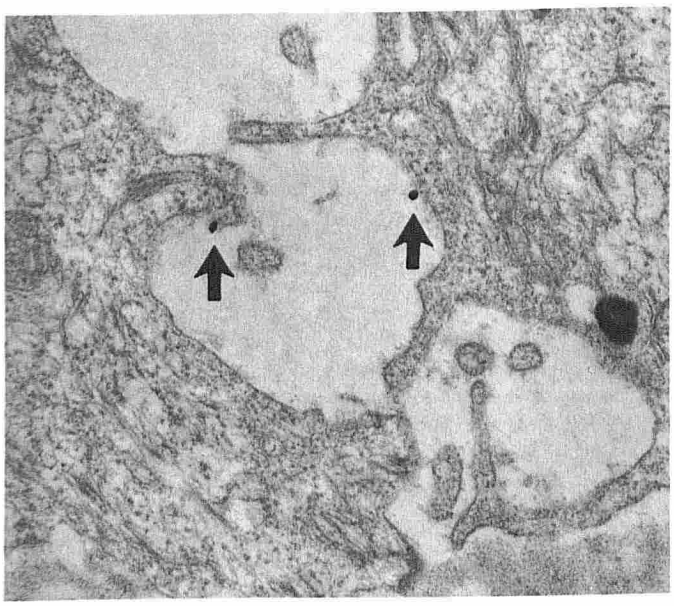

FIG 2. Electron micrograph showing the representative immunocytochemical labeling pattern of normal human epidermis (s. basalis) after incubation in anti-EGF receptor serum and pA-gold. × 47,500.

Background pA-gold Labeling

Extensive analyses of nonspecific labeling were made to evaluate the feasibility of the pA-gold method for postembedment localization of antigens against EGF receptors. Non-pA-complexed gold did not bind or adhere to thin sections. No labeling was observed in thin sections incubated solely with anti-EGF receptor antibody or uncomplexed colloidal gold.

Additional checks of the postembedment staining technique were performed. Background labeling was assessed and found to be negligible in Epon plastic areas devoid of tissue, in the extracellular space between epidermal cells, and in the dermis. An unexplained finding was some gold label in the nucleus and cytoplasm of cells incubated in the EGF receptor antibody. This intracellular label was extremely variable within each micrograph and within each epidermal layer and showed no correlations as did the membrane labeling. Although EGF receptors are known to cluster and become internalized in lysosomes in vitro, the intracellular gold particles did not cluster or associate with intracellular membrane vesicles. A similar labeling phenomenon was also mentioned by another group using pA-gold techniques [18].

EGF Receptors in Human Epidermis

Distributions of gold particles representing putative EGF receptor sites were determined for each layer of epidermis. The typical plasmalemmal labeling pattern of keratinocytes is displayed in Fig 2.

Computerized morphometric analysis of EGF receptor distribution is shown in Fig 3 and displays the number of EGF

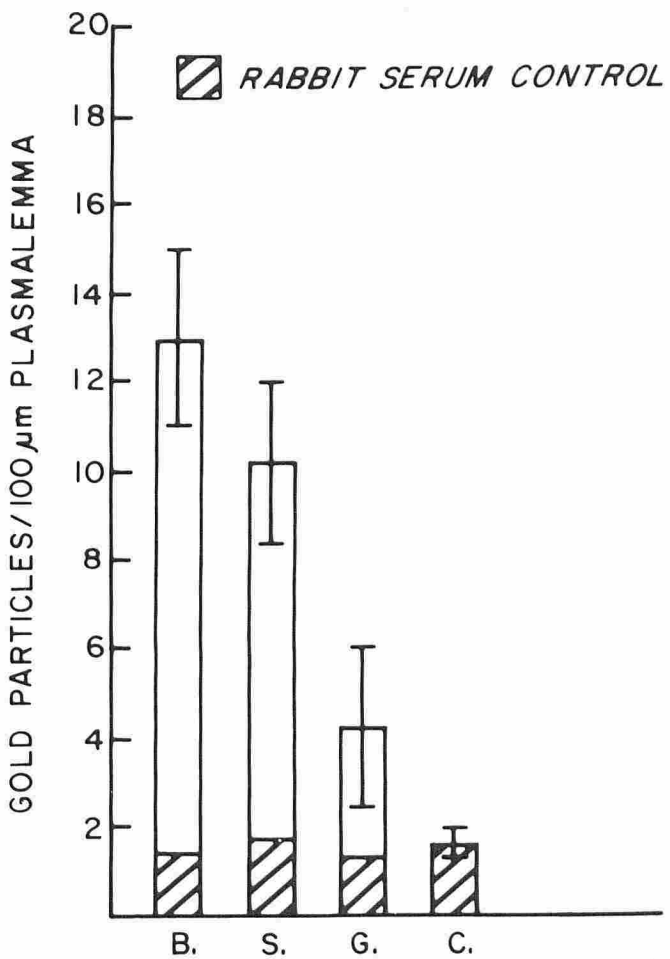

FIG 3. Morphometric analysis of EGF receptor sites along the plasma membrane of normal human epidermis. *B.* = basalis, *S.* = spinosum, *G.* = granulosum, *C.* = corneum, *brackets* = SEM.

receptors along the plasma membrane as correlated to each epidermal layer. Counting of the number of EGF receptors along the membrane in s. basalis confirmed that pA-gold labeling was maximal in this lowest layer (12.9 ± 2). Although EGF receptors were somewhat decreased in s. spinosum (10.19 ± .9), this difference was not statistically significant ($p > .20$) compared to s. basalis. This finding may be due in part to the difficulty in unequivocally determining s. spinosum cells due to obliqueness in the plane of section. A marked decrease in EGF receptors in the s. granulosum and s. corneum was significant in comparisons to s. basalis ($p < .001$ and $p < .0001$, respectively). Thus a progressive decline in gold label (presumed EGF receptor sites) was evident in the more differentiated cell layers in the epidermis.

Experimental Controls

Thin sections incubated in rabbit preimmune serum, as a substitution for monospecific EGF receptor antibody, were used as controls for the specificity of pA-gold labeling. The number of gold particles along the plasma membrane for all epidermal layers was determined (Fig 3). In these control determinations, the amount of nonspecific label was significantly less than the specific label found in EGF receptor antisera treatments for all layers of the epidermis excluding s. corneum ($p < .001$).

DISCUSSION

We developed two independent methods to visualize the distribution of EGF receptors in intact stratified sqaumous epithelium. By using two different methods, we demonstrated that EGF receptors from human skin (1) were present in epidermis, and (2) were present in comparatively greater numbers in the lower as compared to the upper keratinocyte layers. The autoradiography method is more visually obvious than the other method, but less precise. Independently performed [^{125}I] EGF binding studies are in agreement with these two anatomical localization methods [12].

The ultrastructural postembedment localization of antigens using the pA-gold technique has rapidly gained in use since it was first employed for demonstration of peptide hormones and enzymes in pancreatic tissue [17]. The pA-gold method has now been used in studies of vascular permeability [20], antigenic sites on lymphocytes, red blood cells, and platelets [21], localization of polysaccharides and glycoproteins on yeast cell walls and RBC membranes [22], intracellular localizations of ACTH in pituitary cells [18], and other applications [19–25]. Postembedment immunolocalization (pA-gold method) has multiple advantages. The primary advantage is the ability to label tissue sections after optimal fixation and yet still render all layers of the skin accessible to immunocytochemical reagents. Other factors such as ease of preparation, relative inexpense, ease of visualization at low TEM magnification, and small amount of antisera required are also compelling considerations. One major disadvantage of the pA-gold technique is its lack of one-to-one correlation between the gold marker and the binding site. Protein A has 4 Fc binding regions per molecule and is theoretically able to react with 4 Fc portions from 4 different IgG molecules [26,27]. Also, the number of pA molecules absorbed to a gold particle is believed to be greater than one. In addition, it is well recognized that some loss of antigenicity in the tissues occurs during exposure to harsh treatments during fixation, dehydration, and embedding [28]. Therefore, this procedure can be useful for quantifying relative differences but may not be very sensitive or precise at either very high or low concentrations of binding sites.

A scarcity of knowledge exists concerning the physiologic role of EGF in skin. It is unclear whether EGF directly stimulates keratinization or whether its effect on differentiation is due to increased proliferation. In vivo studies have shown that EGF increased the number of mitotic epidermal cells [29]; the dry weight, DNA, and RNA content of the epidermis [30]; the disulfide content of the epidermis [31]; and the activity of an epidermal enzyme, ornithine decarboxylase [32,33]. In addition, thyroid hormone effects on neonatal skin maturation may be mediated by EGF [34]. Topical applications of EGF also increased the rate of reepithelization in healing skin wounds [35–37].

Although the earliest EGF observations were made in skin, the major focus of the work has turned to its in vitro effects. EGF stimulates the proliferation of epidermal cells in organ cultures of chick embryo skin [38] and human fetal head skin [39]. This peptide hormone increased ornithine decarboxylase activity, DNA, RNA, and protein synthesis, cell division, and, eventually, keratinization [38,40]. Since these EGF effects were shown either in the presence of "killed" dermis or in the absence of dermis, it appears that EGF acts directly on the epidermis. Within 3 h after an injection of [^{125}I]EGF in rats or rabbits, radioactivity was concentrated 300% in epidermis compared to blood [41]. Specific binding sites for [^{125}I]EGF in neonatal epidermis [11] and cultured keratinocytes [12] have been reported.

In cultured keratinocytes, morphologic changes and keratin production were associated with decreased binding of EGF and presumably EGF receptors [12]. In the present in vivo study, EGF receptors either decreased in number or became masked as cells differentiated and moved toward the surface. EGF receptors are not unique in this respect. In vivo and in vitro evidence is accumulating which links decreased expression of epidermal cytoplasmic antigens with keratinocyte differentiation [42,43]. Certain proteins such as keratin polypeptide [44] and calcium-binding proteins [45] have also been linked to the stage and type of keratinocyte differentiation. Thus the inverse relationship between the number of EGF receptors and the degree of epidermal differentiation and/or keratinization may suggest a physiologic role for EGF in human epidermis.

REFERENCES

1. Carpenter G, Cohen S: Epidermal growth factor. Annu Rev Biochem 48:193–216, 1979
2. King LE, Carpenter GF: Epidermal growth factor, In: Biochemistry and Physiology of the Skin. Edited by L Goldsmith. New York/Oxford, Oxford University Press, 1983, pp 269–281
3. Hollenberg MD, Cuatrecasas P: Epidermal growth factor: receptors in human fibroblasts and modulation by cholera toxin. Proc Natl Acad Sci USA 70:2964–2968, 1973
4. Cohen S, Carpenter G, Lembach KJ: Interaction of epidermal growth factor with cultured fibroblasts. Adv Metab Disord 8:265–285, 1975
5. Haigler HT, McKanna JA, Cohen S: Direct visualization of binding and internalization of a ferritin conjugate of epidermal growth factor in human carcinoma cells A-431. J Cell Biol 81:382–395, 1979
6. Schlessinger J, Schechter Y, Willingham MC, Pasten I: Direct visualization of binding, aggregation, and internalization of insulin and epidermal growth factor on living fibroblastic cells. Proc Natl Acad Sci USA 75:2659–2663, 1978
7. Gordon P, Carpenter JL, Cohen S, Orci L: Epidermal growth factor: morphological demonstration of binding, internalization, and lysosomal association in human fiobroblasts. Proc Natl Acad Sci USA 75:5025–5029, 1978
8. Fabricant RN, DeLarco JE, Todaro GJ: Nerve growth factor receptors on human melanoma cells in culture. Proc Natl Acad Sci USA 74:565–569, 1977
9. Haigler H, Ash JF, Singer SJ, Cohen S: Visualization by fluorescence of the binding and internalization of epidermal growth factor on human carcinoma cells A-431. Proc Natl Acad Sci USA 75:3317–3321, 1978
10. Cohen S, Elliott GA: The stimulation of epidermal keratinization by a protein isolated from the submaxillary gland of the mouse. J Invest Dermatol 40:1–5, 1963
11. O'Keefe E, Hollenberg MD, Cuatrecasas P: Epidermal growth factor: characteristics of specific binding in membranes from liver, placenta, and other target tissues. Arch Biochem Biophys 164:518–526, 1974
12. O'Keefe E: Epidermal growth factor receptors in human epidermal cells (abstr). J Invest Dermatol 76:311, 1981
13. O'Keefe EJ: Modulation of epidermal growth factor receptor number by calcium. J Cell Biol 95:420a, 1982

14. Stoscheck C, Carpenter G: Characteristics of antibodies to the epidermal growth factor receptor—kinase. Arch Biochem Biophys, in press
15. Haigler HT, Carpenter G: Production and characterization of antibody blocking epidermal growth factor: receptor interactions. Biochim Biophys Acta 598:314–325, 1980
16. Carpenter G, Cohen S: ^{125}I-labelled human epidermal growth factor. J Cell Biol 71:159–171, 1976
17. Roth J, Bendayan M, Orci L: Ultrastructural localization of intracellular antigens by the use of protein A-gold complex. J Histochem Cytochem 26:1074–1081, 1978
18. Batten FC, Hopkins CR: Use of protein A coated colloidal gold particles for immunoelectron microscopic localization of ACTH on ultrathin sections. Histochemistry 60: 317–320, 1979
19. Bendayan M: Double immunocytochemical labeling applying the protein A-gold technique. J Histochem Cytochem 30:81–85, 1982
20. Bendayan M: Use of the protein A-gold technique for the morphological study of vascular permeability. J Histochem Cytochem 28:1251–1254, 1980
21. Romano EL, Romano M: Staphylococcal protein A bound to colloidal gold: a useful reagent to label antigen-antibody sites in electron microscopy. Immunochemistry 14:711–715, 1977
22. Horisberger M, Rosset J: Colloidal gold, a useful marker for transmission electron microscopy. J Histochem Cytochem 25:295–305, 1977
23. Doerr-Schott J, Garand JC: Ultrastructural identification of gastrin-like immunoreactive nerve fibers in the brain of Zenopus laevis by means of colloidal gold and ferritin immunocytochemical methods. Cell Tissue Res 216:581–589, 1981
24. McKanna JA, Haigler HT, Cohen S: Hormone receptor topology and dynamics: morphological analysis using ferritin-labeled epidermal growth factor. Proc Natl Acad Sci USA 76:5689–5693, 1979
25. Dickson RB, Willingham MC, Pastan I: Alpha$_2$ macroglobulin absorbed to colloidal gold: a new probe in the study of receptor-mediated endocytosis. J Cell Biol 89:29–34, 1981
26. Sjoquist J, Meloun B, Hjelm J: Protein A isolated from *Staphylococcus aureus* after digestion with lysostaphin. Eur J Biochem 29:572–578, 1972
27. Hjelm J, Sjodahl J, Sjoquist J: Immunologically active and structurally similar fragments of protein A from *Staphylococcus aureus*. Eur J Biochem 57:395–403, 1975
28. Kuhlmannn WD, Peschke P: Advances in ultrastructural post-embedment localization of antigens in Epon sections with peroxidase labeled antibodies. Histochemistry 75:151–161, 1982
29. Birbaum JE, Sapp TM, Moore JB: Effects of reserpine, epidermal growth factor and cyclic nucleotide modulators on epidermal mitosis. J Invest Dermatol 66:313–318, 1976
30. Angeletti PU, Salvi ML, Chesanow RL, Cohen S: Azione dell epidermal growth factor - sulla sintesti di acidi nucleici e priteine dell'epitelio cutaneo. Experientia 20:1–6, 1964
31. Frati C, Covelli I, Mozzi R, Frati L: Mechanism of action of epidermal growth factor: effect on the sulfhydryl and disulfide groups content of mouse epidermis during keratinization. Cell Differ 1:239–244, 1972
32. Stastny M, Coehn S: Epidermal growth factor IV. The induction of ornithine decarboxylase. Biochim Biophys Acta 204:578–589, 1970
33. Blosse PT, Fenton EL, Henningsson S, Kahlson G, Rogengren E: Activities of decarboxylases of histidine and ornithine in young-male mice after injection of epidermal growth factor. Experientia 30:22–23, 1974
34. Hoath SB, Lakshmanan J, Scott SM, Fisher DA: Effect of thyroid hormones on epidermal growth factor concentration in neonatal mouse skin. Endocrinology 112:308–311, 1983
35. Frati C: L'axione dell'E.G.F. sulla pelle di topo in condizioni fisiologiche e patologische. Giornale Italiano di Dermatologia 46:205–209, 1971
36. Frati C, Scarpa C: Trattamento dell'ustione hel topo con l'E.G.F. applicato localmente sotto forma di lozione. Giornale Italiano di Dermatologia 46:73–76, 1971
37. Franklin JD, Lynch JB:Effects of topical applications of epidermal growth factor on wound healing. Plast Reconstr Surg 64:766–770, 1979
38. Cohen S: The stimulation of epidermal proliferation by a specific protein (EGF). Dev Biol 12:394–407, 1965
39. Cohen S, Savage CR: Part II. Recent studies on the chemistry and biology of epidermal growth factor. Recent Prog Horm Res 30:551–574, 1974
40. Hoober JK, Cohen S: Epidermal growth factor. I. The stimulation of protein and ribonucleic acid synthesis in chick embryo epidermis. Biochim Biophys Acta 138:357–368, 1967
41. Covelli I, Rossi R, Mozzi R, Frati L: Synthesis of bioactive ^{131}I-labeled epidermal growth factor and its distribution in rat tissues. Eur J Biochem 27:225–230, 1972
42. Faure M, Eisinger M, Bystryn J-C: Decreased expression of epidermal cytoplasmic antigens in cultured human keratinocytes. J Invest Dermatol 76:347–352, 1981
43. Didierjean L, Woodley D, Regnier M, Prunieras M, Saurat JH: Skin explant cultures: expression of cytoplasmic differentiation antigens in outgrowth cells. J Invest Dermatol 76:38–41, 1981
44. Leoning T, Staquet MJ, Schmitt D, Thivolet J: Immunocytochemical and ultrastructural localization of keratin polypeptides in normal epidermis and mucosal cells and tissues. J Invest Dermatol 78:44–47, 1982
45. Saurat JH, Didierjean L, Pavlovich JH, Laouari D, Dalsan S: Skin calcium binding protein is localized in the cytoplasm of the basal layer of the epidermis. J Invest Dermatol 76:221–223, 1981