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Modification of Polypeptide Composition in Keratinocyte Fibrous Protein

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The thick paw epidermis of the rat and the guinea pig showed a relatively increased amount of the heaviest fibrous polypeptide compared to the thinner epidermis of back and ear. The hyperplastic epidermis of mouse and rat induced by abrasion or painting with 12-0-tetradecanoyl-phorbal-13-acetate (TPA) also revealed an increase in the heaviest polypeptide. The fibrous polypeptides of epidermis from scarred skin lacked components present in normal epidermis. Cultured epidermal cells showed a different fibrous polypeptide composition than the parent tissue which was altered when the cells were transplanted to an animal or grown on a dermal substrate in culture. Thus multiple factors such as hyperplasia, the dermis and cellular environment may modify the polypeptides synthesized by the keratinocyte.

The fibrous protein of epidermis has been shown to consist of 3 polypeptide chains with a type of α helical configuration [1-3]. Considerable heterogeneity has been observed in the protein extracted from one area of skin in a given animal with up to 7 different polypeptides being demonstrated [4,5]. Variation in 2 different sites, bovine peri-hoof and snout epidermis, has also been observed [6]. The polypeptides also appear to vary between different animals but generally have molecular weights in the range of 40,000 to 70,000 daltons [7].

It has been suggested that the helical portion of the polypeptides is rather constant and variation occurs in the nonhelical segment representing about half the molecule, but this has not been established. No difference in the molecular structure or supramolecular organization of the fibrous protein has been observed when proteins with different polypeptides are compared. Thus, no explanation for this variation in terms of structure and function of the epidermis has been presented.

This study presents further data on the factors related to heterogeneity of epidermal fibrous protein. Changes in polypeptide composition have been observed in different body areas, with hyperplasia, with alterations in the dermis and in different environments.

METHODS

Hair was removed from the bodies of CD Fisher rats and Hartley guinea pigs (Charles River Farms) by plucking and the skin was excised after sacrificing the animals. Skin was also excised from the paws and both surfaces of the ears. Human skin was obtained from surgical specimens that otherwise would have been discarded and the epidermis was removed by heating the skin to 50°C for 30 seconds and scraping with a scalpel. The epidermis was obtained from cow snouts and peri-hoof skin by slicing parallel to the surface with a blade.

A 9 cm^2 area of epilated skin of a guinea pig was frozen with liquid nitrogen under ether anaesthesia. Full thickness necrosis of skin resulted which healed within 1 mo with a hairless scar which was much smaller in area as a result of wound contracture. After sacrificing the animals the scar was excised and the epidermis removed by heating and scraping.

Abrasion was done on the chemically depilated skin of anesthetized mice (CD-1 female, Charles River Farms) and rats (male albino, Tacome Farms) using a felt wheel on a Dremol Moto-tool (8). Treatment with 12-0-tetradecanoyl-phorbal-13-acetate (TPA) was done by applying 27 nm in .2 ml acetone to the back of animals which had been clipped 2 days previously. The epidermis was removed by scraping and processed as described by Argyris [9].

The epidermis was finely minced, suspended in 0.25 M sucrose (10.0 ml/.1 gm wet wt of tissue) and ground in a VirTis homogenizer for 4 min at 4°C to extract the soluble proteins. After centrifugation at 35,000 g at 4°C the pellet was homogenized in 0.1 M citrate buffer, pH 2.65, for 4 min and the mixture stirred for 1 hr at 4°C. After centrifugation the acid soluble fibrous protein, prekeratin, was purified by precipitation at pH 7.0. In some experiments the sucrose washed tissue was extracted directly with 0.1 M Tris, pH 9.0, 8 M urea and 0.1 M mercaptoethanol (TUM) for 25 hr at room T° under nitrogen and the suspension centrifuged. The resulting solution was purified by precipitation when dialyzed against 0.1 M Tris, pH 7.0.

Culture of epidermis was done as previously described [10] using cells obtained after treating excised skin with .025% trypsin at 4°C for 16 hr. In some experiments the cultured cells were also grown on 1 cm squares of human skin from which the epidermis had been removed by prolonged soaking in phosphate buffered saline. These were stored frozen and made available by Dr. M. Prunieras.

SDS polyacrylamide gel electrophoresis (PAGE) was done after equilibrating the samples using the system described by Neville [11]. Cow prekeratin or molecular weight markers were present in each run as standards as reported previously [6]. The 40,000-70,000 region containing fibrous polypeptides is indicated in the figures by *arrowheads*.

RESULTS

Variation of the SDS PAGE pattern of fibrous protein has been investigated in the CD Fisher rat and guinea pig using body, ear and paw epidermis. The fur bearing area of the body had the same pattern in various sites but differed from ear and paw as shown in Fig 1 and 2. In both animals there was an increase in the relative amount of the slowest running component (heaviest molecular weight) in the thick paw epidermis. The malpighian layer thickness was 1 to 3 cells for body skin, 5 to 10 cells for ear skin and 15 to 20 cells for paw. The guinea pig hairless snout and lip epidermis pattern were similar to the ear, although these were thicker.

Since these data suggested that differences in thickness of the epidermis were important in determining the type of pattern observed, we looked at several models of epidermal hyperplasia. Abrasion of the back of CD-1 mice with a felt wheel as described by Argyris [8] caused a reproducible thickening of the epidermis which was at a maximum by the 5th or 6th day and altered the electrophoretic pattern (Fig 3) from the third through the seventh day following treatment. Treatment of the skin with TPA had a similar effect in the same time frame (Fig 4). There was an increase in the relative amount of the polypeptide of highest molecular weight. The same results were observed following abrasion of Tacome Farms rat skin (Fig 5).

In order to evaluate if changes in the dermis had an effect on the polypeptide pattern, epidermis on the scar produced by liquid nitrogen treatment was studied. Because the amount of epidermis obtained was small, it was extracted with TUM directly after the sucrose wash. The SDS PAGE pattern was compared with that of normal back TUM soluble protein and was the same except for the absence of 2 bands (Fig 6). This was observed in scars produced in several animals.

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Abbreviations:
PAGE: SDS polyacrylamide gel electrophoresis
TPA: 12-0-tetra-decanoyl-phorbal-13-acetate
TUM: 0.1 M tris, 8 M urea, and 1 M mercaptoethanol

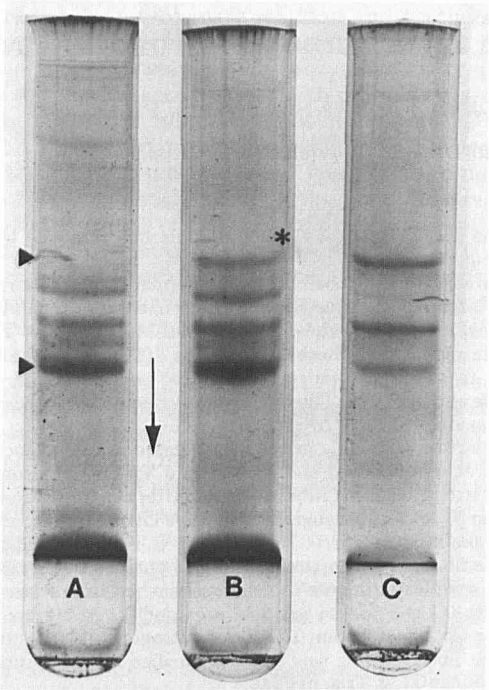

FIG 1. SDS PAGE patterns of citrate soluble fibrous polypeptides of the CD Fisher rat. *A* is from back, *B* from ear and *C* from paw. The region between ▶ is the location of the polypeptides and the heavy component present in increased amount is *.

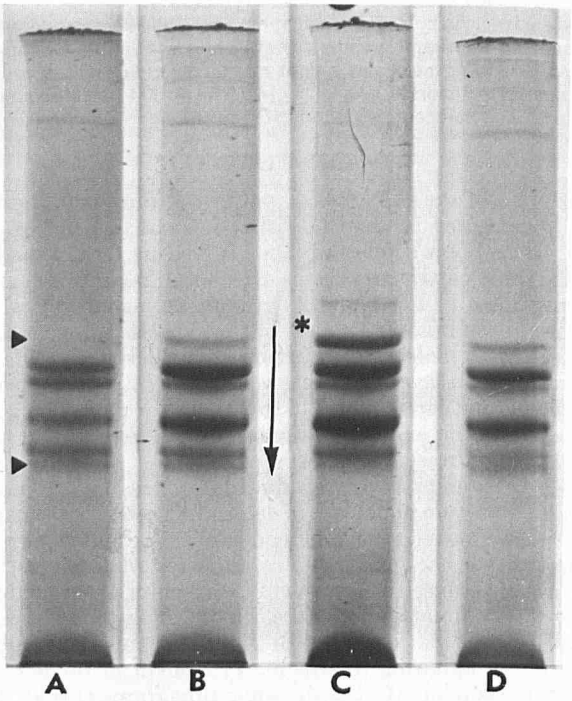

FIG 2. SDS PAGE patterns of citrate soluble fibrous polypeptides of guinea pig epidermis. *A* is from back, *B* from ear, *C* from paw and *D* from snout. The region between ▶ is the location of the polypeptides and the heavy component present in increased amount is *.

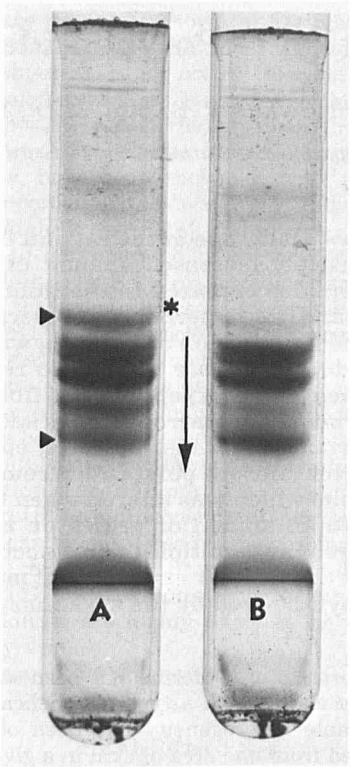

FIG 3. SDS PAGE patterns of citrate soluble fibrous polypeptides of epidermis from 7 day post-abraded (*A*) and pre-abraded (*B*) CD-1 mouse skin. The region between ▶ is the location of the polypeptides and the heavy component present in increased amount is *.

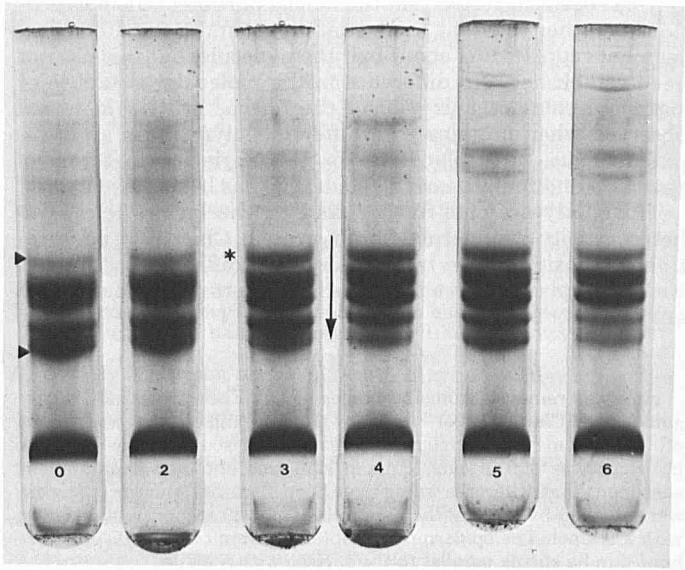

FIG 4. SDS PAGE patterns of citrate soluble fibrous polypeptides of CD-1 mouse epidermis treated with TPA. The time in days is indicated. The region between ▶ is the location of the polypeptides and the heavy component present in increased amount is *.

We previously have reported that cultured cells from human and cow snout epidermis have a different SDS PAGE pattern than the original tissues [10]. Cow peri-hoof epidermis, which has a different pattern than cow snout [6], was cultured and the pattern was different than the original tissue but similar to that of cultured snout epidermal cells. Finally, cultured CD Fisher rat back epidermis gave a pattern different than the starting epidermis (Fig 7). In all cases, the cultured cells showed bands present in the initial tissue but the relative proportion was different and some components were missing.

In order to determine if the SDS PAGE pattern of cultured cells was fixed, tissue was transplanted back to an animal. First passage human keratinocytes were scraped as a layer from a Petri dish and placed on the dorsal surface of a guinea pig ear from which the skin had been excised to the depth of the cartilage. The graft was covered with petrolatum impregnated

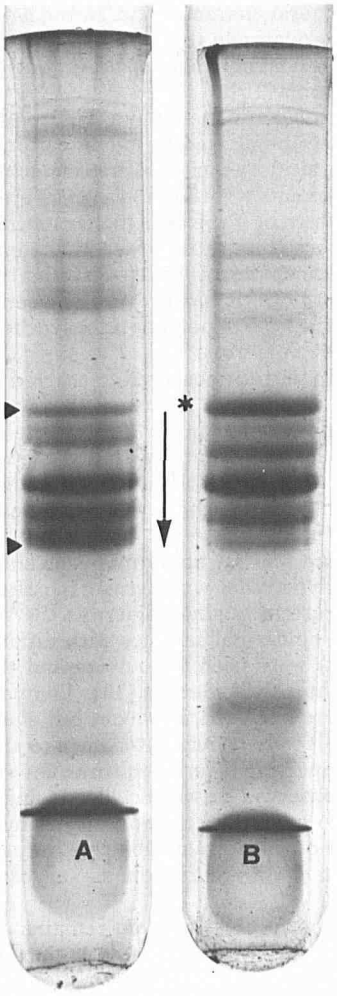

FIG 5. SDS PAGE patterns of citrate soluble fibrous polypeptides of epidermis from pre-abraded (*A*) and 7 day post-abraded (*B*) Tacome Farms rat skin. The region between ▶ is the location of the polypeptides and the heavy component present in increased amount is *.

gauze, covered with dry gauze and sealed with cyanoacrylate glue. After 7 days, before the graft could be rejected, the dressing was removed and the epidermis pulled off and the fibrous polypeptides extracted. The other ear had the same surgery and dressing but no cultured cells were applied; there was some regeneration of epidermis at the edge of the wound and it was scraped off. The electrophoretic pattern of transplanted keratinocytes changed and showed 3 of the bands observed in foreskin epidermis, but was missing the 4th (Fig 8). The regenerating guinea pig epidermis gave an SDS PAGE prekeratin pattern identical to that of normal guinea pig ear epidermis and this was different than the cultured transplanted human epidermis.

Further evidence for the capacity of cells to change their pattern of fibrous polypeptides was obtained by growing cultured keratinocytes on human dermis. As shown in Fig 9, the SDS PAGE pattern of human epidermal cells growing on the dermis was different than cells growing in the petri dish around the dermis. Since the latter cells had the same pattern as cells grown in dishes without a specimen of dermis, the effect of the dermis must result from direct contact with the keratinocytes. SDS PAGE of extracts from dermis not covered with keratinocytes showed no fibrous polypeptides.

DISCUSSION

In this study we have clearly established that the fibrous polypeptides synthesized in the epidermis may differ in various

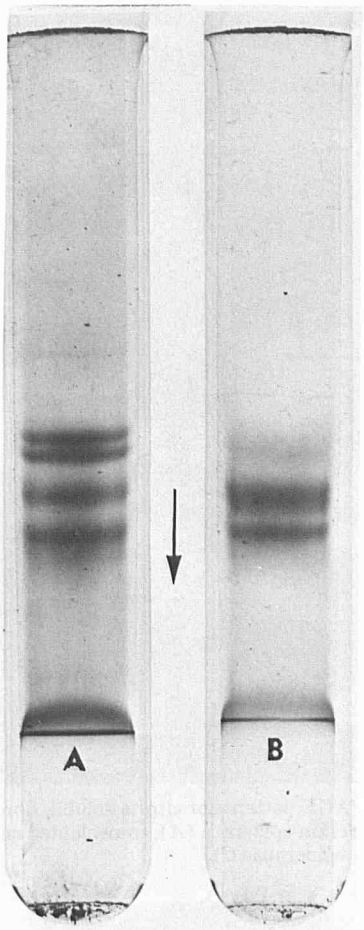

FIG 6. SDS PAGE patterns of TUM soluble fibrous polypeptides from normal (*A*) and scarred (*B*) guinea pig skin.

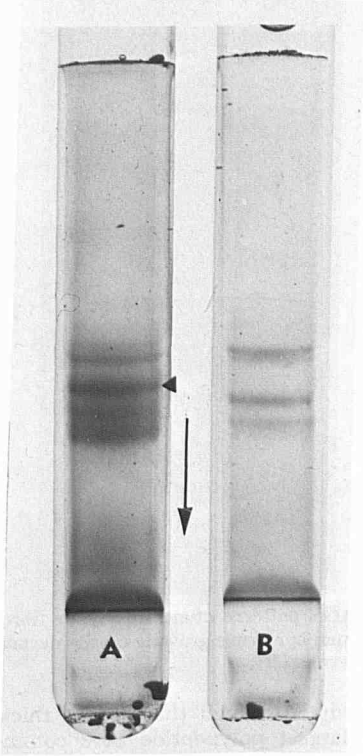

FIG 7. SDS PAGE patterns of citrate soluble fibrous polypeptides from CD Fisher rat back epidermis (*A*) and cultured rat back epidermis (*B*). A band ◀ is missing from the cultured cells.

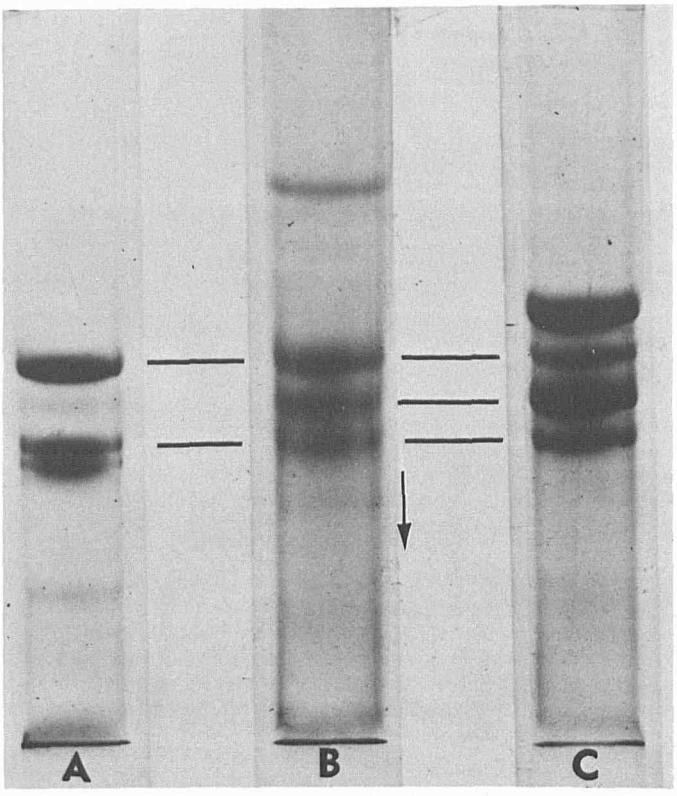

FIG 8. SDS PAGE patterns of citrate soluble fibrous polypeptides from cultured foreskin epidermis (*A*), transplanted cultured epidermis (*B*) and foreskin epidermis (*C*).

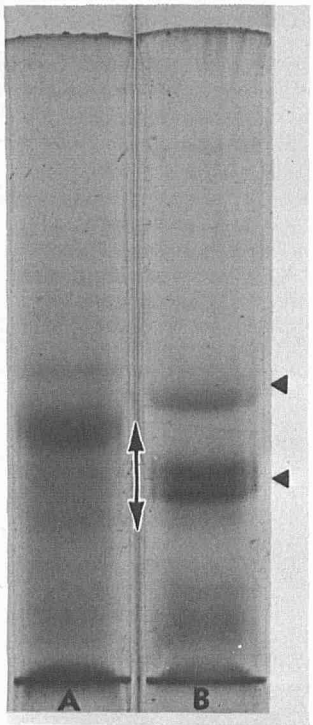

FIG 9. SDS PAGE patterns of citrate soluble fibrous polypeptides from cultured human epidermis growing on the dermis (*A*) and in the dish around the dermis (*B*).

areas of the body. Although the area of thickest epidermis contained the largest polypeptide in 2 common laboratory rodents, a quite different pattern was previously observed in bovine tissues [6]. It is very likely, therefore, that the correlation of a higher molecular weight polypeptide with thicker epidermis may not be a general characteristic. In the case of human skin, for example, the relatively thin epidermis of the foreskin has a unique pattern which includes the largest polypeptide observed in human epidermis [10].

The polypeptide components in a particular area of skin are not fixed, but can be altered by modification of the tissue by chemical or physical means. It is not clear from the abrasion and TPA experiments whether all the cells are making the new polypeptide pattern or if the cells higher in the epidermis make a different pattern than those lower in the epidermis. Studies of cultured mouse epidermis have suggested the latter possibility [12]. Furthermore, it has previously been shown in snake epidermis that the type of polypeptides synthesized was related to position in the epidermis [13].

The change in pattern observed in scarred skin clearly indicates that the dermis can influence the synthesis of keratin polypeptides, and this may in part explain the regional variation observed. This leads to the idea that the environment of the epidermal cell is important in influencing the type of keratin polypeptide synthesized, which was most clearly elucidated in the cell culture studies.

Bovine, human and rat epidermal cells showed a change in their fibrous polypeptides when grown in culture on 3T3 feeder layers. In the case of bovine epidermis the same pattern was observed when epidermal samples with different patterns (eg. hoof and snout) were used for culture and this has also been observed with human epidermis [10]. The pattern of cultured human cells were not fixed, however, but changed again when the cultured cells were transplanted back to an animal or grown on a dermis supporting layer in culture. The striking difference in the pattern observed between cells growing on the bottom of the Petri dish compared to those on the dermis further supports the importance of the dermal substrate in regulating the products of epidermal differentiation as suggested by Prunieras [14].

A persistent perplexing problem remains unsolved, namely, how do the differences in polypeptide composition of the fibrous protein relate to the structure and function of the cell. Since x-ray diffraction studies have indicated that fibrous proteins of different polypeptide composition have the same molecular configuration, any differences must be related to filament structure, packing and organization. The recent demonstrations that stratum corneum basic protein interacts with the filamentous protein [15] and that cations [16] can modify the organization of the fibrous protein suggests that such effects could be modified by polypeptide composition.

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