ELECTROPHORESIS OF SOLUBLE PROTEINS EXTRACTED FROM CELL SUSPENSIONS OF NEONATAL RAT EPIDERMIS*

IRVING B. STERN, D.D.S., KALLIOPE H. SEKERI-PATARYAS, PH.D., AND MYRON RABIN, M.S.

ABSTRACT

During keratinization basal cells differentiate sequentially into spinous, granular, and keratinized cells. One method of studying keratinization involves separating the tissue into cell types of its component strata and examining them for differences in synthetic activity. Another approach involves analyzing the preparations in order to determine what proteins are present at each stage of differentiation. This study deals with polyacrylamide disc gel electrophoretic analysis of neutral salt soluble proteins obtained from the four cell populations. In the basal, spinous, and granular cell gels there are some 18 electrophoretic bands. Using the most prominent band as reference ($R$, 53–57), there are eight faster-moving and 10 slower-moving bands. In electrophoretograms of protein from the keratinized cells, on the other hand, only four prominent bands are present and of these two are new. The other bands are present as traces or are lacking. They apparently represent substances which diminish during differentiation or are precursors of new protein. The alteration in soluble protein content in the differentiated as opposed to the undifferentiated cell gels may be utilized to study sequential changes which occur during keratinization.

The morphology of the differentiating keratinocyte alters progressively in its migration from the basal layer toward the surface. By the time it reaches the stratum corneum, the keratinocyte has become keratinized. Epidermal keratin may be viewed as the end product of a series of biochemical reactions starting in the basal layer and continuing in the succeeding strata. Presumably there are soluble protein precursors which are ultimately transformed into insoluble keratin and/or there are soluble proteins essential for the conversion of insoluble prekeratins into keratin.

A technique has been reported for obtaining preparations of cells from each of the epidermal layers [1]. Studies of incorporation of labeled amino acids and/or analysis of the proteins present at different stages are means of defining the role of the keratinocyte in the process of keratinization. This report deals with the polyacrylamide disc gel electrophoretic patterns of neutral salt soluble protein fractions obtained from preparations of basal, spinous, granular, and keratinized cells of newborn rat epidermis. Comparison of these patterns permits the identification of regions in the electrophoretograms where changes have occurred. Such changes may indicate the levels at which new proteins may have been synthesized, or where proteins may have become depleted in vivo.

MATERIALS AND METHODS

The dorsal skin of 25–30 Sprague-Dawley, Berkeley-strain rats was taken from 2–3 litters ranging from newborn to one day of age. Four fractions of epidermal cells were obtained by the method referred to above. These cells were suspended [10$^6$ cells/ml] in Earle's balanced salt solution (EBSS), pH 7.4, and sonicated for 60 sec in 15-sec pulses, separated by 10-sec intervals, using a Biosonic sonicator at a probe intensity of 70. The homogenates were centrifuged at 1000 $\times$ g for 30 min. Residues and supernatant fractions were separated. The supernatant fractions were dialyzed against distilled water overnight. Dialysates were then lyophilized, resolubilized, and their protein concentration determined by the method of Lowry et al [2]. Polyacrylamide gel electrophoresis was carried out using the Buchler analytical apparatus in the anionic system of Davis [3]. A total volume of 150 ml of sample large-pore mixture was used which contained 0.3–0.6 mg protein as determined above. Electrophoresis was done at 3 mA per gel and was terminated when the Bromphenol blue marker had migrated approximately 5.5–6.0 cm through the separation gel, i.e., in about 1.5 hr. After electrophoresis, the gels were stained with 1% amido black in 7% acetic acid and destained in 7% acetic acid. Gels were scanned on a Gilford spectrophotometer at 550 nm. In addition, several gels were run in a slab gel apparatus with albumin as a marker. Prominent bands were recorded on the basis of the $R_d$ (distance traveled by a band/distance traveled by tracking dye). The $R_d$ was calculated for 16 representative electrophoretograms, 4 for each cell layer, and other gels were compared for reproducibility.

RESULTS

The data in this experiment were obtained from samples of the percentage purity indicated in Table I.

Proteins soluble in EBSS were extracted from epidermal cell suspensions and subjected to separation by disc gel electrophoresis. Patterns of

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TABLE 1  
Percentage of cells found in each cell fraction

<table>
<thead>
<tr>
<th>Cell Fraction</th>
<th>Basal Cells</th>
<th>Spinous Cells</th>
<th>Granular Cells</th>
<th>Cornified Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>basal</td>
<td>spinous</td>
<td>granular</td>
<td>basal + spinous</td>
</tr>
<tr>
<td>Basal Cells</td>
<td>80.5 ± 2.6</td>
<td>15.5 ± 4.4</td>
<td>3.2 ± 2.8</td>
<td>3.0 ± 2.2</td>
</tr>
<tr>
<td>Spinous Cells</td>
<td>basal</td>
<td>spinous</td>
<td>granular</td>
<td>granular</td>
</tr>
<tr>
<td></td>
<td>35.5 ± 6.7</td>
<td>59.3 ± 4.5</td>
<td>4.9 ± 2.0</td>
<td>68.5 ± 4.0</td>
</tr>
<tr>
<td>Granular Cells</td>
<td>basal</td>
<td>spinous</td>
<td>granular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.0 ± 1.7</td>
<td>15.7 ± 0.8</td>
<td>68.5 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>Cornified Cells</td>
<td>basal</td>
<td>spinous</td>
<td>granular</td>
<td>keratinized</td>
</tr>
<tr>
<td></td>
<td>~3.0 ± 2.2</td>
<td>4.0 ± 2.2</td>
<td>94.5 ± 4.2</td>
<td></td>
</tr>
</tbody>
</table>

migration obtained by densitometric scanning of the gels are shown in the Figure. The patterns for basal (1A), spinous (1B), and granular cells (1C) are similar. In all, there were 17-18 bands for each cell layer except for the keratinized cells where there are fewer and less distinct bands. There were as many as 7-8 faster-moving bands including “f,” the largest and most dense band, and as many as 10 slower-moving bands. This “f” band corresponded in slab gel electrophoretograms of all four fractions and consequently it, rather than the tracking dye, was used for alignment. Corrections for differences in gel length were made to each side of this reference band. The bands were labeled with letters (a–p) for convenience. The approximate $R_f$ of these bands are given in Table II. Bands b, c, e, f, h, i, and l tend to be the most prominent. The major difference appears among the patterns for the keratinized cell fraction and the others. Based upon absorbancy data, the bands of the keratinized cells with the exception of f, l, and two new bands (one in the g–h region and the other in advance of b) have the least protein. The new bands are marked with † in Fig. 1D. The increased prominence of the four bands and the decreased prominence of the i band are notable. The b, c, d, e, g, h, i, j, k, m, n, o, and p bands may be present as traces or are totally lacking. On the other hand, the h, m, n, and o bands in the basal layer and the k, m, and n bands in the spinous layer also occur in trace amounts. Human albumin migrates with a major peak $R_f$ of .66–.68 in this system and a minor peak of .43–.44. Bands a to d migrate more rapidly than the major band of albumin.

**DISCUSSION**

Flesch [4] pointed out that “in order to study the source and fate of epidermal keratin, one would have to isolate and analyze the keratin-forming cells and compare them with their product. . . .” Techniques are available for the preparation of basal cell suspensions of a high degree of purity [5–8], of proliferative and nonproliferative populations [6–8], of mixed granular and cornified cells [9, 10], and of granular cells [11]. In the present study fractions containing a predominance of one cell type were employed [1]. A higher degree of purity of the spinous and granular cell preparations would be desirable; however, when cell volume [1] as well as percentage of each cell type in these preparations are taken into account, the amount of protein derived from the spinous and granular cells is probably greater than their percentage would indicate. The separation is adequate to provide representative samples of proteins found in each of the four strata. The electrophoretograms obtained from the basal, spinous,
and granular cell preparations resemble those obtained by Leonhardi et al [12] who depict some 20 bands for normal and psoriatic human whole epidermis. They state that 2-10 percent of the soluble protein is made up of the fast-moving bands in front of albumin and, of the five bands in this zone, they characterize four as being nucleoprotein. In the present study almost all the bands that occur in advance of albumin in gels of the lower layers are absent in the cornified layer and therefore may also be nucleoprotein. There is one band in this zone which could not be identified in the lower layers and it has an $R_f$ slightly greater than that of the b band. A new band is also found in the g-h region. There is in toto less soluble protein in extracts of keratinized cells than in extracts of other layers.

Studies of soluble proteins obtained from whole epidermis using various electrophoretic and immunochecmical methods have shown that they are for the most part dissimilar to serum proteins [12-19] and therefore, a priori, may be products of the epidermal cells. There are differences in the electrophoretic patterns of the soluble proteins of normal and psoriatic epidermis and tongue epithelium [14, 16-18] indicating that protein synthesis may vary with state of health and with the type of epidermis. Differences found in the electrophoretic patterns of the keratinized cells may relate to the process of keratinization, and the similarity in
patterns of the undifferentiated cells may indicate that these soluble proteins are either not significantly altered in the lower strata or that their pool size remains relatively constant. On the other hand, neutral soluble precursors have not yet been identified, while insoluble precursors have been correlated with prekeratin [20].

The examination of the electrophoretic migration of proteins of the epidermal layers provides another means by which one may analyze the process of keratinization. In this study similarities and differences between electrophoretic patterns of the soluble proteins of the four cell strata have been found. The regions of difference are perhaps the more important. The significance of these differences remains to be demonstrated. The question of whether keratin synthesis is a continuous synthesis from preexisting peptides or whether synthesis occurs de novo in the upper layers is a cogent one which is not resolved in this study. However, the strong similarity of most bands in the first three layers and the reduction in the number of bands in the keratinized layer, is consistent with the idea that some preexisting peptides are utilized in the process. It is now necessary to identify the types of proteins that these bands represent and, where possible, to correlate soluble with insoluble proteins in order to elucidate the sequential changes that occur during epidermal keratinization. These studies are now under way.

REFERENCES

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