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REGIONAL DIFFERENCES IN THE THICKNESS (CELL LAYERS) OF THE HUMAN STRATUM CORNEUM: AN ULTRASTRUCTURAL ANALYSIS*

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ABSTRACT

The importance of the stratum corneum as the rate-limiting barrier to percutaneous penetration has been well documented in the literature. Data have also been reported which suggest that the barrier function of this zone varies among different regions of the body. However, little attention has been given to regional variation of two morphologic parameters known to affect permeability—thickness and number of cell layers. In the present study, significant regional variation in both the mean thickness and the mean number of cell layers has been documented for four selected, sample regions of the body of a population of six adult volunteers, and for two more homogeneous subgroups separated by sex and age. While the pooled data from the total population and the pooled data from the male and female subgroups are in general agreement, it has been shown that there is also marked individual variation within a region that is characteristic and specific for each individual. The number of cell layers appears to account for the variation in thickness.

Widespread interest in the permeability of human skin has generated a plethora of experimental investigations using in vivo and in vitro techniques. Several of the in vivo studies were carried out by measuring the diffusion of a radioactively labeled compound through the epidermis and dermis into the blood vascular system of living subjects [1, 2]. Other investigators have used in vitro model systems in their experimentation [3]. In the latter method, permeability of human cadaver skin is measured. The measurements of the kinetics of transport that are obtained from both experimental approaches would not be expected to differ significantly on the basis that only the nonviable cells of the stratum corneum serve as the rate-limiting barrier to the passage of substances across the epidermis [4].

The rate of diffusion of molecules is, in part, dependent upon the length of the diffusion pathway, which in this instance is the thickness of the stratum corneum. However, data on the thickness of the stratum corneum are limited and generalized and do not systematically take into account the possibility of significant regional variations which might be implied from the demonstration of regional variation in percutaneous absorption [1, 2].

The studies to investigate the differences in thickness and numbers of cell layers of the stratum corneum from different regions of the body have failed to establish controls for preservation of the full thickness of the samples [5, 6]. Nonetheless, the importance of these two parameters of the stratum corneum to the interpretation of permeability data has been recognized: "Strictly speaking, such comparisons of [rates of permeation] are not valid unless one corrects for horny layer thickness or alternatively for number of cell layers" [7].

It was, therefore, the objective of the present study to apply methods that would assure preservation of an intact, full-thickness stratum corneum and to evaluate quantitatively the regional variation in total thickness and numbers of cell layers of this epidermal zone using electron microscopy.

MATERIALS AND METHODS

Specimens were obtained from the abdomen, flexor forearm, anterior thigh, and posterior inferior iliac region of six, healthy, human volunteer subjects, three white males and three white females within the age group of 25–31. The areas to be sampled were gently cleansed by a single stroke with a 70% alcohol wipe prior to biopsy with a high-speed, electric rotary drill fitted with a 2.5-mm biopsy punch. The tissue "plug" obtained was then marked in a manner to assure the preservation of full-thickness stratum corneum during further processing. Small sheets of lens paper were placed above and below the core of tissue. The "sandwiched" preparation was then set onto a hollow plastic cylinder (prepared by trimming away the bottom of a BEEM capsule) and secured in place with an open-top cap (Fig. 1). The assembled capsule with the paper-enclosed biopsy sample was carried through fixation, dehydration, and embedment for electron microscopy. Thus, cell layers which might have become detached during processing were trapped beneath the lens paper and could be included in the measurements of thickness and the counts of cell layers (Figs. 2a,b). In pilot assays of this method, marking the surface with $AgNO_3$ and particulate carbon prior to removal of the biopsy specimen established that the technique of biopsying did not remove the outer layer of the stratum corneum.

Tissue was fixed for electron microscopy in 2 parts of 2% osmium tetroxide buffered with 1 part of 0.2 M

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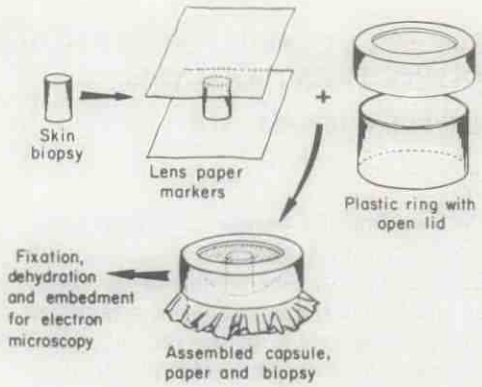
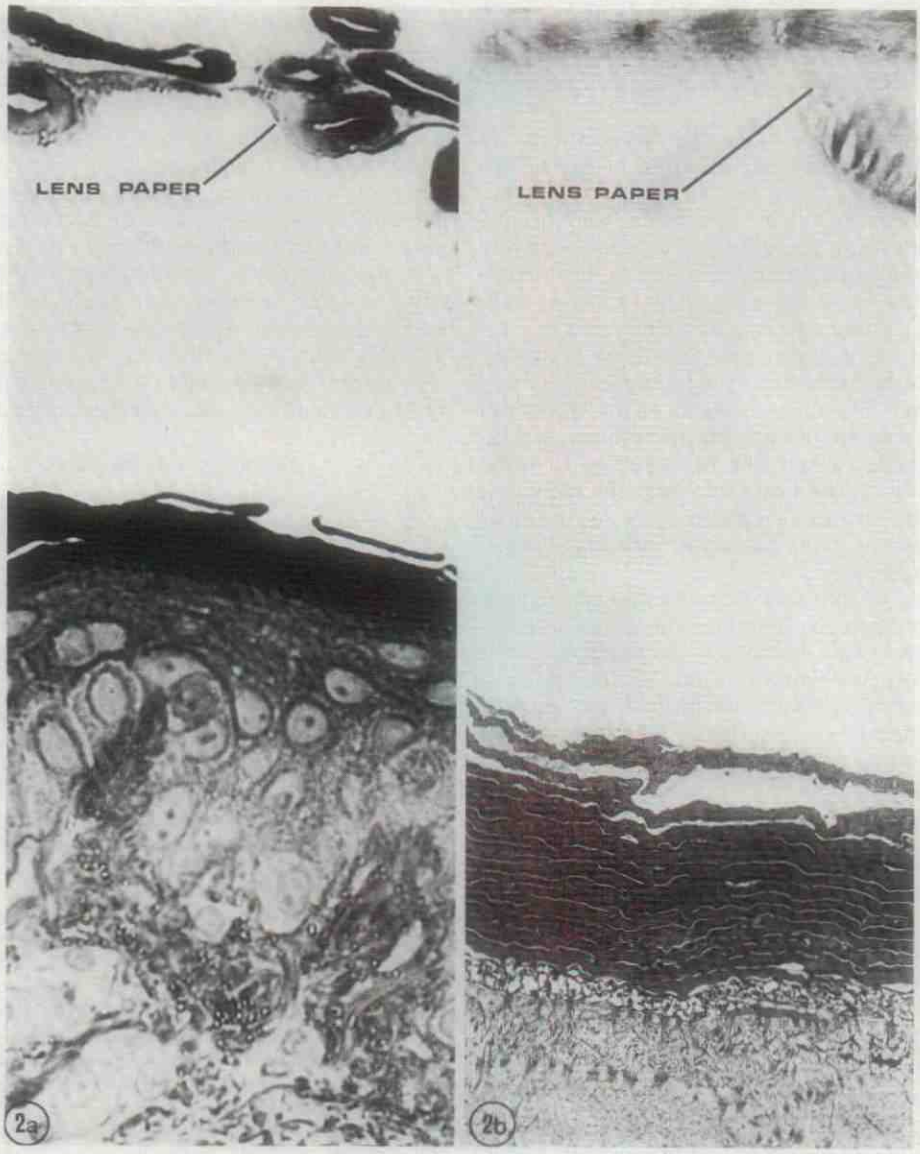


Epon 812 [8]. Following polymerization, the plastic cylinder was cut away leaving the tissue and enclosing paper to be oriented on metal stubs with the stratum corneum positioned parallel to the axis of the microtome chuck such that tissue sections would be cut in a plane perpendicular to the skin surface. Thick sections (1.5 μm) were prepared with glass knives and thin sections, in the silver interference color range, were cut through both the tissue and lens paper with a diamond knife mounted on a Reichert Om U2 ultramicrotome. Several thin sections were cut at each of 8–10 uniformly separated intervals

FIG. 1: Technique designed by M. Hoff to assure preservation of full-thickness stratum corneum during processing of tissue for electron microscopy.



through the entire biopsy specimen. All sections were double stained with saturated uranyl acetate and Reynold's [9] lead citrate. Sections were examined in a Philips 200 electron microscope.

Fifty electron micrographs were photographed from each skin sample. It was estimated that measurements were made over a total of 20–25 lineal mm of the stratum corneum. For the purpose of evaluating the thickness, a line was drawn through the stratum corneum perpendicular to the skin surface on each micrograph and

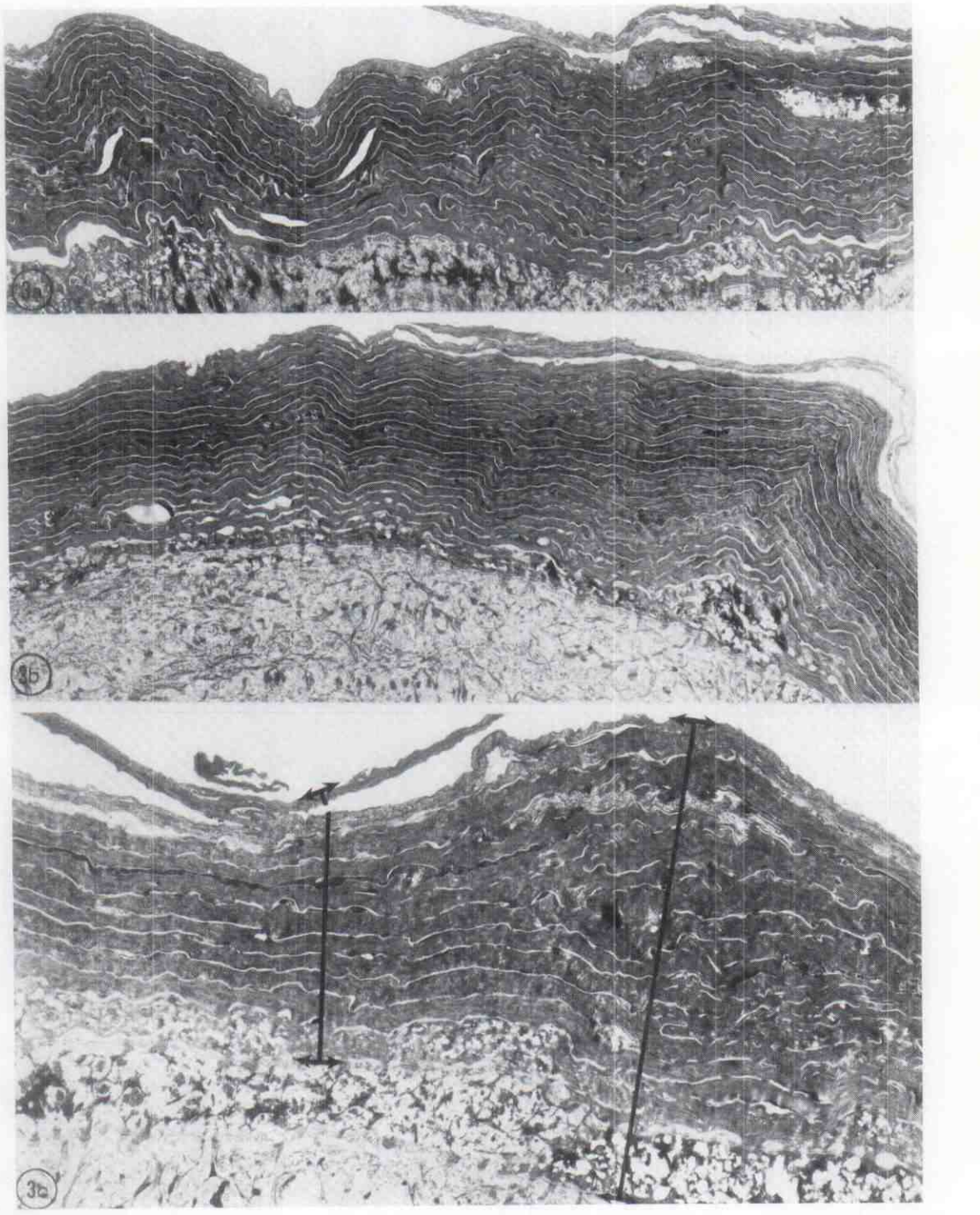


FIG. 3. (a, b) Stratum corneum from the posterior inferior iliac region. Note the variation in the thickness along the

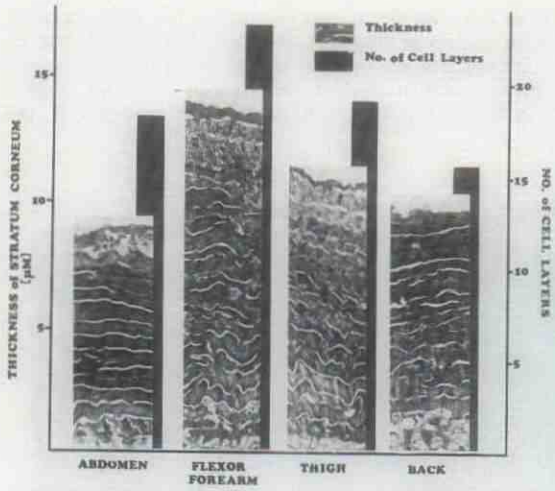


FIG. 4: Mean thickness and mean numbers of cell layers of the stratum corneum for the four regions sampled from six volunteer subjects. (Electron micrograph segments × 6,635)

measured from the deepest layers to the most superficial. The number of cells intersected by that line were then counted (Fig. 3c). On several micrographs more than one measurement was obtained when the thickness of the stratum corneum showed obvious variation along its photographed length (Figs. 3a,b).

The measurements were then typed onto IBM computer cards and submitted to the CDC 6400 computer for statistical analyses. Mean values, standard deviations, and p-values of comparisons were computed using version 2.3 of the SPSS Statistical Package for the Social Sciences [10].

RESULTS

It was the primary objective of this study to document regional variation in the thickness and number of cell layers of the stratum corneum, first for a selected sample population and then for two, more homogeneous subgroups of individuals within that population. The criteria used to establish the latter groups were sex and age. The three male subjects selected were of ages 25–27 and the three female subjects were 30–31 years of age. The consistency of the data from each region and each individual was also considered in the interpretation of results.

Pooled Data from All Subjects

Figure 4 is a graphic representation of the mean values for both thickness and numbers of cell layers of the stratum corneum from the four body regions tested. Each bar represents a composite mean value that was obtained as an average value of the individual means of the data from the six subjects selected. With the mean value of the abdominal stratum corneum serving as the standard for comparison, both thickness and numbers of cell layers from the flexor forearm, thigh, and

level of $p < .0001$. The large standard deviations calculated for these pooled data reflect two features: (1) that the stratum corneum in a given sampled region is variable in both thickness and numbers of cell layers among the subjects studied, and (2) that a sample length of stratum corneum measured from any region of each individual is not consistent in thickness and numbers of cell layers (Figs. 3a,b). The individual data from each body region of each subject are expressed in Tables I (abdomen), II (flexor forearm), III (thigh), and IV (back).

It was also found that the thickness of the stratum corneum appears to vary proportionately with the number of cell layers comprising that zone of the epidermis. This proportionality has been calculated by dividing the mean thickness of each region by the mean number of cell layers for that region. The resulting value also provides an average dimension for individual cell thickness. In three of the four regions the cell thickness was determined to be .17–.18 μm. However, by this

TABLE I

Mean thickness (μm) and mean number of cell layers of the stratum corneum from the abdomen of six human subjects

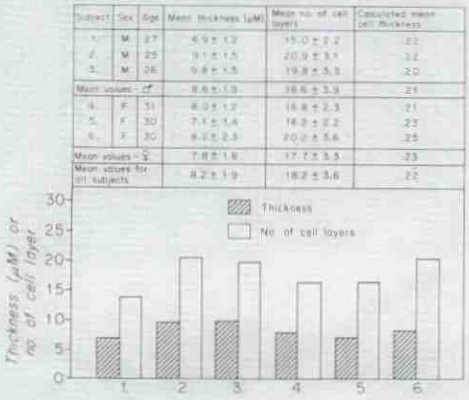
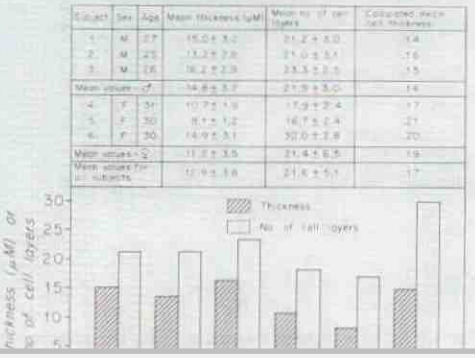


TABLE II

Mean thickness (μm) and mean number of cell layers of the stratum corneum from the flexor forearm of six human subjects



method of calculation the cells of the abdomen were estimated to have a greater average thickness of .22 μm. These results appear to support a generalization that regional variation in the thickness of stratum corneum is related to the number of cell layers that comprise it within a given region; the data appear to lend less support to an alternate explanation of the regional differences as a consequence of extensive variation in individual cell thickness. These conclusions, although drawn from mean values computed from the total population of subjects, are borne out by the data that were summarized independently for the male and female subgroups.

Pooled Data from Male and Female Subgroups

Figure 5 graphically displays the mean values for stratum corneum thickness and numbers of cell layers of all female subjects. The same data are illustrated for the male subjects in Figure 6. In both groups, the mean thickness of the stratum

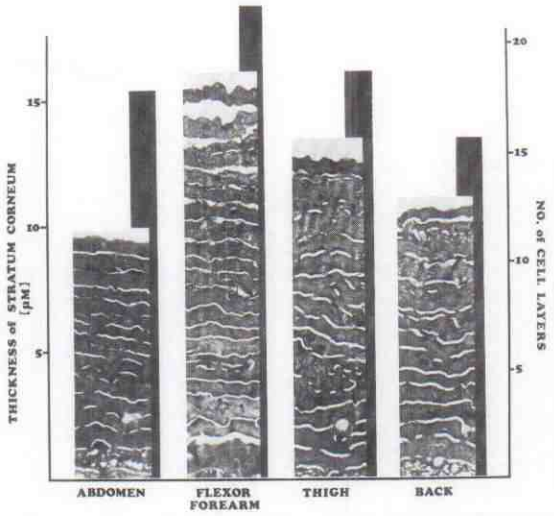


FIG. 5: Mean thickness and mean number of cell layers of the stratum corneum for the four regions sampled from the male subgroup. (Electron micrograph segments × 6,365)

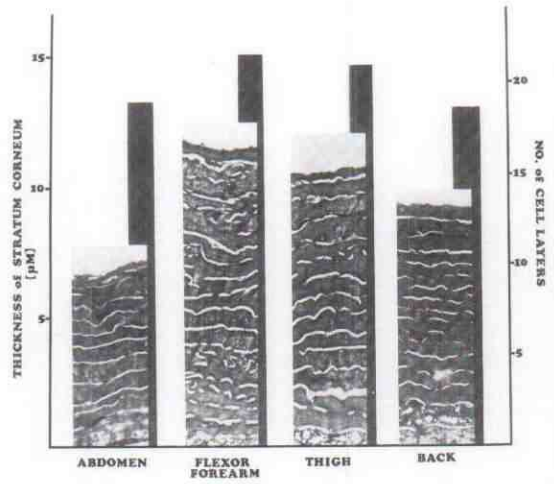


FIG. 6: Mean thickness and mean number of cell layers of the stratum corneum for the four regions sampled from the female subgroup. (Electron micrograph segments × 6,365)

TABLE III

Mean thickness (μm) and mean number of cell layers of the stratum corneum from the thigh of six human subjects

Subject	Sex	Age	Mean thickness (μM)	Mean no. of cell layers	Calculated mean cell thickness
1.	M	27	7.7 ± 1.4	14.3 ± 2.6	.19
2.	M	25	10.3 ± 1.8	17.5 ± 2.7	.17
3.	M	26	15.3 ± 3.1	22.7 ± 4.0	.15
Mean values - ♂			10.9 ± 3.8	18.0 ± 4.5	.17
4.	F	31	10.1 ± 2.0	20.1 ± 2.5	.20
5.	F	30	7.7 ± 1.4	21.2 ± 3.0	.28
6.	F	30	11.9 ± 1.5	20.7 ± 2.4	.17
Mean values - ♀			11.0 ± 2.0	20.7 ± 2.7	.22
Mean values for all subjects			10.9 ± 3.1	19.3 ± 4.0	.18

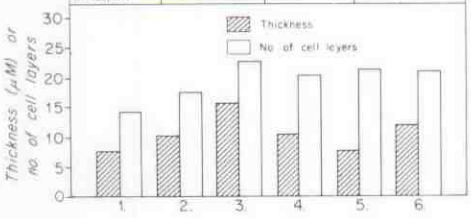
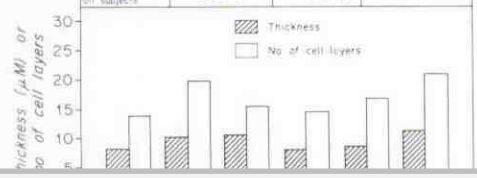


TABLE IV

Mean thickness (μm) and mean number of cell layers of the stratum corneum from the back of six human subjects

Subject	Sex	Age	Mean thickness (μM)	Mean no. of cell layers	Calculated mean cell thickness
1.	M	27	8.3 ± 1.8	14.0 ± 2.4	.17
2.	M	25	10.3 ± 2.4	19.9 ± 3.9	.19
3.	M	26	10.6 ± 1.7	15.6 ± 2.5	.15
Mean values - ♂			9.6 ± 2.2	16.3 ± 3.9	.17
4.	F	31	8.2 ± 1.9	14.4 ± 2.6	.18
5.	F	30	8.6 ± 1.3	17.2 ± 2.7	.20
6.	F	30	11.3 ± 1.9	21.1 ± 3.3	.19
Mean values - ♀			9.2 ± 2.2	17.2 ± 3.9	.19
Mean values for all subjects			9.4 ± 2.2	15.8 ± 3.9	.17



corneum is significantly different ($p < .0001$) when compared with the mean abdominal dimensions. The mean numbers of cell layers were also significantly different ($p < .0001$) among the four regions of the male subjects, but did not differ significantly ($p = .154$) in a comparison of the abdomen and back of female subjects. It may be noted that the calculated individual cell thickness is greater for the female subjects as a group and for each female subject individually when compared with the group of males and with the individual male subjects. Again, male and female group data indicate that there are thicker cells in the abdominal region. The pooled data from the total popula-

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