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Percutaneous absorption and the surface area of occluded skin

A SCANNING ELECTRON MICROSCOPIC STUDY

DAVID R.HARRIS*, CHRISTOPHER M.PAPA AND RAYMOND STANTON

Letterman Army Institute of Research, Presidio of San Francisco, and †Dermatological Division, Johnson and Johnson, New Brunswick, New Jersey, and Rutgers Medical School, Piscataway, New Jersey, U.S.A.

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SUMMARY

Scanning electron microscopy of samples of human skin occluded for 72 h, revealed that the hydrated stratum corneum not only swells, but develops multiple folds. Surface area estimations of such stratum corneum, utilizing stereo pairs of the photomicrographs, indicated a 37% increase over the normal, non-occluded horny layer values. It is speculated that the increase in absorptive area contributes to the increased skin permeability following occlusion.

The most widely used method of increasing percutaneous absorption of topically applied therapeutic agents is by the use of occlusive plastic films (Garb, 1960; Hall-Smith, 1962; Scholtz, 1961; Sulzberger & Witten, 1961). With this technique, absorption of some substances can be increased by as much as 100 times, in addition to the formation of a long-lasting reservoir within the stratum corneum (Bettley, 1970). Fritsch & Stoughton (1963) have shown that, in occlusive environments approaching 100% relative humidity, increased penetrability is due almost entirely to the hydration of the stratum corneum.

In the course of studies in which skin was occluded for 72 h and prepared for examination with the scanning electron microscope (SEM), the present authors noted remarkable alterations in the appearance of the horny layer of the occluded skin. The possible influence of such changes on percutaneous absorption seemed obvious, since the area of the absorptive surface was apparently increased. The potential increment was assessed using SEM photomicrographs and mathematical estimation techniques.

* Present address: Chief of Dermatology Service, Veterans Administration Hospital, Palo Alto, California, and Assistant Professor of Dermatology, Stanford University School of Medicine, U.S.A.

Reprint requests to: Christopher M. Papa, M.D., 501 George Street, New Brunswick, New Jersey 08903, U.S.A.

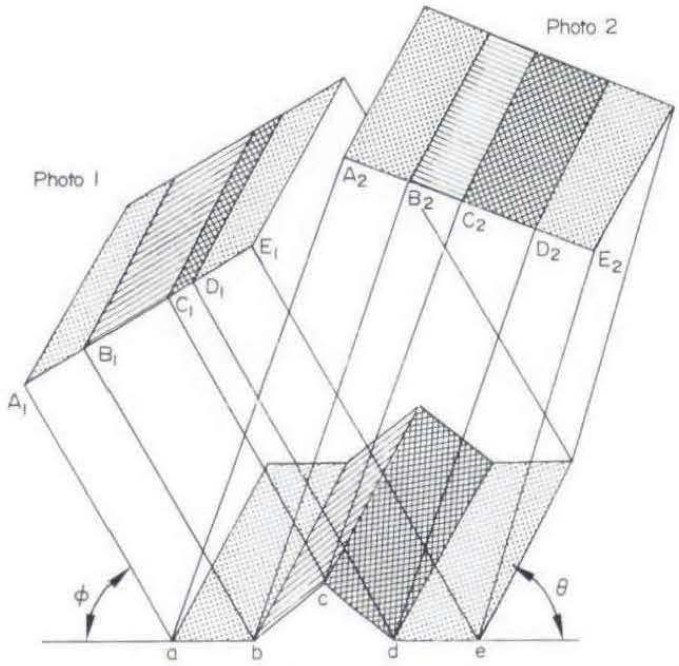

FIGURE 1. Diagrammatic representation of the principle of photogrammetric estimation, where two photographs of the same object taken at different angles are used to calculate measurements of height.

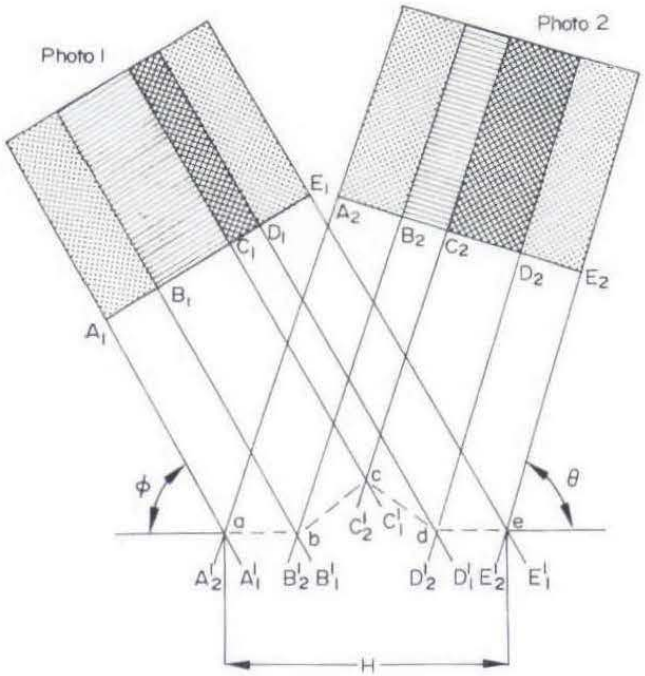

FIGURE 2. Simplified diagram of a planimetric technique where the photographic series is arranged in such a way that the intersections of perpendicular lines through known points completes a topographical profile.

METHODS

Subjects

Ten healthy male volunteers were included, none of whom had previous skin disease.

Histological preparations

Two 3 mm dermal punch biopsies were taken from each subject; one from a site occluded for 72 h with firmly affixed polyethylene film, and one from an un-occluded site. The specimens were dehydrated in graded alcohols, air dried and fixed to mounting blocks with the horny layer surfaces facing upward. The tissues were coated with gold–palladium in a vacuum evaporator and examined in a Jelco JSM-2 Scanning Electron Microscope. Photographs of the resulting images were taken of the samples at angles of 0°, 5°, 10° and 15°.

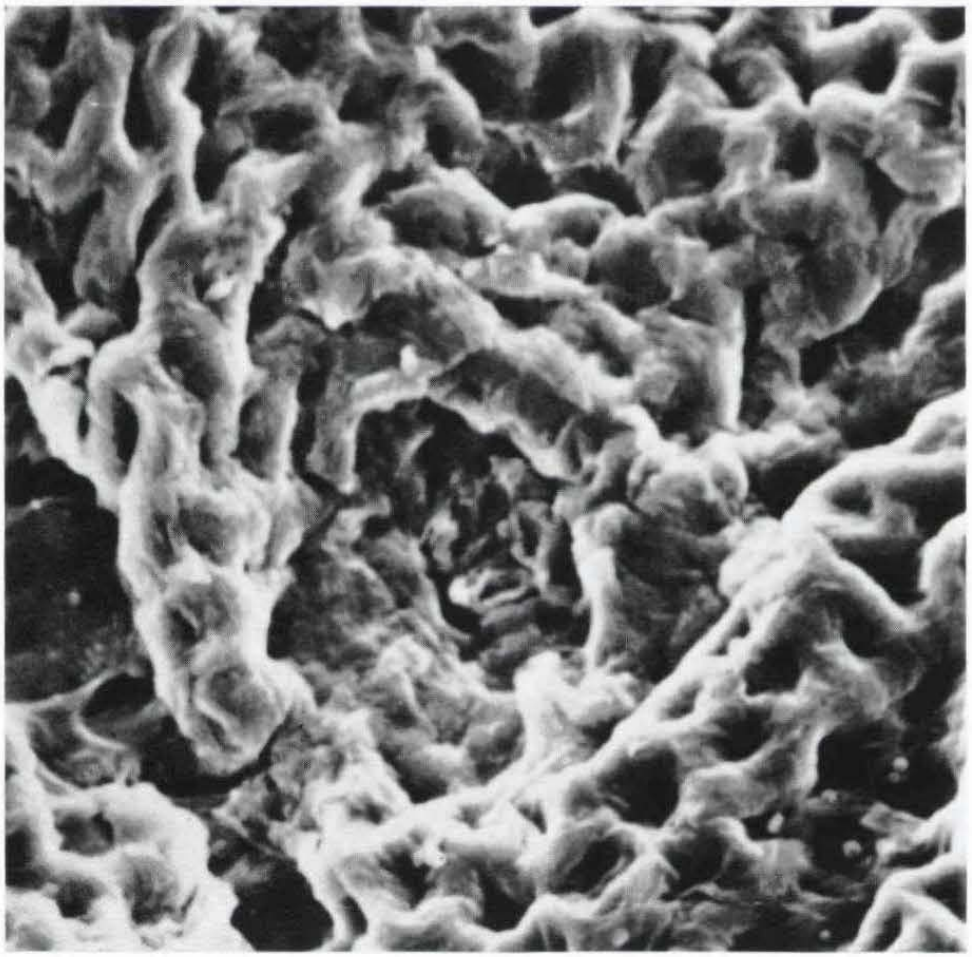

FIGURE 3. Skin occluded for 72 h. The horny layer is swollen and thrown into folds. Individual, desquamating cells are difficult to visualize. The oval structure in the centre of the field is an eccrine sweat pore (× 500).

Estimation of surface area

The basic approach was to use two photographs of the same object taken from different angles (Fig. 1)

and geometrically calculate measurements of height from them. When making topographical maps, the photographs are taken from two different locations, usually from an aeroplane (Thompson, 1966). In our case, the camera was fixed and the prepared sample was rotated to obtain different views.

FIGURE 4. Non-occluded skin. The relatively flat surface and shedding of stratum corneum cells is the normal picture. The central oval structure is an eccrine pore (× 500).

Resulting photographs were then used to plot a series of profiles or cross-sections taken through the sample perpendicular to the main plane of the surface. A line was drawn in the same place on each photograph through which a profile was to be made. These are shown as lines A_1–E_1 and A_2–E_2. The photographs were laid out on a drawing board as shown in a simplified profile (Fig. 2), such that angles ϕ and θ are the same as ϕ and θ in Fig. 1. Lines A_1–$A_1{}^1$ to E_1–$E_1{}^1$, and A_2–$A_2{}^1$ to E_2–$E_2{}^1$ were drawn perpendicular to the lines A_1–E_1 and A_2–E_2. The point *a* on the profile is determined by the intersection of lines A_1–$A_1{}^1$ and A_2–$A_2{}^1$ and point *b* is determined by the intersection of lines B_1–$B_1{}^1$ and B_2–$B_2{}^1$, etc. In this way, a series of known points on the profile were located. Using the photo-

graphs as a reference, a line was then drawn which connects all the points, thus completing the profile (Thompson, 1966; Robinson, 1969).

In the simplified example shown in Figs. 1 and 2, all profiles taken parallel to a–e would be identical, but with the more complicated surfaces of the stratum corneum samples, this was not the case. Therefore, several different profiles were made. The length of the line forming each profile was measured and divided by the horizontal length (H), giving a unit profile length (L). An average L value for both the un-occluded and occluded samples was calculated. An approximation of the unit surface area was found by squaring the resulting average L values. The ratio of L^2 for occluded stratum corneum to L^2 for un-occluded stratum corneum would thus be an approximation of the ratio of the two areas.

RESULTS

Skin occluded for 72 h becomes swollen and thrown into undulating folds (Fig. 3). This picture is an obvious distortion of the normal, predominantly flat or plate-like, layered texture of the stratum corneum seen in SEM images of un-occluded skin (Fig. 4).

The ratio (L_o) of the average surface area calculated for the occluded specimens compared to an ideal horizontal line (H) was 1·30. For the un-occluded specimens the ratio (L_u) was 1·11. The ratio of these average L values $1.30^2/1{\cdot}11^2$ was found to be 1·37. In other words, according to these calculations, the occluded sites had 37% more surface area than the un-occluded.

DISCUSSION

When the stratum corneum becomes hydrated under an occlusive dressing, the tissue changes from one containing little water (5–15%) to one which may contain as much as 50% water (Blank & Scheuplein, 1964). For the most part, the water is held in spaces between fibre bundles, swelling the interfibrillary structure of the stratum corneum, straightening the polar side-chains, and forming a more or less continuous aqueous pathway through part of it (Yates, 1971). Virtually all of the resulting increased rate of transport of water-soluble substances has been attributed to this internal change in the physical structure of the membrane (Fritsch & Stoughton, 1963; Blank & Scheuplein, 1964; Yates, 1971).

However, even cursory examination of the SEM images (Figs. 3 and 4) reveals that stratum corneum hydrated by polyethylene occlusion has a greater surface area than un-occluded specimens. It is reasonable to assume that the increased surface area contributes to increased cutaneous penetrability which follows occlusion.

It is worth noting that the simple, graded alcohol preparation of skin for SEM preserves the swollen, convoluted morphology of the occluded samples. The end point of preparation, dryness, does not restore their structure to the flat surfaces of non-occluded skin. As has been previously noted, the fixation which attends gradual dehydration of skin apparently minimizes the production of artifacts (Papa & Farber, 1971).

Our mathematical determination of the relative increase in surface area of occluded skin is, at best, a crude estimate. Equipment similar to that used for converting aerial photographs of earth surface terrain into line topographical maps (stereo comparagraph) could be used to obtain more precise information.

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