Skin Replication Procedure for the Scanning Electron Microscope

Abstract. *An improved method for preparing polyethylene replicas of skin from silicone rubber molds was developed for examination in the scanning electron microscope. Electron micrographs of the replicas compare favorably to those of fixed tissues from the same animal. Because of the great depth of field of the scanning electron microscope, both loosely attached epidermal cells and the background epidermal surface can be seen simultaneously in sharp focus, thus providing a more complete picture of the topography of skin.*

As the protective integument that shields the body from its environment, the skin has long been the subject of extensive study (*1*, *2*). Although the external surface of skin is accessible for examination and experimentation, surprisingly little attention has been paid to it as compared to the deeper layers. Relatively far more is known about the ultrastructure of the underlying cells partly because of technical difficulties inherent in the use of conventional light and electron microscopy for investigating the surface of skin.

The newly emerging technique of scanning electron microscopy (SEM) overcomes many of these difficulties. Because of the great depth of field (approximately 1600 μ at $\times$ 100), topographical relationships not seen with light optics at high magnification become apparent with the SEM, and photographs of unsectioned specimens are given a three-dimensional effect.

Yet the need remains for a reliable replication technique for use with the SEM. Such replicas obviate the need of taking biopsies, and, by allowing successive replicas to be made at the same site, allow comparative observations to be made with ease. Various techniques for making replicas of skin surfaces (*3*) are known. However, use of the SEM imposes requirements on the making of these replicas, in that they must resist heat distortion both in the vacuum evaporator and under the electron beam of the SEM.

In order to meet these requirements, the following replication procedure was developed after experimentation with different casting materials. Skin replicas prepared by this method, when used with the metal shadowing techniques and under the SEM conditions described below, have given the most satisfactory results to date. The technique and examples described apply to the inner aspect of the human forearm and to the clipped and epilated back of an albino guinea pig.

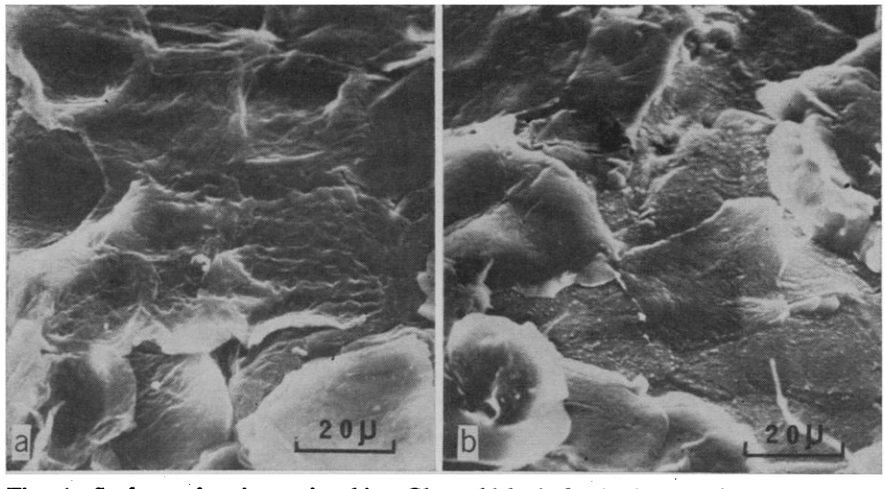

Fig. 1. Surface of guinea pig skin. Glutaraldehyde-fixed, frozen-dried biopsy (a) compared with polyethylene replica (b) at $\times$ 1000.

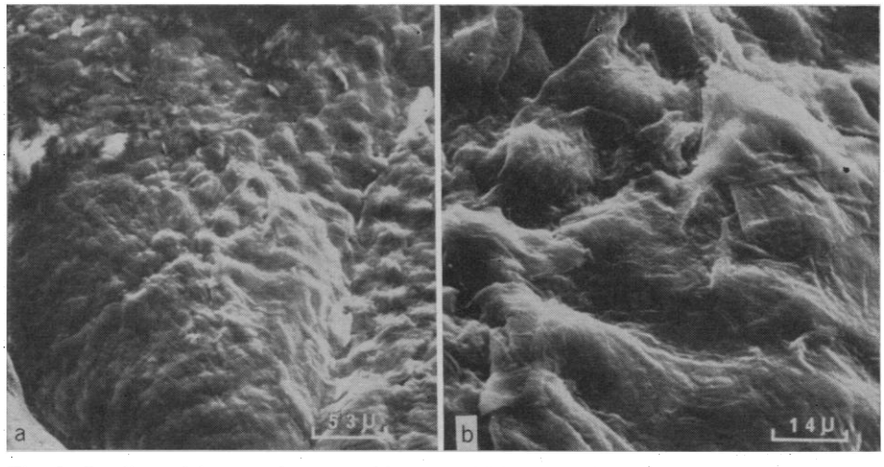

Fig. 2. Replica of human forearm skin at $\times$ 375 (a) and $\times$ 1400 (b). Some loosely attached epidermal cells in a field of more firmly attached cells appearing as protuberances are seen in (a) and in greater detail in (b).

Negative replication involved mixing Dow Corning Silastic A RTV mold-making silicone rubber (10 g) with Dow Corning RTV thinner (1 ml) immediately before it was to be used. Six drops of undiluted Nuocure 28 (Tenneco Chemicals, Inc.), a stannous octoate catalyst, were added, and the mixture was stirred rapidly for 45 seconds. It was then quickly placed in a vacuum desiccator in which the pressure was reduced by a mechanical pump in order to remove air introduced into the mixture by stirring. This degassing procedure was repeated two more times. When the crest of resulting bubbles had receded, the Silastic was removed from the desiccator and applied to the skin. Care was taken to avoid entrapment of air during the application and to ensure that the skin and Silastic were motionless during the subsequent setting time. After 3 to 5 minutes the Silastic was no longer tacky, and 5 minutes after that (about 12 minutes after addition of catalyst) the Silastic was gently lifted from the skin and cured in an oven at 175°C for 1 hour. The cured replica can be stored indefinitely before positive casts are made.

To make a positive cast, the negative replica was cut and fitted into a

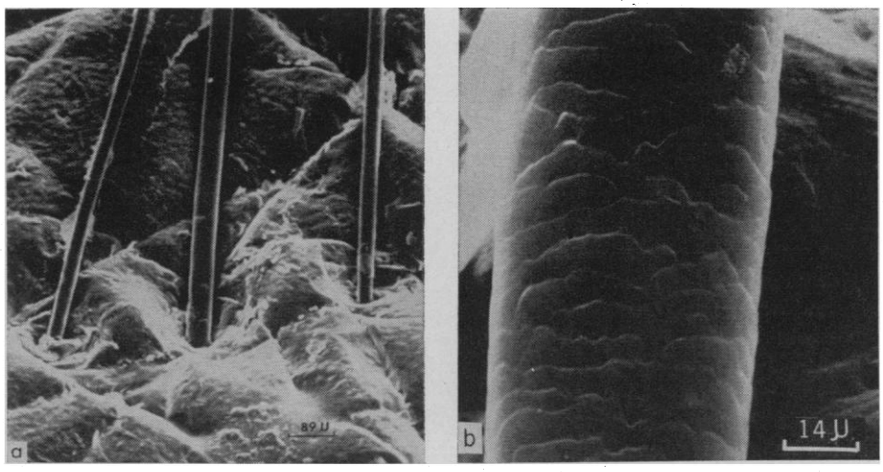

Fig. 3. (a) Polyethylene replica of the surface of human forearm skin (× 225), showing three hairs emerging from the skin. (b) The middle hair of (a) shown at higher magnification (× 1400).

rectangular brass frame (17 by 38 mm). A layer of polyethylene pellets (Union Carbide DYLT) was placed on the surface of the negative Silastic mold which was then put in a vacuum oven in which the temperature was slowly raised to 180°C. The polyethylene melted and flowed evenly over the surface of the Silastic and at the same time the oven was evacuated to about 10 torr until the air bubbles on the surface of the polyethylene had collapsed (about 5 minutes). The vacuum was then opened to air. Intermittent evacuation was repeated until essentially all entrapped air was removed from the polyethylene-Silastic interface.

The entire mold was removed from the oven and cooled to room temperature. The polyethylene was separated from the Silastic, and this first positive cast containing nonreplicated hair and loose debris removed from the surface of the Silastic was discarded. A second cast for viewing in the SEM was made in an identical manner.

In preparing the cast for SEM micrography, 9-mm punches were taken from the center and were mounted on specimen blocks with silver-containing cement (electronic grade). These punches were shadowed at 2 to 3 × 10^{-5} torr with 7 to 8 cm of 8-mil Au-Pd wire wrapped around a tungsten filament at a distance of 12.5 cm from the specimens. (Average thickness of Au-Pd on the surface of the replicas was approximately 150 Å.) The block was inclined at an angle of 45° toward the electron beam of a Jeolco JSM-2 scanning electron microscope which was usually operated with a beam accelerating voltage of 25 kv.

The faithfulness of replications made by this method is illustrated by a comparison of a replica of guinea pig skin with a glutaraldehyde-fixed and frozen-dried specimen taken from adjacent sites of the body (Fig. 1). The shape and appearance of the epidermal cells in the replicated and fixed specimens are similar. Minute detail and dimensions are also preserved in the replica of human forearm skin, as illustrated by the loosened polygonal cells (Fig. 2b) which average about 30 to 40 µ in width, a measurement in agreement with values reported for fixed specimens (*4*). At this higher magnification, both the underlying, still firmly attached, epidermal cells (appearing as protuberances) and the loosened, overlying squamous cells are visible. Because of the greater depth of field, the squamous cells, which appear flat when viewed through a light microscope, are seen in their true three-dimensional form.

In other replicas of human forearm skin containing hair fibers, not only are the same epidermal patterns faithfully reproduced, but so are the details of the hair shafts (Fig. 3, a and b). The size and spacing of the cuticle scales on these hairs are the same as those observed on actual hairs by other microscopic techniques.

This new replication technique, used in combination with metal shadowing and scanning microscopy, provides a very useful addition to existing methods for the study of the skin surface.

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Paradoxical Fear-Increasing Effects of Tranquilizers: Evidence of Repression of Memory in the Rat

Abstract. *Conditioned suppression of feeding, an index of fear, was increased rather than decreased by the administration of benzodiazepine tranquilizers or amobarbital. The drug-induced increase in conditioned fear varied directly with the intensity of the shock used in fear conditioning. The drugs had no fear-increasing effect in unshocked controls or in rats made amnesic by electroconvulsive shock given immediately after fear conditioning. These observations in animals are reminiscent of clinical reports that intraveneous amobarbital facilitates the recall of repressed traumatic experiences. The retrieval of painful memories may be inhibited or repressed in animals as well as in humans. In both cases, tranquilizers may counteract repression by disinhibition of the act of retrieval.*

Minor tranquilizers are used in clinical practice to alleviate symptoms of anxiety and tension (*1*). Laboratory studies in animals generally parallel these indications of anxiety-reducing activity in man (*2*, *3*). In the experiments reported here, however, tranquilizers increased rather than decreased conditioned fear in the rat. These paradoxical fear-increasing effects sug-