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Ultrastructural studies of barrier restoration in epidermis of hairless mice following dimethyl sulfoxide application

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Sixteen hairless mice were studied by transmission electron microscopy to determine the ultrastructural changes in epidermal barrier restoration following topical applications of dimethyl sulfoxide (DMSO) to back skin. Samples of experimental skin were examined at 30 min, 1 h, 2 h and 3 h after the initial DMSO treatment and compared with that of control animals. At earlier time periods (30 min-1 h) the DMSO treated epidermis showed greatly expanded intercellular spaces, disrupted desmosomal attachments, and desmosomal remnants lying free within the intercellular space. Mitochondria contained droplets of lipoid material which reached maximum size in the spinous and granular layers. Cytoplasmic vacuoles were particularly prominent in the 30-min samples. Large numbers of membrane coating granules (MCG) emerged in the 1 h specimens. The MCG were laden with numerous electron-dense inner membranes which crisscrossed at various angles. Many of the MCG were observed in the process of fusion with plasma membranes of granular cells adjacent to the stratum corneum. At 2 h a process of recovery from the effects of DMSO was evident and at 3 h there was little or no difference between experimental and control epidermis.

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This investigation was prompted by the observation that the epidermal barrier to percutaneous passage of fluorescent dyes could be compromised temporarily, simply by treating the skin with dimethyl sulfoxide (DMSO) prior to application of the dye (1). In this study 64 hairless mice were used to establish periods of maximum penetration of the fluorescent probe, ethidium bromide, and the subsequent recovery from the effects of DMSO. The fluorescent probe easily penetrated the epidermis for up to 2 h following DMSO pretreatment, but the solvent effects were greatly reduced after 3 h.

The present study was undertaken using transmission electron microscopy (TEM) to determine the ultrastructural changes in hairless mouse epidermis occurring during the period of barrier repair which follows DMSO treatment. The principal objectives of the study were to elucidate further the nature of the epidermal barrier as well as to understand better the mechanism by which DMSO disrupts the barrier.

Material and methods

Sixteen hairless mice were used in the investigation, including 4 controls and 12 experimental animals. The mice receiving DMSO were swabbed over a 2 cm area of the back adjacent to the tail. The cotton swabs were saturated with about 0.2 ml of full strength DMSO, and each animal was swabbed 3 times at 10 min intervals.

Mice receiving the DMSO were killed by cervical dislocation at intervals of 30 min, 1 h, 2 h and 3 h after the initial DMSO treatment. Thus, the 30-minute samples were taken 10 min after the final DMSO swabbing.

Strips of skin (2 mm × 1 cm) were removed from the back area immediately after death, straightened with pins on a piece of styrofoam and emersed in 2% phosphate buffered glutaraldehyde (pH 7.2). Specimens were post-fixed in 1% phosphate-buffered osmium tetroxide (pH 7.2) and embedded in Spurr's resin for thin sectioning. Control and experimental skin specimens were taken from the same back areas and processed for TEM identically. Thin sections of all specimens were mounted on copper grids and examined with a Philips 301 electron microscope.

Results

Control sections of hairless mouse epidermis showed a stratum corneum composed of 6–8 layers of dead cells. The stratum granulosum, stratum spinosum and stratum basale consisted of one cell layer each, although the stratum spinosum was somewhat variable in thickness. Intercellular spaces between living keratinocytes contained cytoplasmic folds projecting into the spaces with desmosomal attachments joining the folds of adjacent cells. The basal plasma membrane of the stratum basale was marked by numerous hemidesmosomes.

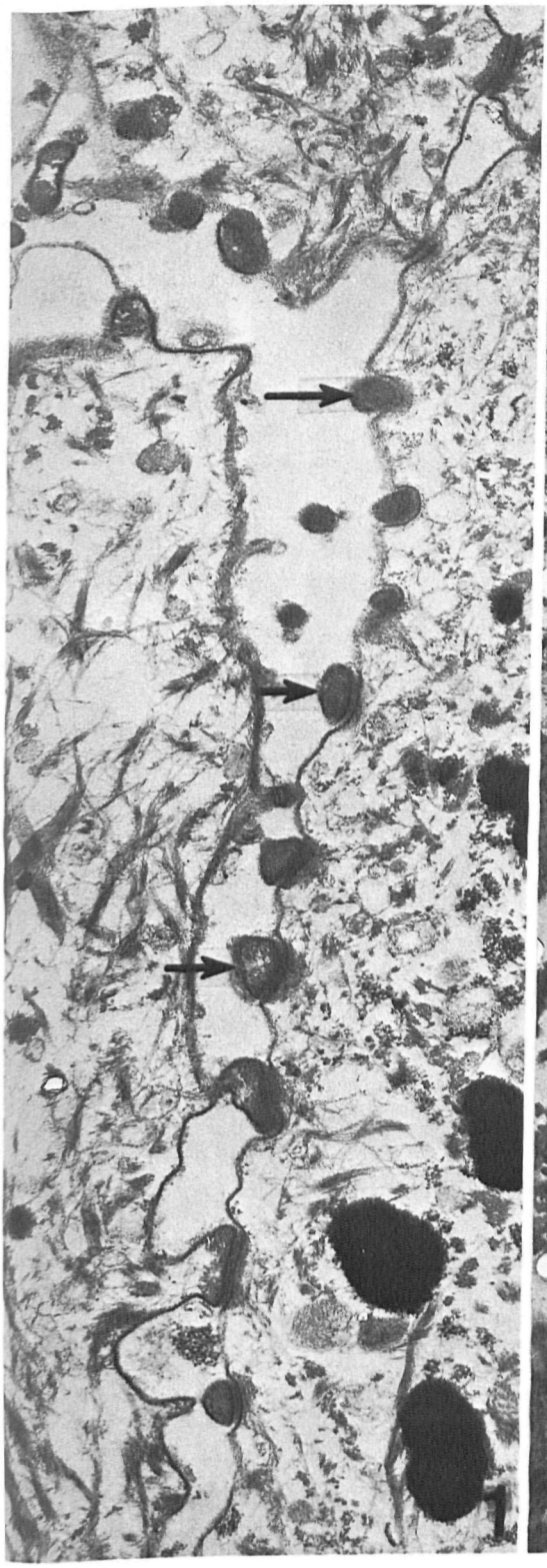
Mitochondria encircled cell nuclei or were scattered between an abundant display of tonofibrils, especially in the stratum basale and stratum spinosum. In the stratum granulosum, mitochondria and tonofilaments were intermingled with the keratohyalin granules. Here, tonofibrils as well as numerous ribosomes showed intimate relationships to keratohyalin granules. Membrane coating granules (MCG) were present in spinous and granulosa layers. Most of the MCG appeared to fuse with plasma membranes of granular cells adjacent to the horny layer, but the best evidence for intercellular dispersion of the MCG was seen in the experimental animals after treatment with DMSO.

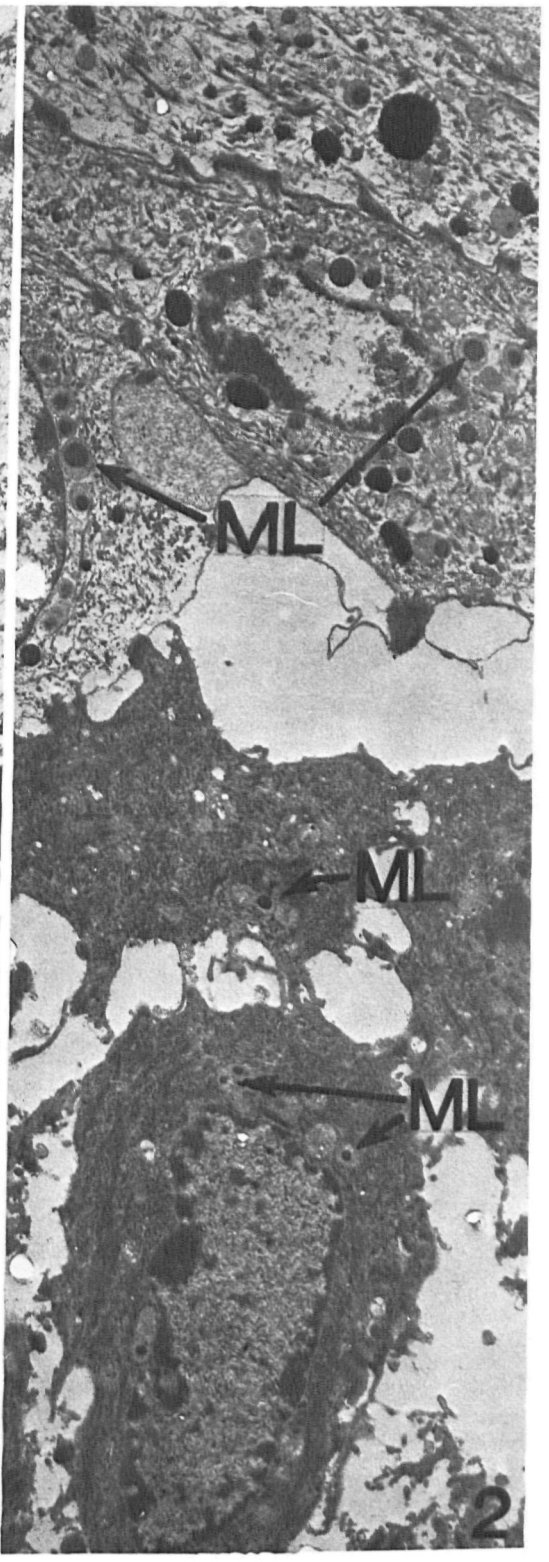
The above description of hairless mouse epidermis, although necessarry for purposes of comparison with the experimental skin, is intended to be brief and is by no means exhaustive. Subsequent data on the ultrastructural effects of DMSO will relate primarily to those epidermal components mentioned in the control specimens.

Skin samples taken 30 min after the first exposure to DMSO showed greatly expanded intercellular spaces with attenuated cytoplasmic processes extending to the desmosomal attachments. In many instances, the

Fig. 1. Electron micrograph of specimen taken 30 min after DMSO treatment. Notice disrupted desmosomal attachments (at arrows) of granular cells (×21,300).

Fig. 2. Electron micrograph of specimen taken 30 min after DMSO treatment. Notice enlarged intercellular spaces and mitochondrial lipid (ML). Droplets of mitochondrial lipid are smaller in stratum basale cells (lower portion of micrograph) as compared with those of the granulosal cells (upper portion of micrograph) (×7,500).

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attenuated attachment areas were separated from their cellular origins. The broken areas persisted as oval or irregularly shaped densities either partially attached to the cell by its desmosomal remnant or lying free in the intercellular space (Fig. 1). This phenomenon was observed through all layers of the epidermis except for the horny layer. It was most severe, however, in the stratum granulosum.

Mitochondria of the 30-minute samples contained small to large droplets of lipoid material. These droplets were smallest in mitochondria of the stratum basale and reached maximum size in the spinous and granular layers. (Fig. 2). Mitochondria of the basal layer exhibited well preserved internal structure whereas those of the spinous and granular layers, showing a state of lipoidal engorgement, were identified by their double surface membranes and remnants of a few remaining cristae (Figs. 3, 4, 5). Aside from the appearance of the mitochondria, the expanded intercellular spaces and the disrupted desmosomal attachments, large cytoplasmic vacuoles gave evidence of a remarkable degree of fluid imbibition by the epidermal cells. The normal structure of the nuclei was often distorted by deeply invaginating vacuoles, although these were never observed to penetrate the envelope or to become incorporated in the nucleus itself. The cellular changes observed, while widespread, were less severe in some areas. Recognizable membrane coating granules could not be identified in the 30-min specimens.

The most remarkable change that occurred in the 1-h specimens, relates to the emergence of membrane coating granules in large numbers (Fig. 6). In this case, the MCG were laden with numerous, electron-dense inner membranes which, with due respect to plane of sectioning, appeared to crisscross at various angles (Figs. 6, 7). Many of the MCG were seen in the process of fusion with plasma membranes of granular cells adjacent to the stratum corneum. Remnants of MCG were also seen in the intercellar spaces between the stratum granulosum and stratum corneum. At the point of fusion, the outer membrane of the MCG became continuous with that of the keratinocyte (Fig. 7).

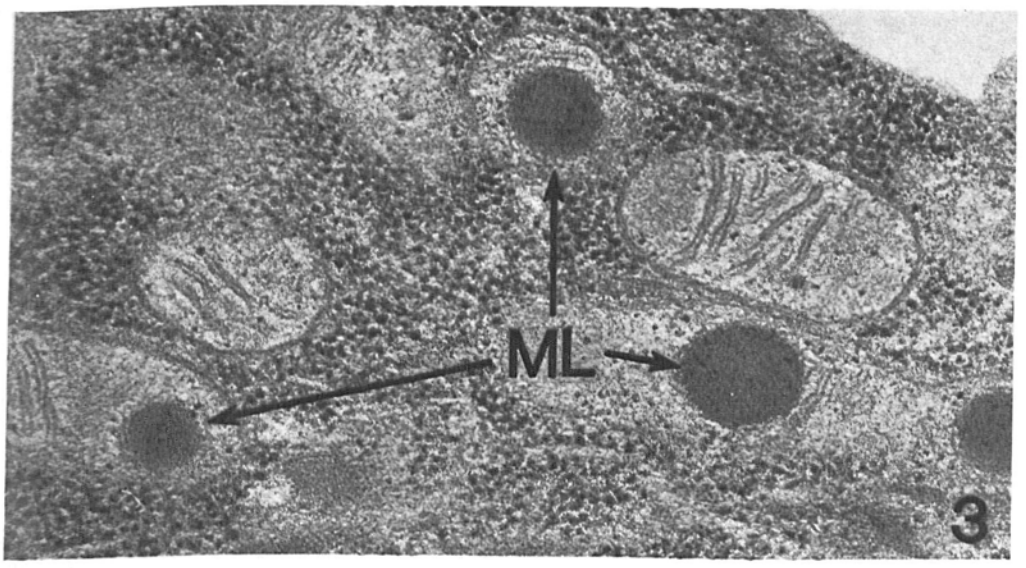
In samples of skin taken 1 h from the start of the experiment the changes described above were greatly diminished. Survey electron micrographs showed a return to near normal appearance for the keratinocytes (Fig. 8). Lipid-containing mitochondria were present but these were mostly limited to cells of the stratum granulosum. Attenuated cytoplasmic processes and broken desmosomal attachments were also reduced in numbers. Cytoplasmic vacuolation, although occasionally present, was greatly diminished.

Recovery from the effects of DMSO was essentially completed in the 2-h specimens. Occasionally, the process of continued repair was evidenced by an expanded intercellular space or a lipid-containing mitochondrion, but the overall appearance of the 2-h specimen was close to that of control epidermis (Fig. 9).

Fig. 3. Electron micrograph of stratum basale mitochondria. Lipid droplets (ML) are clearly present within mitochondrial matrices. Sample taken 30 min after DMSO treatment (×75,000).

Fig. 4. Electron micrograph of stratum granulosum. Mitochondria scattered between keratohyalin granules (KG) contain droplets of lipid material (ML). Sample taken 30 min after DMSO treatment (×27,300).

Fig. 5. Electron micrograph of cell in stratum spinosum. Note engorgement of mitochondria with lipid material. Mitochondrial cristae are poorly preserved in this spinosal cell. Sample taken 30 min after DMSO treatment (×57,000).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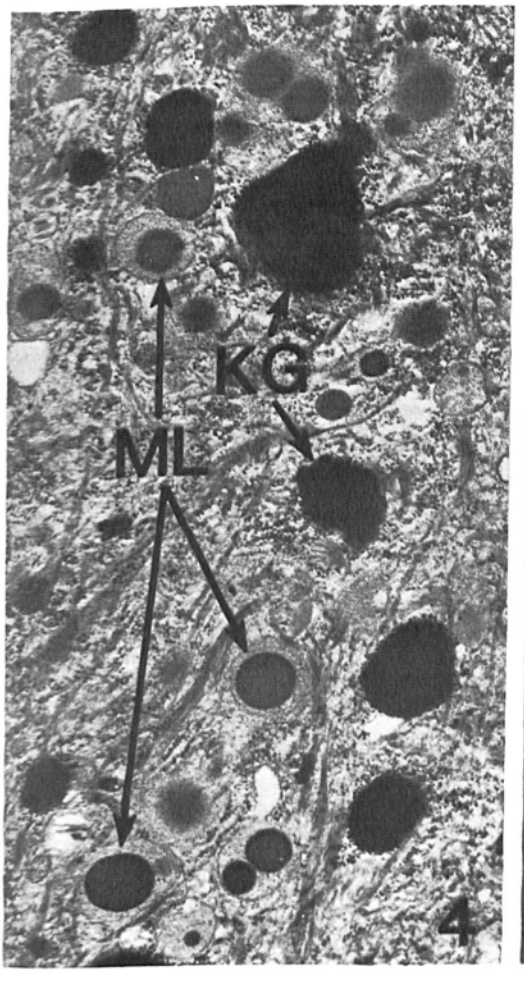
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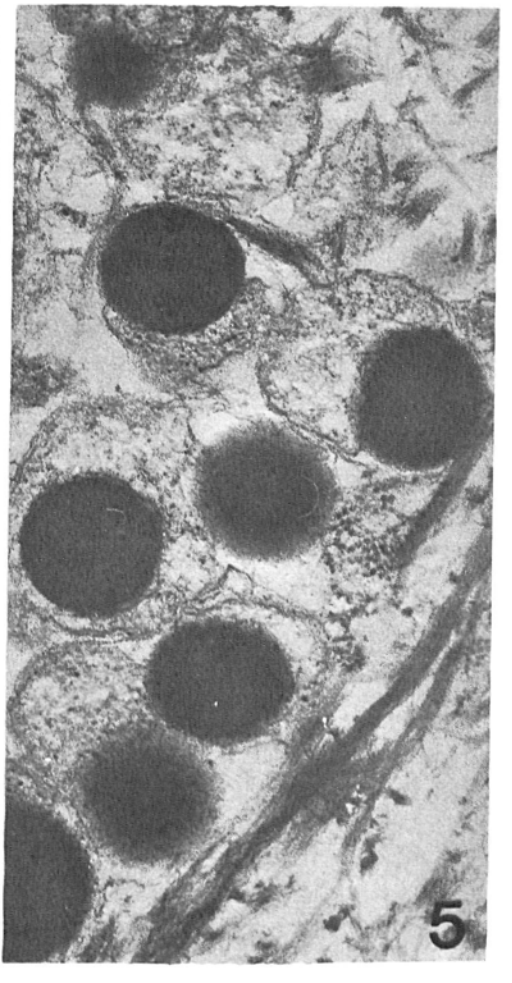
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The same was true of the 3-h specimens. This is not to imply that differences did not persist in the pattern of fibrillar keratin of the stratum corneum, but here there is no mechanism for repair as compared with that of the living keratinocytes.

Discussion

The present studies have shown that treatment of the skin with DMSO results in acute disruption of the epidermis with expanded intercellular spaces, desmosomal disruption, lipid-containing mitochondria, and intracellular vacuoles. This is followed by a period of recovery characterized by elaboration of many membrane-coating granules.

In the present investigation, specimens were taken at the same time intervals after DMSO treatment as those used previously to evaluate fluorescent probe penetration of the skin (1). Thus, the acute phase of disruption in the present studies correlated with the period following DMSO during which the fluorescent probe penetrated rapidly after application to the skin. Furthermore, the apparent damage was reversible, and the barrier returned to normal within 3-h after the DMSO was applied in both studies.

The transmission electron micrographs of skin specimens taken during the period of barrier recovery show a number of changes which are interpreted as mechanisms of barrier recovery. The earliest specimens (after DMSO exposure) showed a greatly altered cellular environment including broken desmosomal attachments, expanded intercellular spaces and evidence of fluid imbibition in the form of cytoplasmic vacuolation.

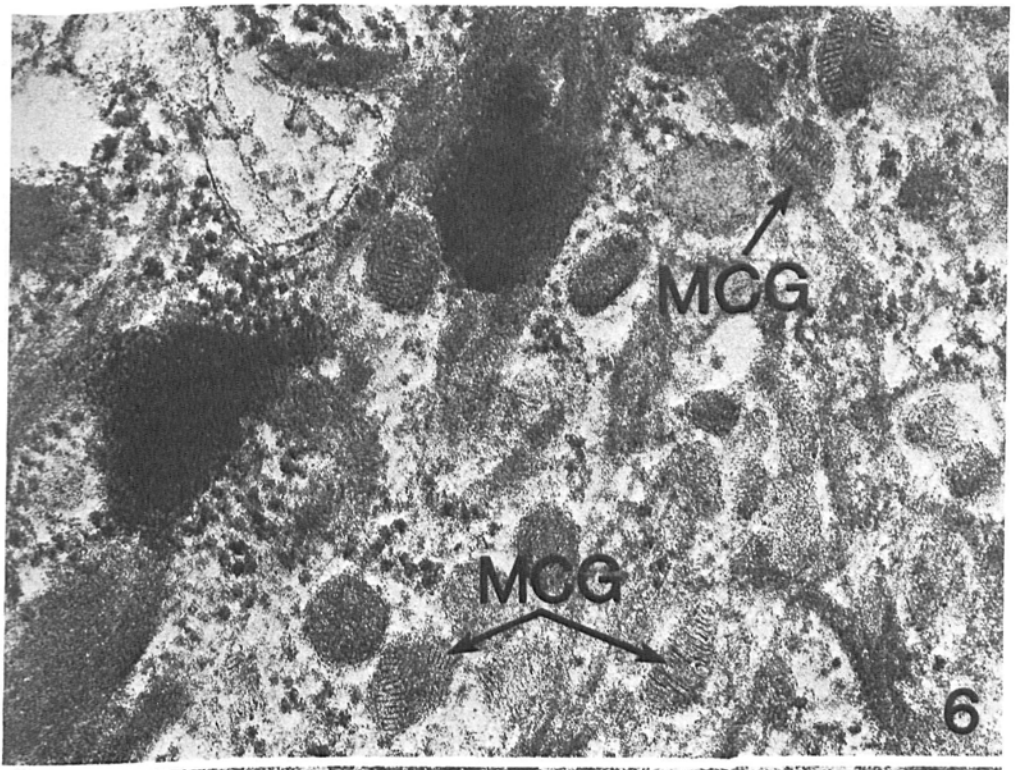
The lipoid material seen within mitochondrial matrices is interpreted as an effort by these organelles to regulate environmental changes in the cytoplasm. It is apparent that the mitochondria take in DMSO solubilized lipids, among other substances, and concentrate them into recognizable droplets within the mitochondrial matrix. As the process of lipophanerosis continues, the droplets grow in size until the mitochondria become completely engorged with these substances.

The DMSO, replete with solubilized lipid from the stratum corneum, reaches the mitochondria of the stratum granulosum first, and it is here that the matrix droplets are largest. Mitochondrial droplets are smallest in the stratum basale because it is believed that the more superifical mitochondria have been efficient in removing the higher concentrations of solubilized lipid. Gradual diminution of the lipid-containing mitochondria as the time increases, indicates that the lipid is either metabolized by the mitochondria or is somehow released from the mitochondrial matrix. At 2 h (post-DMSO treatment) lipid-containing mitochondria are rarely seen.

At 1 h post-DMSO, the most striking change occurs in the stratum granulosum. At this point, membrane-coating granules (MCG) are present in large numbers, many of which can be seen fusing with the surface plasma membrane adjacent to the stratum corneum. This observation supports that of Matoltsy and Parakkal (2) as to the fate of MCG in spreading their content into the intercellular spaces. The authors further suggest

Fig. 6. Electron micrograph of specimen taken 1 h after DMSO application. Note abundant membrane coating granules (×102,000).

Fig. 7. Electron micrograph of membrane coating granules. Note crisscross appearance of inner membranes and apparent continuity of outer membranes with plasma membrane (at right of micrograph). Darker and thicker membrane at far right belongs to a stratum corneum cell. One h post-DMSO (×213,000).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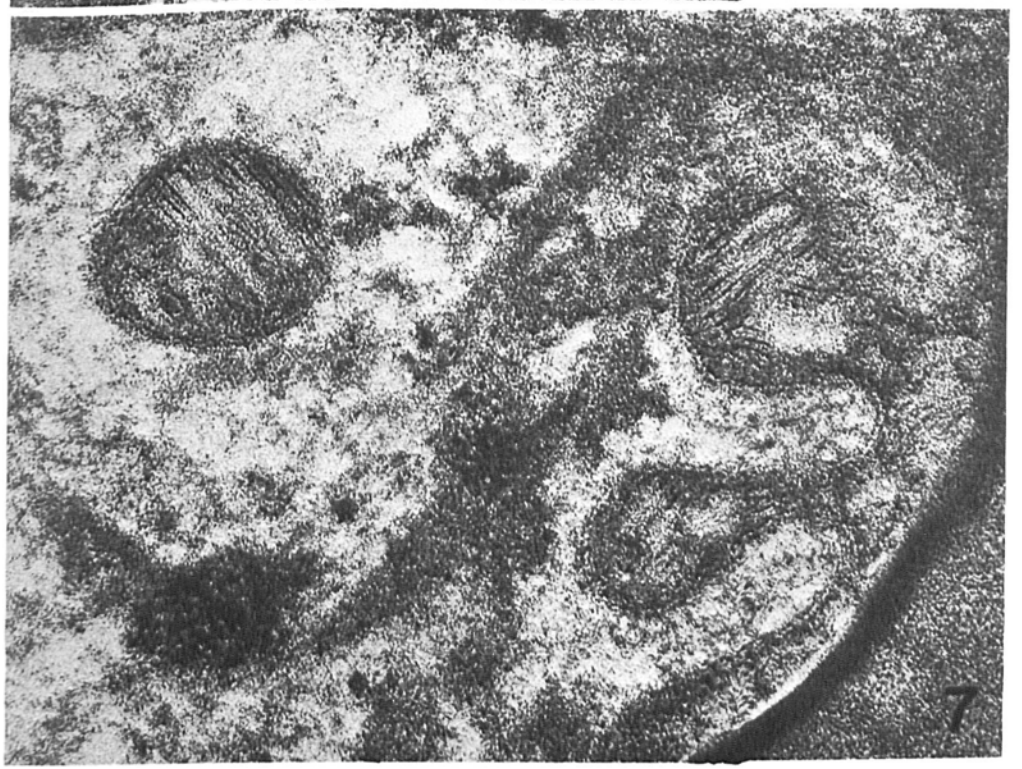
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that the MCG play a role in the development of a protective layer. A more recent study of neonatal mouse skin by Grayson, Johnson-Winegar and Elias (3) reports a lipid-to-protein ratio of 40–60 in isolated MCG (lamellar bodies) and presumes the lipid component of the MCG to be important in barrier function. Also, in view of Scheuplein's observation (4) that solvents such as DMSO can destroy the barrier properties of the stratum corneum by removing lipids and leaving a porous tissue, it seems appropriate to conclude that the MCG are restoring lipids, and possibly other barrier properties, to the horny layer.

The disrupted desmosomal attachments seen in the DMSO-treated epidermis evidently repaired efficiently and rapidly. No effort was made to classify the types of domain turnover of junctional membranes although similar changes have been categorized in pathologic skin (5). Domain turnover refers to destructive changes occurring in cell-to-cell attachments, e.g., DMSO effects on the desmosomes of the present study. There were variations in the pattern of junctional disruptions which seemed to depend on cellular levels within the epidermis, but a detailed study of these variations would require a separate investigation.

It is recognized that the response of living epidermal cells to barrier disruption is only a part of a complex biological story involving other tissues and their products. The process of epidermal exsorption, for example, involving the extravasation of biologically active substances, has been shown to be enhanced by topical applications of DMSO (6).

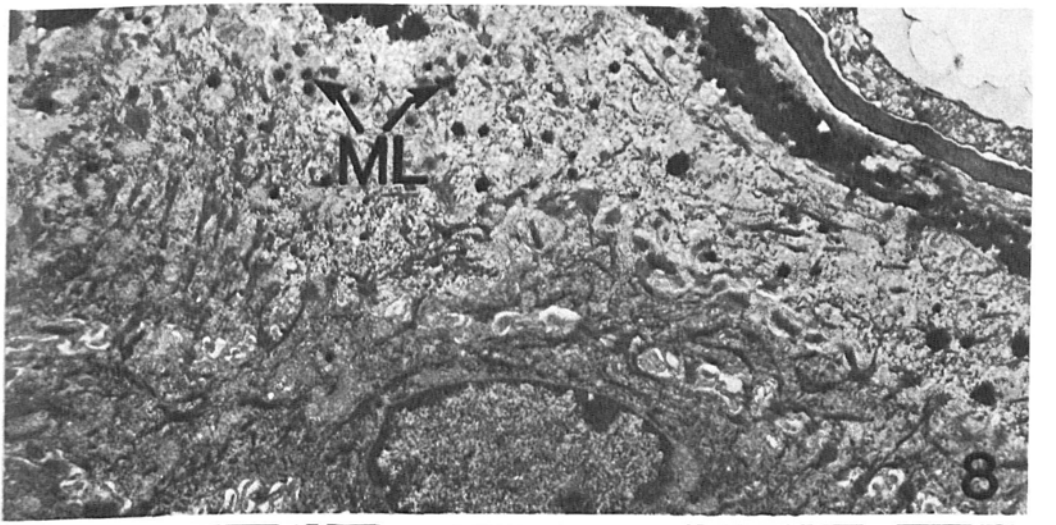
There are numerous published works reporting the effects of various vehicles in percutaneous absorption which include DMSO among other solvents (7, 8, 9). It is common knowledge in most communities in the USA that the general public can purchase DMSO (sold as a degreaser) to rub on painful jaints and tendons. The fact that this actually occurs with a high degree of frequency adds a dimension to the study of DMSO, that does not apply equally to other solvents.

DMSO is referred to variously as an aprotic solvent, a hydroxyl radical scavenger and a membrane stabilizer. It has been successfully employed in experimental animals to prevent irreversible ischemia of the kidney (10), and intestine (11), to prevent brain damage after occlusion of cerebral blood vessels (12), to suppress atherosclerosis in cholesterolemic rabbits (13), and to open the blood-brain barrier (14). These are only a few of the many studies which guarantee the continued widespread use and controversy regarding DMSO.

An ultrastructural study on the effects of DMSO on guinea-pigs (15), and one cited by Idson (8) on the effects of chloroform:methanol as well as DMSO, emphasize changes in the stratum corneum. The present work, however, concentrates on the reaction of living keratinacytes to DMSO. The short period required for recovery from the effects of DMSO in hairless mouse skin, as indicated by the penetrability of the DNA ligand, ethidium bromide (1), led to the belief that living keratinocytes can in some measure repair the damaged stratum corneum. According to this hypothesis, based on individu-

Fig. 8. Electron micrograph of specimen taken 1 h after DMSO treatment. Granular and spinous areas show partial recovery from effects of DMSO. A few droplets of mitochondrial lipid (ML) are present in the granular cells (×7,500).

Fig. 9. Electron micrograph taken 2 h after DMSO application. Most of the epidermis has returned to near normal appearance. Intercellular spaces, desmosomal attachments and mitochondria closely resemble that of control epidermis (×7,500).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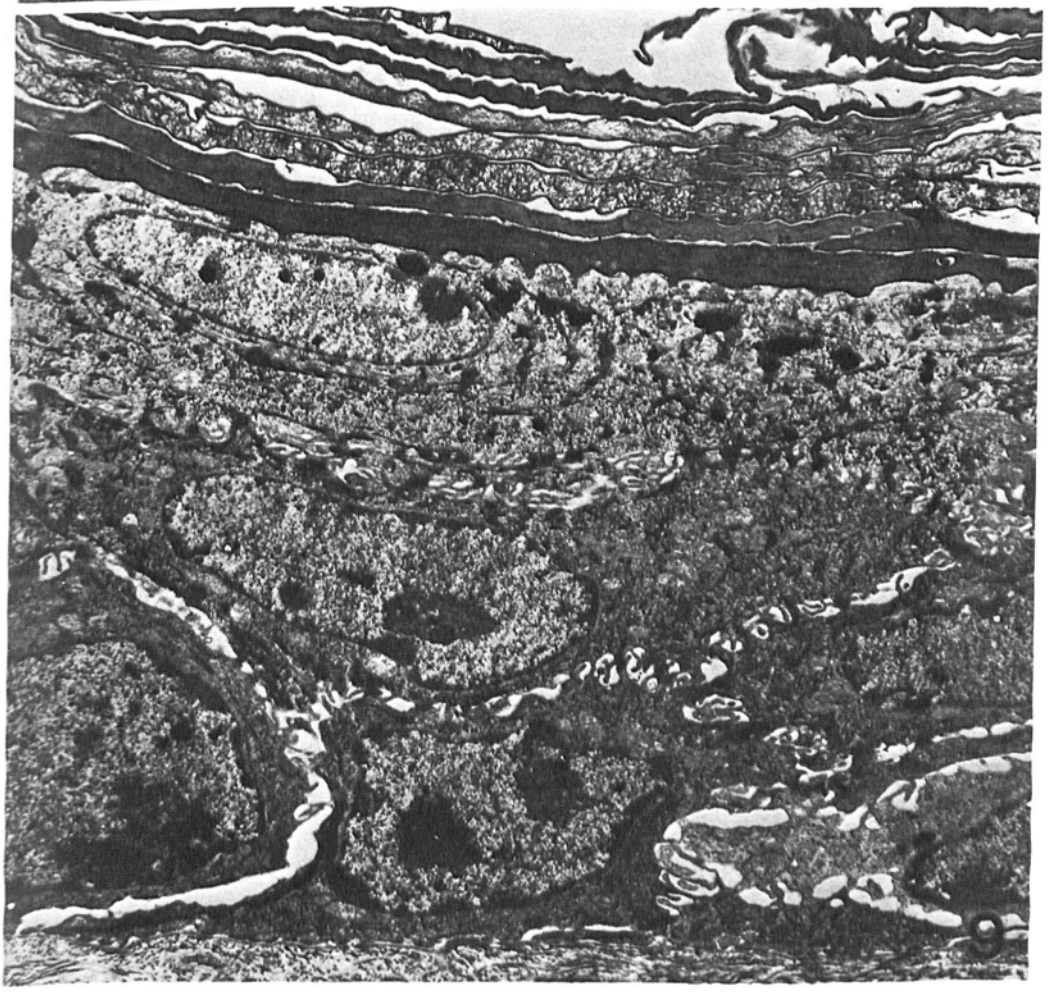
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ally scored slides and the technique of microspectrofluorometry (6), the time span of 3 h was selected for the TEM studies.

Others may have missed the ultrastructural changes reported here for various reasons but the rapidity of recovery from the effects of DMSO seems foremost among them. That is not to say, of course, that chemicals other than ethidium bromide, may have continued to penetrate the epidermis for longer periods if applied under similar circumstances. Morphologic recovery, however, as well as evidence of some repair to the stratum corneum, correlated well with the fluorescent dye studies.

Acknowledgements

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References

1. Shackleford JM, Yielding, KL. Unpublished data.
2. Matoltsy AG, Parakkal PF. Membrane-coating granules of Keratinizing epithelia. J Cell Biol 1965: **24:** 297.
3. Grayson S, Johnson-Winegar AD, Elias PM. Isolation of lamellar bodies from neonatal mouse epidermis by selected sequential filtration. Science 1983: **221:** 962.
4. Scheuplein RJ. Percutaneous absorption after twenty-five years: or "old wine in new wineskins." J Invest Dermatol 1976: **67:** 31.
5. Mahrle G. Domain turnover of junctional membrane areas in the epidermis. Arch Dermatol Res 1982: **274:** 93.
6. Shackleford JM, Yielding KL. Observations on epidermal exsorption in mice following injections of procion dyes and ethidium bromide and topically applied dimethyl sulfoxide. J Invest Dermatol 1984: in press.
7. Wester RC, Maibach HI. Cutaneous pharmacokinetics: 10 steps to percutaneous absorption. Drug Metab Rev 1983: **14:** 169.
8. Idson B. Vehicle effects in percutaneous absorption. Drug Metab Rev 1983: **14:** 207.
9. Scheuplein RJ, Bronaugh RL. Percutaneous absorption, In: Goldsmith LA, ed. Biochemistry and physiology of the skin. New York: Oxford University Press, 1983: 1255.
10. Kedar I, Jacob NB, Ravid M. Dimethyl sulfoxide in acute ischemia of the kidney. Ann NY Acad Sci 1983: **411:** 131.
11. Ravid M, Van-Dyk D, Bernheim J, Kedar I. The protective effect of dimethyl sulfoxide in experimental ischemia of the intestine. Ann NY Acad Sci 1983: **411:** 110.
12. Rosenblum W. Dimethyl sulfoxide effects on platelet aggregation and vascular reactivity in pial microcirculation. Ann NY Acad Sci 1983: **411:** 110.
13. Layman DL, Alam SS, Newcomb KC. Suppression of atherosclerosis in cholesterolemic rabbits by dimethyl sulfoxide. Ann NY Acad Sci 1983: **411:** 336.
14. Broadwell RD, Saleman M, Kaplan RS. Morphological effect of dimethyl sulfoxide on the blood-brain barrier. Science 1982: **217:** 164.
15. Montes LF, Day JI, Wand CJ, Kennedy L. Ultrastructure changes in the horny layer following local application of dimethyl sulfoxide. J Invest Dermatol 1967: **48:** 184.

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