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Effect of Sulphur Mustard on Mouse Skin—an Electron Microscopic Evaluation

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Effect of Sulphur Mustard, 2-2'dichlorodiethyl sulphide exposure on human skin results in the formation of blisters which are slow to heal (Frank 1967). It is reported that the pathology of Sulphur Mustard (SM) exposed human skin bears resemblance to animal skin to a certain extent (Renshaw 1946). Subsequently many novel in vitro models have been developed to study aspects of SM behaviour and toxicity on animal skin (Papirmeister et al 1991; Riviere et al 1991). Response of animal skin to SM exposure has two phases. The immediate phase is characterized by injury to the fibroblast and selective granulocyte infiltration. The delayed phase is characterized by the death of basal epidermal cells accompanied by vascular leakage (Vogt 1984). Cellular injury by SM, recorded by electron microscopy showed condensation of heterochromatin, blebbing of the nuclear membrane and formation of large perinuclear and cytoplasmic vacuoles (Papirmeister et al 1984). Characteristically similar ultrastructural changes have been observed in the SM exposed isolated perfused porcine skin flap (Riviere et al 1991). Most of these studies have described the pathogenicity of SM with reference to degenerative changes in cells and their organelles. The epidermal layer along with the extracellular matrix (ECM) has not been given due attention for electron microscopic studies. It is recognised that the reepithelization of wounded skin takes place only when there is an underlying bed of ECM in the form of the dermal support (Yannas 1990). It is hypothesised that for a lipid soluble substance like SM, the stratum corneum may function as a `Sponge' and can hold a quantity of SM in the stratified layers (Kao 1989). Studies on the skin penetration and distribution of radioactive Butyl-2-chloroethyl sulphide, an analogue of SM in rats revealed that at 24 h the epidermis contained about 25% of the radioactivity (Klain et al 1989). The stratified keratinocyte

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cultures (representing the epidermis) have been employed in studies with sulphur mustard,but these have not been characterized ultrastructurally (Papirmeister et al 1991). Considering these factors a study was taken up to focus attention on the microenvironment of the epidermis along with the surrounding ECM to follow the course of injury due to SM and subsequently the initiations of recovery in the mouse skin.

MATERIALS AND METHODS

Swiss Albino male mice weighing 25±3 g were kept in polypropylene cages. The animals had free access to food and water. Sulphur Mustard (98% pure) synthesized in the Chemistry division of the Defence R & D Establishment was applied on the clipped area on the dorso caudal surface of the animals. A single dose of SM (LD_{50}: 154.7 mg/kg percutaneous) was applied on an area of approximately one square cm. The animals were sacrificed on day 1, 3 and 6 after the treatment. In each group six animals were taken. The control sample was taken from the other side of the dorsal portion of the corresponding animals. However only one representative control micrograph has been given in this study. Skin tissues of animals were processed for routine electron microscopy. Initially, thick sections were cut to scan the tissues having similar skin thickness as well as gross histological features. Ultrathin sections from selected tissues were cut in Ultracut E, stained with Uranyl acetate and lead citrate before observing in a JEOL JEM-1200EX transmission electron microscope at 80 kV. Wax blocks were also prepared from the other set of the same tissues, after fixing them in formaldehyde glutaraldehyde fixative (Karnovsky 1965). 12-15 micron thick sections were cut and cleared of the wax by xylene. They were sputter coated with gold before observing in a JSM-840 scanning electron microscope.

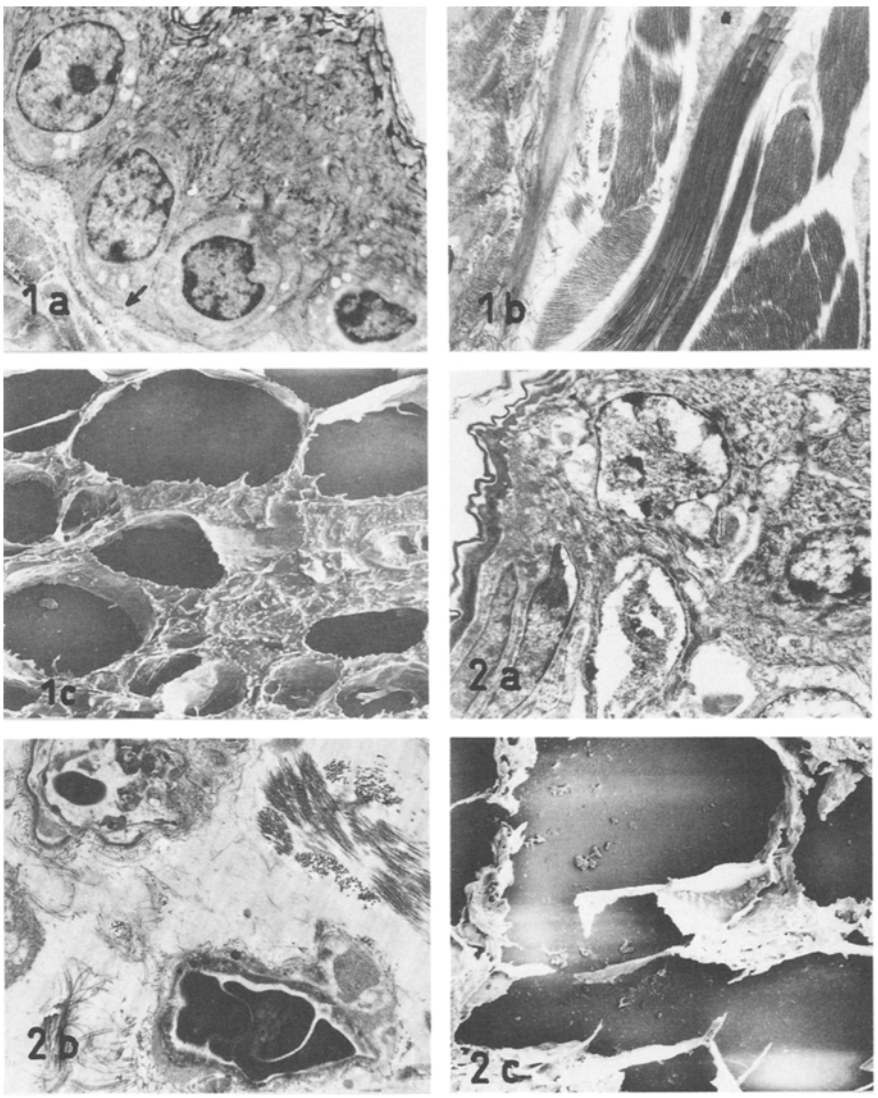
RESULTS AND DISCUSSION

Since epidermis and dermis containing cells and extra-cellular matrix form a functional unit, changes at both levels were described. Most of the micrographs were taken at relatively moderate magnifications to demonstrate the overall changes. Skin from the unexposed portion of the animal showed normal cell structure, specifically intact epidermal layers, cell surface as well as the intercellular junction between the collagenous dermis and the epidermis (Fig 1a). The morphological details adjacent to the epidermis of the control skin demonstrated a well arranged compact collagen fibre bundles both in the longitudinal as well as transverse directions (Fig 1b). Fig. 1c shows the

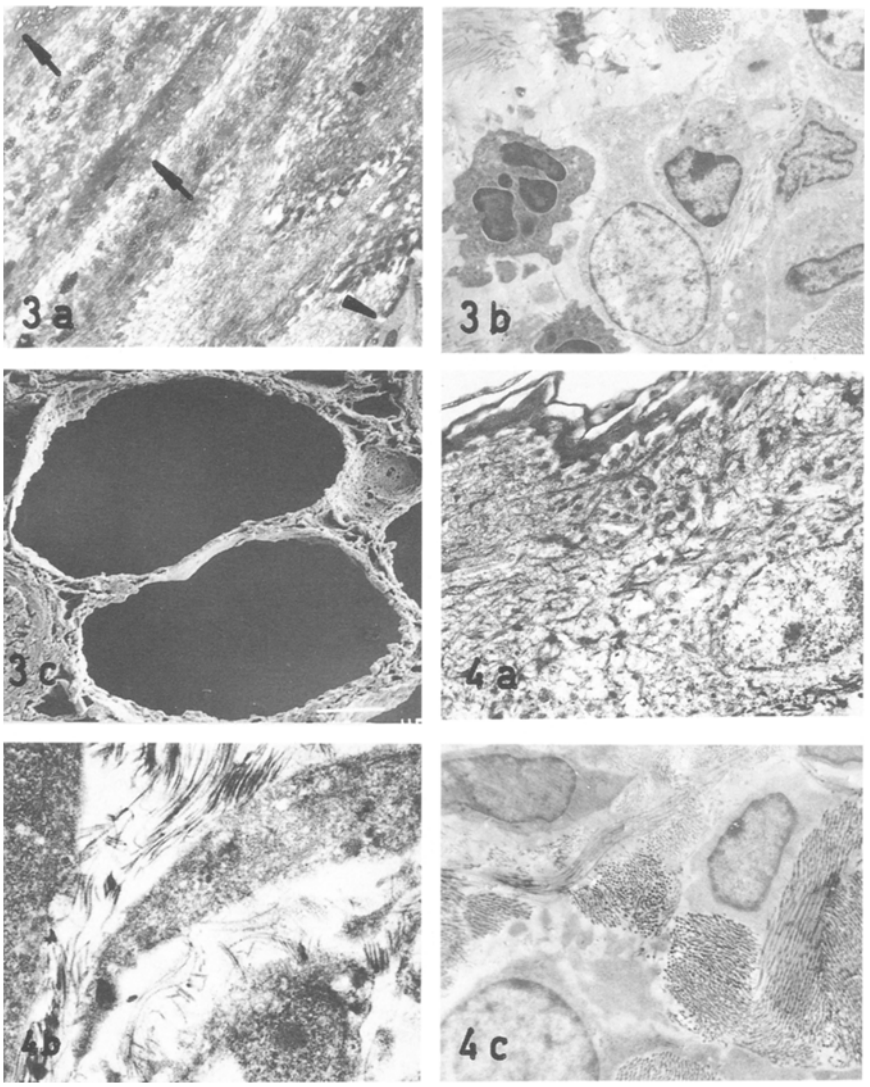
intact lipocyte boundaries in the control specimen. The architectural order of the epidermal region was disturbed 24 h after the application of SM. The discrete cell boundaries were distorted. Fig 2a shows these necrotic epidermal cells with cytoplasmic vacuoles. Fig 2b shows the intercellular gap formed due to the retention of edematous fluid. The increased intercellular gap results in the damage to the collagenous matrix. The bundles of collagen fibres were disrupted. The blood vessels were observed to be dilated and walls disintegrated to various extents. One of the striking changes observed was the breaking of delicate collagenous structure surrounding the Lipocytes. This was more frequently observed for Lipocytes having large area and thin wall. The isolated small lipocytes surrounded by thick ECM were seen intact (Fig 2c).

Histopathological changes in skin on the third day after the exposure revealed contiguous microholes in the stratified layers of the epidermis (Fig 3a). These microholes were noticed in the layers of the epidermis and indicate the weakening of the structure between the epidermis and the dermis. The nuclear membrane at the upper dermis showed loss of integrity and cytoplasmic vacuoles were seen frequently. These cytoplasmic as well as nuclear changes observed in the present study are in agreement with the published work though on different animal models (Papirmeister et al 1984; Riviere et al 1991). The inflammatory cells like histiocytes and mast cells registered an increase over the one day old lesion (Fig 3b). The network of lipocyte boundaries reappeared at many places (Fig 3c). Besides these two changes namely the formation of lipocyte boundaries and an increase in the number of inflammatory cells no other features revealed any sign of recovery untill the third day. Fig 4a shows the epidermis on the sixth day after the exposure, with a kind of alignment in the epidermal layers resulting in the restoration of order. The cells started appearing at the lower epidermal layers. The intercellular gap in the region adjoining the epidermis was filled with a dark stained material (Fig 4b). The collagen bundles had acquired more orderly arrangement at a few places (Fig 4c).

In vitro experiments on biologically active analogues of ECM indicated that the filling up of the ECM plays a pivotal role in the remodelling of skin (Yannas 1990). Studies on the effect of SM in organ culture from rabbit skin indicated that the healing began on the 6th day after the exposure. The healing occured under the crust by migration of epidermal cells from the undamaged skin as well as from hair follicle cells. The



Figures 1a-2d; 1a:3000X, Normal epidermal cells of control skin with intact cell boundaries, distinct basal membrane (arrow); 1b:3000X, Ordered intercellular arrangement of collagen bundles in longitudinal as well as transverse direction; 1c:750X, Intact lipocyte cell boundaries in the control mouse skin; 2a:3000X, Morphological changes after 24 h, necrotic cells with cytoplasmic vacuoles; 2b:3000X, Increased intercellular gap; 2c:750X; Scanning micrograph showing broken collagenous walls surrounding lipocytes after 24 h.



Figures 3a-4c;3a:3000X, Array of microholes in the epidermal layers on the third day (arrow) indicating initiation of the detachment process of the layers of epidermis from the dermis (arrow head); 3b: 3000X, Increased intercellular gap persisted, showing presence of inflammatory cells; 3c:1500X, Formed lipocyte outer boundary; 4a:4000X, Alignment of epidermal layers parallel to the upper dermis, cell boundary started appearing; 4b:4000X, Dark-stained material occupying intercellular space on the sixth day; 4c: 4000X, Compact and ordered collagen bundles at the upper dermis.

organ culture of SM exposed skin biopsies showed a reduction in weight untill the third day when compared with the normal skin culture. Subsequently the weight increased for 6 and 10 day old lesions. The increase in weight was detrmined to be due to the change of ground substance from the sol state of acute inflammation to the gel state. These data on organ cultures from rabbit skin correlated well with the histopathology of the rabbit skin (Dannenberg et al 1985).

In our study the dark stained material which appeared on the sixth day as the intercellular space could be the normal gel state of protein which had taken the electron-dense stain. The other extracellular material which attained ordered configuration is the fibrous collagen (Fig 4c).

This study indicates that concurrent changes at the epidermal layer, ground substances as well as formation of lipocyte boundaries could be taken as indicators of recovery, though the extent of transformation may vary in these components. Ultrastructurally there are indications that the process of recovery starts on the third day. The recovery became perceptible on the sixth day when the epidermal tissues became aligned in parallel layers and the extracellular matrix acquired a relatively compact configuration.

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