days showed abundant desmosomes, tonofilaments, and mitochondria in the basal layer (Fig. 7), and electron-dense granules, morphologically similar to in situ keratohyalin granules, and cells resembling those in the in situ stratum corneum were evident in the upper layers of lifted cultures at this age (Fig. 8). Many of these epidermal markers do not develop in submerged cultures.

IV. EXPERIMENTATION WITH SULFUR MUSTARD IN CUTANEOUS TOXICITY STUDIES USING THE EPIDERMAL CULTURE

A. Topical Application of a Xenobiotic to the Surface of the Lifted Cultures

This section describes the experimentation to measure the toxic response of epidermal cultures after topical application of the sulfur mustard, bis(β-chloroethyl)sulfide (BCES). Details of the methodology have been reported (28). Aliquots of BCES dissolved in methylene chloride (MC) (10 mg/ml) were further diluted to selected concentrations in one of the following solvents: 2,4-pentanedione (acetyl acetone), ethanol (ETOH), hexane, or dimethyl sulfoxide (DMSO). A total of 0.04 ml solvent or of solvent containing BCES was applied to the surface of an epidermal culture grown on a 13-mm disk of P200 and lifted for 14 days. This amount covered most of the culture surface without flowing over the side onto the supporting glass-fiber filter. The amount of material applied was expressed as nanomoles per square centimeter of the culture surface ($nmol/cm^2$). Solvent or solvent–BCES solutions were removed from the culture surface by gently washing in Earle's balanced salt solution (EBSS). The washed cultures were placed on the surface of supporting glass-fiber filters saturated with fresh growth medium and incubation continued for preselected periods. The cultures were next transferred to the appropriate medium containing radiolabeled precursors when experiments were conducted to observe effects on macromolecular metabolism subsequent to various treatment (28).

Data were obtained from preliminary experiments to select an appropriate solvent for topical application of xenobiotics to the surface of epidermal cultures. Acetyl acetone, hexane, and MC were all highly toxic as indicated by significant reductions in the incorporation of [^{3}H] thymidine into DNA, when compared with untreated controls. In further studies, it was observed that dilutions of ETOH and DMSO in distilled water were much less toxic and were also appropriate solvents for BCES. Table 2 shows the results of both [^{3}H] thymidine and [^{14}C] leucine incorporation into DNA and