stage of differentiation are called *granular cells* because they contain keratohyalin granules, an important structure in the formation of keratin. The cells at the terminal stage of differentiation are the *cornified cells* that form the outermost desquamated layers of the epidermis. It is believed that tonofilaments and keratohyalin granules combine in some way to form the keratin that characterizes the terminally differentiated keratinocyte. Another characteristic unique to the epidermis is the formation of *desmosomes*—structures that form very tenacious bonds between the cells in various layers. *Hemidesmosomes* are bonds between the basal keratinocyte and the basement membrane.

Therefore, any chemical that contacts the epidermis is presented with all of the cellular components of the epidermis. We have developed a differentiated keratinocyte culture system that forms a stratified squamous epithelium with morphological and biochemical markers like those observed in an epidermis formed in situ. Keratinocytes are obtained as primary isolates (to retain as much of the original metabolic activity as possible), seeded on an appropriate substratum, allowed to attach and proliferate, then, raised to the air–medium interface to stimulate further growth and differentiation. This method creates a microenvironment similar to that in which a normal epidermis develops. As a result, the cells differentiate and form a homeostatic relationship with each other that results in a tissue that would be expected to react to a xenobiotic applied to its surface in a manner similar to the epidermis. We have also developed methods of applying test substances to the surface of this keratinocyte culture system and have obtained data concerning the effects that certain classes of chemicals have on its metabolism of macromolecules. Epidermal cultures of this type have been established using both rat and human basal keratinocytes isolated from epidermal fragments. The system currently used for studying the effects of xenobiotics applied to the surface of the culture has as its substratum a commercially available nylon membrane, rather than biological materials such as collagen.

The early, initial molecular events that take place after topical application of bis(β-chloroethyl)sulfide (BCES) to the surface of the culture has been identified using this culture system.

II. DEVELOPING CULTIVATION PROCEDURES

A. Isolation of Rat or Human Keratinocytes for Cultivation

Two types of cells are the major structural components of the normal mammalian full-thickness skin: fibroblasts and keratinocytes. *Fibroblasts*, found in the dermis and responsible for the formation