

of collagen, can be cultured *in vitro* and subcultured in a basal medium with serum as the only supplement (9). *Keratinocytes*, which form the epidermal structure, do not proliferate as well *in vitro* in primary cultivation or after passage when serum is the only supplement to basal medium. Only the basal cells harvested from the living epidermis engage in proliferative activity (10,11). The more differentiated cells appear to have active DNA that engaged in repair but not semiconservative synthesis (12).

Several systems have been developed to promote increased serial cultivation of keratinocytes. Improvements resulted when cells were grown on collagen (13,14), on feeder layers of irradiated fibroblasts (15), or in conditioned medium (16). Later reports indicate that these systems may not be necessary (16-18) and that serial cultivation of keratinocytes can be achieved by supplementing the medium with growth promoters such as epidermal growth factor (19,20) and hormones (17,21). We reported a description of the morphological and cultural characteristics of rat keratinocytes after supplementing the growth medium with various concentrations and combinations of hydrocortisone and insulin (22).

In our investigations into successfully cultivating keratinocytes, full-thickness skins from both newborn rats and human biopsy specimens were obtained from which the desired cells were isolated, using essentially the same procedures. The skins were aseptically removed, placed in a petri dish and loose connective tissue and blood vessels scraped from the underside of the dermis. This scraping also caused the skin to stretch slightly. The resulting tissue was chilled to 4°C and covered with crude trypsin (1:250) pre-chilled to the same temperature. This combination of stretching and trypsinizing the skin at 4°C caused the dermis and epidermis to separate at the basement membrane and the loosening of desmosomal attachments between basal and lower spinous cells. These cells, mostly in the form of aggregates, were removed from the epidermal fragment by brushing them into growth medium containing fetal bovine serum (FBS). The remaining epidermal fragments containing differentiated cells and stratum corneum were discarded. Brushing the cellular components into growth medium also suspended single basal cells, fibroblasts, and cellular debris. These were removed by layering the resulting suspension on 10% Ficoll followed by centrifugation for 5 min at $13 \times g$ (22). The pellet containing aggregates of basal cells was resuspended in fresh medium, and this washing procedure repeated seven times to remove most of the single cells and debris. Cell aggregate viability was determined using a dye exclusion test with 0.5% erythrosin B (23). The viability of these suspensions was routinely greater than 98% as indicated by this method.