

58.3.1.2 Alkali

58.3.1.2.1 Ammonium Hydroxide

Ammonium hydroxide (NH_4OH) has a large concentration of undissociated NH_4OH and NH_3 molecules, which penetrate quickly and elevate pH to about 12. Baumberger [1] found the optimum choice i.e., the use of isotonic NH_4OH applied for 35 minutes.

58.3.1.2.2 Sodium Hydroxide

Felsher [12] verified the effect of sodium hydroxide (NaOH) and alkali salt mixtures, observing that the time required for separation of the epidermis decreases as the pH and swelling effect increased. It was unfeasible to utilize solutions below pH 10; pH 12 was recommended. Bases not only separated epidermis from dermis but caused the separation between epidermal cells.

58.3.1.3 Neutral Salts

Felsher [12] demonstrated that 2N sodium thiocyanate, iodide, and bromide caused rapid epidermal swelling and glazing, yielding ease of separation. Diaz et al. [7] extended Felsher's sodium thiocyanate separation method. Immunofluorescence and electron microscopy verified that a clean cleavage occurred at the lamina. Separation of epidermis by neutral salts resembles that caused by acids; however, edema between individual collagen fibrils is not as marked as in the acetic acid method. Epidermal separation occurs exactly at the EDJ and the basal cells remain preserved.

58.3.2 ENZYME DIGESTION

Several enzymes separate the epidermis from dermis (Table 58.2). The enzymes produce separation at the EDJ by digesting different structures specifically.

58.3.2.1 Trypsin

Basement membrane elastic fibers play an important part in anchoring the epidermis to the underlying tissue. Medawer [28] observed human skin incubated in trypsin and found that it is possible to disengage the epidermis in the form of an intact sheet. Klein et al. [21] separated mouse epidermis using trypsin and elastase. A shorter incubation period was required for elastase than trypsin. Ease or difficulty of separation was directly related to the total amount and dimensions of skin samples being incubated. Trypsin separation remains widely used, and it is reported to be less damaging to keratinocytes than the other physical and chemical procedures [9]. Separation predominantly occurs between basal and suprabasal cells by disruption of desmosomes, which may cause basal cells to remain loosely attached to the basement membrane [26]. Trypsinization causes loosening of the keratinocytes' intercellular connections. Therefore, vigorous washing of the isolated epidermal sheet to remove contaminating dermal fibroblasts also dissociates keratinocytes. Slight modification of trypsinization conditions also causes separation at the suprabasal layer.

58.3.2.2 Pancreatin

Becker et al. [2] described the pancreatic enzyme method; results resembled those of Medawer [28] obtained with trypsin. Fan [11] showed that crystalline trypsin and purified trypsin separated epidermis using an equal or smaller amount than pancreatin. Purified trypsin freed the epidermis the most efficiently.

58.3.2.3 Pronase

Einbinder et al. [8] compared pronase, a broad-spectrum proteinase, and the relative digestive action with five other proteolytic enzymes, namely trypsin, collagenase, pancreatic elastase, fungal