had a greater percentage of applied dose penetration into tissues with low doses than high doses, suggesting a fixed absorption rate. This was also seen for PNP, but only under occluded conditions. Neither phenol dose, vehicle, nor occlusion had a significant effect on the labeled phenol seen in the stratum corneum or on time of peak flux, a finding that limits the usefulness of noninvasive stratum corneum sampling to assess topical penetration. Neither PNP dose, vehicle, nor occlusion had a significant effect on total recovery of labeled PNP. The researchers suggested that comparative absorption of phenol and PNP are vehicle, occlusion, and penetrant dependent.

In contrast to most penetration studies, which use mainly occlusive application of drug formulations onto the skin, Cevc and Blume (46) highlighted the importance of the nonocclusive application of drug-loaded deformable vesicles, Transfersomes, onto the skin to preserve the "hydrational driving force" across the epidermis, which should, according to the authors, "push" the deformable vesicles across the epidermis. According to Cevc and Blume (45), the osmotic gradient, which is created by the difference in the total water concentrations between the skin surface and the skin interior, provides one possible source of such driving force. This force would be, according to the authors, sufficiently strong to "push" at least 0.5 mg of lipids per hour and cm^2 through the skin permeability barrier into the region of the stratum corneum (45). Thus, the ability of deformable vesicles to penetrate the intact skin, carrying their encapsulated drugs, is attributed to their xerophobia (tendency to avoid dry surroundings and hence move along with the hydration gradient) and to their deformation ability to "pull" through the skin under nonocclusion (46–48, 55, 56). It has also been shown in murine stratum corneum ex vivo and in vivo that Transfersomes, after nonocclusive application, penetrate into the stratum corneum through preexisting channels, i.e., through the "intercluster" pathway (mostly) between groups of corneocytes that only partly overlap (low-resistance route) with a barrier maximum at around 10 µm and the "intercorneocyte" pathway between individual corneocytes (high-resistance route) with a barrier maximum at around 4 to 7 µm (57). Occlusion would therefore be detrimental for vesicle penetration into intact skin and their penetration-enhancing effect.

El Maghraby et al. (58) investigated in vitro in human skin the transdermal delivery of estradiol from ultra-deformable liposomes after their occlusive and nonocclusive application (using an "open stratum corneum protocol," thus preserving the transepidermal hydration gradient). Occlusion resulted in a significant reduction of the transdermal flux of the drug, which confirms the importance of the "open-application protocol" for the transdermal drug delivery from ultra-deformable vesicles. Therefore, as occlusion is believed to abolish the driving force for the penetration of deformable vesicles into the skin, these vesicles (e.g., Transfersomes, invasomes, ethosomes, etc.) are applied in skin penetration studies, as well as for the evaluation of their therapeutic effectiveness, always under nonocclusive condition (58–67). Thus, when applied onto the skin only under nonocclusion, drug-loaded ultra-deformable vesicles achieve their therapeutic effectiveness, as shown in many studies, such as for the antiacne effect of retinoic acid (66), antiinflammatory effect of ammonium glycyrrhizinate (67), antiinflammatory effect of ketoprofen (56, 65, 68), hypoglycemic effect of insulin (69), etc. For more information on the application conditions for different deformable vesicles and their mechanism of action, one should refer to Refs. (48, 63, 70–75).

14.3 PERCUTANEOUS ABSORPTION IN VIVO

14.3.1 Animals

Bronaugh et al. (34) measured the percutaneous absorption of cosmetic fragrance materials safrole and cinnamyl anthranilate, as well as of cinnamic alcohol and cinnamic acid, at occluded and nonoccluded application sites over a 24-hour period. They determined the absorption in the rhesus monkey in vivo and also measured the absorption value through excised human skin in diffusion cells. Each radiolabeled compound was applied in an acetone vehicle at a concentration of 4 $\mu g/cm^2$. Occlusion was accomplished by taping plastic wrap to the skin application site for