testosterone exceeds by several-fold that of the dermis, but because of the many-fold thickness of the dermis, far larger amounts are associatively bound from which it is eluted in body fluids. It is plasma protein bound, and therefore its absorption is enhanced. Malathion (36), hair dyes (37), and vitamin E (38) are examples of the many compounds shown to be bound to stratum corneum components.

4.11 VIABLE EPIDERMIS AND DERMAL RESERVOIR

Most studies have emphasized the stratum corneum as a reservoir. However, the viable epidermis, dermis, and underlying tissues may themselves also act as reservoirs. Baker et al. (39) have reported binding of topical steroids to epidermal tissue. The effect of viable epidermal and dermal tissues as reservoirs may be limited by the often-extensive metabolism, which can occur in viable tissue and its location. For beta-estradiol, metabolic activity mainly resided in the basal layer of the viable epidermis (40). Accumulation and the concentration in the basal cell layer of the epidermis are important for many solutes, and it has been suggested that it is the free drug concentration that exerts a pharmacological effect (41). Accumulation in the viable epidermis can be deduced using the model described in Figure 4.6, and this has been used to examine epidermal concentrations of steroids (14). Walter and Kurz (42) have reported that the binding of 10 drugs to both epidermis and dermis was related to the lipophilicity of the drugs. Yagi et al. (33) have commented that binding of beta-blockers to viable epidermis components is an important determinant of the residence time in viable skin. In addition to binding, the rate of removal by the perfusing blood should be a determinant of the extent of reservoir formation in these tissues, as is evident by prolonged anesthesia when a vasoconstrictor is included with a local anesthetic in intradermal injections. Dermal and lower tissue level concentrations are apparent when a drug is applied together with a vasoconstrictor (43).

As an illustration of this principle, the dermal clearance and retention of diclofenac can be shown to depend on both binding to dermal tissues and to blood flow, and this effect is most evident when binding protein is not present in the blood (44).

When dextran rather than albumin is used as the perfusate, diclofenac is retained in the skin after dermal application, and the retention sites are the dermis and subcutaneous tissue (Figure 4.14).

The expression for the retention half-life ($t_{0.5}$) for solutes in the dermis and other tissues can be related to three key parameters: binding to plasma components (fraction unbound [fu_p]), binding to the dermis (fu_T), and the blood flow to the topical site (Q_p), as well as the relative volumes for solute distribution in the plasma (V_p) and in extravascular tissue space (V_{TE}) (44). Drugs such as diclofenac show much greater binding to dermis (and plasma) than some other solutes and therefore are preferentially retained.

4.12 IN VITRO–IN VIVO CORRELATIONS

Recently considerable interest has concerned the skin reservoir associated with in vitro skin penetration studies (45). Conflict presently exists as to whether or not skin levels remaining in the stratum corneum and epidermis/dermis at the end of an in vitro study should be included in the overall estimation of absorbed material. As Yourick et al. (45) point out, guidelines issued by COLIPA and the Scientific Committee on Cosmetic Product and Non-food Products suggest that material remaining in the stratum corneum at the end of a study should not be considered as systemically available, whereas that in the viable epidermis and dermis should. In contrast, the European Centre for Ecotoxicology and Toxicology of Chemicals suggests that percutaneous absorption should be based on receptor fluid concentrations only. The draft Organization for Economic Co-operation and Development guidelines take an intermediate position, namely that the amount of material should include both the skin and receptor fluid unless additional studies can demonstrate that the material in the skin is effectively not available.