P450 enzymes that are also found in the liver (4). Skin protein expression levels of alcohol dehydrogenase, aldehyde dehydrogenase, oxidases, hydrolases, carboxylesterase, and glutathione S-transferase were generally fourfold to tenfold lower than in the liver. Skin cytochrome P450 protein levels were nearly 300-fold lower than those measured in liver. This indicates that cytochrome P450 enzymes will minimally affect chemical metabolism during skin absorption (5).

As alternative methods are developed to reduce animal usage for toxicity testing, in vitro human skin and animal skin models are being increasingly utilized for skin absorption and biochemical function studies. It is important to understand how similar in vitro human skin models are to intact human skin for barrier function and metabolic capacity. Understanding the pathways of chemical metabolism and the metabolic capacity of human skin is needed given the increased use of alternative methods. In EpiSkin it was found that 61 phase I and II enzyme mRNA expression levels were similar to the human epidermis (6). There was reduced expression of cytochrome P450s and flavin monooxygenase in EpiSkin, whereas phase II enzyme expression was present at higher background levels. A proteomics method was used to further characterize the profile of chemical metabolizing enzymes in human skin and other in vitro skin models (5). Four in vitro human skin models (EpiDerm, EpiSkin, an in vitro reconstructed human epidermis [RHE], and the HaCaT cell line) have metabolic protein expression profiles that are comparable to enzyme activities found in intact human skin. Furthermore, when the expression of 139 genes related to metabolic enzymes were compared in EpiDerm and intact human skin, it was found that there was an 87% homology of expressed genes (7). Even though there was low expression of cytochrome P450s in both intact human skin and EpiDerm, it is possible to induce cytochrome P450 expression and activity with 3-methylcholanthrene (7). Animal skin metabolic capacity will be discussed further later.

19.2 SKIN VIABILITY

The use and maintenance of viable skin in absorption studies is a requirement for studying metabolism of compounds diffusing through skin. In fact, it is stated in the Organisation for Economic Co-operation and Development (OECD) guideline for in vitro skin absorption studies that "if metabolism is being studied, the receptor fluid must support skin viability throughout the experiment" (8). Early on it was demonstrated that benzo[a]pyrene mouse skin penetration was increased after induction of epidermal oxidase activity and was reduced using previously frozen skin, which had decreased viability (3).

Several improvements and advances have been developed to better maintain in vitro skin disc viability over the years. For example, pig skin viability was maintained using an oxygenated (95% O_2:5% CO_2) tissue culture medium as the receptor fluid for 50 hours (9). As an indirect way to prove viability, the pig skin discs were removed from the in vitro penetration-evaporation cells after 50 hours and were successfully grafted onto athymic nude mice.

The viability of skin in flow-through diffusion cells was thoroughly examined (10). Skin viability can be determined by measuring lactate found in the receptor fluid as a measure of skin glucose utilization. Skin viability can be assessed in this manner throughout the time course of an experiment. Many different tissue culture media or water/solvent mixes have been used by different investigators for receptor fluids. It was shown that a HEPES-buffered Hanks' balanced salt solution (HHBSS) was equivalent to minimal essential media in maintaining skin viability for at least 24 hours after mounting a skin disc in a diffusion cell. Skin viability was also confirmed by examining the cellular ultrastructure by electron microscopy and by the maintenance of estradiol and testosterone skin metabolism.

The use of the 3-[4,5- dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT) assay for determining skin viability has been defined. Sometimes it is necessary to add bovine serum albumin (BSA) to the receptor fluid to enhance chemical partitioning from skin into the receptor fluid. However, the addition of 4% BSA to an HHBSS-based receptor fluid interfered with the lactate assay (11). Therefore, the MTT assay was adapted to assess skin viability during this in vitro absorption study. The viability of human, fuzzy rat, and hairless guinea pig skin was found to be maintained