Widening of intercellular spaces was observed (75), with some desmosomal alteration, but these modifications were transient. The assumption that intercellular cavities were secondary to cavitation is doubtful, since the occurrence of cavitation is uncertain at the frequency and intensity used (18). It is thus more likely that widening of the intercellular space was secondary to mechanical stress causing disruption of the lipid bilayers. This is supported by a further study carried out on fish below the cavitational threshold at 3 MHz and 2 W/cm^2, which demonstrated intensity dose-dependent intercellular widening, while cavitation was undetectable (71). The maximum disorganization affecting the outer layers of the epidermis was found with a 45-degree angle of the incidence radiation, suggesting a role of a transverse wave in the occurrence of cell-to-cell disruption.

43.5.2.2 Low-Frequency Ultrasound

Human SC exposed to 168 kHz at 1.2 W/cm^2 for 15 minutes was examined with epifluorescence microscopy: 20 micrometer cavities were seen and considered to be the consequence of cavitation. As expected, the attenuation coefficient of SC was increased and assumed to be induced by multiplication of cell-to-cell interfaces, intercellular lipids, and entrapped air pockets (61). Confocal images were used to trace sonophoresis (20 kHz) of a hydrophobic and fluorescent compound and showed a marked but spatially discontinuous increase in transepidermal transport. The areas made permeable could not be identified to detect the anatomic structure, but there was focal in-depth penetration of the tracer, whereas despite being located within ultrasound field, adjacent areas did not display any penetration of the tracer. These modifications were restricted to a 1 cm^2 surface below the ultrasound probe (45). In a study combining low-frequency sonophoresis with tape stripping or oleic acid as a chemical enhancer, the penetration of lanthanum nitrate solution through an intercellular pathway was evidenced using transmission electron microscopy (66).

43.5.2.3 Dual-Frequency Ultrasound

Dual-frequency ultrasound, utilizing 20-kHz and 1-MHz wavelengths simultaneously, was found to significantly enhance the size of localized transport regions (LTRs) in both *in vitro* and *in vivo* models (76). The flux of 4 kDa dextran was 3.5- and 7.1-fold greater using 20 kHz + 1 MHz at 6 and 8 minutes, respectively, compared to the use of 20 kHz alone.

43.5.3 Skin Tolerance to Ultrasound

High-intensity focused ultrasound (HIFU) is used to treat and destroy tumors (77). High-intensity ultrasound (in general greater than 5 W/cm^2) can produce coagulation necrosis in biological tissue, and this is the phenomenon mainly exploited in the HIFU ablation technique. In contrast, low-intensity ultrasound (0.125 to 3 W/cm^2) leads to nondestructive heating and can be used for other clinical applications. However, some studies performed on excised skin have used intensities greater than 15 W/cm^2. Therefore, special attention has to be paid to tolerance of skin exposed to medium- and low-frequency ultrasound in the conditions used in transdermal transport studies. As seen earlier, parameters such as intensity, frequency, mode, and duration of ultrasound exposure are of importance, since it is expected that cavitation again can be a key issue in damaging living cells. With medium- and high-frequency ultrasound (1 to 3 MHz) and intensities ranging from 2 to 3 W/cm^2, we observed macroscopic changes in human skin *in vitro* (39). Histological studies have shown multiple areas of keratinocyte necrosis, with epidermal detachment, edema, and degeneration of collagen fibers in the upper part of the dermis, whereas heating alone produced no histological alterations (39, 48, 74). Transmission electron microscopy additionally revealed alterations of intracytoplasmic organites (39), and holes could be demonstrated on the skin surface using scanning electron microscopy (cf. Section 5.1) (41).

In vitro experiments using hairless mice skin exposed to low-frequency ultrasound (20 kHz continuous mode for 4 hours) showed epidermal and dermal lesions despite relatively low intensity (0.2 W/cm^2). Lesions were less marked using pulsed mode (28). Using human skin *in vitro*, we observed normal appearance of skin exposed to 2.5 W/cm^2 and dose-dependent severity of skin