the increase in temperature induced by ultrasound within the donor compartment, control (or not) of the integrity of the skin by clinical and histological studies, and the origin of the membranes used (isolated epidermis, controlled skin thickness using a dermatome, or full-thickness skin). It can be concluded that the increase in percutaneous flux still remains moderate (enhanced ratio generally between 1 and 5) when ultrasound is applied over a short time (5 to 15 minutes) (19, 20). Increasing the exposure time from 10 to 60 minutes has been reported to result in a twofold to fivefold increase in transdermal diffusion of prednisolone *in vitro* with continuous application of 1 MHz ultrasound at 4.3 W/cm^2 (21). Interestingly, in the latter study no ultrasound-induced transdermal transport was found after removing the SC, demonstrating that ultrasound modified SC permeability. With longer exposure of skin to ultrasound (up to 20 hours), providing periodic replacement of donor and receptor compartment solutions to ensure sufficient dissolved gas concentrations and hence to maintain the cavitation activity, permeability may be increased across isolated epidermal sheets from 1 to 13 (22, 23). This was explained by the enhancement of diffusivity of the drug within the SC rather than an increase in the partition coefficient (24). However, it must be emphasized that these latter *in vitro* operating conditions are far from being applicable *in vivo*.

43.3.1.2 *In vivo* Studies

Studies conducted with various animal species have generally shown a significant effect of ultrasound on transdermal transport (Table 43.2). The technique became popular in humans in the United States (14). The efficacy of the technique overall is believed to be explained both by the physical effect of ultrasound itself on subcutaneous injured tissues and by enhancement of transdermal transport (4, 5). However, *in vivo* controlled studies have provided conflicting results (4, 5, 25) (Table 43.3).

In summary, it is clear that high- and medium-frequency ultrasound can increase both *in vitro* and *in vivo* transdermal transport of medium-sized molecules (<500 Da) that are currently used in clinical practice without ultrasound. Increasing locoregional diffusion of nonsteroidal antiinflammatory drugs twofold to tenfold in synovial tissue or muscles may be specifically helpful in sports medicine. However, when it does exist, such enhancement remains moderate or needs a long time exposure to ultrasound, making the use of this range of frequency for systemic transdermal delivery questionable.

TABLE 43.2
***In vivo* Studies of High- and Medium-Frequency Phonophoresis in Animals**

Author and Year (Ref.)	F (kHz)	I (W/cm^2)	Mode	Duration (minutes)	Molecule	Animal	Effect
Vyas 1995 (83)	20000	3	P	15	Diclofenac	rats	Reduction in provoked paw edema
Bommannan 1992 (15)	2000 10000 16000	0.2	C	20	Salicylic acid	guinea pigs	Urinary excretion increase at 10 MHz (×4)and 16 MHz (×2.5) but not at 2
Griffin 1963 (84)	1000	1–3	C	5	Cortisol	swine	Intramuscular concentration ×3
Levy 1989 (85)	1000 1000	1.5 3	C P	3 5	D-mannitol	rats	Increased diffusion ×5–20
Asano 1997 (48)	1000	1–2.5	P C	10–19 10	Indomethacin	rats	Mild increase in blood concentrations
Liao 2016 (82)	1000	2	P	5	Diclofenac	Rat	Reduction in provoked arthritis 70% with ultrasound 90% with ultrasound and microbubbles