ELSEVIER

International Journal of Pharmaceutics 118 (1995) 129–149

international journal of pharmaceutics

Invited Review

Phonophoresis – is it a reality?

V.M. Meidan [a,*], A.D. Walmsley [b], W.J. Irwin [a,*]

[a] *Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET, UK*
[b] *School of Dentistry, University of Birmingham, St. Chad's Queensway, Birmingham B4 6NN, UK*

Received 28 August 1994; revised 17 October 1994; accepted 27 October 1994

Abstract

Phonophoresis – the application of ultrasound to enhance percutaneous drug delivery – has been administered by physiotherapists for over 30 years. However, since the treatment has been conducted on a highly subjective and non-quantitative basis, no clear consensus exists on the effectiveness of the technique nor on the nature of the phonophoretic mechanism. Ultrasonic energy can perturb mammalian tissue via its heating, radiation pressure, cavitation and acoustic microstreaming effects and each of these is discussed in turn in relation to topical drug delivery. Evidence from the literature is reviewed from the separate perspectives of in vitro research, animal studies and human volunteer trials. It may be concluded that phonophoresis is indeed a reality for certain molecules under certain conditions and that ultrasonic heating is its main though not exclusive mechanism of action. However, at present the therapeutic value of the technique is still under question.

Keywords: Enhancement; Mechanism; Percutaneous absorption; Phonophoresis; Sonophoresis; Topical delivery

1. Introduction

The low permeability of the epidermis, and in particular of the stratum corneum (Scheuplein, 1978a,b; Wentz and Downing, 1989), limits the percutaneous delivery of drugs. The facilitation of transdermal drug delivery is an important area of pharmaceutical research and strategies include structural modification to optimise the physicochemical properties of the drug (Chan and Li Wan Po, 1989; Sloan, 1992), diverse pharmaceutical formulations with a range of vehicles (Barry, 1983; Cooper, 1985), the use of permeability enhancers (Barry, 1991) and occlusion (Bucks et al., 1989). Iontophoresis, in which drug flux is promoted by an electrical potential, also attracts considerable interest (Tyle and Kari, 1988). One further approach involves the application of ultrasound to increase the penetration of drugs into tissues – a phenomenon termed phonophoresis (Griffin et al., 1967; Tyle and Agrawala, 1989; Singh and Singh, 1990) or sonophoresis (Bommannan et al., 1992a,b).

For over 30 years, physiotherapists have used the combination of ultrasound plus steroid or analgesic in order to treat a variety of muscular and arthritic conditions. Unfortunately, most of this treatment has been conducted on a subjective and non-quantitative basis (Williams, 1983).

* Corresponding authors.

SSDI 0378-5173(94)00371-8

Frequent limitations of the literature reports have included a lack of proper controls, incomplete accounts of the dosimetry and protocols employed, and the non-calibration of the ultrasound source. Consequently, much of the available data from these studies are inadequate and even contradictory (Famaey, 1985; Pottenger and Karalfa, 1989; Mohl et al., 1990). In the last few years, some higher quality studies have been conducted by both pharmaceutical scientists and medical physicists. However, because individual research groups have used different methods and models, the nature of the ultrasound effect on percutaneous drug penetration is still not fully understood (Newman et al., 1992; Saxena et al., 1993).

It is the aim of this review is to discuss the proposed mechanisms for phonophoresis and review the evidence for the occurrence of phonophoresis from the separate perspectives of in vitro research, animal studies, and human volunteer trials.

2. The biological interactions of ultrasound

2.1. Ultrasound dosimetry

Therapeutic ultrasound is normally generated by a transducer that converts electrical energy to ultrasound by utilising the piezoelectric principle. This effect describes the behaviour of certain ceramics, most commonly formulated of lead zirconate, titanate and crystalline quartz, which expand or contract when a voltage is applied across them. For continuous wave (c.w.) ultrasound production, an alternating voltage of the appropriate frequency is applied to the transducer resulting in the continuous emission of ultrasound of the same frequency. To produce pulsed ultrasound, short bursts of alternating voltage are repeatedly applied to the transducer. The ultrasound emitted by the transducer propagates away from the front face in the direction in which the transducer is pointing. The ultrasound beam can be considered to consist of two regions (Fig. 1). These are the near-field or Fresnel zone and the far-field or Fraunhofer zone. The near field is a cylindrical beam of spatially fluctuating acoustic intensity

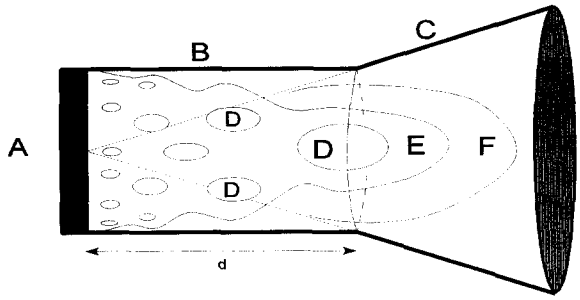

Fig. 1. Ultrasonic profile showing diagrammatic representation of beam shapes and intensities produced from a large transducer typically used in phonophoresis (adapted from Williams, 1983). A, ultrasound generator; B, cylindrical beam in Fresnel zone or near field; C, diverging beam in Frauenhofer zone or far field; D, E, F, acoustic energy contours such that D > E > F. Angle subtended by the divergent beam at the generator surface is 2θ.

caused by the constructive and destructive interference of ultrasonic waves. The far field is a diverging beam exhibiting a central acoustic intensity peak in the centre of the beam which smoothly falls off at either side. The boundary between these two zones occurs at a distance d from the transducer which may be determined from the equation:

$$d = \frac{r^2}{\lambda}$$

where r is the transducer radius and λ represents the wavelength. As long as $r > 5\lambda$, which usually holds true, the beam diverges in the far zone with divergence angle θ (in °):

$$\theta = \frac{35\lambda}{r}$$

Acoustic intensity is not homogeneously distributed throughout the beam space or in time (in the case of pulsed ultrasound) and several intensity parameters have been defined. The spatial-average temporal-average (SATA) intensity is the most commonly employed parameter. It is calculated by dividing the total power in an ultrasonic beam by the beam area and, in the case of pulsed ultrasound, averaged over the pulse repetition

cycle. However, it has to be remembered that at certain sites, much greater ultrasound intensities will develop. The most intense hot spot within an ultrasonic beam occurs in the centre of the beginning of the far zone.

Ultrasonic dosimetry is necessary to quantify the ultrasonic energy emitted by a specific transducer at a set driving voltage, and to gain some idea of the size and location of acoustic hot spots. Perhaps the most direct measurement method involves the use of hydrophones. These are tiny piezoelectric transducers, which are small compared to λ – typically, wavelengths are near to 1.5 mm at 1 MHz for typical propagation velocities of 1500 m s^{-1} in soft tissues and aqueous media. They re-convert the mechanical energy of ultrasound back to electrical potential which can then be visually displayed. These instruments can be used for the absolute determination of intensity at a point but are more commonly used to locate the size and position of hot spots within the beam. Materials do not transmit ultrasound with 100% efficiency and some of the ultrasonic energy is consequently removed from the beam and is converted into thermal energy. This process is termed attenuation. Certain substances such as sound-absorbing rubber (SOAB), castor oil, and carbon tetrachloride attenuate virtually all the energy of an applied beam into heat. One simple method to determine the total acoustic power output of a transducer is to simply direct the ultrasonic beam through a chamber containing castor oil and then to measure the resulting temperature increase with sensitive thermocouples. Alternatively, thermocouple junctions embedded within a small volume of ultrasound-absorbing material can act as point detectors to map out the distribution of ultrasonic energy within a field.

An alternative dosimetry technique involves measuring radiation pressure effects. Any medium or object which is in the path of ultrasound irradiation and absorbs some or all of the beam energy is subjected to a steady force termed the radiation pressure force. This force acts to push the material in the direction in which the wave is propagated. For an object which completely absorbs ultrasound waves, the resultant radiation pressure force it is subjected to is related to intensity according to the relationship:

$$F = \frac{P}{v}$$

where F is the radiation pressure force (in N), P denotes the intensity (in W) and v is the velocity of ultrasound in that medium (in m s^{-1}). The radiation pressure force is greatest in a strongly absorbing medium. Consequently, any liquid with less than 100% transmission efficiency in front of a transducer tends to be pushed away from it so that a continuous circulation is established. This steady circulation is clearly seen in a tank of water if it contains some suspended particles and it is termed quartz wind streaming. Radiation pressure can be quantified by simply measuring the force exerted on a virtually perfectly sound-absorbing target by an ultrasound beam. This can be accomplished by using various types of mechanical systems which are commercially available. In the simplest such detection device, a block of SOAB rubber is mounted on one arm of a sensitive analytical balance and immersed in a tank of degassed water. An acoustically transparent screen is interposed between the ultrasound transducer and the absorber. This prevents the absorber being displaced by quartz wind streaming. Weights are added to counterbalance the pan until the pan containing the absorber returns to the position it occupied before the ultrasound was switched on. So long as the absorber is large compared to the ultrasonic beam then this type of detector measures the total radiation force emitted by the transducer. However, in order to determine the radiation pressure force at any specific point within an ultrasonic field, a ball radiometer is commonly employed. A stainless-steel ball, usually 2–4 mm in diameter is suspended from two thin nylon threads which are designed so that the sphere may be displaced away from the transducer but cannot be displaced from side to side. The deflection of the ball bearing is measured with a travelling microscope and consequently an absolute value of the radiation pressure force over the area of the sphere is obtained.

The mechanism by which ultrasound may enhance percutaneous drug penetration is complex.

When ultrasound interacts with biological tissues it will produce changes by one of four methods. These are heating, radiation pressure, cavitation and acoustic microstreaming (Fig. 2).

2.2. Heating

Ultrasound does not pass through tissues with 100% efficiency and much of the energy is attenuated by the dual processes of scatter and absorption. The amount of heat absorbed depends on the absorption characteristics of the tissue being irradiated and the amount of ultrasonic energy passing through it. The intensities used by ultrasonic therapy devices do heat tissues by a few degrees centigrade and this is thought to be a major factor of any biological effect.

Absorption of the ultrasound depends upon the molecular weight of material and its physical properties. For instance, the absorption of the individual amino acids of haemoglobin is much less than the larger combined structure. Furthermore, absorption increases in a non-linear manner with increasing concentration. Tissues of high collagen content, such as bone and joint capsular structures, undergo greater attenuation, and therefore greater heating, than skin and subcutaneous fat. This is because the attenuation coefficient increases with increasing structural protein content and with decreasing water content. The intensity of the ultrasonic beam decreases exponentially with the depth of tissue. The tissue structure will also scatter and absorb the energy

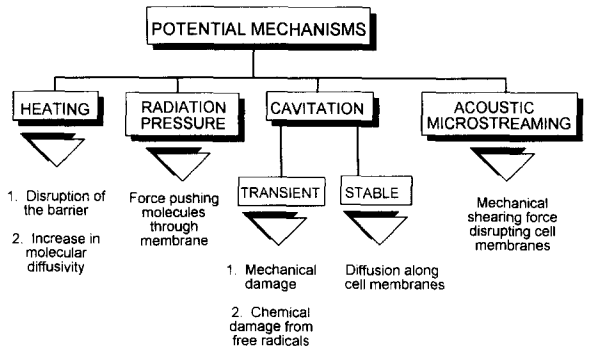

Fig. 2. The potential mechanisms through which phonophoretic enhancement of percutaneous absorption may be mediated.

Table 1
Attenuation of a 1 MHz ultrasound beam (from Ziskin and Michlovitch, 1986)

Tissue	Attenuation (% cm^{-1})
Blood	3
Fat	13
Muscle	24
Blood vessel	32
Skin	39
Tendon	59
Cartilage	68
Bone	96

and this is influenced by frequency with greater attenuation developing at higher frequencies. In the frequency range 0.5–10 MHz, the attenuation coefficients of various tissues have been measured in detail (Goss et al., 1979) and are shown in Table 1. However, attenuation also depends upon frequency, with greater attenuation developing at higher frequencies. In the frequency range 0.5–10 MHz, the attenuation coefficients for tissues other than bone can be fairly well approximated by the relationship:

$$\alpha = \alpha_1 \cdot f^{1.1}$$

where α_1 is the attenuation coefficient at 1 MHz, f represents the frequency in MHz, and α is the attenuation coefficient at the specified frequency (Goss et al., 1979).

An ultrasonic beam induces a rise in the average temperature of the sonicated tissue. A temperature gradient results and heat diffuses away. This temperature rise initiates physiological reflexes resulting in the dilation of blood vessels and an increase in the rate of blood flow through that tissue. This, in turn, will lead to an increase in the rate at which the excess heat is carried away. Heating of tissues can enhance drug transport by directly increasing molecular diffusivity. Furthermore, ultrasound energy will pass into deeper tissues and will induce penetrating hyperthermia. This may increase the solubility of drugs as well as lead to the increased blood flow described above. In vivo, all of these processes can facilitate drug delivery. In in vivo work with rats (Walmsley and Squier, 1991), it was established that 0.7 W cm^{-2} c.w. ultrasound induced a skin

temperature which ranged from 28.5 to 63°C. This heating could be reduced to a cutaneous increase of only 4°C by interposing a circulating water bag (28°C) between the skin and ultrasound transducer. In a sequential study (Walmsley et al., 1992), these two protocols were compared in terms of their effect on triamcinolone acetonide transdermal delivery. It was found that steroid penetration was reduced when the beam had passed through the water path. This work was only conducted as a pilot study and further work is required, preferably including non-sonicated control treatments, in order to elucidate the magnitude of the thermal mechanism.

Redistribution of energy occurs at an interface and this is more pronounced at large mismatches of acoustic impedance such as tissue/bone or tissue/air interfaces. Soft tissues behave like liquids and have a limited ability to conduct shear waves even though their attenuation coefficient for these waves is extremely high. Thus, such ultrasound waves are rapidly absorbed and converted into heat. When an ultrasonic wave meets a tissue/bone interface, part of the wave is reflected into the tissue. The reflected wave is composed of both longitudinal and transverse waves. Any transverse wave component is not propagated and will be deposited as heat at the bone/soft tissue interface. The region close to the bone is the periosteum and this tissue will be rapidly heated and this will be perceived as painful to the patient. Such periosteal pain limits the amount of ultrasonic exposure over bony anatomy. Heat will be dissipated over areas where there is a thick layer of absorbent tissue and this will enable the patient to tolerate the heating. A further area of mismatch is the contact between transducer and skin. A contact gel is usually used and heating will also develop at this interface.

If phonophoresis is caused purely by ultrasonic heating then clearly the process is of no great therapeutic interest since the same penetration enhancement effect can be obtained by using any applied heat source. Ultrasonic heating can be minimised by applying the beam in the form of millisecond on-off pulses or alternatively by applying c.w. ultrasound from a transducer which is continually in motion over the skin surface.

Ultrasonic machines used by physiotherapists generally suffer from poor calibration and the amount of sound energy being emitted may not be truly reflected by the control dial on the machine. Manufacturers often produce a sound-wave signal generation which is a square wave and thus electrically rounded off to produce the sine wave. Any non-sine sound waves will be of high frequency and will be rapidly absorbed by the soft tissues giving an artificially high skin temperature. Pulsed beams of ultrasound will also tend to contain a high component of non-sine sound waves. Such artificial effects will contribute to ultrasonic heating.

2.3. Radiation pressure

Any medium or object which absorbs some or part of a beam of energy is subjected to a force, the radiation pressure force, which tends to push that material in the direction of wave propagation. This will be greatest in a strongly absorbing medium.

At therapeutic intensities, the primary radiation pressure forces are small and are unlikely to damage well-anchored soft tissues. However, such forces are enhanced if a standing wave field is set up. For example if a strong reflector such as bone is present, the ultrasonic beam is reflected back on itself. The reflected wave may interfere with the incident wave to produce a standing wave field. Any resultant radiation pressure forces act over a distance of 0.4 mm in soft tissue at 1 MHz so that unanchored particles which are denser than their suspending medium are pushed to the zones of maximum acoustic pressure. This process has been observed within small blood vessels where blood cells have accumulated together in discrete stationary bands separated by clear plasma (Dyson et al., 1974). This impairment in microvascular blood perfusion can mean that heat is removed less efficiently from sonicated tissues, thus promoting the thermal phonophoretic mechanism.

A common view is that an induced radiation pressure, acting on the penetrant molecules, 'pushes' the drug through the skin, thus accounting for the main non-thermal phonophoretic ef-

fect (Skauen and Zentner, 1984; Rolf, 1988; Singh and Singh, 1990). However, most of the evidence for this hypothesis is derived from studies involving artificial membranes. One group of investigators concluded that radiation pressure played a part in the observed ultrasound-accelerated transport of electrolytes through cellophane membranes (Lenart and Auslander, 1980). A model system of the diffusion of molecules through a water-cooled agar gel demonstrated differences in the phonophoretic effect of ultrasound. (Williams et al., 1990). Sodium diethylenetriaminepentaacetic acid underwent ultrasound-accelerated diffusion whilst sodium pertechnetate did not; both were coordinated to ^{99m}Tc. It has been previously shown in vitro that the absorption of ultrasound by molecules can increase with increasing molecular weight (Kremkau and Cowgill, 1984). The authors suggested that the larger DTPA molecule may be consequently subjected to a greater radiation pressure force which increases its diffusion rate through gel although charge and other differences are also apparent.

As a consequence of radiation pressure, any liquid which is not a perfect transmitter in front of a transducer tends to be pushed away from it so that a continuous circulation is produced. This quartz wind streaming has been implicated as a mechanism in ultrasound-enhanced oxygen diffusion through frog skin (Mortimer et al., 1988) although this may not be an ideal model for poorly permeable human skin. The frog skin was maintained at a fixed temperature during sonication and the deduction that quartz wind streaming was responsible was made by comparing the effect of pulsed ultrasound with c.w. ultrasound.

2.4. Cavitation

Cavitational activity encompasses a wide variety of bubble behaviour ranging from the relatively gentle linear pulsations of gas-filled bodies to the violent and highly destructive formation and collapse of vapour-filled voids and cavities (Flynn, 1964; Nyborg, 1977; Williams, 1983). These bubbles are powered by the energy from the incident acoustic field, about 10% of which may be re-radiated from the bubble as an outgoing spherical wave (Nyborg, 1977). The remainder may be converted irreversibly into heat, or may reappear as shock waves or hydrodynamic shear fields to disrupt biological tissues (Nyborg, 1977; Williams, 1983).

The intensity threshold for the development of cavitation increases with increasing frequency in the MHz range (Esche, 1952). For example, at frequencies up to 0.1 MHz, the threshold intensity in degassed aqueous media is approx. 1 W cm^{-2} (0.1 W cm^{-2} for an aerated sample) whereas at 1 MHz, the corresponding values are in the region 10^2–10^3 W cm^{-2} (Williams, 1983). One explanation for this relationship is that, at higher frequencies, there is not enough time for gas molecules to diffuse into cavities during the extremely short period of the rarefaction phase of the acoustic cycle. A tendency to inhibit cavitation development can also be obtained at lower frequencies if the same quantity of ultrasonic energy is beamed in the form of numerous short but intense pulses. This protocol allows time for any gaseous inclusions which may have begun to grow during the 'on-time' to readily dissipate. In general, the threshold for transient cavitation is higher than for stable cavitation.

In studies involving synthetic membranes, cavitational processes have been claimed to play a part in ultrasound-promoted transport (Lenart and Auslander, 1980; Levy et al., 1989). Also, in in vitro research with frog skin, cavitation was claimed to be primarily responsible for the observed ultrasound effects on ion transport (Dinno et al., 1989). This was deduced from the fact that when cavitation was inhibited by employing degassed bathing fluid, the electrophysiological changes induced by sonication were also suppressed.

Using the intensities and frequencies employed by physiotherapists, cavitational events can develop in mammalian tissues (Ter Haar and Daniels, 1981; Ter Haar et al., 1982). It has been suggested that the phenomenon may play a part in the mechanism of phonophoresis (Tyle and Agrawala, 1989). It has been proposed that the application of higher frequency ultrasound results in proportionally greater energy deposition within the stratum corneum such that this layer is per-

meabilised to drug diffusion by cavitational damage (Bommannan et al., 1992a,b). The authors used 2, 10 and 16 MHz frequencies at 0.2 W cm^{-2} although there is no detailed reference to calibration of the ultrasound source nor the mode used. Following sonication of guinea pig skin at both 10 and 16 MHz, electron microscopy revealed altered cellular morphology in the stratum corneum. Evidence for cavitation was shown as voids within cell tissues. The diameter of these voids was 4 μm. It is unlikely, however, that the process is cavitation as its threshold increases dramatically at the high frequencies employed in the study. Changes in the stratum corneum are probably the result of some other mechanism such as radiation pressure. There is a need for further work in this area where calibration of the source and better understanding of the potential biophysical interaction of the cavitational process is brought to bear on experimental design.

2.5. *Acoustic microstreaming*

It has been demonstrated that a complex steady-state or time-independent streaming pattern is established around a stationary solid cylinder immersed in a fluid through which an acoustic wave is propagating (Holtzmark et al., 1954). These patterns can be exactly duplicated if the ultrasound is switched off and the cylinder is driven to oscillate at exactly the same acoustic frequency. The dimensions of the streaming patterns are determined by the boundary layer thickness, the size of which is estimated to be about 3.6 m in water at an ultrasonic frequency of 0.025 MHz. These small dimensions mean that the velocity gradients, the rate of change of velocity with distance, and hence the hydrodynamic shear stresses are large even though the actual streaming velocities which produce them are relatively low (of the order of a few cm s^{-1}). Consequently, at ultrasonic frequencies, the streaming patterns are usually not visible and the process is termed acoustic microstreaming. Microstreaming can also develop secondarily to cavitation, when the rapid cyclical volume pulsation of a gas bubble results in an unequal distribution of time-independent forces around the bubble surface.

In research with artificial membranes, it has been shown that acoustic microstirring can decrease the concentration gradient in the vicinity of the membrane. Such a mechanism was found to be responsible for the ultrasound-promoted transfer of sodium chloride across a commercial dialysis membrane (Howkins, 1969) as well as being the major factor responsible for the enhanced diffusion of sodium, potassium and calcium chlorides through synthetic cellophane membranes under the influence of 1 MHz ultrasound (Lenart and Auslander, 1980).

Acoustic microstreaming has been generated experimentally in order to determine its biological effects. In practice, a small exponentially tapered metal probe with known tip geometry and diameter is driven to oscillate at the required frequency. Typically, the effect of this application on cellular and intracellular structures is analysed by light microscopy. Using such techniques, it has been demonstrated that the shearing forces associated with acoustic microstreaming can, under certain conditions, perturb intracellular contents, rupture cell membranes or disaggregate clumps of cells. For example, in in vitro experiments it was demonstrated that human platelets could be ruptured by induced acoustic microcurrents (Williams, 1974). Other workers have shown that acoustic microstreaming can disaggregate sheets of human cervical epithelial cells in suspension (Parry et al., 1971). It has been postulated that such shear forces generated by acoustic microstreaming can perturb the skin barrier and thus may play a role in enhancing skin diffusion during phonophoresis (Ziskin and Michlovitch, 1986).

3. The clinical use of phonophoresis

Over the past 30 years, a general method for co-administering drug and ultrasound has gradually been established in physiotherapy clinics. The technique involves applying the therapeutic agent within a viscous ultrasonic coupling medium to the treatment site such that good contact is maintained between the skin surface and ultrasound transducer. The transducer emits the ultrasound beam through the drug layer into the tissue.

Individual therapists prefer different frequencies and intensities. Generally, the ultrasonic frequency applied is between 0.020 and 3 MHz while intensity is rarely greater than 3 W cm^{-2} Frequently, physiotherapists will increase the intensity setting, stopping just short of the patient beginning to feel pain. Alternatively, the transducer can be set into circular or stroking motion over the skin surface, thus reducing the energy deposition per unit area. Sometimes, a beam of pulsed ultrasound is used to achieve the same purpose. The treatment time can be up to 10 min and can be made more complex by administering the drug and ultrasound in a non-simultaneous manner. It may be concluded that in the physiotherapeutic setting, the treatment parameters are highly dependent upon the subjective ideas of individual clinics and therapists.

Phonophoresis in vivo represents a complex system. Ultrasound energy can be partially reflected off bones and from the interfaces between two different tissue types, thus resulting in standing-wave production. It would seem probable that the extent to which this phenomenon occurs may depend to a large extent on the anatomy of the site which is being exposed to phonophoresis. However, to date, no research has been published on the effect of ultrasound standing waves on drug migration, either in vivo or in vitro. One other crucial aspect is the fact that in most phonophoresis work, researchers have neglected to map out the ultrasonic intensities at different points within the beam. Consequently, it is quite possible that certain irradiated tissues have been subjected to ultrasound intensities that are either much lower or much higher than the intensity stated by the authors. This is particularly true if the skin lies within the near zone of ultrasound which is frequently the case in much of the literature reports. Some workers have applied phonophoresis by having the transducer in continuous motion over a small specific area of the skin surface, a protocol which, to some extent overcomes this problem. Another important factor is the ultrasound effect on drug stability and on the relative partition coefficient of the drug between vehicle and skin. All these issues are commonly overlooked in much of the literature.

One further neglected variable relates to the use of a coupling or contact agent which is placed between the ultrasound transducer and the skin surface. This coupling medium is required so that an efficient transfer of ultrasonic energy between the transducer and the skin surface can take place. This is because air is a very poor medium for ultrasound propagation. An ideal coupling agent should exhibit an absorption coefficient similar to that of water and retain a gel or paste consistency at body temperature so that contact is maintained between the transducer and the skin. The presence of air bubbles in the contact medium obviously reduces ultrasound transmission and a good coupling agent will exhibit a low capacity for dissolved gases. In some cases, topical pharmaceutical products containing the active drug have been used as the coupling material. However, the problem with this approach is that, unlike contact media designed specifically for that purpose, topical pharmaceutical products are generally not formulated to optimise their efficiency as ultrasound couplants. Consequently, much of the ultrasound energy may be lost before it reaches the skin. In this situation, the actual amount of energy that reaches the skin can be measured by placing calibrated hydrophones on the skin surface.

One group of investigators examined the transmission of ultrasound energy at frequencies of 0.75, 1.5 and 3 MHz through 41 different common topical pharmaceuticals (Benson and McElnay, 1988). Large variations in ultrasound transmissions were found between different products, as well as in some cases, between the same products at different frequencies. Specifically formulated ultrasound coupling agents generally exhibited better transmission than the other products. Another discernible trend was the fact that gel-type products on the whole, displayed better transmissitivity than other formulations. Seven products were found not to transmit ultrasound at all at any of the tested frequencies. Recently, one American group (Cameron and Monroe, 1992) compared the relative transmission of 25 popularly used coupling products with that of degassed water at a frequency of 1 MHz. The products tested could be grouped together into

two classes; those where transmission was greater than 80% of water (six products) and those where transmission was less than 40% of that of water (13 products). The media that transmitted ultrasound well included products specifically designed as ultrasound couplants, mineral oil, a corticosteroid gel and a methyl salicylate cream. Most of the creams and ointments tested exhibited poor transmission. Furthermore, it was found that adding an equal amount of medium that transmits well to a medium that transmits poorly does not improve transmission.

The importance of vehicular effects has been demonstrated very recently in experiments where hairless mice were immersed in either lignocaine gel or aqueous lignocaine solution and exposed to 0.048 MHz ultrasound at 0.17 W cm^{-2} (Tachibana and Tachibana, 1993). Anaesthetic absorption was evaluated by counting the number of animal reactions to electrical stimulation, both before and after drug application. Enhanced delivery was observed from the aqueous solution but not from the gel base. Unfortunately, the reasons for this difference were not explored.

4. In vitro phonophoresis

In order to gain a greater understanding of the parameters which may influence phonophoresis, workers have conducted in vitro experiments using modified diffusion cells. The advantage of these systems is that a more controlled environment can be developed in which the key variables can be more precisely identified, measured, and controlled. Some researchers have attempted to minimise or control for ultrasonic heating (Brucks et al., 1989; Al Suwayeh and Hikal, 1991) whilst others have not (Kost et al., 1986; Nanavaty et al., 1989; Pinton et al., 1991). Many different drug formulations have been used for in vitro work and a brief description of each type and the results obtained are outlined below.

4.1. Ibuprofen

The Franz diffusion apparatus was specially modified so that a stationary ultrasound transducer could be positioned on top. The epidermis strips were then sonicated for 30 min at both time zero and at 6 h. 1 MHz c.w. ultrasound was used at an intensity of 1 W cm^{-2}. In order to control for ultrasonic heating, two controls were employed: one with no ultrasound and another where heat alone (simulating the ultrasonic heating profile) was applied. The penetration of [^{14}C]ibuprofen was determined by scintillation counting of samples taken from the receptor solution.

It was found that ultrasound enhanced ibuprofen permeation through human epidermis to a greater extent than a control representing comparative heating effects with no ultrasound. Furthermore, the workers claimed that this real phonophoretic effect was associated with no permanent epidermal damage. This was deduced from two different observations. Firstly, the increase in ibuprofen transport developed at the beginning of the experiment only when ultrasound or heat was applied and remained for 10 min after the energy source was switched off. After this time period, drug transport in each of the three protocols was virtually the same. In addition, the measured skin temperature increases induced by sonication did not visually damage the skin and were not of a magnitude that would be expected from theory to be destructive to tissue.

Interestingly, the lag time of drug delivery was not reduced in either the ultrasound or heat application controls. This contradicts the findings from in vivo research with rats and guinea pigs (Kost et al., 1986; Levy et al., 1989). Brucks and co-workers have proposed that ultrasound could enhance drug penetration through hair follicles to a greater extent than through the bulk stratum corneum. The greater number of hair follicles in animal skin as compared to the human breast samples could therefore explain this discrepancy. An observation of this in vitro research was that the phonophoretic effect was less pronounced when the skin/vehicle partition coefficient was reduced following the evaporation of aqueous vehicle components. Unfortunately, the authors did not explore this issue further by actually making measurements of partition coefficient changes. This study may be criticised for having

several technical shortcomings which perhaps cast doubt on some of the conclusions reached by the authors. For example, the distance between the transducer and epidermis sample was not stated while the skin temperature may have been not adequately monitored as the thermocouple was placed behind the epidermis. In addition, the calibration of the ultrasound source was not measured and the likely possibility of standing wave production was not explored.

The same group of investigators have also examined in vitro the effect of sonication on the structure of the epidermis (Nanavaty et al., 1989). Human epidermis was irradiated with 2 W cm^{-2}, 1 MHz c.w. ultrasound for 30 min. The technique of attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was employed to compare sonicated epidermis with non-sonicated epidermis. No control of ultrasonic heating effects was reportedly carried out in this study. Ultrasound induced no major irreversible changes in the epidermis samples but it did cause minor conformational changes in either stratum corneum lipids and/or proteins.

4.2. Indomethacin

Indomethacin was applied in vitro to hairless mouse skin cut from both the thoracic and abdominal areas (Al-Suwayeh and Hikal, 1991) although the authors did not report whether they used full thickness skin samples. The influence of 15 min of 0.48 W cm^{-2} c.w. ultrasound at 1 MHz on indomethacin penetration was investigated by UV. The donor compartment was maintained at pH 6 whilst the receptor compartment was at pH 7.4 thus simulating the in vivo pH gradient. Crucially, ultrasonic heating was minimised by circulating and maintaining both donor and receptor solutions at 37°C. It was reported that indomethacin permeation was increased by 113% during the phonophoresis as compared to the flux of drug before ultrasound exposure. The researchers also examined the system for synergism between phonophoresis and chemical enhancers. In the non-sonicated system, the addition of 5% ethanol or 50% DMSO did not affect indomethacin transport. However, these chemicals did promote drug penetration when combined with ultrasound but it is unclear if this was statistically significant.

4.3. Digoxin

An intensity-drug flux response has been demonstrated by another research team also employing a modified Franz diffusion cell system. (Pinton et al., 1991). Liquid scintillation counting was used to monitor the diffusion of tritiated digoxin through hairless mouse skin under the influence of 10 min 3.3 MHz c.w. ultrasound. However, these workers did not attempt any control of ultrasonic heating. Sonication at 3 W cm^{-2} resulted in increased drug permeation but application of 1 W cm^{-2} ultrasound did not. The authors speculated that this may be due to thermal and/or cavitational effects.

4.4. Mannitol

Successful in vitro phonophoresis of tritiated mannitol through animal skin has been demonstrated (Kost et al., 1986). Dorsal skin samples obtained from rats and hairless mice were placed in a diffusion cell system with the stratum corneum facing a donor solution of tritiated mannitol. Ultrasound was irradiated for 2 h at a frequency of 0.075 MHz. It was determined that, compared to a non-sonicated control, ultrasound enhanced permeation in both rat and mouse skin. Again, no control of ultrasonic heating was attempted but more fundamentally the intensity and mode of the applied ultrasound was not reported.

5. In vivo phonophoresis: the evidence from animal studies

In contrast to work with human volunteers, here it is possible to use more invasive techniques such as tissue excision to derive more information about the nature and mechanisms involved in

ultrasound-enhanced delivery. Numerous phonophoresis animal studies have been carried out over the last 30 years, especially in Eastern Europe (Mayev, 1985). However, in this section, emphasis has been placed on the original work of the 1960s as well as the most recent well-controlled studies. Several different agents have been used and these will be discussed in turn.

5.1. Hydrocortisone

In the 1960s, Griffin and co-workers conducted a series of studies investigating the effect of ultrasound on hydrocortisone delivery through mammalian skin. In their first trial, a hydrocortisone-containing ointment was applied to the shaved paravertebral skin areas of anaesthetised male pigs. Continuous wave 1 MHz ultrasound was then applied for 5 min at intensities of both 1 and 3 W cm^{-2} (Griffin and Touchstone, 1963). The authors concluded that it was possible to drive hydrocortisone ultrasonically into the underlying muscle. In similar further work, the authors stated that hydrocortisone could be delivered to nerve structures by phonophoresis (Griffin et al., 1965). In addition, it was claimed that more drug could be delivered to the underlying nerves and muscle by employing a low-intensity long-duration exposure rather than a high-intensity low-duration exposure (Griffin and Touchstone, 1968). A frequency of 0.25 MHz was stated to be the most effective for hydrocortisone phonophoresis into muscle and nerve (Griffin and Touchstone, 1972). All these results have generated considerable publicity and interest in phonophoresis over the years (Quillen, 1980, 1982; Antich, 1982). However, since 1963 many other researchers have criticised these findings (Williams, 1990). It has been suggested that the chemical assay system employed was inherently variable and consequently, the given results could have been obtained simply by statistical chance. Since the original study did not report information on errors, it is difficult to refute these criticisms.

Hydrocortisone phonophoresis has been examined more recently in a trial involving 15 mongrel dogs (Davick et al., 1988). Samples of cream containing 5 and 10% tritiated hydrocortisone were applied separately to the surface of the medial aspect of the knee of the animals. 0.5 W cm^{-2} c.w. ultrasound was applied at 0.87 MHz for 8 min. A non-sonicated drug application acted as a placebo. The treated skin site was then completely excised and processed so that autoradiography could be used to quantify the presence of hydrocortisone. The application of 10% hydrocortisone with ultrasound resulted in significantly more drug penetration into the viable epidermis than 10% hydrocortisone alone. For the five sonicated animals, hydrocortisone was detected on average in 3.44% of the cells in the viable epidermis as opposed to no steroid detected in three out of four control animals (in one of the control dogs, hydrocortisone was detected in 0.48% of the viable epidermal cells). Unfortunately, no control of induced heating was undertaken in this study. Consequently, the observed increase in drug permeation may have been a purely thermal effect and not necessarily ultrasound specific. Furthermore, as the work was conducted on mongrel dogs, it is likely that the knee dimensions differed between individual animals and this is a major limitation of this study.

The penetration of hydrocortisone into canine knee has also been assessed by Muir et al. (1990) in a refined study involving 24 purebred greyhounds. 2.75 W cm^{-2} ultrasonic energy at 1 MHz frequency was beamed to the surface of canine joints through a layer of ultrasonic coupling gel containing 10% hydrocortisone. Fluorescence polarisation was used as the analytical technique. No statistically significant differences were found between phonophoretic applications and non-sonicated control treatments.

5.2. Mannitol

The effect of ultrasound on the transdermal transport of mannitol through shaved dorsal rat skin has been examined (Levy et al., 1989) using a moving ultrasonic applicator (1 MHz) and operated at an intensity of 1.5 W cm^{-2} c.w. for 3 min. The permeation of tritiated mannitol was determined by liquid scintillation counting of the urine. In an important step, ultrasonic heating was eval-

uated by placing a temperature probe on the treated surface sites immediately after sonication. Ultrasound was found to enhance mannitol absorption by about 11 times compared to a non-sonicated control, within 2 h following treatment. Another effect due to ultrasound was a shortening of the lag time. Sonication increased skin surface temperatures by not more than 1–2°C and consequently cavitation and acoustic microcurrents in the skin were claimed to have caused the promoted drug flux.

5.3. Inulin

In similar rat experiments with inulin – a high molecular weight polysaccharide, 1 MHz pulsed ultrasound was applied to the skin at an intensity of 3 W cm^{-2} for 5 min (Levy et al., 1989). During the first hour, the secretion rate of tritiated inulin was 5-fold higher in the ultrasound-treated group than in the controls. Phonophoretic treatment shortened the lag phase period and caused only small increases in skin surface temperature. Histological evaluation showed no visible differences between ultrasound-exposed and control skin. Again, the authors stated that microstreaming and/or cavitational changes in the skin caused the enhanced delivery.

5.4. Physostigmine

The use of pulsed 1 MHz ultrasound at 3 W cm^{-2} for 5 min has also been shown to significantly promote the dorsal topical absorption in both rats and guinea pigs of physostigmine – a lipophilic anticholinesterase (Levy et al., 1989). Reduced lag times were observed in the guinea pigs but not in the rats where physostigmine absorption was very rapid. The reversible nature of the ultrasound enhancement was deduced from the observation that sonication of guinea pigs 1 h before drug application did not alter the penetration rate of physostigmine through the skin. Also, the absorption rate of physostigmine in guinea pigs 5 h after sonication was the same in both the treated animals and controls, as opposed to the large difference in absorption rate detected just after sonication.

5.5. Salicylic acid and lanthanum hydroxide at high frequencies

The hairless guinea pig model has been employed to investigate the in vivo phonophoresis of salicylic acid (Bommannan et al., 1992b). A gel containing ^{14}C-labelled salicylic acid acted as a coupling agent between the transducer and treatment site. 0.2 W cm^{-2} c.w. ultrasound was then applied at frequencies of 2, 10, and 16 MHz for 5 or 20 min. Drug absorption was quantified by the amount of radiolabelled salicylic acid present in stratum corneum tape strips and urine. Sonication for 20 min at 2 MHz induced no significant increase in salicylic acid penetration over passive diffusion. However, ultrasound treatment at 10 and 16 MHz did significantly promote drug permeation by 4- and 2.5-fold, respectively. The lag time was also reduced with these frequencies. A shorter 5 min period of sonication at the higher frequencies also resulted in enhanced drug delivery as compared to the control although the degree of enhancement was lower than the 20 min treatment. When the skin sites were presonicated at 10 and 16 MHz and then subsequently exposed to the drug, enhanced delivery was also observed indicating that the phonophoretic effect was not immediately reversible.

In a homogeneous medium, high-frequency ultrasound will be attenuated over much shorter distances than lower frequency ultrasound (Williams, 1983). These researchers explained the effectiveness of high frequency ultrasound in terms of this protocol leading to proportionally greater energy deposition in the stratum corneum, thus permeabilising it. However, the authors did not fully substantiate this idea since the skin represents an acoustically heterogeneous medium. Furthermore, the group's finding that surface skin temperature increased by not more than 1°C during sonication to some extent casts doubt on this assumption.

In a follow-up study (Bommannan et al., 1992a), an identical ultrasound protocol was used to determine the nature of the phonophoretic mechanism. Transmission electron microscopy was used to track the skin permeation of the electron-dense tracer colloidal lanthanum hydrox-

ide. Under non-sonicated conditions, the tracer was found not to penetrate the stratum corneum. However, at higher frequencies, the penetration of tracer by an intercellular route through the epidermis to the upper dermis was observed. This enhancement of drug delivery was not associated with any adverse cellular morphology except at the highest frequency (16 MHz) for the longest time period (20 min). Since surface skin temperature increases of not more than 1°C were measured, the authors concluded that cavitational processes must be inducing the detrimental observations. However, it is highly unlikely that cavitation will develop at such intensity and frequency in mammalian tissues and therefore other mechanical effects must be responsible.

Recently, a series of follow-up studies have been published as short abstracts (Menon et al., 1992, 1993a,b). One study, (Menon et al., 1992), reported the effect of 5 min of 0.1 W cm^{-2} ultrasound at 15 MHz on hairless mouse skin. Skin biopsies were taken immediately after irradiation, as well as after 24, 48 and 96 h, and examined by electron microscopy. RuO_4 staining was used to investigate stratum corneum lammellar bilayer structure. Ultrasound was found to alter the usually compact structural organisation of lamellar body derived contents at the stratum granulosum-stratum corneum cell interface, producing distinct domain separation. These changes were present in the 24 h samples but not in the 48 and 96 h samples which were comparable to the non-sonicated controls. The authors noted the potential therapeutic significance of the reversible nature of the ultrasound effect. It was concluded that lamellar phase separation of stratum corneum lipids may be responsible for the phonophoretically enhanced penetration of colloidal lanthanum previously reported. These perturbations may be caused by radiation pressure forces or microstreaming. It has been postulated that the oscillating molecular motion associated directly with the propagation of the ultrasonic wave can liquify thixotropic structures such as the mitotic spindle during cell division (Williams, 1983). The ultrasonically induced changes may be due to similar liquification of thixotropic structures within the epidermis.

In recent work with this model (Menon et al., 1993a,b), the investigators used calcium ion capture cytochemistry to show that phonophoresis reduces the concentrations of epidermal calcium ions. The consequent removal of the calcium gradient results in the promotion of lamellar body secretion from the upper stratum granulosum. In addition, phonophoresis was found not to alter transepidermal water loss. These latest studies on the effect of high frequency ultrasound on mouse skin have been reported only briefly (Menon et al., 1992, 1993a,b). More information on these research techniques is required before a better assessment of the findings can be made.

5.6. Insulin

In the in vivo studies discussed so far, ultrasonic heating may have been monitored but not controlled. One Japanese research team developed an ingenious method in order to minimise ultrasonic heating in their experiments with insulin (Tachibana and Tachibana, 1988, 1991). Fasted, hairless mice were partially immersed in a 100 ml aqueous solution of 20 U ml^{-1} insulin. It was stated that the animals were then exposed for 5 min to 0.048 MHz ultrasound at one of two intensity ranges (3000–5000 and 5000–8000 Pa). The ultrasound mode was not stated but was presumably c.w. The exact intensity being irradiated on to each individual animal was measured by a hydrophone fixed beside each mouse. The controls consisted of insulin immersion with no ultrasound, saline immersion with no ultrasound, and saline immersion with ultrasound. Crucially, all the solutions were at a temperature of 37°C. Drug delivery was evaluated by glucose analysis of blood samples. Compared to the controls, ultrasound plus insulin immersion resulted in statistically significant reductions in blood glucose concentrations which persisted for 4 h. The plasma glucose level decrease was more pronounced at the higher intensity application indicating that the intensity parameter may control permeation.

One of the disadvantages of this study is that water itself acts as a potent transdermal enhancer. Consequently, another more conventional phonophoresis study with insulin was carried out

(Tachibana, 1992). A metal cup that was attached to the ultrasonic transducer was filled with 3 ml of 40 U ml^{-1} insulin solution and placed on the skin sites of anaesthetised diabetic rabbits. Ultrasound (5000 Pa, 0.105 MHz) was then irradiated on to the skin site in pulsed 5 s on, 5 s off mode. The treatment period was 90 min. The transducer and drug reservoir employed were kept at 4°C representing only some control over ultrasound-induced heating. Drug absorption was evaluated by insulin and glucose analysis of blood samples taken in the 6 successive hours following sonication and the authors deduced that ultrasound enhanced the absorption of insulin and decreased blood glucose levels to a statistically significant extent. Furthermore, microscopic analysis of treated skin biopsy specimens indicated that no histological changes had been induced and that the stratum corneum was intact.

It must be noted that in both these articles, the intensity values are stated in Pascals which cannot be directly converted into the more usual W cm^{-2}. Furthermore, the insulin is unlikely to penetrate the skin barrier without substantial enhancement and the above findings could be explained. perhaps, in terms of ultrasonic waves directly causing changes to the islets of Langerhans.

5.7. *Indomethacin*

Indomethacin phonophoresis has been investigated by Miyazaki and co-workers in a series of studies (Miyazaki et al., 1991a,b, 1992b). In these experiments, 1 g of 1% indomethacin ointment was applied to the shaved abdominal areas of anaesthetised rats. 1 MHz c.w. ultrasound was then directed at the treated area for between 5 and 20 min with a range of intensities (0.25, 0.5, 0.75, and 1 W cm^{-2}). Control animals underwent the same procedure except that the ultrasound transducer was not applied. Blood samples were taken by cardiac puncture in the hours following phonophoresis and indomethacin levels were quantified by HPLC. Both skin surface temperature and hypodermis temperature were monitored during the experiment. In addition, microscopic evaluation of excised skin samples were undertaken following their exposure to phonophoresis. It was found that 0.75 W cm^{-2} was the intensity that induced the greatest absorption enhancement, whilst 10 min irradiation was the optimal duration for phonophoresis. However, the authors suggested that 0.5 W cm^{-2} was the preferred intensity since its application for 10 min did not cause marked skin temperature increases or any significant skin tissue damage. With this protocol, indomethacin delivery was enhanced 2.7 times relative to the control. Progressively worsening skin damage was observed as the intensity and duration of ultrasound administration were increased. Thus, epidermal swelling and dermal interstitial degeneration were noted at 0.75 W cm^{-2} for 10 min; whilst 1 W cm^{-2} ultrasound resulted in epidermal atrophy and dermal collagen fibre degeneration, both accompanied by associated necrosis. The authors proposed that this damage at 1 W cm^{-2} reduces skin permeability and explains why this intensity is not as phonophoretically effective as 0.75 W cm^{-2} This is despite the fact that damage generally reduces the effectiveness of the skin as a barrier.

In further work with the same model (Miyazaki et al., 1992a), the effect of 1:3 pulsed output was examined, again at different intensities and durations. Pulsed ultrasound was found to be less effective as an enhancer than the same energy delivered in continuous mode. This is probably due to the pulsed mode causing less tissue heating. In a key step, the experimenters investigated the effect of heat alone on indomethacin penetration. The skin was heated in such a way as to simulate the ultrasonically induced time-temperature profile in the hypodermis. This heating did not influence indomethacin absorption. Consequently, the authors suggested that a non-thermal mechanism was causing the phonophoresis. However, one major limitation of this method is that ultrasonic heating may have developed in the stratum corneum.

The above results somewhat contradict the findings of another indomethacin study where phonophoresis was not observed (Pratzel et al., 1986). These workers applied indomethacin gel to the shaven backs of anaesthetised pigs. Over a 5 h period, ultrasound at 1 W cm^{-2} was adminis-

tered for 30 min at the start of the experiment and subsequently for 10 min at 30 min intervals. The whole experiment lasted for 5 h. Since the ultrasound mode and frequency were not stated, it is difficult to compare the results but such differences in these parameters may explain the discrepancies.

5.8. *Amphotericin B*

In order to assess the effectiveness of phonophoresis, one research team compared ultrasound with an established chemical enhancer (Romanenko and Aravisky, 1991). Amphotericin B ointment was applied to the sides of 24 shaved guinea pigs in vivo. The first group of animals were subjected to 1 W cm^{-2} ultrasound at 2.64 MHz for 15 min at both continuous and pulsed mode. A control group of animals was treated with amphotericin B without ultrasound. A third group of guinea pigs received applications of dimethyl sulphoxide (DMSO) followed by amphotericin. Ultrasound-induced heating was not monitored or controlled. The skin and subcutaneous fatty tissues were sampled at regular time intervals up to 72 h after treatments. Diffusion in agar with test cultivation of *Candida utilis* was employed as the analytic technique. It was found that, compared to the control treatment, both DMSO pre-treatment and phonophoresis resulted in enhanced amphotericin penetration. DMSO treatment was a better enhancer than phonophoresis during the first 48 h but phonophoresis was more effective in maintaining amphotericin levels after this period. The results from this study lead to the idea of combining phonophoresis and DMSO in order to improve drug delivery further.

6. In vivo phonophoresis: the evidence from human volunteer studies

Most human volunteer trials have been conducted on an extremely subjective and non-quantitative basis. For example, in some reports, at least some of the parameters of the applied ultrasound beam have not been stated (Cameroy, 1966; Chatterjee, 1977; Antich et al., 1986; Halle et al., 1986; Moldover and Danish, 1986; Stratford et al., 1989; Roques et al., 1992). Other authors have omitted to conduct controls (Coodley, 1960; Kahn, 1980; Wing, 1982; Smith et al., 1986).

In one trial involving 102 arthritic patients (Griffin et al., 1967), hydrocortisone with 1 MHz ultrasound was compared to 1 MHz ultrasound alone. Treatment efficacy was evaluated by pain and range of motion criteria that assessed limb movement. It was determined that the combination of ultrasound plus steroid was more effective than ultrasound alone ($P = 0.001$, χ^2 test). In a retrospective study of 285 patients treated for several different types of inflammatory conditions (Kleinkort and Wood, 1975), the authors compared 1 MHz ultrasound plus 10% hydrocortisone versus 1 MHz ultrasound plus 1% hydrocortisone. Statistical analysis indicated that phonophoresis of 10% steroid was superior to phonophoresis of 1% steroid. However, in both studies, there were no non-sonicated control groups and the beam intensity was varied according to individual patient tolerance. Moll divided 52 patients, each suffering from various painful conditions, into three groups (Moll, 1977). One group received 0.87 MHz ultrasound together with a gel containing 32 mg of dexamethasone and 60 ml of 2% lignocaine jelly. Another group was treated with ultrasound plus a placebo whilst yet a third group received sham ultrasound with placebo gel. It was found that the use of lignocaine and dexamethasone with ultrasound was the most beneficial protocol but much larger scale trials would be required in order to establish conclusive statistical significance.

In recent years, a range of superior studies has been conducted by McElnay and co-workers. In one trial (McElnay et al., 1985a, 1987), 3 g of 0.025% fluocinolone acetonide gel was applied to the flexor surface of the forearms of 12 human volunteers. 2 W cm^{-2} pulsed output ultrasound at 0.87 MHz was administered for 5 min. The study was of a double blind, sham ultrasound controlled, cross-over nature. The skin blanching test was used to evaluate drug absorption. It was found that ultrasound treatment resulted in sig-

Table 2
A summary of the findings from the phonophoretic literature

Compound	Molecular mass (Da)	υ (MHz)	Intensity (W cm^{-2})	% maximal enhancement	End-point evaluation	Reference
Amphotericin B	945	2.64	P 1	36	skin concentrations 1 h after application	Romanenko and Aravisky (1991)
Benzocaine/benzethonium chloride	165.2/448.1	1.1	0.25 [a]	0	electrical sensory perception	Williams (1990)
Benzydamine HCl	345.9	0.75	1.5	0	residual amounts in vehicle	Benson et al. (1989)
		1.5	1.5	0		
		3	1.5	0		
			P 1	0		
Dibucaine	379.9	1.1	0.25 [a]	0	electrical sensory perception	Williams (1990)
Ethyl nicotinate	151.2	3	1	79	AUC vasodilation from LDV	Benson et al. (1991)
Fluocinolone acetonide	425.5	0.87	P 2	32	AUC from skin blanching	McElnay et al. (1987)
Hexyl nicotinate	207.3	3	1	0	AUC vasodilation from LDV	Benson et al. (1991)
Hydrocortisone	362.5	0.87	1	272	liver concentrations 30 min after application	Gatev (1970)
			0.5	617	epidermal cell radiograph	Davick et al. (1988)
		1	2.75	0	blood concentrations 10 min after application	Muir et al. (1990)
Ibuprofen	206.3	1	1	82	flux rate over 30min	Brucks et al. (1989)
Indomethacin	357.8	1	0.75	240	AUC blood concentrations	Miyazaki et al. (1992a)
			0.48 [a]	113	mean flux over 90 min	Al-Suwayeh and Hikal (1991)
Insulin (neutral)	6000	0.105		684	maximum blood drug concentration during experiment	Tachibana (1992)
Inulin	5200	1	P 3	400	urinary secretion	Levy et al. (1989)
Lignocaine	288.8	0.87	P 2	0	time to onset of anaesthesia	McElnay et al. (1985b)
Lignocaine/prilocaine	288.8/257	1.1	0.25 [a]	0	electrical sensory perception	Williams (1990)
		1.5	P 1	56	time to total sensation recovery	Benson et al. (1988)
		3	P 1	101	time to partial sensation recovery	Benson et al. (1988)
Mannitol	182.17	1	1.5	10200	urinary secretion	Levy et al. (1989)
Methyl nicotinate	137.1	3	1	59	AUC vasodilation from LDV	Benson et al. (1991)
				104	AUC vasodilation from LDV	McElnay et al. (1993)
Salicylic acid	138.1	2	0.2	0	stratum corneum concentration from tape strips	Bommannan et al. (1992b)
		10	0.2	315		
		16	0.2	153		

[a] Denotes that controlling for thermal effects was conducted by minimising ultrasonic heating. P indicates pulsed ultrasound otherwise continuous mode ultrasound was used.

nificantly enhanced steroid penetration. Unfortunately, the magnitude of the effect was small and as such it is not likely to result in greater therapeutic efficacy in the clinical setting.

Similarly controlled studies on lignocaine phonophoresis in human forearm skin have been conducted using a 2 W cm^{-2} pulsed output at 0.87 MHz for 5 min (McElnay et al., 1985b). Compared to non-sonicated applications of drug, a statistically insignificant increase in anaesthesia onset rate was reported when ultrasound energy was applied. In sequential work with a cream containing both lignocaine and prilocaine (Benson et al., 1988), these workers identified a significant increase in anaesthesia duration.

Recently, this group (Benson et al., 1991) investigated the influence of 5 min of 1 W cm^{-2}, 3 MHz c.w. ultrasound on the percutaneous absorption of ethyl nicotinate, methyl nicotinate, and hexyl nicotinate across human forearm skin. The workers employed laser Doppler velocimetry in order to measure nicotinate absorption. All three nicotinate esters were found to undergo ultrasound-enhanced delivery, though for hexyl nicotinate the difference was not statistically significant. In further trials with methyl and hexyl nicotinates (Murphy and Hadgraft, 1990), a method of sonication followed by drug application was compared to drug application followed by sonication. For hexyl nicotinate, which is lipophilic, the rate-limiting step was assumed to be partitioning from the lipid-rich stratum corneum. This process was not affected by ultrasound pre-treatment but was enhanced by ultrasound postreatment. The authors suggested that ultrasound may be able to enhance the rate of partitioning of drug out of the intercellular lipids once a reservoir has been formed. For methyl nicotinate, the major barrier to penetration are the structured stratum corneum lipids and thus both ultrasonic treatments similarly enhanced methyl nicotinate absorption. The authors postulated that this may be due to the fluidisation of the stratum corneum lipids by ultrasound.

In other double-blind, placebo-controlled trials (Benson et al., 1986, 1989), these workers evaluated the effect of ultrasound at different modes, intensities and frequencies on benzydamine absorption. It was found that sonication did not influence benzydamine absorption across human flexor surface skin. Negative findings have also been reported in recent attempts to phonophoretically deliver trolamine salicylate (Oziomek et al., 1991).

In all of the above trials, ultrasonic heating was not monitored or controlled thus yielding no information on the mechanisms involved in any observed phonophoresis. One research team developed a sensory perception method in which anaesthetic absorption could be reproducibly quantified by measuring the pain threshold associated with an applied small electric shock (Williams et al., 1987). In a follow up study (Williams, 1990), this system was used to determine the effect of 50 min of 0.25 W cm^{-2}, 1.1 MHz ultrasound on the penetration of Americaine, containing 20% benzocaine and 0.1% benzethonium; Emla cream, containing 2.5% lignocaine and 2.5% prilocaine; and Nupercainal containing 1% dibucaine. A water path maintained at 37°C was placed between the transducer and skin in order to minimise the thermal effects of sonication. It was found that ultrasound induced no detectable effects upon the penetration rates of any of the agents tested.

7. Conclusions

A summarised compilation of important studies is presented in Table 2. Only those which have incorporated adequate controls and a comprehensive account of the applied ultrasonic protocols have been included. The enhancement values represent the percentage increase over the control value so that 100% enhancement indicates a doubling in drug absorption as measured by the appropriate end-point. Where a research group has conducted more than one method or experimental type, the enhancement figure listed is the maximal value obtained using the most optimal conditions. It can be seen that due to the multiplicity of drugs, methods, models and endpoint evaluation techniques used, it is difficult to discern any trends between ultrasonic frequency, intensity, molecular structure and the degree of

enhancement. The authors of this review have attempted to fit various mathematical models to these data but no meaningful relationship could be found between the ultrasonic/molecular parameters and the magnitude of the enhancement effect.

It can be seen that the most dramatic enhancement effects have been documented with molecules that are either too large (inulin, insulin) or too hydrophilic (mannitol) to normally permeate through the stratum corneum – perhaps suggesting that transfollicular pathways are more susceptible to ultrasonic enhancement than are transcellular processes. However, these observations have not been confirmed elsewhere and they may be due to poor experimental design such as animal ingestion of the applied substance, or direct ultrasonic effects on pancreatic activity in the case of insulin.

Nevertheless, there are a large number of studies which report modest but significant enhancement for drugs that can normally penetrate the skin. This indicates that phonophoresis is indeed a reality for certain molecules under specific conditions. The main component of phonophoresis is ultrasonic heating. It has been established that a skin surface temperature increase of 10°C will double water permeability and enhance the absorption of a range of substances by between 1.4 and 3 times in vivo (Scheuplein, 1978a). Ultrasonic heating on a smaller magnitude is largely responsible for the modest improvements in drug delivery reported in the literature. As well as intensity, frequency and mode, the degree of heating is greatly influenced by subtle factors such as transducer motion, anatomical site as well as the quantity and type of vehicle/coupling medium. Variations in all these explain the conflicting results obtained by different workers. It is interesting to note that when heating was removed as an artifact of phonophoresis, the absorption of five different anaesthetic molecules was not affected by ultrasound (Williams, 1990).

However, ultrasound-enhanced delivery has also been documented in experiments where negligible ultrasonic heating developed (Al-Suwayeh and Hikal, 1991; Bommannan et al., 1992b). This indicates the existence of other phonophoretic mechanisms. It has been shown that the enhanced absorption of methyl nicotinate, hexyl nicotinate and salicylic acid could be obtained by sonicating tissues prior to drug application (Murphy and Hadgraft, 1990; Bommannan et al., 1992b; McElnay et al., 1993). This means that drug molecules are not being pushed through by radiation pressure since accelerated delivery occurred after the ultrasound had been switched off. In these situations, radiation pressure or other effects such as first order forces, acoustic microstreaming (and stable cavitation at lower frequencies) are perturbing the stratum corneum structure, thus permeabilising it to drug delivery. There is good evidence that these changes are reversible and hence the process is potentially useful within the clinical setting.

It can be concluded that phonophoresis does exist and that it operates by several different mechanisms. However, at present its therapeutic value is still under question. Clearly, more research needs to be conducted in order to identify the role of the various parameters which influence phonophoresis so that the process may be optimised. This can best be achieved by carrying out in vitro skin transport studies in conjunction with analytical techniques such as ATR-FTIR spectroscopy and differential scanning calorimetry. In particular, there is a need for greater collaboration between medical physicists and pharmaceutical scientists so that a knowledge of the biophysical interactions of ultrasound can be linked to established pharmaceutical models and principles.

Acknowledgements

We are grateful to Dr A.R. Williams (University of Manchester) for helpful discussion.

References

Al-Suwayeh, S. and Hikal, A.H., Influence of ultrasound on in vitro diffusion of indomethacin through hairless mouse skin. *Pharm. Res.* 8 (1991) S-140.

Antich, T.J., Phonophoresis: The principles of the ultrasonic driving force and efficacy in treatment of common orthopaedic diagnoses. *J. Orthop. Sports Phys. Ther.*, 4 (1982) 99–102.

Antich, T.J., Randall, C.C., Westbrook, R.A., Morrisey, M.C. and Brewster, C.E., Physical therapy treatment of knee extensor mechanism disorders: Comparison of four treatment modalities. *J. Orth. Sports Phys. Ther.*, 8 (1986) 255–259.

Barry, B.W., *Dermatological Formulations: Percutaneous Absorption*, Dekker, New York, 1983.

Barry, B.W., Modern methods of promoting drug absorption through skin. *Mol. Aspects Med.*, 12 (1991) 195–241.

Benson, H.A.E. and McElnay, J.C., Transmission of ultrasound energy through topical pharmaceutical products. *Physiotherapy*, 74 (1988) 587–589.

Benson, H.A.E., McElnay, J.C. and Harland, R., Phonophoresis of lignocaine and prilocaine from Emla cream. *Int. J. Pharm.*, 44 (1988) 65–69.

Benson, H.A.E., McElnay, J.C. and Harland, R., Use of ultrasound to enhance percutaneous absorption of benzydamine. *Phys. Ther.*, 69 (1989) 113–118.

Benson, H.A.E., McElnay, J.C., Harland, R. and Hadgraft, J., Influence of ultrasound on the percutaneous absorption of nicotinate esters. *Pharm. Res.*, 8 (1991) 204–209.

Benson, H.A.E., McElnay, J.C., Whiteman, J. and Harland, R., Lack of effect of ultrasound on the percutaneous absorption of benzydamine. *J. Pharm. Pharmacol.*, 38 (1986) 73P.

Bommannan, D., Menon, G.K., Okuyuma, H., Elias, P.M. and Guy, R.H., Sonophoresis: 2. Examination of the mechanism(s) of ultrasound enhanced transdermal drug delivery. *Pharm. Res.*, 9 (1992a) 1043–1047.

Bommannan, D., Okuyama, H., Stauffer, P. and Guy, R.H., Sonophoresis: 1. The use of high frequency ultrasound to enhance transdermal drug delivery. *Pharm. Res.*, 9 (1992b) 559–564.

Brucks, R., Nanavaty, M., Jung, D. and Siegel, F., The effect of ultrasound on the in vitro penetration of ibuprofen through human epidermis. *Pharm. Res.*, 6 (1989) 697–701.

Bucks, D.A.W., Maibach, H.I. and Guy, R.H., Occlusion does not uniformly enhance penetration in vivo. In Bronaugh, R.L. and Maibach, H.I. (Eds), *Percutaneous Absorption. Mechanisms – Methodology – Drug Delivery*, 2nd Edn, Dekker, New York, 1989, pp. 77–89.

Cameron, M.H. and Monroe, L.G., Relative transmission of ultrasound by media customarily used for phonophoresis. *Phys. Ther.*, 72 (1992) 142–148.

Cameroy, B.M., Ultrasound enhanced local anesthesia. *Am. J. Orthop.*, 8 (1966) 47.

Chan, S.Y. and Li Wan Po, A., Prodrugs for dermal delivery. *Int. J. Pharm.*, 55 (1989) 1–16.

Chatterjee, D.S., A double-blind clinical study with benzydamine 3% cream on soft tissue injuries in an occupational health centre. *J. Int. Med. Res.*, 5 (1977) 450–458.

Coodley, E.L., Bursitis and post-traumatic lesions: Management with combined use of ultrasound and intra-articular hydrocortisone. *Am. Pract.*, 11 (1960) 181–188.

Cooper, E., Vehicle effects on skin penetration. In Bronaugh, R.L. and Maibach, H.I. (Eds), *Percutaneous Absorption. Mechanisms – Methodology – Drug Delivery*, 1st Edn, Dekker, New York, 1985, pp. 525–540.

Davick, J.P., Martin, R.K. and Albright, J.P., Distribution and deposition of tritiated cortisol using phonophoresis. *Phys. Ther.*, 68 (1988) 1672–1675.

Dinno, M.A., Crum, L.A. and Wu, J., The effect of therapeutic ultrasound on electrophysiological parameters of frog skin. *Ultrasound Med. Biol.*, 15 (1989) 461–470.

Dyson, M., Pond, J.B., Woodward, B. and Broadbent, J., The production of red blood cell stasis and endothelial damage in the blood vessels of chick embryo heated with ultrasound as a stationary wave field. *Ultrasound Med. Biol.*, 1 (1974) 133–148.

Esche, R., Untersuchungen der Schwingungskavitation in Flussigkeiten. *Acustica*, 2 (1952) 208–218.

Famaey, J.P., Medical phonophoresis with anti-inflammatories. *J. Belg. Med. Phys. Rehabil.*, 8 (1985) 179–183.

Flynn, H.G., Physics and acoustic cavitation in liquids. In Mason, W.P. (Ed.), *Physical Acoustics*, Vol. 1B, Academic Press, New York, 1964, pp. 57–172.

Gatev, S.P., Ultraphonophoresis and its effect on the distribution and deposition of hydrocortisone-^{14}C in experiments. *Vor. Kurortol. Fizioter. Lech. Fiz. Kult.*, 35 (1970) 42–45.

Goss, S.A., Frizzel, L.A. and Dunn, F., Ultrasonic absorption and attenuation in mammalian tissues. *Ultrasound Med. Biol.*, 63 (1979) 181–186.

Griffin, J.E. and Touchstone, J.C., Effects of ultrasonic frequency on phonophoresis of cortisol into swine tissues. *Am. J. Phys. Med.*, 51 (1972) 62–78.

Griffin, J.E. and Touchstone, J.C., Low-intensity phonophoresis of cortisol in swine. *Phys. Ther.*, 48 (1968) 1336–1344.

Griffin, J.E. and Touchstone, J.C., Ultrasonic movement of cortisol into pig tissues. 1. Movement into skeletal muscle. *Am. J. Phys.*, 42 (1963) 77–85.

Griffin, J.E., Enternach, J.L., Price, R.E. and Touchstone, J.C., Patients treated with ultrasonic driven hydrocortisone and with ultrasound alone. *Phys. Ther.*, 47 (1967) 594–601

Griffin, J.E., Touchstone, J.C. and Liu, A.C.-Y., Ultrasonic movement of cortisol into pig tissue. 2. Movement into paravertebral nerve. *Am. J. Phys. Med.*, 44 (1965) 20–25.

Halle, J.S., Franklin, R.J. and Karalfa, B.L., Comparison of four treatment approaches for lateral epicondylitis of the elbow. *J. Orth. Sports Phys. Ther.*, 8 (1986) 62–69.

Holtzmark, J., Johnsen, I., Sikkeland, T. and Skavlem, S., Boundary layer flow near a cylindrical obstacle in an oscillating incompressible fluid. *J. Acoust. Soc. Am.*, 26 (1954) 26–39.

Howkins, S.D., Diffusion rates and the effect of ultrasound. *Ultrasonics*, 8 (1969) 348–351.

Kahn, J., Iontophoresis and ultrasound for post-surgical temporomandibular trismus and paraesthesia. *Phys. Ther.*, 60 (1980) 307–308.

Kleinkort, J. and Wood, F., Phonophoresis with 1 percent versus 10 percent hydrocortisone. *Phys. Ther.*, 55 (1975) 1320–1324.

Kost, J., Levy, D. and Langer, R., Ultrasound effect on transdermal drug delivery. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 13 (1986) 177–178.

Kremkau, F.W. and Cowgill, R.W., The role of molecular weight and structure in the absorption of ultrasound by proteins. *Proc. IEEE Ultrasonics Symp.*, (1984) 696–698.

Lenart, I. and Auslander, D., The effect of ultrasound on diffusion through membranes. *Ultrasonics*, 18 (1980) 216–218.

Levy, D., Kost, J., Meshulam, Y. and Langer, R., Effect of ultrasound on transdermal drug delivery to rats and guinea pigs. *J. Clin. Invest.*, 83 (1989) 2074–2078.

Mayev, R., Development of ultrasonic investigations in medicine and biology in the USSR. *Ultrasound Med. Biol.*, 11 (1985) 1–11.

McElnay, J.C., Benson, H.A.E., Harland, R. and Hadgraft, J., Phonophoresis of methyl nicotinate: A preliminary study to elucidate the mechanism of action. *Pharm. Res.*, 10 (1993) 1726–1731.

McElnay, J.C., Kennedy, T.A. and Harland, R., Enhancement of the percutaneous absorption of fluocinolone using ultrasound. *Br. J. Clin. Pharmacol.*, 21 (1985a) 609P–610P.

McElnay, J.C., Kennedy, T.A. and Harland, R., The influence of ultrasound on the percutaneous absorption of fluocinolone acetonide. *Int. J. Pharm.*, 40 (1987) 105–110.

McElnay, J.C., Matthews, M.P., Harland, R. and McCafferty, D.F., The effect of ultrasound on the percutaneous absorption of lignocaine. *Br. J. Clin. Pharmacol.*, 20 (1985b) 421–424.

Menon, G.K., Bommannan, D.B. and Elias, P.M., Sonophoresis-induced changes in stratum corneum membrane bilayer organisation and permeation pathways. *Pharm. Res.*, 9 (Suppl.) (1992) S-192.

Menon, G.K., Bommannan, D.B., Price, L.F., Feingold, K.R. and Elias, P.M., Sonophoresis disrupts stratum corneum intercellular bilayers and the epidermal calcium gradient. *Clin. Res.*, 41 (1993a) 404A.

Menon, G.K., Bommannan, D.B., Price, L.F., Feingold, K.R. and Elias, P.M., Sonophoresis disrupts stratum corneum intercellular bilayers and the epidermal calcium gradient. *J. Invest. Dermatol.*, 100 (Abstr.) (1993b) 497.

Miyazaki, S., Kohata, Y. and Takada, M., Effect of ultrasound on the transdermal absorption of indomethacin – Continuous mode and pulsed mode. *Yakuzaigaku*, 52 (1992a) 264–271.

Miyazaki, S., Kohata, Y., Mizuoka, H., Oda, M. and Takada, M., External control of drug delivery: Enhancement of transdermal absorption of indomethacin by ultrasound. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 18 (1991a) 525–526.

Miyazaki, S., Mizuoka, H., Kohata, Y. and Takada, M., External control of drug release and penetration: 6. Enhancing effect of ultrasound on the transdermal absorption of indomethacin from an ointment in rats. *Chem. Pharm. Bull.*, 40 (1992b) 2826–2830.

Miyazaki, S., Mizuoka, H., Oda, M. and Takada, M., External control of drug release and penetration: enhancement of the transdermal absorption of indomethacin by ultrasound irradiation. *J. Pharm. Pharmacol.*, 43 (1991b) 115–116.

Mohl, N.D., Ohrbach, R.K. and Crow, H.C. and Gross, A.J., Devices for the diagnosis and treatment of temperomandibular disorders: 3. Thermography, ultrasound, electrical stimulation and electromyographic feedback. *J. Prosthet. Dent.*, 63 (1990) 472–476.

Moldover, J.R. and Danish, D., Phonophoresis with hydrocortisone: Effect on serum cortisol levels. *Arch. Phys. Med. Rehabil.*, 67 (1986) 689.

Moll, J.M., A new approach to pain: Lidocaine and decadron with ultrasound. *USAF Med. Serv. Dig.*, 30 (1977) 8–11.

Mortimer, A.J., Trollope, B.J., Villeneuve, E.J. and Roy, O.Z., Ultrasound-enhanced diffusion through isolated frog skin. *Ultrasonics*, 26 (1988) 348–351.

Muir, W.S., Magee, F.P., Longo, J.A., Karpman, R.R. and Finley, P.R., Comparison of ultrasonically applied vs intra-articular injected hydrocortisone levels in canine knees. *Orthopaed. Rev.*, 19 (1990) 351–356.

Murphy, T.M. and Hadgraft, J., A physico-chemical interpretation of phonophoresis in skin penetration enhancement. In Scott, R.C., Guy, R.H. and Hadgraft, J. (Eds), *Prediction of Percutaneous Penetration, Methods, Measurements and Modelling*, IBC, London, 1990, pp. 333–336.

Nanavaty, M., Brucks, R., Grimes, H. and Siegel, F.P., An ATR-FTIR approach to study the effect of ultrasound on human skin. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 16 (1989) 310–311.

Newman, J.T., Nellermoe, M.D. and Carnett, J.L., Hydrocortisone phonophoresis – A literature review. *J. Am. Pod. Med. Assoc.*, 82 (1992) 432–435.

Nyborg, W.L., Physical mechanisms for biological effects of ultrasound. *HEW Publication (FDA)* (1977) 78-8062.

Oziomek, R.S., Perrin, D.H., Herold, D.A. and Craig, C.R., Effect of phonophoresis on serum salicylate levels. *Med. Sci. Sports Med.*, 23 (1991) 397–401.

Parry, J.S., Cleary, B.K., Williams, A.R. and Evans, D., Ultrasonic dispersal of cervical cell aggregates. *Acta Cytol.*, 15 (1971) 163–166.

Pinton, J., Valliant, L., Machet, C., Patat, F., Lethiecq, M., Pourcellot, L., Guilloteau, D. and Lorette, G., Does phonophoresis increase the ex-vivo percutaneous absorption? *J. Invest. Dermatol.*, 96 (Abstr.) (1991) 650.

Pottenger, F.J. and Karalfa, B.L., Utilisation of hydrocortisone phonophoresis in United States army physical therapy clinics. *Milit. Med.*, 154 (1989) 355-358.

Pratzel, H., Dittrich, P. and Kukovetz, W., Spontaneous and forced cutaneous absorption of indomethacin in pigs and humans. *J. Rheumatol.*, 13 (1986) 1122–1125.

Quillen, W.S., Phonophoresis: A review of the literature and technique. *Athl. Train.*, 15 (1980) 109–110.

Quillen, W.S., Ultrasonic phonophoresis. *Phys. Sportsmed.*, 10 (1982) 211.

Rolf, D., Chemical and physical methods of enhancing transdermal drug delivery. *Pharm. Technol.*, 12 (1988) 130–140.

Romanenko, I.M. and Aravisky, R.H., Comparable levels of

amphotericin B in the skin and subcutaneous fatty tissue after local application of amphotericin ointment and with preliminary treatment by dimethylsulphoxide. *Antibiot. Khimioter.*, 36 (1991) 29–31.

Roques, C.F., Cotonat, J., Marque, P., Chatain, M. and Fellez, A., NSAID blood rates after transdermal application using manual massage, ultrasound therapy and iontophoresis. *Br. J. Rheumatol.*, 31 S2 (Meeting Abstr.) (1992) 171.

Saxena, J., Sharma, N., Makoid, M.C. and Banakar, U.V., Ultrasonically mediated drug delivery. *J. Biomater. Appl.*, 7 (1993) 277–296.

Scheuplein, R., Site variations in diffusion and permeability. In Jarret, A. (Ed.), *The Physiology and Pathophysiology of Skin*, Academic Press, London, Vol. 5, 1978b, pp. 1731–1752.

Scheuplein, R., The skin as a barrier. In Jarret, A. (Ed.), *The Physiology and Pathophysiology of Skin*, Academic Press, London, Vol. 5, 1978a, pp. 1669–1692.

Singh, S. and Singh, J., Transdermal delivery of drugs by phonophoresis. A review. *Drug Des. Del.*, 5 (1990) 259–265.

Skauen, D.M. and Zentner, G.M., Phonophoresis. *Int. J. Pharm.*, 20 (1984) 235–245.

Sloan, K.B., Prodrugs. *Topical and Ocular Drug Delivery, Drugs and the Pharmaceutical Sciences*, Vol. 53, Dekker, New York, 1992.

Smith, W., Winn, F. and Parette, R., Comparative study using four modalities in shin splint treatments. *J. Orthop. Sports Phys. Ther.*, 8 (1986) 77–80.

Stratford, P.W., Levy, D.R., Gauldie, S., Miseferi, D. and Levy, K., The evaluation of phonophoresis and friction massage as treatments for extensor carpi radialis tendinitis: a randomized controlled trial. *Physiother. Can.*, 41 (1989) 93–99.

Tachibana, K., Transdermal delivery of insulin to alloxan-diabetic rabbits by ultrasound exposure. *Pharm. Res.*, 9 (1992) 952–954.

Tachibana, K. and Tachibana, S., Transdermal delivery of insulin by ultrasonic vibration. *J. Pharm. Pharmacol.*, 43 (1991) 270–271.

Tachibana, K. and Tachibana, Usage of ultrasonic vibration as a new transdermal insulin delivery system. *Diab. Res. Clin. Pract.*, S166 (Suppl.) (1988) 1–5.

Tachibana, K. and Tachibana, S., Use of ultrasound to enhance the local anesthetic effect of topically applied aqueous lidocaine. *Anesthesiology*, 78 (1993) 1091–1096.

Ter Haar, G.R. and Daniels, S., Evidence for ultrasonically induced cavitation in vivo. *Phys. Med. Biol.*, 26 (1981) 1145–1149.

Ter Haar, G.R., Daniels, S., Easthaugh, K.C. and Hill, C.R., Ultrasonically induced cavitation in vivo. *Br. J. Cancer*, 45 (1982) 151–155.

Tyle, P. and Agrawala, P., Drug delivery by phonophoresis. *Pharm. Res.*, 6 (1989) 355–361.

Tyle, P. and Kari, B., Iontophoretic devices. In Tyle, P. (Ed.), *Drug Delivery Devices. Fundamentals and Applications*, Dekker, New York, 1988, pp. 421–454.

Walmsley, A.D. and Squier, C.A., Role of ultrasonics in facilitating cutaneous drug penetration. *J. Dent. Res.*, 70 (1991) 720.

Walmsley, A.D., Squier, C.A. and Kremer, M., The thermal effect of ultrasound during phonophoresis in vivo. IADR Meeting, Glasgow, July, 1992. Abstr. No. 566. *J. Dent. Res.*, 71 (1992) 586.

Wentz, P.W. and Downing, D.T., Stratum corneum: biological and biochemical considerations. In Hadgraft, J. and Guy, R.H. (Eds), *Transdermal Drug Delivery. Developmental Issues and Research Initiatives*, Dekker, New York, (1989) pp. 1–22.

Williams, A.R., Phonophoresis: an in vivo evaluation using three topical anaesthetic preparations. *Ultrasonics*, 28 (1990) 137–140.

Williams, A.R., Release of serotonin from human platelets by acoustic microstreaming. *J. Acoust. Soc. Am.*, 56 (1974) 1640–1643.

Williams, A.R., *Ultrasound: Biological Effects and Potential Hazards*, Academic Press, London, 1983.

Williams, A.R., McHale, J., Bowditch, M., Miller, D.L. and Reed, B., Effects of MHz ultrasound on electrical pain threshold perception in humans. *Ultrasound Med. Biol.*, 13 (1987) 249–258.

Williams, A.R., Rosenfield, E.H. and Williams, K.A., Gel-sectioning technique to evaluate phonophoresis in vitro. *Ultrasonics*, 28 (1990) 132–136.

Wing, M., Phonophoresis with hydrocortisone in the treatment of temporomandibular joint dysfunction. *Phys. Ther.*, 62 (1982) 32–33.

Ziskin, M.C. and Michlovitch, S.L., Therapeutic ultrasound. In Michlovitch, S.L. (Ed.), *Contemporary Perspectives in Rehabilitation, Vol. 1, Thermal Agents in Rehabilitation*, F.A. Davis, Philadelphia, Ch. 7, pp. 1986, 141–176.