Ultrasonic imaging system for the study of decompression-induced gas bubbles

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Daniels, S., W. D. M. Paton, and E. B. Smith. 1979. Ultrasonic imaging system for the study of decompression-induced gas bubbles. Undersea Biomed. Res. 6(2):197–207. — An high-resolution pulse echo ultrasonic imaging system has been developed to study decompression-induced gas bubbles. It was considered necessary that the system be capable of detecting gas bubbles with a diameter of 10 µm and up and be able to monitor the growth of these bubbles. In addition the system needed to be capable of distinguishing separate gas bubbles from within an area containing a number of bubbles and allowing their position to be accurately located. The current system is capable of detecting bubbles as small as 10 µm and of resolving bubbles separated by 0.8 mm in azimuth and 0.4 mm in range, and these values correspond to the maximum accuracy of location. Finally, it has been shown that the technique is extremely unlikely to induce any bubble formation by means of cavitation or thermal mechanisms. It is concluded that the system represents a powerful method for studying the factors controlling bubble formation.

decompression sickness
ultrasonic imaging
bubble detection

Gas bubbles produced by rapid decompression of gas-saturated tissues are generally believed to be the cause of the various symptoms that comprise decompression sickness, and even symptomless decompressions have been shown to result in bubble formation (Hempleman 1957; Spencer, Campbell, Sealey, Henry, and Lindbergh 1969; Griffiths, Miller, Paton, and Smith 1971). Furthermore, the formation and movement of gas bubbles might be an important factor in the elimination of excess gas; this is contrary to most current theories of decompression that are based on the avoidance of bubble formation (Boycott, Damant, and Haldane 1908; Hempleman 1952; Hills 1966). Since ultrasound is strongly attenuated by gas bubbles, various ultrasonic techniques based on either transmission (Welsby 1968; Manley 1969) or reflection (Walder, Evans, and Hempleman 1968; Spencer, Simmons, and Clarke 1971; Rubissow and Mackay 1971) have been advocated for the study of bubble formation in vivo. The most widely used technique is that based on the Doppler shift in the frequency that is observed when ultrasound is reflected from moving bubbles (Smith and Spencer 1970;
Spencer et al. 1971; Powell 1972). However, a potentially more powerful technique, that of pulse-echo imaging, was suggested by Mackay (1963). The use of this technique enables both moving and stationary bubbles to be detected, as has been demonstrated by Rubissow and Mackay (1971). However, the technique has not been employed by other workers and some controversy exists about the minimum size at which bubbles can be detected by it. Rubissow and Mackay (1974) have reported that using ultrasound of wavelength 200 μm with their apparatus they could detect bubbles as small as 0.5 μm in diameter. On theoretical grounds (Rayleigh 1878; Morse 1948) this claim appeared improbable and it has been challenged by various authors (Nishi 1977; Evans 1977). This study is primarily designed to examine the characteristics of a pulse-echo system designed for the study of gas bubbles after decompression.

The ultrasound system

The system was based on a Kretztechnik SHB4100MGB module and a Kretztechnik SHB4100MG Combison Module. These units have been modified to operate at a frequency of 8 MHz in combination with a 5-mm diameter PZT-5A transducer, focused using a plano-concave epoxy lens bonded to the surface of the transducer. At this frequency the absorption coefficient, as calculated from figures quoted by Wells (1969), is expected to lie between 4 and 28 dB cm⁻¹ for soft tissue. To obtain maximum resolution and detection sensitivity, the filtering circuits and amplitude threshold for display have been removed, allowing the ultrasound image to be displayed directly by the modulation of the z axis, using an X–Y storage display oscilloscope. In addition, the electronic display magnification has been modified to give three ranges, X1, X2, and X4. The pulse repetition frequency has been increased from a maximum of 1 kHz to a maximum of 2.5 kHz; above this frequency the cycle time of the capacitors supplying the pulse to the transducer is exceeded. This has resulted in a brighter, more stable image using the normal scanning frequency of 1 Hz. The position of the transducer is derived by using a plastic film sin/cos potentiometer, which, in addition to supplying the angular coordinates of the transducer as it scans, also provides a pulse to trigger an auto-erase circuit and a trigger pulse for the shutter of the camera used to record the ultrasound images. These last two functions act to prevent the storage of successive scans, thus preventing blurring of the image, and to allow the photographic recording of alternate images for subsequent analysis. This last facility is vital to identify small changes against the background echo pattern. A diagram of the system is shown in Fig. 1.

The scanning assembly was designed to fit inside a 36-liter decompression chamber. Simple sector scanning is employed. This was achieved using an eccentric cam and follower driven by a variable speed motor. The sin/cos potentiometer, drive system, and transducer are all linked via a common shaft. This entire assembly is mounted on a rigid brass framework that fits onto a rectangular perspex tank into which is placed the object or animal under study. The transducer is acoustically coupled to the target by filling the tank with a liquid acoustic coupling medium, in the case of in vitro experiments degassed distilled water and for the study of in vivo bubble formation, a solution of sodium and potassium chlorides and magnesium sulphate in physiological concentrations. The support framework was designed to allow movement in all three directions, permitting the correct location of the transducer in relation to the target. The acoustic coupling medium can be thermostatted at 37° by means of the waterproof heating coil fitted to the bottom of the scanning tank. The scanning assembly is shown in Fig. 2.
Fig. 1. Schematic diagram of the 8 MHz pulsed-echo ultrasonic imaging system.

Fig. 2. Scanning assembly for pulse-echo imaging system. Brass framework holding transducer probe allows movement in all three directions, with precise vertical movement accomplished with the scissor jacks attached at either side of the framework. Unit comprises ultrasound probe, sin/cos potentiometer, and motor drive system, which produces a simple sector scanning mode.
Resolution of the ultrasound system

Resolution is defined as the minimum distance separating two targets at which they can be observed to be separate. The lateral resolution will be determined by the beam width, which in turn depends both on the distance from the transducer and the receiver gain. The radius of the focal plane for an acoustic beam focused by a lens, a mirror, or by curving the transducer, may be estimated from

\[ r_{\text{focal}} = 0.61\lambda f R \]

where \( \lambda \) is the acoustic wavelength, \( f \) is the focal length of the lens, mirror, or transducer, and \( R \) is the radius of the transducer (Gooberman 1968). The focal length of the lens used in the study was quoted by the manufacturers (Kretztechnik) as being between 16 and 21 mm. The wavelength of sound used is 0.187 mm and the radius of the transducer is 2.5 mm. Thus the radius of the focal plane is estimated to lie between 0.7 mm and 0.96 mm, giving a beam width of between 1.4 and 1.92 mm.

The lateral resolution was measured, as a function of distance from the transducer and receiver gain, by moving a 10-\( \mu \)m diameter steel wire across the ultrasound beam and measuring the distance between the points at which the target is just detected. The steel wire was held with a Prior micromanipulator and the transducer was kept stationary. The acoustic coupling medium was water. The beam width could be measured to \( \pm 0.05 \) mm. Eight receiver gains were measured, in 3-dB steps, from the maximum (80 dB) down to the minimum gain at which the target could be detected (59 dB). Receiver gain was determined by means of a calibrated attenuator positioned between the transducer and receiving amplifier. Measurements of receiver gain could be made to an accuracy of \( \pm 0.5 \) dB. Measurements of beam width were made at distances of from 10 to 50 mm from the transducer. The 3-dB contour plot of beam width is shown in Fig. 3. The focal plane of this transducer lies 18 mm from the transducer, with a minimum beam width of 0.8 mm at a gain of 59 dB and a maximum beam width of 1.5 mm. The maximum beam width is close to the predicted beam width of 1.6 mm. The beam width is almost constant between 16 and 20 mm from the transducer, with the minimum 0.8 mm and the maximum 1.7 mm. The optimum lateral resolution is thus 0.8 mm over a range of 4 mm from a point 16 mm from the transducer.

The range resolution is generally far better than the lateral resolution. The range resolution is determined by the precision with which the position in time of the arrival of an echo can be measured. This depends on the shape of the pulse, and the gain, dynamic range, and bandwidth of the receiver. Range resolution is ultimately limited by the pulse width of the received pulse.
echo. The pulse width has been recorded from three different targets, a 10-μm diameter steel wire, a 25-μm diameter steel wire, and a flat brass plate. With each of the targets positioned 20 mm from the transducer and using water as the acoustic coupling medium, the received pulse width was 0.5 μs for receiver gains from 70 dB to 75 dB. Below 70 dB the received pulse width fell and at the point at which the target was detected the pulse width was 0.1 μs. The distance from the transducer did not affect the pulse width, provided the effective gain did not fall below 70 dB. Figure 3 shows the beam profile. For a 10-μm target, the limit of constant range resolution was approximately 40 mm from the transducer; moving the target farther from the transducer than this caused an apparent increase in resolution until the target could no longer be detected. The reflectivity of the 25-μm wire and the brass plate were greater, which allowed movement farther from the transducer before any change in range resolution was apparent. Thus over the range of receiver gains and target distances, 70–78 dB and 15–40 mm from the transducer, the limit of the range resolution is determined by the received pulse width of 0.5 μs. This time interval corresponds to a distance of about 0.37 mm. To ascertain the overall range resolution of the system, two 10-μm steel wires were held vertically in the beam and slowly moved together until the images on the B-scan display could not be distinguished as separate. The minimum possible separation of the wires, at a receiver gain of 78 dB, was measured using a stereoscopic microscope and was determined to be 0.4 mm.

The detection threshold for single gas bubbles

Although it had already been ascertained that a 10 μm diameter steel wire can be detected, it was necessary to determine the detection threshold for single gas bubbles. This was accomplished by simultaneously observing the decay of gas bubbles ultrasonically and by optical microscopy. The bubbles were held in the center of the ultrasound beam by locating them in a cavity under a gelatin block. The gelatin blocks were prepared from a 4% gelatin solution and care was taken to ensure that they were bubble-free. Gelatin was chosen for this purpose because it is both optically and acoustically transparent when immersed in water. The decay time of the bubble was found to be reasonably short, provided that the initial bubble diameter did not exceed about 350 μm and that the surrounding acoustic coupling medium was degassed before use. In addition to measuring the detection threshold, this experiment allowed a test of the Epstein-Plesset equation of bubble decay (Epstein and Plesset 1950) to be made. This expression

\[(R/R_0)^2 = 1 - (\alpha/R_0^2)t\]

related the square of bubble radius to time. It has been shown (Beck, Daniels, Paton, and Smith 1978) that this relationship satisfactorily described the decay of small air bubbles to diameters of 10 μm, within the experimental limits of the system. It was found that bubbles of < 10 μm could not be observed microscopically even when detergent or surfactant material was added to the surrounding medium, or when the surrounding medium was saturated with gas to reduce the rate of decay of bubbles below 40 μm in diameter. It was observed that, as the bubble diameter reached 10 μm, the bubble disappeared. This in fact is not surprising if the critical radius of a bubble in solution is considered. The critical radius of a bubble in an infinite medium, i.e., the radius at which a bubble will be stable with respect to either growth or resolution, is given by

\[r_c = \frac{2 \gamma}{P_0 - (P_b - P_f)}\]
where $\gamma$ is the surface tension, $P_a$ is the dissolved gas tension, $P_h$ is the hydrostatic pressure, and $P_v$ is the vapor pressure of the liquid medium. For a gas bubble in saturated water and atmospheric pressure, the term $P_a - (P_h - P_v)$ simplifies to just $P_v$. The vapor pressure of water at room temperature is approximately 20 mmHg. Thus if a bubble of 10-\(\mu\)m radius was to be stable, the surface tension needed can be calculated to be

$$\gamma = \frac{0.001 \times 20 \times 1318}{2} = 13.18 \text{ dynes cm}^{-1}$$

given that 1 mmHg = 1318 dynes cm\(^{-2}\).

The surface tension of pure water is approximately 72 dynes cm\(^{-1}\). The critical radius given above is, of course, unstable because any slight perturbation will cause the bubble either to grow or to resolve.

The detection threshold has been measured by two different methods: 1) by holding the transducer stationary and plotting the minimum bubble diameter detected against receiver gain; and b) by scanning the bubble during its decay and recording the images, which were subsequently measured, and plotting image size against bubble size as determined by microscopic measurement. For the first method, the point at which the bubble was not detected was defined as the receiver gain when the echo amplitude fell below that necessary for registration on the image display. The plot of effective receiver gain against minimum bubble size detected is shown in Fig. 4. Two points can be made concerning this experiment. First, a best line fitted to the data points by a least squares regression analysis gave a slope of $-0.053$, with a correlation coefficient of $-0.996$. By considering

$$\text{gain (dB)} = 10 \log \frac{P_2}{P_1}$$

where $P_2 = \text{transmitted power}$ and $P_1 = \text{detected power (} x \text{ reflected power)}$.

Hence

$$\text{gradient} = \frac{\log D}{10 \log \left( \frac{P_2}{P_1} \right)} = -0.053$$

Fig. 4. Minimum detectable bubble diameter plotted against effective receiver amplification. Bubble diameter was determined by optical microscopy; error bars represent $\pm 10 \mu m$. The effective receiver amplification was measured to an accuracy of $\pm 1 \text{ dB}$. A least-squares linear regression gave a correlation coefficient of $-0.996$. 
where $D$ = bubble diameter.

Then, if transmitted power ($P_t$) is constant

$$P_t \propto D^{0.8}$$

This is a surprising result, indicating that for the bubble diameters measured, the bubble is acting as a geometric reflector down to a diameter of 50 $\mu$m. At this diameter the factor $2\pi r/\lambda = 0.8$ (where $\lambda$ is the acoustic wavelength), and from the theoretical treatment for spherical reflectors (Morse 1948) such a bubble would not be expected to act as a geometric reflector. A bubble is only expected to be a geometric reflector when $2\pi r/\lambda >> 1$, which corresponds to a considerably larger bubble diameter. The second point to be made is that the detection threshold obtained by extrapolation of the regression line to the maximum gain of 80 dB gives a detection threshold of 10 $\mu$m. However, this extrapolation is considered unsafe because the theoretical expression for back-scattered sound suggests that the radius squared relationship should, as the diameter of the bubble becomes very small and $2\pi r/\lambda << 1$, give way to a relationship in which the back-scattering is proportional to the sixth power of the bubble radius (Rayleigh 1878; Morse 1948).

2) Because of the uncertainty in the relationship between image size and bubble size between the limits $2\pi r/\lambda >> 1$ and $2\pi r/\lambda << 1$, a second, and perhaps more pertinent, method of measuring the relationship between bubble radius and image size has been used. The range of bubble radii in question is precisely the range of most interest as regards the early development and growth of bubbles after decompression, i.e., 3–300 $\mu$m in radius. To examine this range, single gas bubbles trapped beneath a cavity in a gelatin block were ultrasonically scanned during their decay. Gelatin is both acoustically and optically transparent and thus simultaneous measurements of bubble radius could be made using optical microscopy. The results of such experiments have been reported in Beck et al. (1978), and an example is shown in Fig. 5. It was shown that bubbles down to 10 $\mu$m in diameter (5-$\mu$m radius) could be detected using a gain of 80 dB. The detection threshold seen in Fig. 5, measured at a receiver gain of 77 dB, was a bubble of diameter 16 $\mu$m. As expected, however, no uniform relationship

![Fig. 5. Plot of bubble decay produced by following decay simultaneously by optical microscopy and ultrasonic scanning. Ultrasonic images were recorded using a gain of 77 dB and image size was determined from photographic record of images taken during decay.](image-url)
between bubble radius and image size was observed. As can be seen in Fig. 5 (and also in Beck et al. 1978), for a larger bubble using a lower gain, more than one solution exists for the relationship between image size and bubble radius, and also the rate of change of image size with bubble radius is either too fast or too slow for comparison. This result does not rule out a semi-quantitative approach; although bubble radii cannot be measured precisely, size ranges can be estimated, e.g., diameters < 100 µm and diameters between 100 and 400 µm. Above diameters of 400 µm, it has been shown (Beck et al. 1978) that image size is linearly related to bubble diameter, with a slope of approximately 4.9.

The intensity of the ultrasound beam

The time-averaged intensity of the ultrasound beam has been measured, at a pulse repetition frequency of 1.2 kHz, by measuring the displacement of a freely suspended glass bead held in the center of the beam (Fig. 6). Although the intensity of the beam will reach a maximum at the focal point (18 mm from the transducer) the measurement of intensity was actually made at a distance of 15 mm from the transducer. This is the point at which the skin surface of the guinea-pig leg is located for studies of bubble formation after decompression. The observed displacement of the bead has two components: the radiation pressure experienced by the bead, and a force due to streaming of the acoustic coupling medium. In practice these effects can be separated by measuring the displacement within 5 s of switching on the ultrasound, because the streaming effect requires a time interval before steady-state conditions are reached.

The force due to acoustic radiation pressure acting on a sphere placed in a radiation field of 1 W·cm⁻² has been given by Fox (1940) as:

\[ F = \pi \rho_r \frac{2}{3} \left[ 1 - 0.719 \left( \frac{2\pi r}{\lambda} \right)^{-1/3} \right] \text{ Newtons} \]

![Fig. 6. Schematic diagram of arrangement for measuring intensity of ultrasound by observing displacement of a glass bead due to the radiation pressure exerted by the incident ultrasonic beam. Displacement of bead was measured by optical microscopy within 5 s of switching on the ultrasound.](image-url)
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where \( r_b \) is the radius of the glass sphere, \( c \) is the acoustic velocity, and \( \lambda \) the acoustic wavelength. This expression is only strictly valid when \( 2\pi r_b/\lambda >> 1 \), in this case \( 2\pi r_b/\lambda \approx 7 \).

The force acting on the glass bead and its suspension thread to oppose the radiation pressure is

\[
F = \frac{dg}{L} \left( m_b + m_r/2 - \rho_w \left[ \frac{4}{3}\pi r_b^3 + \frac{1}{2}\pi r_f^2 L \right] \right)
\]

where \( d \) is the displacement of the bead, \( g \) is the acceleration due to gravity, \( L \) is the length of the thread, \( m_b \) is the mass of the bead, \( m_r \) is the mass of the thread, \( \rho_w \) is the density of the coupling medium (water), \( r_b \) is the radius of the glass bead, and \( r_f \) is the radius of the thread.

Using a glass sphere of 200-\( \mu \)m radius, mass 0.15 mg, suspended with a thread 6.25 cm long, radius 37.5 \( \mu \)m, and mass 0.9 mg, the displacement due to radiation pressure was measured as 275 \( \mu \)m. This gives the intensity of the ultrasound as 23 mW/cm\(^2\). This compares to an intensity threshold for cavitation of 120 W/cm\(^2\) (Briggs, Johnson, and Mason 1947; Coakley 1971). In addition, the thermal effect will be negligible, given that the heat equivalent is 0.0115 cal/s/cm\(^2\) (Wells 1969).

DISCUSSION

The aim of this study was to evaluate the potential of the pulse-echo imaging system for studying bubble formation after decompression. Initially it was planned to use small animals as experimental subjects for decompression studies, and therefore a high resolution and detection threshold could be attained at the expense of penetration. Before drawing any conclusions regarding the physical performance of this system, it is necessary to consider what is required. The available evidence concerning bubble formation suggests that bubbles form from the growth of micronuclei (Harvey 1951; Evans and Walder 1969; Vann and Clark 1975) rather than as a result of de novo formation via cavitation or other suitable mechanism. We found that in free solution, even in the presence of surfactant (di-palmityl lecithin), bubbles of diameter \(< 10 \ \mu m\) dissolved so rapidly that they appeared to vanish. It would seem that to stabilize gas volumes equivalent to a 10 \( \mu \)m bubble or less requires some additional stabilizing factors. Philip, Schacham, and Gowdey (1971) reported that bubbles found in vivo are surrounded by a "platelet skin," which was suggested to stabilize small bubbles by a combination of reduced surface tension and the provision of a physical barrier to gas diffusion. The ability of platelets to form a structured surface around bubbles of \(< 10 \ \mu m\) diameter appears doubtful, although it is possible that very small bubbles may be stabilized by, for example, fibrinogen adhesion to the surface. Alternatively, Harvey (1951) envisaged stabilization as being provided by inclusion of gas in "hydrophobic cracks" in particles, the resulting concave boundary reducing surface tension sufficiently to stabilize the bubble. However, such sites have not been identified, although it is possible that they may exist within the lumen of blood vessels or indeed in some extravascular tissue sites. Stabilization by virtue of tissue structure would imply that any micronuclei existing at those sites would be stationary and would not therefore contribute any active transport of gas. Furthermore, if, as appears to be the case, these micronuclei exist before any decompression occurs, it is presumed that they do not cause any abnormal physiological effect. It is only when the free gas volume increases due to the supersaturation of blood and tissues that an effect may be observed. If the special features required for stabilization of bubbles less than 10 \( \mu m\) in diameter are considered and the otherwise harmless existence of micronuclei is accepted, it appears that a detection threshold of 10 \( \mu m\) would provide the ability to observe the growth of bubbles from a size close to that of
the pre-existing micronucleus. Precise measurements of rates of growth of bubbles less than 300 \( \mu \)m in diameter cannot be made, but semi-quantitative estimates of rates of change are possible.

The resolution of the ultrasound system allows individual gas bubbles to be distinguished, provided they are separated by at least 0.8 mm in azimuth and 0.4 mm in range. At spacings closer than this, a group of bubbles would be observed as though they were a single bubble of diameter equivalent to the combined cross-sections plus the spacing. The position of gas bubbles within an area of tissue can be located to an accuracy corresponding to the resolution limits of the system, i.e., a maximum of 0.8 mm in azimuth and 0.4 mm in range. Finally, from the measured intensity, 23 mW cm\(^{-2}\), the possibility of inducing bubble formation by ultrasound scanning, via cavitation, thermal, or other mechanisms, appears remote.

In conclusion, this pulse-echo ultrasonic imaging system is believed to be a powerful method for detecting the formation of both moving and stationary gas bubbles. The factors affecting formation, growth, and transport can be directly investigated and the effects on gas elimination of bubbles can be measured semi-quantitatively. Thus the possibility of altering the decompression to facilitate gas elimination via mobile, intravascular bubbles, while reducing the extent of stationary bubble formation and thus maintaining normal tissue perfusion, can be investigated. Furthermore, the differences in response to decompression observed when using different inert gases can be examined in relation to possible variation not only in the density of bubble formation but in the location and nature of any bubbles formed.

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Daniels, S., W. D. M. Patton, and E. B. Smith. 1979. Systeme de detection ultrasonique pour l'étude de bulles gazeuses produites au cours de la decompression. Undersea Biomed. Res. 6(2): 197–207.—On rapporte la mise au point d'un systeme a ultrasons tres sensible, destine a l'étude des bulles gazeuses produites par la décompression. Un tel systeme doit être capable de detecler des bulles gazeuses a partir d'un diametre de 10 \( \mu \)m, et de suivre leur croissance. En plus, le systeme doit distinguer les bulles à l'intérieur d'un champ qui en contient beaucoup, et indiquer leurs positions. Le travail actuel décrit un systeme capable de detecter des bulles a partir d'un diametre de 10 \( \mu \)m, et de résoudre des bulles separées de 0,8 mm d'azimut et 0,4 mm de rangée; ces valeurs correspondent au maximum de precision. Enfin, on a démontre que la technique ne provoque pas elle-même la formation de bulles par cavitation ni par mécanisme thermal. Le système sera très utile pour l'étude des facteurs qui determinent la formation des bulles.

maladie de décompression
détection par ultrasons
détection de bulles

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