Reply to Frizzell *et al.*'s comment to our comment

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This is a reply to the preceding letter [Frizzell *et al.*, J. Acoust. Soc. Am. **110**, 1738–1739 (2001)].
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I agree with O'Brien *et al.*'s comment to our comment[1] of their paper that in order for there to be inertial cavitation, the nuclei must be smaller than resonant size, and therefore less than 2 μm in diameter, much smaller than the mean alveolar diameter. Yet it seems entirely unreasonable to suggest that *not a single* such small nuclei exist in the immediate vicinity of the alveoli sacs, which are saturated with gas. It only takes a single such site to act as a nuclei. Furthermore, it is well known that once a site undergoes inertial cavitation, it will produce other small bubble fragments that can themselves act as inertial cavitation nucleation sites. Recent studies support the contention that small nuclei do occur in lung tissue.[2]

It is true that gas equilibration will take place fairly rapidly in the circulation system of the animal. In unpublished tests of decompression in mice that I ran with Dr. Karl Schaefer many years ago, the nominal time for saturation of the animals in a decompression chamber was about 20 min. The slowest tissues, of course, are the fatty tissues, which, in our decompression studies, often were the site of hind limb paralysis of the mice. If overpressure is to produce a strengthening of the animal to cavitation, it must compress the existing sites such that the internal gas in the bubbles has time to dissolve into the surrounding tissue. That dissolution time must be shorter than the saturation time, which depends both on perfusion and diffusion. So it may well be that where the perfusion is slower, as in fatty tissues, overpressure may be successful in reducing bubble nuclei size, whereas where the perfusion and diffusion are faster, as in the lung, this does not take place.

Finally, in their paper, O'Brien *et al.* choose to adopt a negative approach in saying that a particular model does *not* explain their experimental results. They give no convincing model of what *is* responsible for the effects they have observed. When such a negative approach is adopted (and is actually emphasized in the title of their paper), it puts a very high standard on the proof of the contention, a standard they do not reach. Saying that it is unlikely that cavitation nuclei of the appropriate size exist in the lung is, once again, another negative statement that is unverified by any direct evidence, and furthermore is contradicted by the work of others. If any such nuclei exist, then the explanation I have given in my comment (which is *not* contradicted in the paper or in the present comments by O'Brien *et al.*) offers a reasonable explanation for much of the data from their careful experiments.

To illustrate why the standard on the proof of a contention is greater, consider two of the references given in their comment to my comment that they say support their argument. In both, a raised hydrostatic pressure leads to raised cavitation thresholds. In the paper by Hill,[3] a polystyrene tube with the sample under consideration is in an aqueous bath. The pressure of the entire environmental chamber is increased by up to 0.75 atmospheres for anywhere between 5 min and 3 h, and in all cases the cavitation threshold goes up, suggesting that nuclei are removed or at least reduced in size. In this situation one must rely on diffusion through the outer bath and then to the tube opening in order for gas to diffuse into the tube. Diffusion is a very slow process, and so it is not surprising that before gas could diffuse into the tube, many of the small gas nuclei could have dissolved entirely into solution. Furthermore, in this situation, given the distance that gas would have to diffuse and given the relatively small pressure increment of 0.75 atmospheres, even 3 h might not be sufficient for total saturation of the gas in the tube.

In a second quoted paper of Frizzell *et al.*,[4] the hind limb paralysis of neonatal mice was observed. Increased hydrostatic pressure reduced this paralysis leading the authors to conclude that cavitation was involved in causing the paralysis (because it could be suppressed by raising the hydrostatic pressure). An important detail of this experiment was that the mice were kept at 10 °C. I have performed tests with liquid breathing mice kept at 18 °C, and in those circumstances the respiration rate and heart rate are enormously reduced. Working at 10 °C permitted Frizzell *et al.* to avoid chemical anesthesia. The neonatal mice were virtually in suspended animation, a conclusion echoed by Dalecki *et al.*[5] In this case it is certain that the perfusion of blood is negligible compared to the present paper where adult mice are held at 30 °C. (Dalecki *et al.* also find that for adult mice, the lesion area in the lung is very much smaller in the case of mice at 37 °C as compared to those for which the blood flow is significant reduced at 10 °C.[6]) Also, the hind limb paralysis in the neonatal mice study is likely caused by bubbles that are not carried by the blood but rather that are situated stably in other tissue, such as fat, putting pressure on the nervous system. This tissue will not saturate with the speed of lung tissue.

These two studies illustrate an essential message: When discussing cavitation mechanisms, the devil is often in the

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details. In some cases hydrostatic pressure will inhibit cavitation and in other it will not. It is very hard to make generalizations. And the generalization about inertial cavitation not being a mechanism in lung hemorrhage in the paper under contention is simply not supported by the data or the arguments given in the paper or in the authors' comments to my comment. In fact, a mechanism of inertial cavitation is consistent with the arguments given in my comment.

[1] R. E. Apfel, "Comment on 'Ultrasound-induced lung hemorrhage is not caused by inertial cavitation' " [J. Acoust. Soc. Am. **108**, 1290–1297 (2000)], J. Acoust. Soc. Am. **110**, 1737 (2001).

[2] See, for example, C. K. Holland, R. A. Roy, R. W. Biddinger, C. J. Dimile, and C. Cawood, "Cavitation mediated rat lung bioeffects from diagnostic ultrasound," J. Acoust. Soc. Am. **109**, 2433(A) (2001).

[3] R. Hill, "Ultrasound exposure threshold changes in cells and tissues," J. Acoust. Soc. Am. **52**, 667–672 (1972).

[4] L. A. Frizzell, S. S. Lee, P. D. Aschenbach, M. J. Borrelli, R. S. Morimoto, and F. Dunn, "Involvement of ultrasonically induced cavitation in the production of hind limb paralysis of the mouse neonate," J. Acoust. Soc. Am. **74**, 1062–1065 (1983).

[5] D. Dalecki, S. Z. Child, C. H. Raeman, C. Cox, and E. L. Carstensen, "Age dependence of ultrasonically induced lung hemorrhage in mice," Ultrasound Med. Biol. **23**, 767–776 (1997), p. 775.

[6] *ibid.*