Response to "Comment on 'Ultrasound-induced lung hemorrhage is not caused by inertial cavitation'" [J. Acoust. Soc. Am. 110, 1737 (2001)]

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We reply to the preceding letter [R. E. Apfel, J. Acoust. Soc. Am. **110**, 1737 (2001)].
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The comments presented in the letter from Robert Apfel[1] raise an important issue. If there is a difference in the behavior of inertial cavitation nuclei in lung versus other tissues such that overpressure does not affect the inertial cavitation threshold in lung, then there would be an impact on the conclusion in our publication.[2] To examine this question, let us first be clear as to what we believe would and would not constitute inertial cavitation nuclei. We do not believe that inertial cavitation nuclei are the relatively large gas bodies in the adult mouse's lung called alveoli (mean alveolar diameter:[3–5] 38–49 μm). The alveolar spaces essentially fill the lung and are separated by thin layers of tissue called alveolar septa, like the air pockets in bubble wrap. Each alveolus is surrounded by other air-filled alveoli connected to each other by Pores of Kohn, and thus would not exhibit the behavior of a bubble surrounded by liquid. Instead, we believe that any existing inertial cavitation nuclei would be much smaller stabilized gas pockets (with diameters likely less than 1 μm) within the blood in the vascular system or possibly in other tissues or liquid layers within the lung.

If cavitation nuclei were to be present in and/or around the lung, and we know of no support for this premise, three anatomic locations could be hypothesized, viz., the monolayer of surfactant within alveoli, the capillary beds within the septa and visceral pleura, and within the tissue layers forming the visceral pleura. Molecular gases found in the surfactant, such as oxygen, carbon dioxide, and nitrogen, do not exist as micron size bubbles but as diffuse gas molecules. In fact, the blood-gas barrier that exists between the alveolar space and the alveolar capillaries is less than 1 μm thick. Physiologically, micron size bubbles in the surfactant and surrounding tissue would impede the very rapid transport of these gases across the surfactant layer, alveolar wall, and red blood cells (all of which occurs in less than 1 s), thereby severely affecting respiration.

We would expect the gas pressures within the alveolar spaces to closely follow the increases in the ambient air pressure within the pressure chamber. We also agree with Dr. Apfel that it is likely that there would be equilibration, in a short period of time, of gas pressure within the tissues immediately surrounding the alveoli and "other tissues in proximity to the circulation system."[1] In fact, given that most gas exchange and equilibration processes take place in the capillary bed of the lung and other tissues throughout the body, we would expect the same equilibration to take place fairly rapidly within other tissues in the body. That is, equilibration in the lung would raise the partial gas pressures in the blood. These partial gas pressures would be relatively unaffected during transport in the large vessels until reaching the capillary beds of other tissues where equilibration again takes place raising the partial gas pressure in the surrounding tissues. Thus the state of blood-born nuclei would be similar in all tissues of the body. Also, it should be noted that very little oxygen or carbon dioxide is dissolved in plasma which is why we have red blood cells for transport of these gases and that nitrogen equilibration takes much longer.

The central question is "what is the influence of the overpressure on inertial cavitation thresholds in the lung if there has been sufficient time for increases in the partial gas pressures in the blood and other tissues of the lung?" Some insight can be gained from first considering an *in vitro* study that showed that overpressure increased the threshold for inertial cavitation.[6] These *in vitro* results also showed that there was no significant cavitation threshold change under overpressure regardless of the time period between when the overpressure was applied and when the ultrasound was applied (between 5 min and 3 h), suggesting that equilibration of partial pressures of dissolved gases in the liquid sample had no effect.[6]

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Clearly overpressure has also had a mitigating effect on the severity of damage in other body tissues *in vivo*.[7–9] Further, these *in vivo* results have been accepted as providing the levels at which cavitation is involved in the resultant bioeffect, hind limb paralysis. If the nuclei in that case are blood-born, and we have no proof that is the case, then the argument could be made that we would expect the same effect on blood-born and tissue-born nuclei in the lung. Of course, if the nuclei are not blood-born in one or the other of these tissues, then the comparison becomes much more complex and we cannot rule out the scenario suggested by Dr. Apfel. However, we believe this scenario is unlikely.

Regarding the increased volume of the lesions for animals exposed under overpressure conditions, we do not agree that this lesion would be a result of the bubbles expanding after decompression. The nature of the lesions is that they start to develop at the lung surface and progress into lung, perhaps associated with increased ultrasound penetration as the alveoli fill with blood. If this suggestion by Dr. Apfel were applicable, then the lung damage would not necessarily be as localized as it is. Rather, it is likely that bleeding into alveolar spaces is caused by small injuries to tissues in the pathway of the ultrasound beam originating at the air-blood barrier immediately beneath the visceral pleura. Because we and others have observed no tissue or cellular damage in lung using light microscopy, it is likely that the lesion initiating the hemorrhage is extremely small and not visible with routine histologic assessment of lung lesions induced by ultrasound. Because the lesions and the volume of hemorrhage have a finite limit, it appears that the punctate lesions heal or close rapidly following initial injury. These punctate lesions would allow hemorrhage from capillaries forming the air-blood barrier into contiguous alveolar spaces. It is unlikely that bubbles are formed within the walls of the septa that have no effect until they expand upon decompression to cause hemorrhage into adjacent alveoli.

Finally, even though it does not directly address the issue raised by Dr. Apfel, we think it is important to point out that other pieces of evidence continue to develop showing that lung hemorrhage is not caused by inertial cavitation.[10,11]

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