"Lung Cell Immune System Response Due to Irradiation by Alpha Particles", J.F. Burkhart, R. E. Camley, Proceedings of the 2002 International Radon Symposium, Vol. 1, pp. 51-62, 2002.

Lung Cell Immune System Response Due to Irradiation by Alpha Particles

J. F. Burkhart, B. A. Camley, R. E. Camley
Physics Department, University of Colorado at Colorado Springs
E. Villalobos-Menuey, M. K. Newell
Biology Department, University of Colorado at Colorado Springs

ABSTRACT

The danger from radon is commonly estimated using the linear no-threshold theory, which states that the risk of cancer is simply proportional to the amount of exposure. However, recent studies indicate that the linear theory may underestimate the damage done by radon. Others propose that the immune system response to this damage makes the linear theory an overestimation. This study attempts to address both the issue of damage and the possible immune response to that damage. L1210 mouse leukemia cells, and mouse lung and spleen cells were irradiated at different levels of exposure to alpha particles and their subsequent levels of reactive oxygen and B7-1 were measured through flow cytometry. The results support studies (T. Hei, et al.) showing increased levels of reactive oxygen at low doses of radiation, and thus a greater risk for cancer, but showed mixed evidence of immune system stimulation.

Background

Radon, a naturally occurring, radioactive gas, has been estimated to kill 18,000 to 22,000 people annually in the United States, and affects one home in fifteen. These estimates are based upon the linear no-threshold theory, which projects the danger at low doses from that at high doses. The high dose values were obtained primarily by studies on uranium miners.[1]

A series of studies, most of which were conducted by researchers at Columbia University, have indicated a strong possibility that the damage done by alpha particle irradiation at low doses may be proportionally higher than those at high doses, due to a “bystander effect”.

This would suggest that the estimation of risk from the linear theory is inaccurate.[2] Others have suggested, through epidemiological studies, that this bystander effect, while present, is insignificant.[3]

However, another school of thought involves the idea of "radiation hormesis" – the stimulation of the immune system, as well as additional benefits, through low-level radiation. Several studies have shown the presence of immune system stimulation with low doses of radon (Shu-Zheng Liu, et. al, S. Hattori)[4,5]. This might suggest that the linear theory in fact *overestimates* the danger from radon.

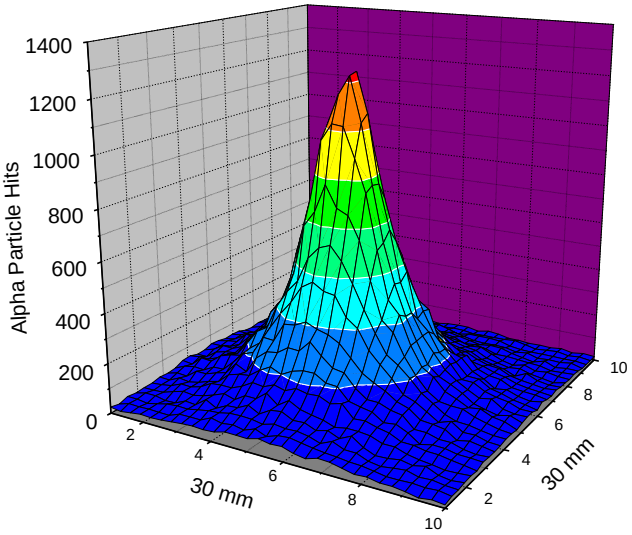
To the best of our knowledge this is the first attempt to study *in vitro* the effect of radon including both damage and immune response. This study uses the technique of flow cytometry to measure the presence of hydrogen peroxide (H_2O_2, a variety of reactive oxygen), and B7-1 (a protein known to provide a co-stimulatory signal necessary for activation of T cells[6]). The presence of reactive oxygen is used to measure oxidative stress, and through this, the potential for genetic mutation, in particular sister chromatid exchanges. The protein B7-1, which can be expressed at the surface of a cell, has been shown to be associated with the activation of the cell-mediated immune response. Without the presence of B7-1 or other co-stimulatory molecules, T cells may not be activated. Therefore, the signal of B7-1 is used as a partial measure of the possible immune system response to the damage.

Procedure and Modeling

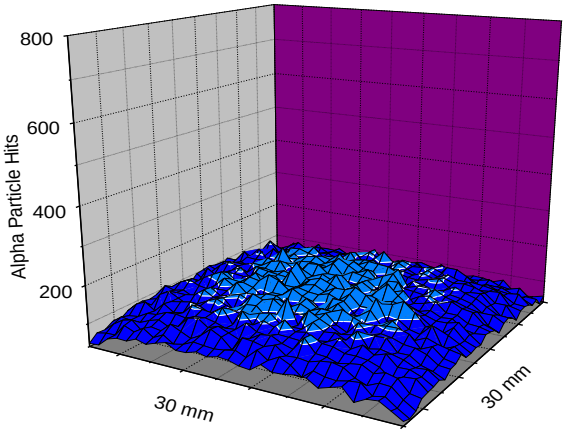
A variety of different types of cells were used in this study. These included mouse lung cells, mouse spleen cells and L1210 mouse blood leukemia cells. These cells were irradiated with alpha particles from three different $RaNO_3$ sources of different concentrations. Since it is known that alpha particles may influence cells by interaction with the liquid media[7], the media was removed prior to irradiation and replaced immediately afterwards.

A computer model was developed to determine the best possible procedure for irradiation. This model predicts the amount of exposure the cells received when exposed to a radiation source. The model randomly generates directions for alpha particles, then traces their paths to find the projected distribution of impacts, when various parameters – such as the geometry of the radiation source, its position, or the time of exposure are varied. As a uniform amount of radiation was desired for all cells, it was important to determine how different source

geometries affected the uniformity of alpha particle impact in the well-plate. This computer model indicated that the most uniform distribution of alpha particles would result from a disc-shaped source. (Fig. 1)



Impact Distribution for Point Source



Impact Distribution for Disc Source

FIG. 1: According to the computer model developed, the alpha impact distribution from a continuous, disc-like source is more uniform than from a point-source.

We made three sources

Low 153,000 hits in 10 minutes

Medium	379,000 hits in 10 minutes
High	760,000 hits in 10 minutes (original value)
	247,000 hits in 10 minutes (current value)

Numerical calculations were in good agreement with the values reported which were measured using alpha track foils. The original "high" value source was measured to be 760,000/10 minutes, but at the conclusion of all experiments the actual value was measured to be 247,000 per 10 minutes. Apparently some of the source was lost during or after the experiment.

We show below the estimated percentage of cells that were impacted by alpha particles for the "low" source when the source is 1.5 cm above the bottom of the well plate:

Cell Type	Number of Cells	Minimum %Hit	Maximum %Hit
Lung	4 million	3	9
Spleen	4 million	3	9
Leukemia	4 million	3	12

Due to the limited penetrating power of alpha particles (on the order of six layers of cells at a maximum), the number of cells that can be hit depends on the number of layers of cells in a well plate. The minimum values in the table above are based on only the top layer being hit, while the maximum values include the possibility that the alpha particle can penetrate 3-4 layers.

Background on Connection of Stress and the Immune System

Previous work by M. K. Newell et al [8,9] has shown that that factors which stress a cell often produce an **increase** in reactive oxygen and a subsequent **increase** in B7-1 as determined from flow cytometry measurements. This has been established for a variety of different types of cells including many tumor cells and neuronal cells. The stresses used were quite varied and included x-ray radiation, chemical stressors, and changes in nutrient availability. It is of interest to see if alpha radiation follows the same pattern, which would indicate that the immune system might compensate for any increased damage at low dosage.

Experimental Results

The first question addressed by this study was: is there a significant impact of the radiation on the cells? We used mouse leukemia cells and only the "low" dose source which was positioned 0.5 cm above the well plate. Death rates measured immediately after irradiation

increased linearly with exposure time for both the irradiated and control samples, but at a much greater rate for the irradiated sample than the control. (Fig. 2) A t-test proved the two means to be significantly different at the 0.05 level.

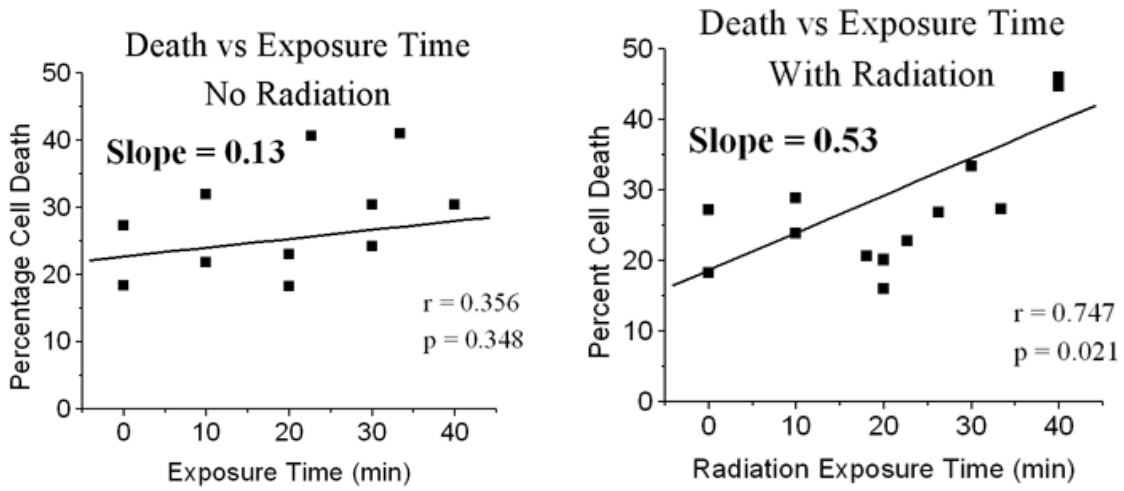


FIG. 2: Death rates are significantly higher on the irradiated sample compared to the control sample

In order to determine the amount of potentially cancer-causing damage, the amount of reactive oxygen in the cells was measured. Levels of hydrogen peroxide were measured at different exposure times, and divided by the levels measured from the control for the same exposure time. (Fig. 3) This revealed that levels of reactive oxygen remained relatively constant when 12% to 24% of the cells were hit by alpha particles (an exposure time of 20-40 minutes) – a result consistent with studies at Columbia. Because the amount of reactive oxygen is the same at relatively low and relatively high doses, it is clearly disproportionately high at low levels. However, when 12% or fewer of the cells were hit by alpha particles, there was a distinct drop in the amount of reactive oxygen present. This seems reasonable, because as the amount of radiation drops to zero, the reactive oxygen of the irradiated sample should approach that of the control.

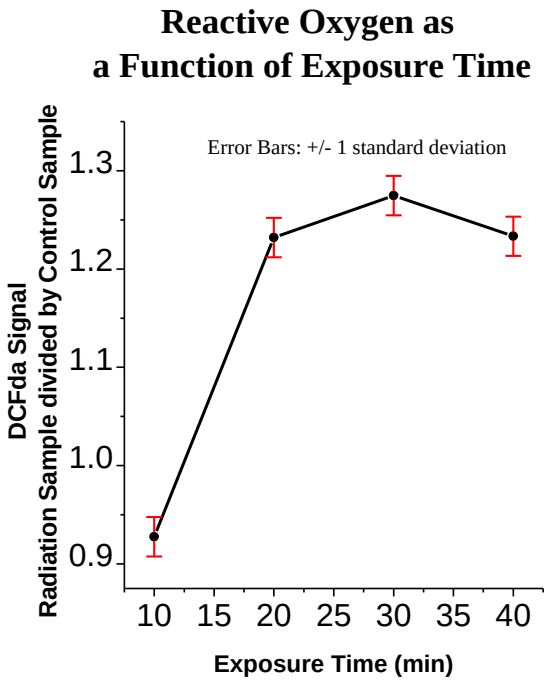


FIG. 3: The reactive oxygen levels saturate after 20 minute exposure time. This corresponds with many other studies.

Levels of B7-1 initially increased slightly, but then decreased. (Fig. 4) This result initially seems unlikely, because it suggests that the immune system does not respond at all to the damage done by radiation. However, there are several possible explanations for this phenomenon. Typically, eighteen to twenty-four hours are required in order to observe the presence of an increased level of B7-1 in the cells, and during this time, many of the cells lysed. As B7-1 is expressed on the surface of the cell, the dead cells which had lysed could not be measured for B7-1. This could have created an effective drop in B7-1 "signal." The possibility of cell lysing is confirmed by microscope observation, as well as a drop in flow-cytometry-measured "death" rates – which do not include lysed

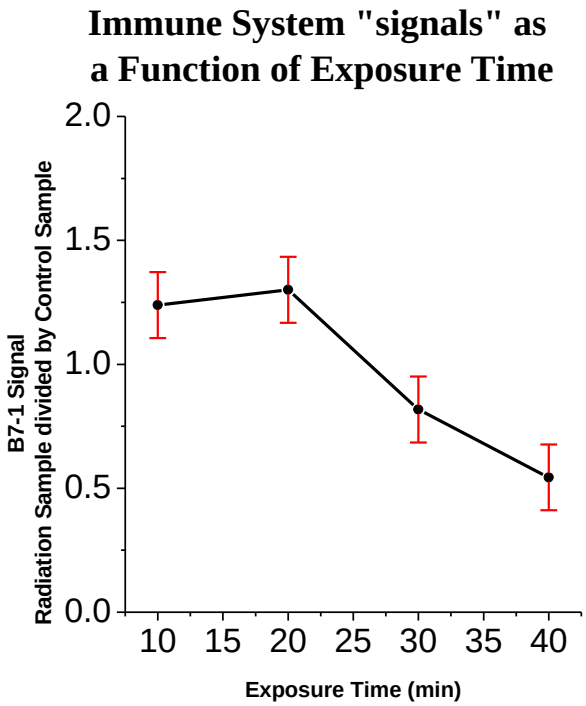


FIG. 4: Levels of B7-1 initially rise with increasing exposure, then drop

cells. However, once the data were controlled for this source of error, the overall picture of B7-1 changes in time did not change substantially. In addition, later experiments showed this pattern in the signal from both the live and dead cells, and the signal from the live cells could not have been affected by cell lysing.

Another possibility for error is that the cells which were exposed for the longest time were returned for incubation for some period of time while shorter exposures were made on other cells. Since the doubling time for these tumor cells is short (14 hours) there could have been substantial growth of new cells which might have lowered the overall amount of B7.

The previous results were for tumor cells and do not necessarily predict how the cells of interest, lung cells, will behave. Strain C57 Black 6 mice were sacrificed to harvest lung and spleen cells. These cells were exposed to the three different levels of alpha particle sources for 10 minutes each. Levels of reactive oxygen were measured immediately after exposure and staining and level of B7.1 were measured 24 hours later. The results are presented below for mouse lung cells; the spleen cells showed no results.

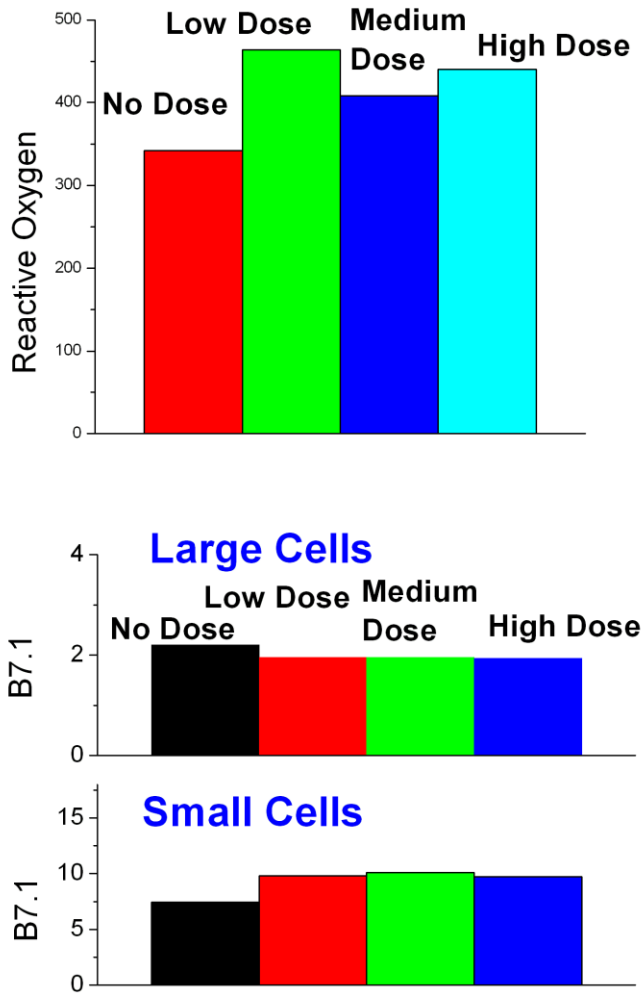


Fig. 5 Reactive oxygen and B7.1 levels on mouse lung cells

We note that the small cells follow the usual pattern, i.e. as reactive oxygen increases there is a corresponding increase in B7.1 levels. It is interesting to note that the B7.1 levels in the large cells basically look unchanged for the different doses. If these results are confirmed it might indicate that some lung cells show increased potential for damage (high reactive oxygen levels) and that the immune system does not respond to the same degree.

The results are inconclusive – the error might be larger than the differences between the different cases (common for very low dose situations). We are unable to provide meaningful error bars for Fig. 5 because the limited number of cells in the experiment did not allow for

multiple measurements. Reactive oxygen seems to increase, in agreement with earlier work, but interesting questions are raised:

Do different cells respond differently?

Do the results depend on time -when the oxygen and B7 levels are measured?

Are alpha particles a different kind of stress than previous stresses?

We have recently revisted the exposure of mouse leukemia cells using all three sources and identical exposure times (15 minutes). The results are presented below in Fig. 6.

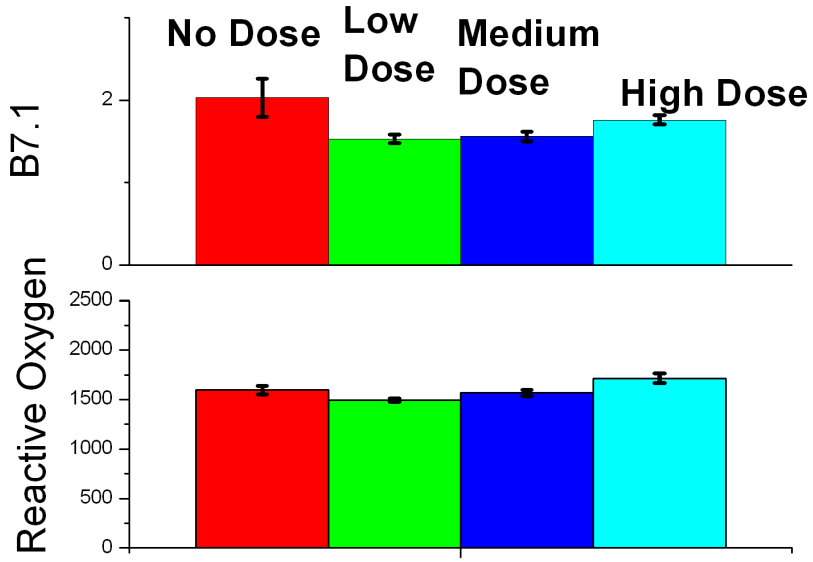


Figure 6: B7.1 levels and reactive oxygen levels measured 4 hours after exposure.

The most immediate observation is that the B7.1 levels seem to track well with reactive oxygen levels. This would argue that the immune system does respond to alpha particle induced stress. It is interesting to note that the "low" dose value for B7.1 is actually below that of the no-dose case.

Discussion

An additional computer model was created in order to explain the results for reactive oxygen in terms of a "bystander effect." This model involved randomly hitting members of a population of cells with alpha particles. When a cell was hit the simulated levels of "reactive oxygen" were increased for both the struck cell and a certain number of its neighbors. At first,

these numbers were chosen arbitrarily – with ten "units" of H_2O_2 added to a cell hit by an alpha particle, and one "unit" added to all bystanders, and twelve "units" of H_2O_2 killing a cell. However, when this was the case, the increase of the average H_2O_2 of each cell was approximately linear – the essential equivalent of no bystander effect. This was at odds with the results of this experiment, and so these values were changed. It was eventually determined that a death level of fifteen units, with ten units added from a direct hit, and five added from a neighboring hit, could produce results in reasonable agreement with the experiments.

The accuracy of these predictions depended on how many cells were counted as "neighboring" cells. In order to produce a model that generally matched the results of this study, and those of T. Hei, et. al[2]., it was found that each alpha particle must affect all cells within a radius of about three cells – or about the nearest twenty cells. This produces results in which there is little increase in reactive oxygen after about 20% of the cells are irradiated. (Fig. 7)

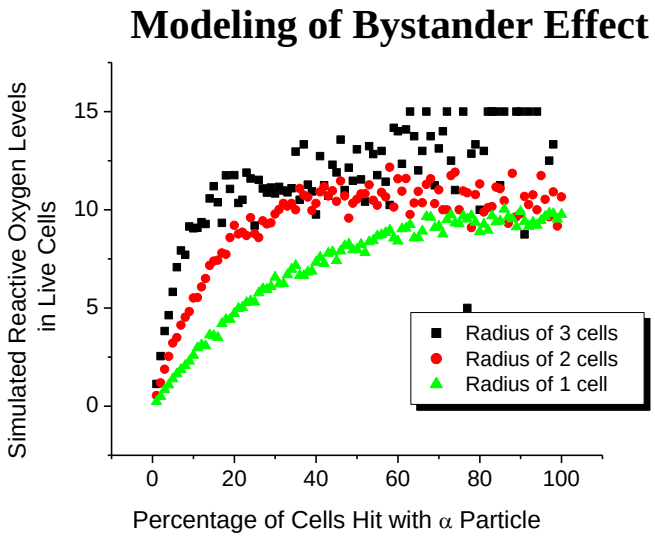


FIG. 7:Simulated H_2O_2 levels increase in a manner consistent with experimental results, and results of T. Hei, et al., when the bystander effect has a radius of three cells.

Though the suggested number of twenty bystander cells affected, is in conflict with other studies (Little and Wakeford[3] suggested lower values), this may be due to different types of cells studied. In addition the model used here only deals with the bystander effect in two dimensions and the result may be different for three. One particular study has indicated that there are

significantly different probabilities of alpha particles impacting the nucleus for different types of cells.[10] As an alpha particle impact to the nucleus could easily kill the cell, and change the data, this suggests that this study be extended to human lung cells.

One way to explain the reactive oxygen data is to invoke a bystander effect, one large enough to saturate the cells in a small region even if only a relatively low precentage of cells are hit. *In vitro*, hitting twenty percent of cells is possible. However, in real life, this is unlikely, especially as the length of time that excess reactive oxygen is produced is uncertain. Despite this, the modeling and *in vitro* experiments indicate that the bystander effect is substantial *in vivo* .

Conclusion

The results to this point are tantalizing but inconclusive. Some of our experiments show that there is an increased co-stimulatory signal produced when increased reactive oxygen levels are measured. On the other hand, some data suggests that different types of cells might respond differently. In particular the large lung cells seem to show almost no change in B7.1 with levels of exposure. This is a very preliminary result and it is clear that more work is needed. Among the factors which need to be considered to improve the quality of the data are the need to work with individual cell types, the need to do time response studies and identify how B7.1 and reactive oxygen change over time, and the need to reduce stresses to cells other than that introduced by alpha irradiation.

Acknowledgements: Mr. David Grammer, RAdata Inc. provided the radium sources

References

[1] Environmental Protection Agency (1992) *Technical Support Document for Ctizen's Guide to Radon EPA 400-R-92* (U.S. Environmental Protection Agency, Washington, D.C.)

[2] Hongning Zhou, Masao Suzuki, Gerhard Randers-Pehrson, Diane Vannais, Gang Chen, James E. Trosko, Charles A. Waldren, Tom K. Hei., "Radiation risk to low fluences of α particles may

be greater than we thought" *Proceedings of the National Academy of Sciences,* December 4, 2001.

[3] M.P. Little and R. Wakeford, "The bystander effect in C3H 10T ½ cells and radon-induced lung cancer," *Radiation Research,* December 2001

[4] Shu-Zheng Liu, "Biological Response of the Immune System to Low Dose Radiation," *The Seventh International Conference on Nuclear Engineering Special Symposium: Forum on Radon,* April 21, 1999

[5] S. Hattori, "Research findings on radiation hormesis and radon therapy," *Transactions of the American Nuclear Society*, 1999.

[6] P. Matzinger. "The danger model: a renewed sense of self" *Science* 2002; **296** (5566)**:**301-5.

[7] Los Alamos National Laboratories, "Lab researchers uncover new effects of radon emissions on human cells", [online] http://www.lanl.gov/worldview/news/releases/archive/97-094.html

[8] Newell MK, Villalobos-Menuey EM, Camley R, Celinski Z, Christensen T. Impact of Metabolism on Immune Response: Implications for Neurological Repair. *Coleman Institute Symposium: Given Institute, Aspen, Colorado* 2001.

[9] Harper M-E, Antoniou A, Villalobos-Menuey E, et al. Characterization of a novel metabolic strategy used by drug-resistant tumor cells. *FASEB* 2002;**16:**in press.

[10] D. Nikezic and K.N. Yu, "Alpha hit frequency due to radon decay products in human lung cells," *International Journal of Radiation Biology*, May 2001