

Commentary on Blood Cell Damage after *in vitro* Irradiation of Fresh Whole Blood with 630 nM Laser Light, F. Fischer, et al.

HEMOGLOBIN IS A PHOTSENSITIVE PIGMENT

It is clear from the data of Fischer, et al., that irradiation of whole blood damages white cells and platelets at lower flux intensities than red cells. Eventually, as irradiation intensity is increased, red cell damage occurs. Their data also confirm that red cell damage can be significantly decreased if the sample is cooled. These findings provide excellent quantitation of flux intensities and the point at which damage commences for the various cell types.

What is additionally intriguing about this particular data set is the disjunction between the morphological changes of red cells and the temperature as measured in the irradiation cell. The changes observed in the blood sample are those which characterize blood that has been heated above a threshold level of 49.5C. At this level spectrin "melts" (1) and characteristic microspherules of membrane enclosed hemoglobin are formed. The temperature probe in the flow cell confirms that the blood temperature never exceeded 37C even in the uncooled samples and remained below 30C in the cooled ones. How then were the red cell microspherules produced?

Two possible explanations come to mind. Perhaps there is some, as yet unknown effect of laser irradiation that can mimic heat damage to red cells without a significant corresponding rise in temperature. This is the explanation that Fischer, et al., favor. Alternatively, perhaps the observed damage is, in fact, heat damage of the usual sort but the temperature measurements somehow do not reflect the true temperature to which the red cells were exposed. I believe the

latter to be the likely explanation. Hemoglobin absorbs laser light of the wavelength employed in these experiments (after all, hemoglobin like Photofrin II is also a hematoporphyrin derivative!).

It is known that if an intense enough beam is directed at an isolated red cell in a microscope slide chamber that the rapid heating that results will cause the red cell to explode. Some years ago Marcel Bessis provided some dramatic footage of just such an event in the process of studying the phagocytosis of red cell debris by macrophages, a process he called necrotaxis (2). It seems likely that the same process is going on here. The red cells are being heated by laser light above the melting point of spectrin during the brief time that they are exposed to intense laser light in the irradiation cell. The intracellular temperature rise is not enough to destroy the cell but it is enough to melt the spectrin and thus produce the characteristic red cell spherules.

It is unlikely that the temperature rise within a red cell can be measured directly. The rise is too brief and the red cell too small. Still, rough estimates of the intracellular temperature can be made. The blood in the irradiation chamber in the uncooled samples presumably starts out at room temperature and, as confirmed by the temperature probe, is heated to < 37C. Since the hematocrit of the blood sample being irradiated is approximately 40% and the temperature rise observed was on the order of 9-10C the red cell internal temperature would have risen something like 23-25C. Starting at room temperature the red cells would have been abruptly raised to about 50C, above the melting point of spectrin. The reason that cooling protects would then be simply that the red cells entered the irradiation chamber below room temperature making a larger temperature rise possible before the critical 49.5C threshold was reached.

If correct, this explanation is encouraging for it suggests that precooling the blood sample would protect red cells against intense laser energy fluxes. It is also discouraging, for if the mechanism of virus kill is similar to that of red

cell heating then precooling the sample would provide some protection for the viruses as well as for red cells.

2. Bessis M. A special form of chemotaxis: necrotaxis. *Nouv Rev Fr Hematol* 13(3):285-290, 1973.

REFERENCES

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