Letters to the Editor

Re: Increased Preservation of Ultrastructure
With LR Gold for Immunocytochemistry

Dear Editor,

I was interested to see the contribution by Hildegard Crowley in the January 1999 issue of *Microscopy Today,* concerning the London Resins.

It is good to see that it is now generally acknowledged that aromatic bisphenol A makes a major contribution to the beam resistance of some epoxy resins. It should be noted, however, that bisphenol A is only present in the Araldite resins used in electron microscopy, and not in the Epons.

According to the London Resin company (personal communication), a polyhydroxy-substituted bisphenol A dimethacrylate is a component of both LR Gold and LR White. I am not aware of the evidence that LR Gold has a higher beam stability or that it produces better ultrastructure than LR White and would be interested in seeing it. Newman and Hobot (ref. 1) appear to be misquoted, since they state that the preservation of ultrastructure is very poor with LR Gold, and this has been my own experience.

Tests I have made with Michael Lamvik (by EELS) have shown that LR White is considerably more resistant to beam damage than the other acrylic resins used in EM, such as glycol methacrylate and the Lowicryls, but still comes far behind the epoxy resins. We did not examine LR Gold. The most stable resins are Araldite and Spurr's resin, followed by Epon. I have observed a direct correlation between the stability of the resins under the electron beam and the preservation of ultrastructure. The results of these studies are summarized in Volume 17 of the *Practical Methods in Electron Microscopy* series *Biological Specimen Preparation for Transmission Electron Microscopy,* by Audrey M. Glauert and Peter R. Lewis (Portland Press and Princeton University Press, 1998).

Yours Sincerely,
Audrey Glauert, University of Cambridge, UK

Dear Editor,

I am delighted that such a well known authority has raised questions about this paper. I will respond in three points:

1. Dr. Glauert says that she is not aware that LR Gold has better beam stability than LR White. Newman and Hobot state that "Interestingly, the aromatic backbone that gives LR Gold (as compared to LR White) its increased resistance to the electron beam, bisphenol A, can also be used to produce epoxy resins."
2. Another of Dr. Glauert's comments usefully suggest a change in emphasis regarding the improved ultrastructure of tissue embedded in LR Gold as compared to tissue embedded in LR White. Newman and Hobot say "The only improvement that was observed with LR Gold over LR White was in the preservation of lipid-rich structure in the rat nephron." I was writing about the preservation of membranes and synapses, with are lipid rich.
3. Migheli concludes that "The degree of antigencity of LR Gold-embedded tissue was comparable to that of the LR White embedded one, but the morphological detail was much better preserved." With considerations from this paper and the reference of Berryman, I wrote a protocol suitable for our situation.

Next, we preformed three side-by-side experiments to compare the preservaton of ultrastructural morphology of LR White and LR Gold. We do note that Dr. Glauert has not examined this. Our results showed that not just lipid structures but also general unltrastructure was greatly improved. This coincides with the summary of Berryman and, also, Megheli, who summarizes that "The various cell organelles, particularly the cytoskeletal filaments, rough endoplasmic reticulum, Golgi apparatus and mitochrondria, were generally well preserved. Optimal conservation of the morphologic detail was achieved in neuritic terminals and synapses." We have now totally abandoned LR White as an embedding medium in favor of LR Gold for immunocytochemistry.

I thank Dr. Glauert for raising the opportunity to clarify these issues.

Yours truly,
Hildegard Crowley, University of Denver