

Luft J H. Improvements in epoxy resin embedding methods.

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Epoxy resin embedding methods are presented consisting of variations of the original Araldite procedure of Glauert et al. The advantages of the methods are (1) rapid embedding, (2) easy sectioning of the embedded tissue, (3) good contrast in the electron microscope, and (4) a wide range of hardness, achieved by using two different anhydride curing agents. [The *SCI*[®] indicates that this paper was cited 6,953 times in the period 1961-1975.]

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'It is pleasant to be the author of a paper on a list of 'Citation Classics' and to be invited to comment on it. Part of the enjoyment comes from seeing that the product of one's laboratory works reliably around the world. Perhaps one reason that 'methods' are so frequently in this category is that many more scientists have need for a reproducible procedure rather than a novel idea or concept-it's hard to paraphrase a method. The project arose from the need for a good reliable embedding method so that I could get on with the main work-electric eels. The consequence of success was to find an albatross hung around my neck as a 'methodologist.'

"Back in the bad old days, electron microscopy of thin sections of biological tissue depended a lot on luck. Among other troubles, methacrylate embedding was erratic, requiring that good' embeddings be selected and the 'bad' thrown out. Selection was largely intuitive, which added variety to the final interpretations. In 1956, Audrey Glauert in Britain published promising results with the Ciba epoxy

resin Araldite. ¹ Our experiments with American Araldite gave brittle, useless blocks. A letter to Audrey (in January, 1958) revealed that we were not alone, and that she had struggled with Ciba/Basle to allow some English material sent to their American division. Samples arrived a month later, along with oilier supplies sent by Hugh Huxley, J.T. Randall and M.S.C. Birbeck in England to H.S. Bennett, then Chairman of the Anatomy Department in Seattle. The English material behaved properly, and by December, 1958, we found that a 3-stage cure (35-45-60°) gave equally good results with American Araldite. The clue came from the excellent book on epoxy resins by Lee and Neville.²

"Araldite was viscous, sticky stuff with an affinity for skin and clothes, and we looked for an alternative easier to live with. Shell produced an epoxy resin known as Epon 562 (later Epon 812) but their recipes, designed for maximum mechanical properties, gave blocks impossible to cut. Again we turned to epoxy chemistry in Lee and Neville. Keeping the 35-45-60° cure, we systematically varied the anhydride:epoxy ratio and the amount of accelerator. Each had an independent optimum for cutting. Hardness of the block could be varied over a wide range by any desired mixture of one soft and one hard liquid anhydride, still retaining the optimum anhydride:epoxy ratio. On July 20, 1959, the first embedding with the final recipe was done. Five days earlier, I had completed the epoxy titrations of the Epon 562 and 812 which we used, so that the anhydride:epoxy ratio could be set exactly. (The titration was done in pyridine in an open lab-very popular with the neighbors.) In August, I left for a year in London where I wrote up the paper, while the large and active electron microscopy group in Seattle was testing the method under a variety of conditions. R..L. Wood in Seattle adapted the optimum anhydride:epoxy ratio back to the original Araldite formula, and this was inserted into the manuscript on one of its many round trips between H.S. Bennett in Seattle and myself in London. The approved manuscript and I left London together...."

1. Glauert A M, Rogers G E & Glauert R H. A new embedding medium for electron microscopy. *Nature* **178**:803, 1956.

2. Lee H & Neville K. *Epoxy resins: their application and technology.* New York. McGraw-Hill, 1957.