

Richardson K C, Jarett L & Finke E H. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technol.* 35:313-23, 1960.

[Department of Anatomy, Washington University School of Medicine, St. Louis, MO]

This paper describes detailed methods for the use of American and English Araldite epoxy resin, yielding blocks that trim easily and provide sections of soft tissue of 1-2 μ and ultrathin sections that cut as easily as methacrylate. Araldite produces no damage to tissues and does not sublime or require support of collodion, formvar, or carbon. [The SCI® indicates that this paper has been cited in more than 2,365 publications, making it the most-cited article published in the journal.]

From Embedding to Insulin Action

Leonard Jarett

Department of Pathology and
Laboratory Medicine

Hospital of the University of Pennsylvania

3400 Spruce Street

Philadelphia, PA 19104-4283

In the late 1950s, the use of electron microscopy was reaching a pinnacle as a high resolution microscopic tool for cell biological and diagnostic purposes. One of the rate-limiting factors in exploiting the power of electron microscopy was appropriate embedding materials that allowed application of histochemical and cytochemical techniques common to light microscopy. A.M. and R.H. Glauert¹ first described the use of Araldite, a new epoxy resin. However, the polymerization and infiltration techniques were hard to reproduce and resulted in blocks that easily fractured and that could not be sectioned thick enough for light microscopy. The staff of the anatomy and pathology departments of Washington University at that time were among the world leaders in the use of ultrastructural techniques.

Independently, K.C. Richardson, E.H. Finke, and I were attempting to improve the techniques for the use of Araldite. Richardson was a professor of anatomy, Finke was a technician for Sara Suse, and I was a first-year medical student working in Paul Lacy's laboratory in pathology. (Finke now works as a research associate for

Lacy.) We combined our experience and produced the data presented in this manuscript. The most difficult task was working out the correct mixture of compounds for Araldite made in America versus that made in England, for they differed dramatically. The other major advance that made infiltration of tissue complete was the use of toluene as a clearing agent. In the 30 years since this manuscript was written, a number of new epoxy and polyester resins, as well as methacrylates, have been developed that allow different uses in ultrastructural research, including immunocytochemistry.

This paper was my first one and helped my research career. My research interest and the activities of my own laboratory have been involved in understanding the mechanism of insulin action. One of our efforts, in collaboration with Robert M. Smith, has been to use combined biochemical and ultrastructural (morphological) approaches to characterize insulin internalization and intracellular processing. We have developed two ultrastructural markers for insulin that behave as native monomeric insulin—namely, monomeric ferritin-insulin² and gold-insulin.³ More recently we incorporated electroimmunocytochemistry into our studies. We have found cell-to-cell variation,⁴ with adipocytes having naturally aggregated receptors while all other cell receptors aggregate after occupancy. Internalization occurs through either uncoated invaginations only (adipocytes), coated pits only (mouse preimplantations embryo), or through both (all other cells). There are multiple intracellular routes, leading to retroendocytosis, degradation, or nuclear translocation. Recently, we have shown insulin translocates into the nucleus without its receptor, using a unique routing and nuclear uptake system.⁵

These studies suggest that internalization and intracellular processing are involved in insulin action and nuclear translocation. The latter phenomenon may be related to insulin regulation of cell growth and/or gene transcription.

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Received November 28, 1990