This Week's Citation Classic®

Locke M. The structure and formation of the cuticulin layer in the epicuticle of an insect, *Calpodes ethlius* (Lepidoptera, Hesperiidae). *J. Morphology* 118:461-94, 1966. [Developmental Biology Center, Western Reserve University, Cleveland, OH]

A dense lamina of cuticulin forms the outermost layer of the epicuticle that completely invests an insect. It arises at the plasma membrane surface as the first sign of new cuticle formation. Cuticle patterns arise by expansion and buckling as the cuticulin increases in area. [The *SCI*® indicates that this paper has been cited in over 185 publications.]

Michael Locke
Cell Science Laboratories
Department of Zoology
University of Western Ontario
London, Ontario N6A 5B7
Canada

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On the top floor of the Zoology Department at Cambridge University in the late 1940s there was a consuming interest in insect cuticle, stimulated by the work of V.B. Wigglesworth (later, Sir Vincent) and J.W.L. (Jimmy) Beament (later, Sir James). Hypothetical structures explained the cuticle's peculiar properties until the prewar Siemens electron microscope in the Cavendish laboratory showed, for the first time, what cuticle looked like in thin sections.

Those were the early days of embedding and sectioning. Knives were made by scratching plate glass sheets with diamond scribes. Some researchers favoured the old glass of Victorian mirrors and broke their knives with a glowing molten globule of silica on a natural crack. Others dropped their sheets on the floor and searched among the fragments for the perfect knife. The microtome was a cuticle specimen mounted on a metal rod that rotated around the knife. When placed in the window on a dull day there was just enough thermal expansion of the rod to cut a thin section of cuticle at each pass. Sunny days gave thick sections, but even these showed that the cuticle had a thin dense outer envelope that came to be called the cuticulin layer.[1]

Electron microscopy became easier for me in the 1960s, first at the Rockefeller Institute, where the RCA 2B was still being used by George Palade, and then in Cleveland, at Western Reserve University (later to become Case Western) in Howard Schneiderman's Developmental Biology Center (later to become a parking lot: puzzled motorists now see the vibration-free pad for an electron microscope embedded in the asphalt), where I used an RCA 3F. This instrument had set back the career of many a promising electron microscopist, but I used it in the work discussed here to study the way that the cuticulin layer forms at an insect moult.

The cuticulin layer completely invests an insect although it is like a bacterial cell wall and not much thicker than a plasma membrane. It is the interface with the environment: the first layer of any new cuticle to be secreted and the layer on which all inner cuticle is deposited. It arises directly above the apical plasma membrane of the epidermis, often in relation to plaques at the tips of microvilli,[2] and it seems to self-assemble at that surface and to grow by accretion at the edges and later by intussusception. Expansion and buckling of the newly formed cuticulin layer gives rise to the intricate surface patterning of insects. Its permeability until ecdysis allows the resorption of moulting fluid, and its postecdysial quinone tanning that makes it hydrophobic creates a surface for cuticular lipids to give the extreme resistance to water loss for which insects are renowned.[3]

My 1966 paper is frequently cited because the discovery that cuticle is deposited within a cuticulin envelope explained how cuticle outside the insect could still be within the body metabolic pool.

Subsequent work has shown that the ability to make cuticulin-like envelopes at plasma membrane surfaces may be a fundamental property of cells.[4] Envelopes occur as bacterial cell walls and as cuticles in algae, plants, most or all invertebrates, and even some vertebrates.

1. Locke M. Pore canals and related structures in insect cuticle. *J. Biophys. Biochem. Cytol.* 10:589-618, 1961. (Cited 155 times.)
2. ——————. The role of plasma membrane plaques and Golgi complex vesicles in cuticle deposition during the molt/intermolt cycle. (Hepburn H R, ed.) *The insect integument.* Amsterdam: Elsevier, 1976. p. 237-58. (Cited 45 times.)
3. ——————. The permeability of insect cuticle to water and lipids. *Science* 147:295-8, 1965. (Cited 80 times.)
4. ——————. Envelopes at cell surfaces—a confused area of research of general importance. (Mettrick D F & Desser S S, eds.) *Parasites, their world and ours: proceedings of the Fifth International Congress of Parasitology,* 7-14 August 1982, Toronto, Canada. Amsterdam: Elsevier Biomedical Press, 1982. p. 73-88.

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