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Concluding Statement

In 1991, I reviewed the ultrastructural work concerning basophils and mast cells that I had completed in the previous 20 years (1970–1990) [7]. At that time several large themes ran through these studies, many of which continue to this day. These themes included identification of basophils (and mast cells) in various circumstances and species. This resulted in the identification and description of a new form of cell-mediated immunity, which we named cutaneous basophil hypersensitivity (CBH) [4]. In the present review essentially covering works spanning about 10 years (1991–2001) new identity issues are covered in Chapter 2. Altogether, these ultrastructural studies identified new sources of basophils and mast cells for the future work of our laboratory, as well as in those of numerous investigators. Another large theme presented earlier in detail [7] was our ultrastructural identification of a new form of secretion, which we named piecemeal degranulation (PMD) [6]. In that presentation we established the capacity for vesicular transport of extracellular tracers to reach secretory granules, as part of an effort to implicate vesicular transport as the mechanism for effecting PMD. A large part of the effort of my laboratory since that review has been directed toward proof of principle of this concept [see Chapter 7]. The third large theme covered in the 1991 review [7] involved recognition that mast cells and basophils contained cytoplasmic lipid bodies, in addition to secretory granules. Our early studies implicated lipid bodies in new function(s) as key players in the arachidonic acid cascade(s) in which basophils and mast cells participate [5].

Newer studies which solidify these role(s) for lipid bodies are covered here in Chapter 3. The fourth large theme reviewed in 1991 [7] concerned our ultrastructural observations that basophils and mast cells have the capacity to survive and recover from either explosive degranulation, characterized by AND, or from PMD. An update on these processes using specific labels for granule contents such as histamine [251] and heparin [216] is presented in Chapter 9. Recognition that these recovery events do occur, combined with our ultrastructural recognition that ribosomes populated granule (and lipid body) domains, informed our pursuit of evidence for site-specific synthetic machinery [see Chapter 10].

Finally, a topic not covered at all in the 1991 review [7] blossomed between 1991 and 2001, and is presented in detail in Chapter 8. In this body of work our recognition and definition of a new endothelial cell organelle, which we termed the vesicular-vacuolar organelle (VVO), and its capacity to be altered functionally by potent permeabilizers (of basophil, mast cell, and tumor cell origin) has enhanced our understanding of how mast cell or basophil secretion causes vascular leakage *in vivo* in disease [13–15].

Organelles of interest that have been the focus of many of our ultrastructural studies reviewed here include granules, lipid bodies, and VVOs. We have entertained (and in some cases answered) many questions about the function(s) of these organelles. For secretory granules the question of how they empty is answered in detail by our description of a degranulation model that is a continuum from PMD to AND. If they empty in place and retain their containers, as defines PMD, do these empty containers refill? Substructural labeling of two granule constituents, histamine and heparin, show that they do refill [Chapter 9]. If these containers refill do they have the capacity to act in a synthetic capacity? Our identification of ribosomes and RNA metabolic machinery in and about granules and lipid bodies [16, 17] supports a vast published literature documenting site-specific synthesis in other circumstances [Chapter 10]. Lipid bodies and secretory granules also contain key cytokines [133–135, 137; Chapter 4], hence may participate in biological reactions by release of such potent agents. Another newly recognized organelle of interest, VVOs in endothelial cells, provided an answer to the regulation of leakage from the vascular compartment to the extravascular space in allergic inflammation [Chapter 8].

Studies of these key themes and of organelles of interest in basophils, mast cells and endothelial cells were greatly facilitated by the development and application of new ultrastructural techniques, as well as of older well-documented electron microscopic methods. Chapters 3–6, 8, and 10 describe these methods in detail. Thus, we made extensive use of ultrastructural autoradiography, post-embedding immunogold, pre-embedding immunonanogold and immunoperoxidase, enzyme-affinity-gold, inhibitor-affinity-gold, tracer techniques,

serial sectioning and computer-assisted three-dimensional reconstructions in pursuit of our aims.

Development and application of this myriad of methods has allowed proof of principle for at least three major cell biological mechanisms, which we initially proposed, based on standard electron microscopic preparations and ultrastructural observations. These are as follows: (1) PMD is effected by vesicular transport of granule contents [Chapter 7]; (2) VVOs in endothelial cells are sessile cytoplasmic structures that allow regulated leakage from microvessels [Chapter 8], and (3) machinery for site-specific synthesis is present in and around secretory granules and lipid bodies of HMCs [Chapter 10].