Endothelial cells of all vascular beds form a single cell layer \textit{in vivo} which is exposed to blood on the lumenal side and to interstitial fluid and extracellular matrix on the ablumenal side. When endothelial cells are removed from the inner lining of the vasculature and grown \textit{in vitro}, this defined anatomical position cannot be used as a major criterion for the morphological identification of these cells as endothelium. The growth substrate, the humoral environment on all sides of the cell, and the hydrodynamic conditions in a culture dish differ substantially from those \textit{in vivo}. However, endothelial cells derived from a variety of large vessels retain an important characteristic in culture: they grow as a single layer of cells. The typical ‘cobblestone’ monolayer pattern at stationary density (Fig. 1A) has therefore become a practical, though insufficient, criterion for the identification of cultured vascular cells as being endothelium (6). In contrast to 3T3 cells and other cells exhibiting density inhibition of growth, the addition of fresh serum or of any of the known growth factors does not influence this monolayer pattern of cultured endothelium in its stationary, post-confluent phase of \textit{in vitro} growth (7). Only physical separation of the cells, as in mechanical scraping, induces further endothelial divisions. Toxic influences and drugs affecting the cytoskeleton may also lead to a temporary separation of the endothelial cells in a post-confluent monolayer, eventually followed by a round of cell divisions. As the cells recover and spread again, excess cells, not finding space for sufficient anchorage on the substratum, are expelled into the medium and die. As a result, the monolayer is restored to its original cell density (10).

There are exceptions, however, to this stringent monolayer pattern of endothelial cell growth in culture. The most apparent one is the formation of ‘sprouters’ (Fig. 1B) (1). These cells appear underneath endothelial cell monolayers and assume a wide variety of shapes, but they contain the ultrastructural and immunohistochemical markers of endothelial cells (see below and elsewhere), and they are capable of forming a typical ‘cobblestone’ monolayer again when they are isolated and replated. No \textit{in vivo} correlate of this ‘sprouter’ phenomenon is known. It may be due to the suboptimal culture conditions or may represent variant endothelium (9). In time-lapse video movies the ‘sprouters’ show increased motility similar to that of non-confluent cells, continuously protruding cell extensions as if some, but not sufficient, anchorage sites could be found. Another possible exception to the monolayering growth pattern is sometimes seen in long-term cultures, where multiple layers are observed being separated by large amounts of extracellular matrix. Although later overgrowth by a non-endothelial cell or spontaneous transformation cannot entirely be ruled out (Fig. 1C), the possibility exists that the cells may use excess extracellular matrix as a substrate for a new, partially complete, monolayer in addition to the old one (11). Similarly, when the endothelial cell layer separates from the lateral wall of the culture vessel as a
Fig. 1. Phase contrast light micrographs of bovine aortic endothelium in culture, passage 2. A: typical 'cobblestone' pattern (× 48); B: 'sprouters' (× 48); C: overgrown culture (× 32).