

2.2.2 Assays that measure membrane alterations

In contrast to necrosis, apoptosis occurs without inflammation. In the end stages of apoptosis, apoptotic bodies are engulfed by macrophages and other phagocytic cells² *in vivo*. Thus, apoptotic cells are removed from the population without spilling their contents and eliciting an inflammatory response.

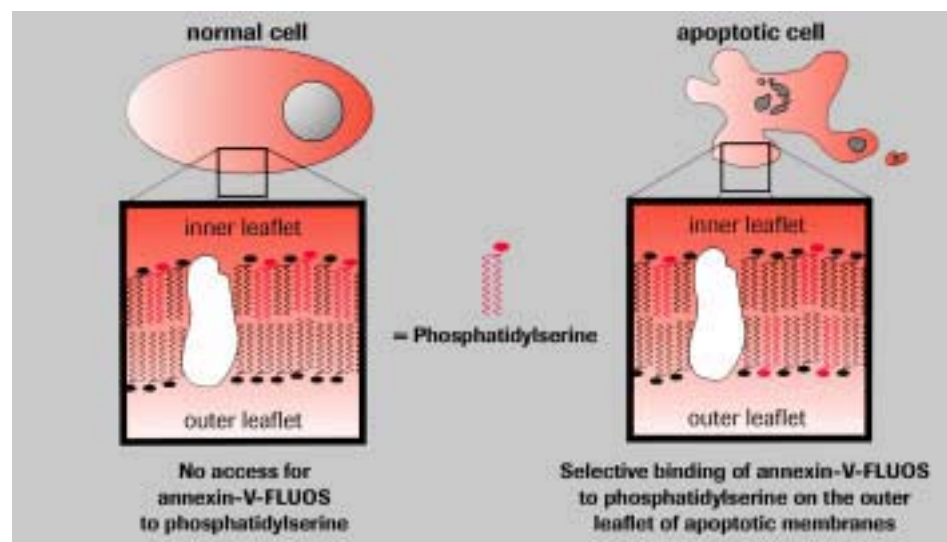
The exact mechanism by which the apoptotic cell becomes a target for phagocytes is unclear. However, it has been shown that a number of changes in cell surface (membrane) markers occur during apoptosis, any one of which may signal “remove now” to the phagocytes. These membrane changes include:

- Loss of terminal sialic acid residues from the side chains of cell surface glycoproteins, exposing new sugar residues^{23,24}.
- Emergence of surface glycoproteins that may serve as receptors for macrophage-secreted adhesive molecules such as thrombospondin²⁵.
- Loss of asymmetry in cell membrane phospholipids, altering both the hydrophobicity and charge of the membrane surface²⁶.

In theory, any of these membrane changes could provide an assay for apoptotic cells. In fact, one of them has – the alteration in phospholipid distribution.

In normal cells (Figure 32, left diagram), the distribution of phospholipids is asymmetric, with the inner membrane containing anionic phospholipids (such as phosphatidylserine) and the outer membrane having mostly neutral phospholipids. In apoptotic cells (Figure 32, right diagram) however, the amount of phosphatidylserine (PS) on the outer surface of the membrane increases, exposing PS to the surrounding liquid²⁷.

Annexin-V, a calcium-dependent phospholipid-binding protein, has a high affinity for PS²⁷. Although it will not bind to normal living cells, Annexin-V will bind to the PS exposed on the surface of apoptotic cells (Figure 33, 34). Thus, Annexin-V has proved suitable for detecting apoptotic cells^{28, 29}. Roche Applied Science supplies a number of products for the detection of PS translocation by Annexin-V.



▲ **Figure 32: Detection of surface morphology changes during apoptosis.** During apoptosis, the distribution of neutral phospholipids (black symbols) and anionic phospholipids such as phosphatidylserine (red symbols) in the cell membrane changes. Phosphatidylserine is present in the outer membrane of apoptotic cells, but not of normal cells. An exogenously added molecule specific for phosphatidylserine, such as Annexin-V-FLUOS, will bind to phosphatidylserine on the outer membrane of apoptotic cells, but cannot react with the phosphatidylserine of normal cells.