**Ankara University Medical Faculty**

**Cell Biology Practice: Programmed Cell Death-Apoptosis Assay**

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Apoptosis is a programmed cell death and generally characterized by distinct morphological characteristics. It acts as a homeostatic mechanism to maintain cell populations in tissues or as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents. Apoptosis involves chromatin condensation and margination, cell shrinkage, membrane blebbing and formation of apoptotic bodies.

With cell shrinkage, the cells are smaller in size, the cytoplasm is dense and the organelles are more tightly packed. The proteases, called nuclease and caspase, cut nucleus and cytoplasmic skeletal proteins. During this process the cell is divided into small fragments containing the nucleus and organelle fragments which called apoptotic bodies.

Light microscopy have detected these morphological changes that occur during apoptosis.

For light microscopic observation, apoptotic cells were stained with Giemsa staining. The apoptotic cell appears as a round or oval mass with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments.

Pre-apoptotic cell

Eraly-apoptotic cell

Late-apoptotic cell

**Procedure:**

1) Cells are washed with PBS.

2) Cells are fixed with a 3: 1 methanol: acetic acid mixture.

* The aim of fixation is capturing and visualizing the state of the relationships between the various cells and the intracellular and extracellular tissue compartments.

3) Cells are spread on glass slide and dried

4) Cells are stained with 5% Giemsa dye for 5-10 minutes.

5) The slides are washed with water after staining.

6) Observe the apoptoic bodies.

7) Draw the displayed structures particularly apoptotic bodies with a microscope magnification.