CELL FRACTIONATION

DR.S.ARULJOTHISELVI
ASSISTANT PROFESSOR

DEPARTMENT OF ZOOLOGY
PERIYAR GOVERNMENT ARTS COLLEGE

- **Cell fractionation:**
- Cell fractionation is a procedure for rupturing cells, separation and
- suspension of cell constituents in isotonic medium in order to study their
- structure, chemical composition and function.
- Cell fractionation involves 3 steps: Extraction, Homogenization and
- **Centrifugation.**
- **1. Extraction:**
- It is the first step toward isolating any sub-cellular structures. In order to
- maintain the biological activity of organelles and bio-molecules, they must be extracted in mild
- conditions called cell-free systems. For these, the cells or tissues are suspended in a solution of appropriate pH and salt content, usually isotonic sucrose (0.25 mol/L) at0-40°C.

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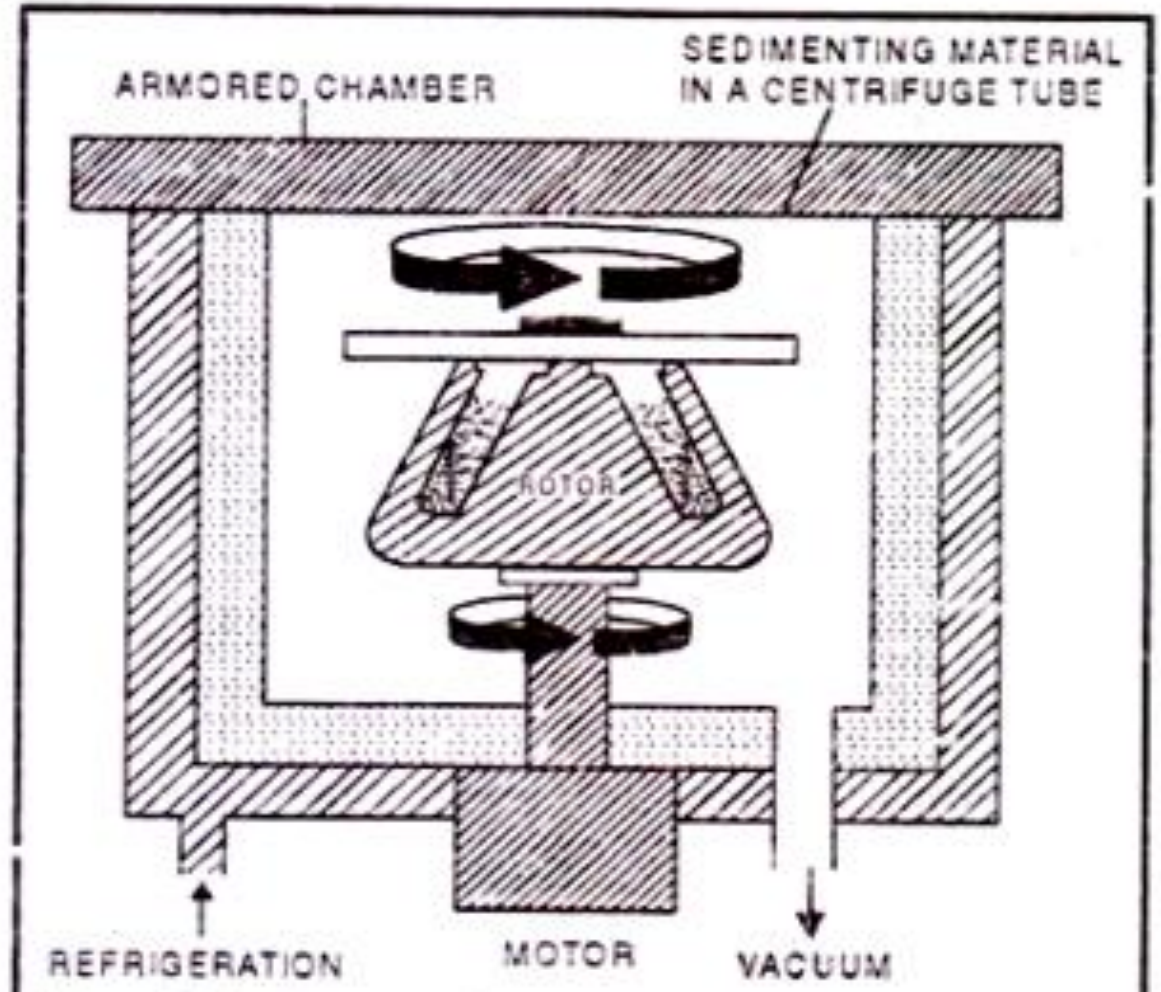
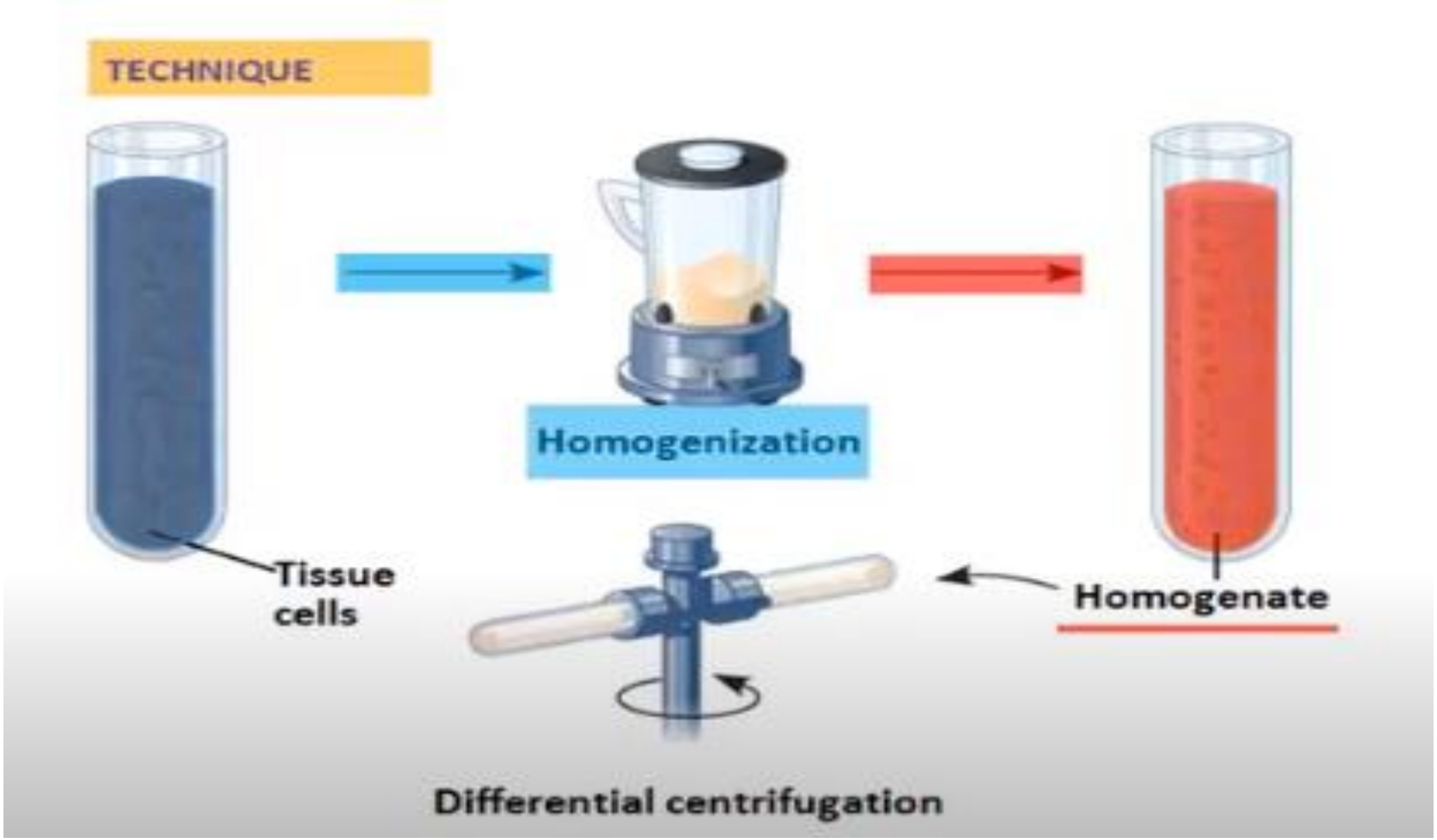


Figure 1.10 A preparative ultracentrifuge, showing details of structure; sample is contained in tubes carried by a rotor, which rotates generating centrifugal force causing particles to sediment; sample is maintained at 4°C; vacuum prevents heating due to friction.


TECHNIQUE
Homogenization
Tissue cells
Homogenate
Differential centrifugation

Cell Fractionation

A two stage process…

1. Homogenisation

2. Differential Centrifugation

Homogeniser
(Science-Blender)

Centrifuge
(Spinny-Thing)

Homogenisation

Breaks open cell membrane / cell wall of sample

Sample must be:

- Ice cold to stop lytic enzyme activity
- pH Buffered to prevent damage to organelles
- Isotonic to prevent osmotic lysis

Differential Centrifugation

Because the organelles have different densities, spinning the sample in a centrifuge allows us to separate out the organelles

The less dense the organelle, the faster the centrifuge must spin in order to separate it from the rest of the sample.

Before we can spin we need to filter…

Differential Centrifugation

First spin at low speed

Supernatent containing less dense organelles

Sediment Pellet containing nuclei

HOMOGENIZERS

- **Homogenization:**

It is a process in which coarse globules in emulsion are converted into smaller globules of uniform composition, so that each measured dose has the same composition.

- **Principle:**

It is based on the principle that when large globules in coarse emulsion are passed under high pressure through a narrow orifice are broken into smaller globules having a greater degree of uniformity and stability.

- **2. Homogenization:**
- The suspended cells are then disrupted by the process of homogenization.
- **It is usually done by:**
- (i) Grinding
- (ii) High Pressure (French Press or Nitrogen Bomb),
- (iii) Osmotic shock,
- (iv) Sonication (ultrasonic vibrations). Grinding is done by pestle and mortar or potter homogenizer (a high-speed blender). The later consists of two cylinders separated by a narrow gap.
- The shearing force produced by the movement of cylinders causes the rupture of ceils. Ultrasonic waves are produced by piezoelectric crystal. They are transmitted to a steel rod placed in the suspension containing cells. Ultrasonic waves produce vibrations which rupture the cells. The liquid containing suspension of cell organelles and ether constituents is called homogenate. Sugar or sucrose solution preserves the cell organelles and prevents their clumping.

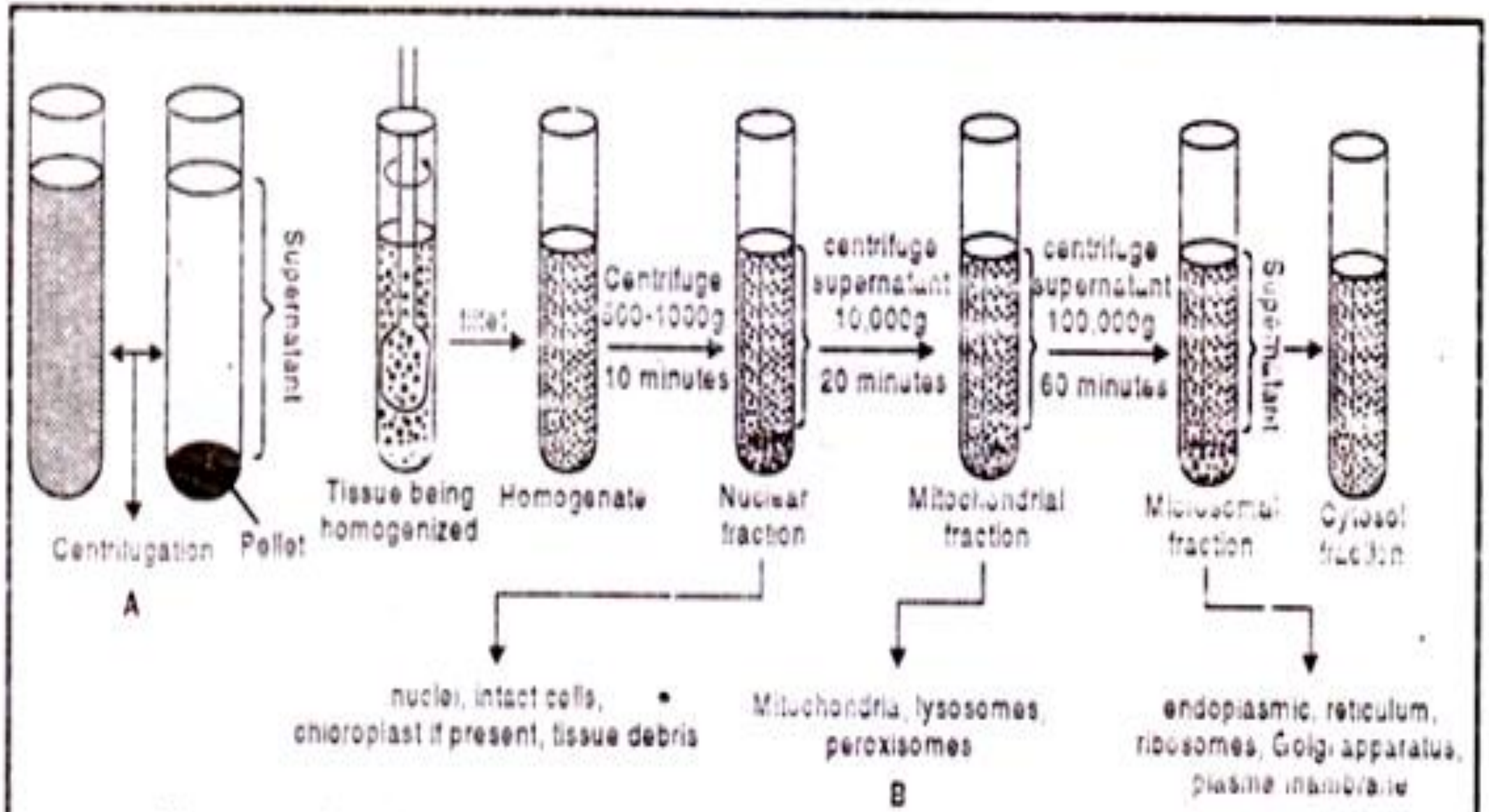
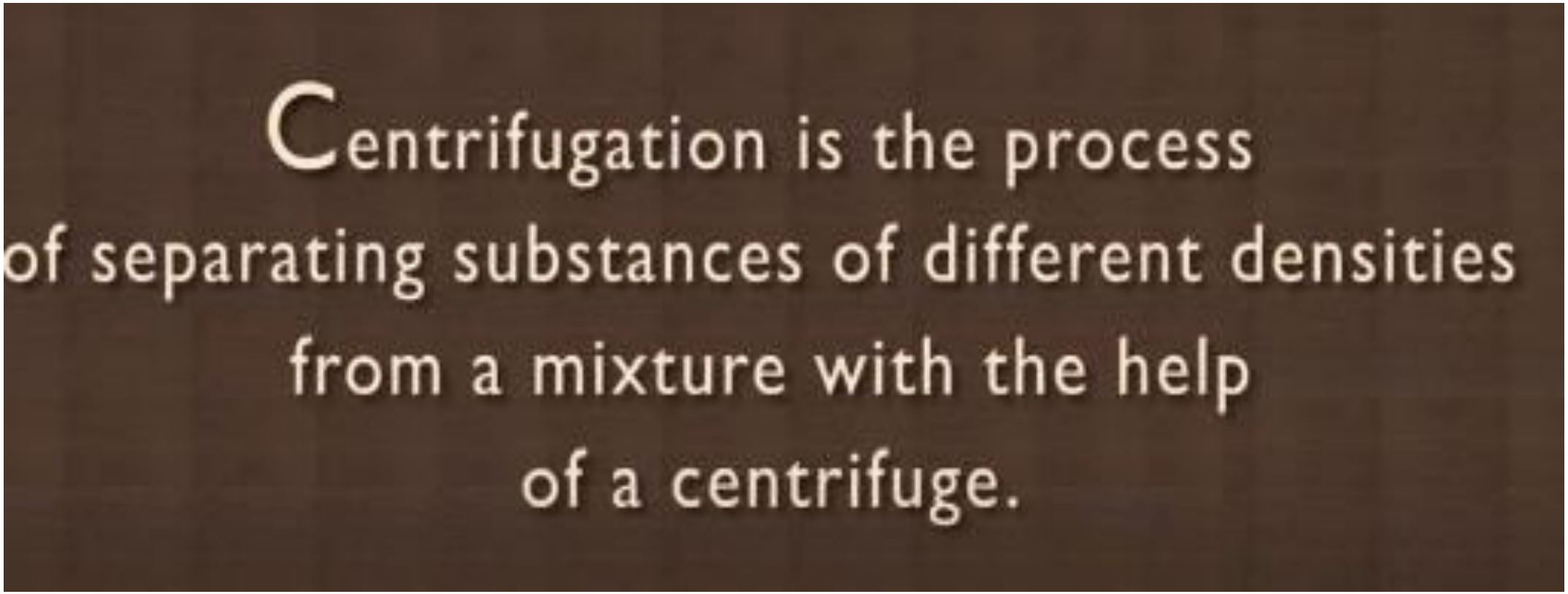


Figure 1.11 Cells fractionation. **(A)** appearance of a sample in the centrifuge tube before and after centrifugation, **(B)** different steps of differential velocity centrifugation.


Centrifugation is the process
of separating substances of different densities
from a mixture with the help
of a centrifuge.

- **3. Centrifugation:**
- The separation (fractionation) of various components of the homogenate is carried out by a series of centrifugations in an instrument called preparative ultracentrifuge.

- The ultracentrifuge has a metal rotor containing cylindrical holes to accommodate centrifuge tubes and a motor that spin the rotor at high speed to generate centrifugal forces.

- Theodor Svedberg (1926) first developed die ultracentrifuge which he used to estimate the molecular weight of hemoglobin.

- Present day ultracentrifuge rotate at speeds up to 80,000 rpm (rpm= rotations per minute) and generates a gravitational pull of about 500,000 g, so that even small molecules like t-RNA, enzymes can sediment and separate from other components.

- The chamber of ultracentrifuge is kept in a high vacuum to reduce friction, prevent heating and maintain the sample at 0-4°C.

- During centrifugation, the rate at which each component settle down depends on its size and shape and described in terms of sedimentation coefficient or Svedberg unit or S-value, where IS = 1×10^{-13} second.

- TYPES OF CENTRIFUGATION
- 1. LOW SPEED CENTRIFUGE
- 2. HIGH SPEED CENTRIFUGES
- 3. ULTRA SPEED CENTRIFUGE

- **LOW SPEED CENTRIFUGE**

- 1) Most laboratories have a standard low-speed centrifuge used for routine sedimentation of heavy particles
- 2) The low-speed centrifuge has a maximum speed of 4000-5000rpm
- 3) These instruments usually operate at room temperatures with no means of temperature control. 4) Two types of rotors are used in it,
- Fixed angle
- Swinging bucket.
- 5) It is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation.

- **HIGH-SPEED CENTRIFUGES**

1. High-speed centrifuges are used in more sophisticated biochemical applications, higher speeds and temperature control of the rotor chamber are essential.
2. The high-speed centrifuge has a maximum speed of 15,000 – 20,000 RPM
3. The operator of this instrument can carefully control speed and temperature which is required for sensitive biological samples.
4. Three types of rotors are available for high-speed centrifugation-

- Fixed angle
- Swinging bucket
- Vertical rotors

ULTRACENTRIFUGES

1.It is the most sophisticated instrument.
2.Ultracentrifuge has a maximum speed of 65,000 RPM (100,000’s x g).
3.Intense heat is generated due to high speed thus the spinning chambers
4. must be refrigerated and kept at a high vacuum.
4.It is used for both preparative work and analytical work.