CENTRIFUGATION TECHNIQUE

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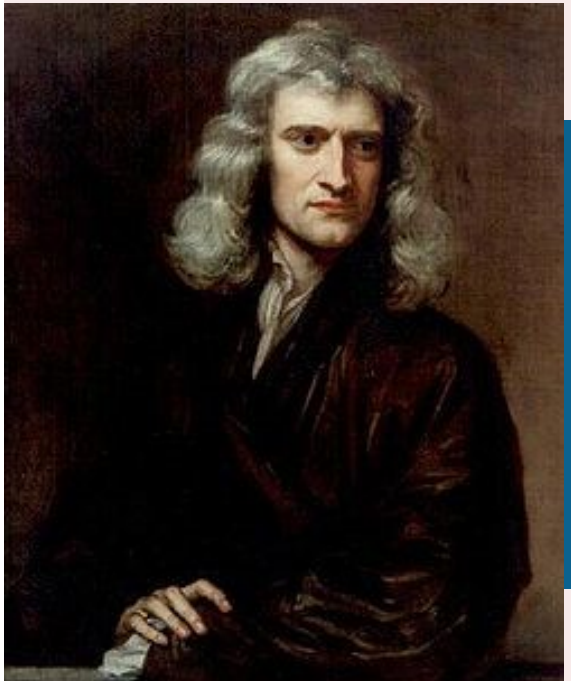
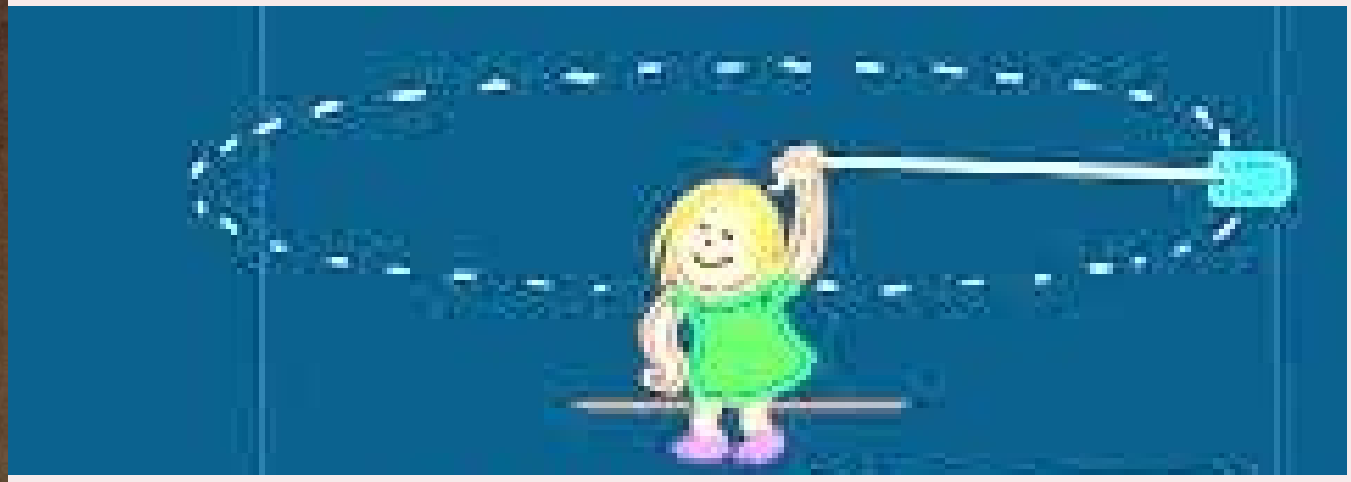




Cited Literature: Book of Biochemical Methods by Wilson and Walker, Cambridge low price edition

What is Centrifugation? What is the use? How it works?

- **A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed**
- The theoretical basis of this technique is the effect of gravity on particles (including macromolecules) in suspension. The particles are sedimented by a greatly increased gravitational field.
- Particles of different masses will settle in a tube at different rates in response to gravity. Centrifugal force is used to increase this settling rate in the centrifuge.
- The gravitational force or centrifugal force (F) is proportional to the square of angular velocity (w^2), which is expressed in revolutions per minute (rpm), times the radial distance r (cm) from the center of rotation.

  $F = (1.12 \times 10^{-5}) (w^2) (r)$
- e.g. rotor with radius of 7 cm and angular velocity of 10,000 rpm, the gravitational force is 8000 times the force of gravity or 8000g.
- In biology, the particles are usually cells, subcellular organelles, viruses, large molecules such as proteins and nucleic acids.

Principles of Centrifugation

Sedimenting force on particle
= Mass x centrifugal field
= $\mathbf{m\omega^2 r}$

where ω = angular velocity of rotor (radians/sec)
r = radius (*ie* distance of particle from axis of rotation)

Relative Centrifugal Force (RCF)

RCF = 1.119 x 10^{-5} x $(rpm)^2$ x r

RCF value reported as "No. x g" (ie multiples of earth's gravitational (force).

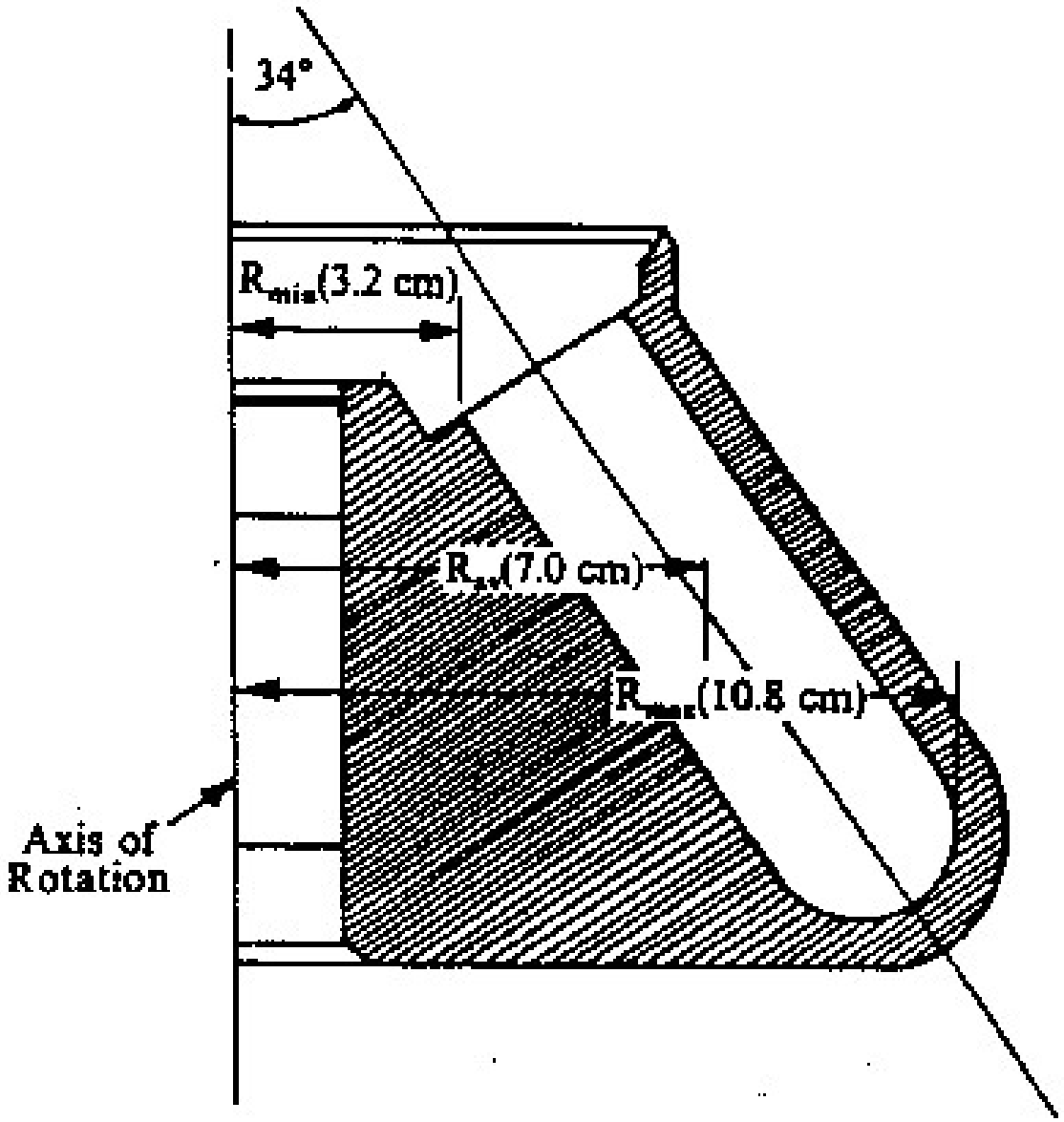
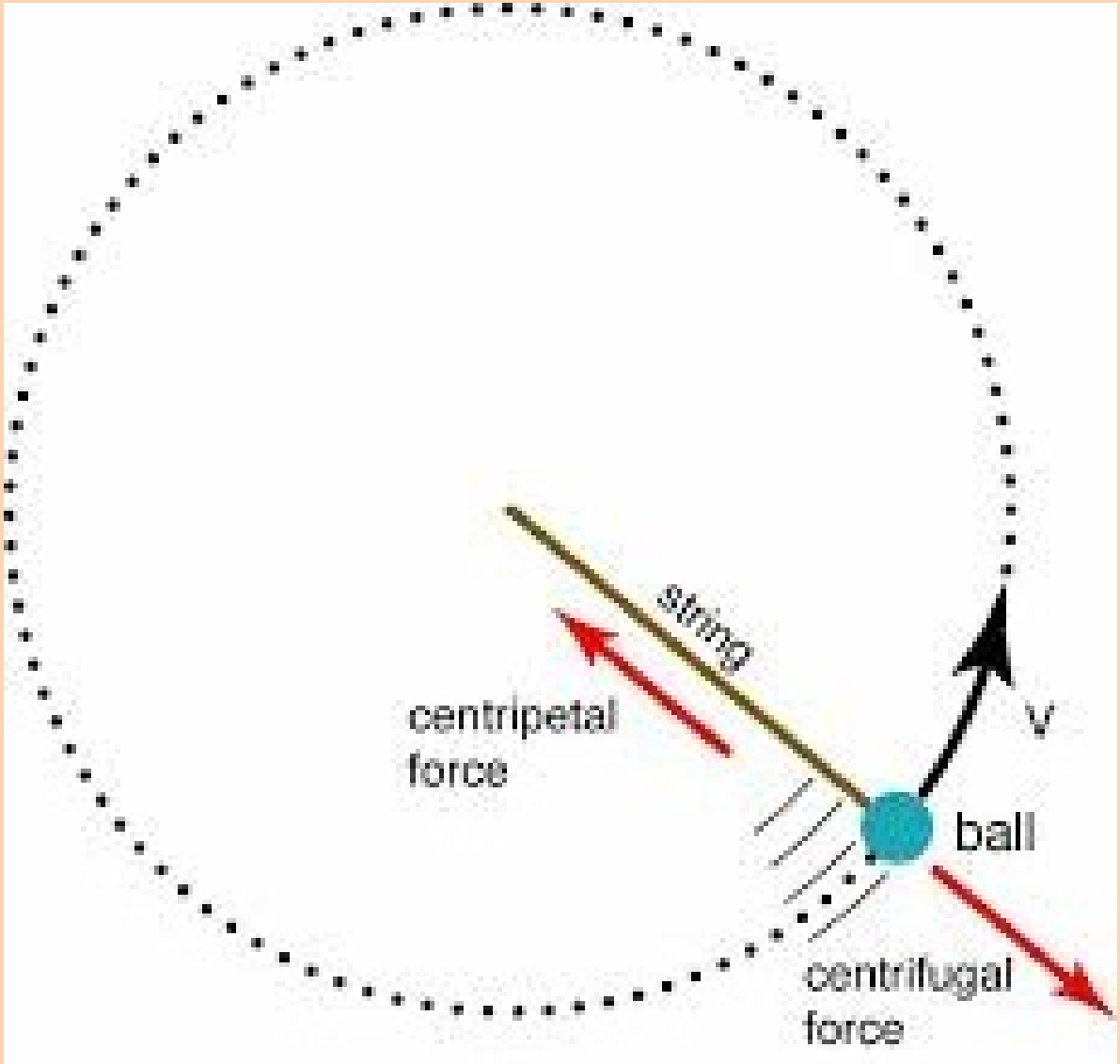
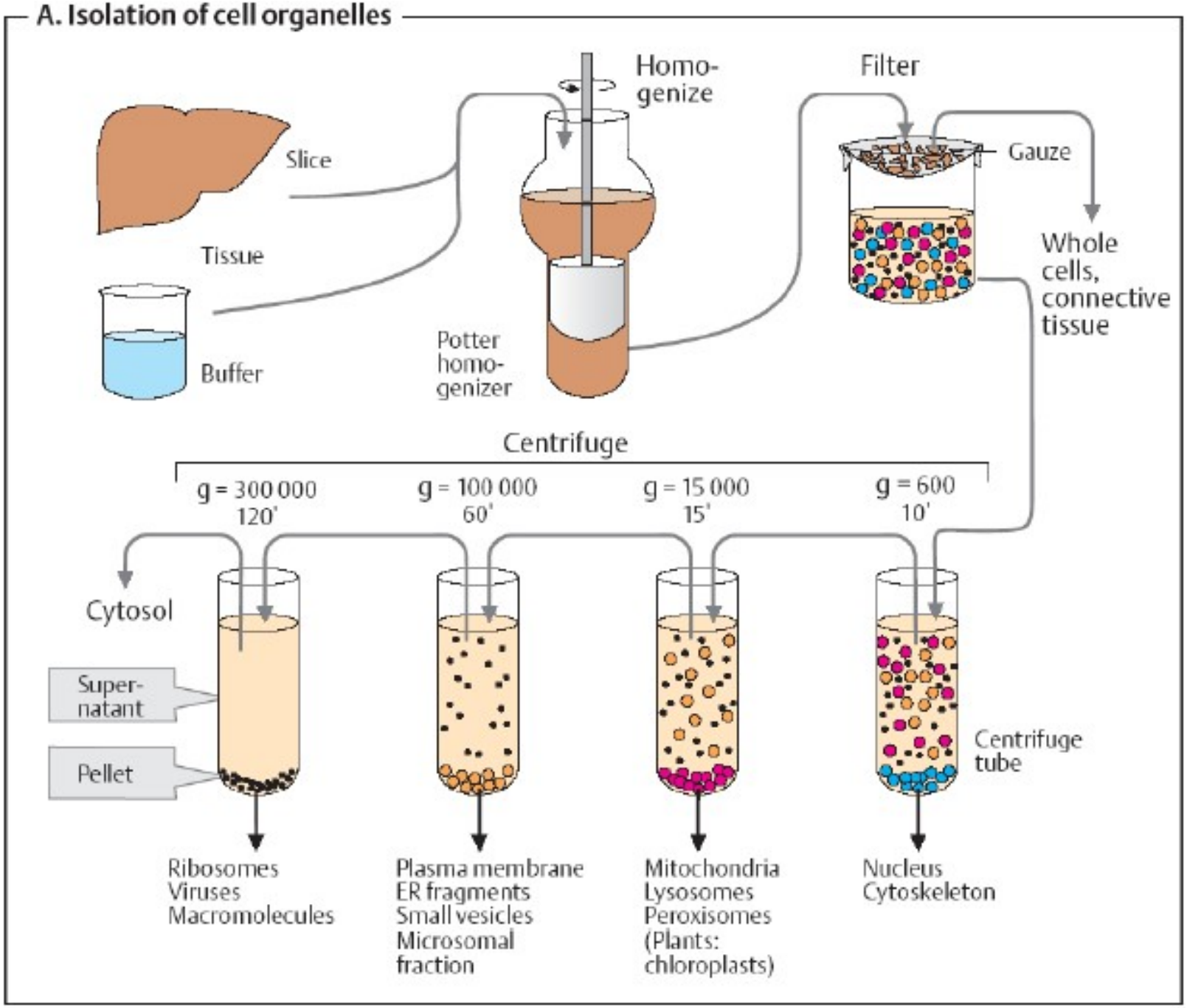


Fig. illustrate the angel of rotation and radius from the axis of rotation

Centripetal and Centrifugal force



- In a solution, particles whose density is higher than that of the solvent **sink (sediment),** and particles that are lighter than it **float** to the top.
- The greater the difference in density, the faster they move.
- If there is no difference in density (**isopyknic conditions**), the particles **hover**. To take advantage of even tiny differences in density to separate various particles in a solution, **gravity** can be replaced with the much more powerful "**centrifugal force**" provided by a centrifuge.


A. Isolation of cell organelles
Slice
Tissue
Buffer
Homo-
genize
Potter
homo-
genizer
Filter
Gauze
Whole
cells,
connective
tissue
Centrifuge
g = 300 000
120'
g = 100 000
60'
g = 15 000
15'
g = 600
10'
Cytosol
Super-
natant
Pellet
Centrifuge
tube
Ribosomes
Viruses
Macromolecules
Plasma membrane
ER fragments
Small vesicles
Microsomal
fraction
Mitochondria
Lysosomes
Peroxisomes
(Plants:
chloroplasts)
Nucleus
Cytoskeleton

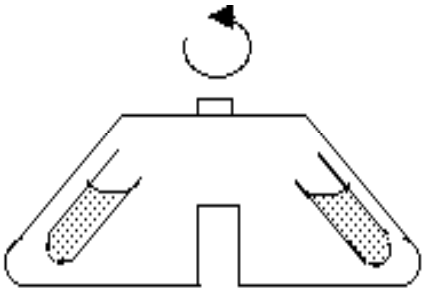
Types of Centrifugation

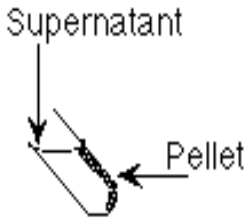
- On the basis of purpose of centrifugation, centrifugation are of two types analytical and preparative
- Analytical centrifugation involves measuring the physical properties of the sedimenting particles such as sedimentation coefficient or molecular weight.
- Optimal methods are used in analytical ultracentrifugation. Molecules are observed by optical system during centrifugation, to allow observation of macromolecules in solution as they move in gravitational field.
- The concentration of the solution at various points in the cell is determined by absorption of a light of the appropriate wavelength (Beer's law is followed).
- The other form of centrifugation is called preparative and the objective is to isolate specific particles which can be reused.

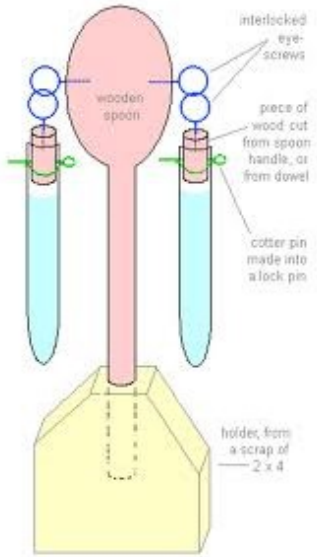
- Clinical centrifuges – it's a tabletop centrifuges that can run at a speed of up to 3000 rpm. These can pellet cells, but not organelles or biomolecules.
- Microfuge - these can typically run at a speed of up to 14,000 rpm, sufficient speed to pellet nucleic acids and denatured proteins. Microfuges are specially adapted for small volumes of sample.
- High speed centrifuges - can run at speeds up to 25,000 rpm, sufficient to pellet cell nuclei and most biomolecules. These are often refrigerated.
- Ultracentrifuges - The ultracentrifuge is capable of reaching even greater velocities and requires a vacuum to reduce friction and heating of the rotor. It can run at speeds up to 75,000 rpm, sufficient to allow fractionation of biomolecules, for example: plasmid DNA, chromosomal DNA, and RNA. Ultracentrifuges are usually refrigerated and are very expensive and delicate pieces of machinery.

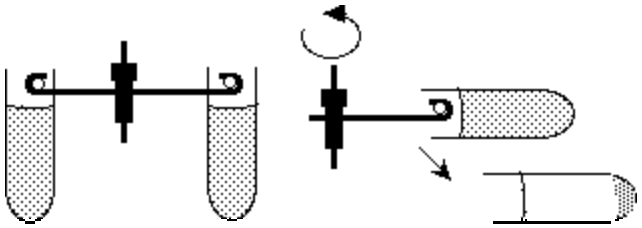
Types of Rotors

- Rotors for a centrifuge are of different types e.g. fixed angles, swinging buckets, continuous flow, or zonal.

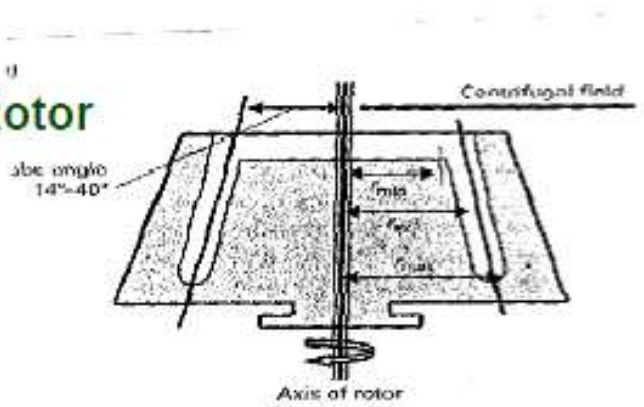


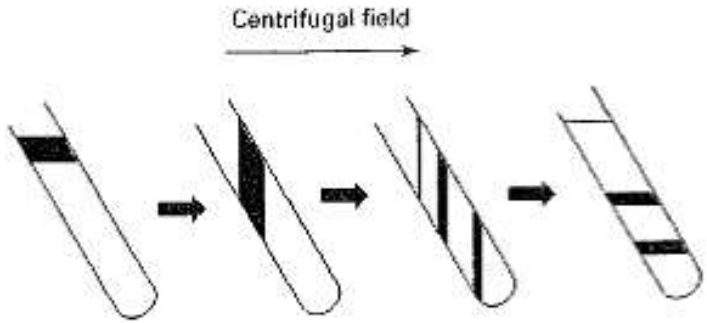




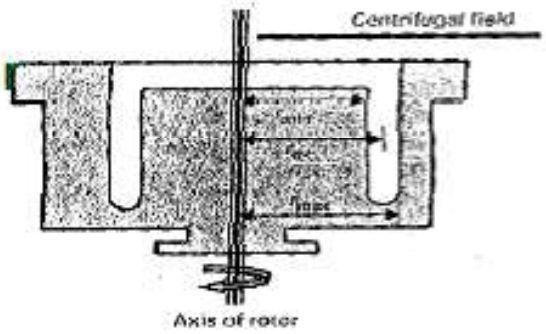


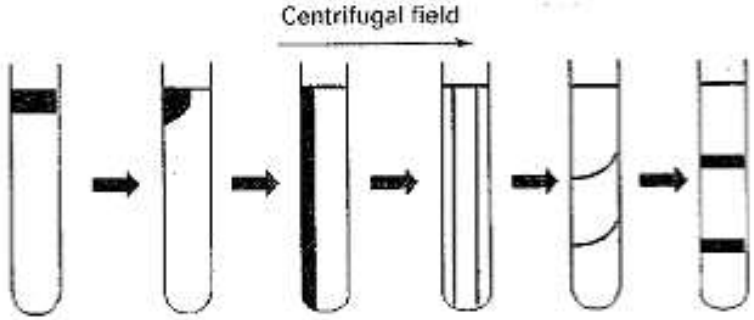
■Fixed Angle Rotor



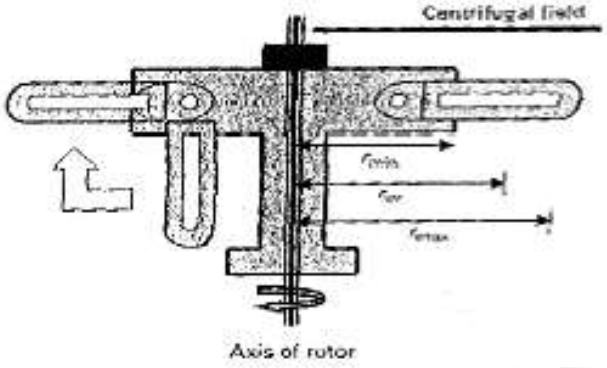


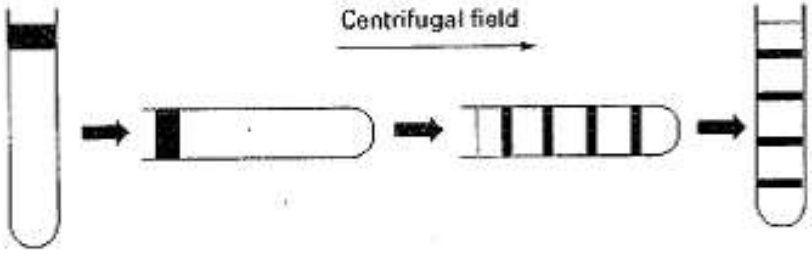
■Vertical Tube Rotor



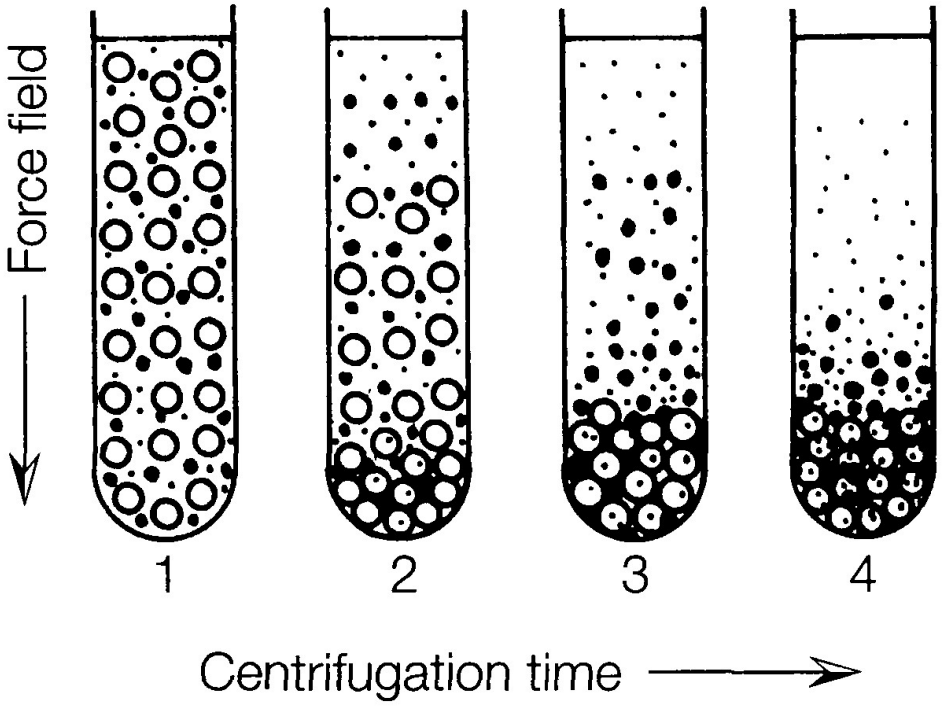


■ Swinging Bucket Rotor





Process of Separation



Cell fractionation and ultracentrifugation to separate cell components

Preparing for centrifugation

- Take one spinach leaf
- Put it into a mortar and add 5 ml cold buffer
- Grind to a green soup (=homogenate)
- Filter through a sieve into a beaker
- Transfer to a centrifuge tube.
- Place tube on ice ready for centrifugation

Centrifugation

Biologists use the technique of centrifugation to extract and isolate pure samples of individual cell organelles for study

Once isolated, the chemistry and functioning of the organelles can be investigated in detail

Two types of centrifugation are possible

Detail not needed (extension)

DIFFERENTIAL CENTRIFUGATION

Isolated organelles are separated on the basis of their weight

The suspension of isolated organelles is spun for different combinations of speed and time

The lowest speed and shortest time separates out the heaviest organelles with high speeds and longer times separating the lightest organelles

DENSITY GRADIENT CENTRIFUGATION

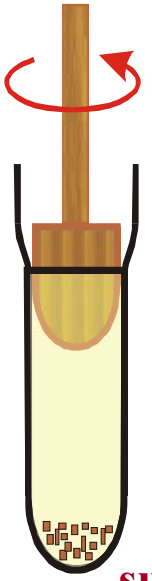
Isolated organelles are separated on the basis of their density

Solutions of increasing density, such as sucrose solutions, are layered into a test tube with the most concentrated solution at the bottom of the tube

The suspension of isolated organelles is pipetted onto the top of the most dilute solution

As the tubes are spun, the organelles collect in the layer which corresponds to their own density

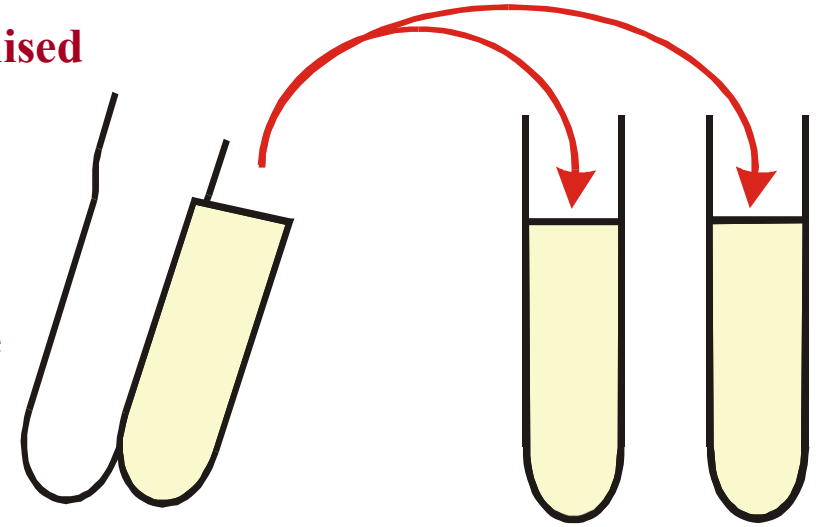
DIFFERENTIAL CENTRIFUGATION



Chopped liver tissue is homogenised in an isotonic salt solution kept at 2°C

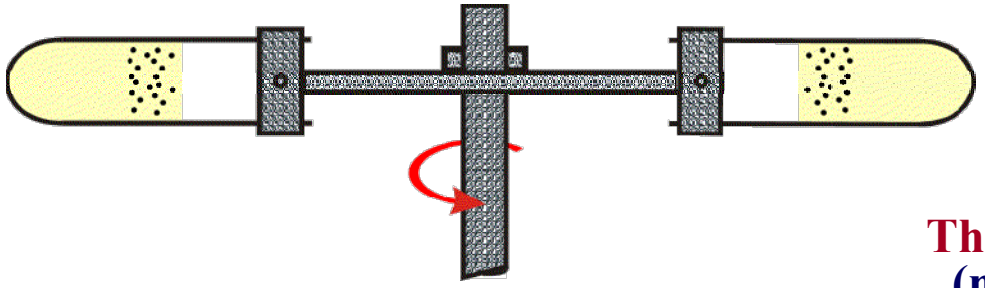
The isotonic solution prevents organelles from shrivelling or bursting due to osmotic effects and the low temperature prevents any cellular chemical activity from taking place

The internal organelles of the cell are released into a suspension called the HOMOGENATE

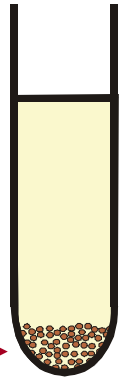


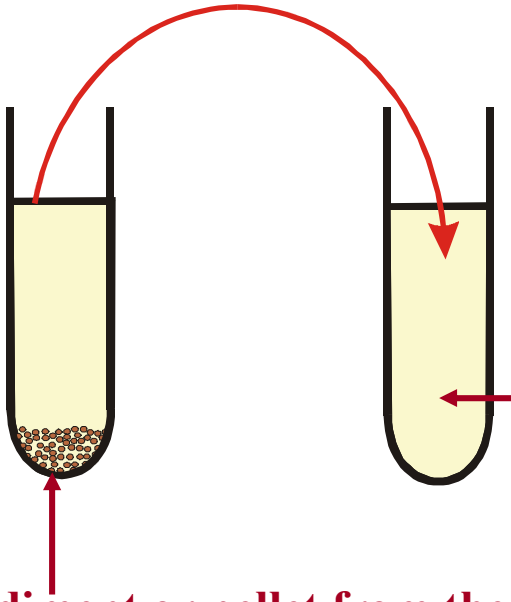
The homogenate is transferred into centrifuge tubes

THIS FIRST SAMPLE IS THEN CENTRIFUGED AT LOW SPEED FOR A SHORT PERIOD OF TIME



The heaviest organelles (nuclei) collect at the bottom of the tube



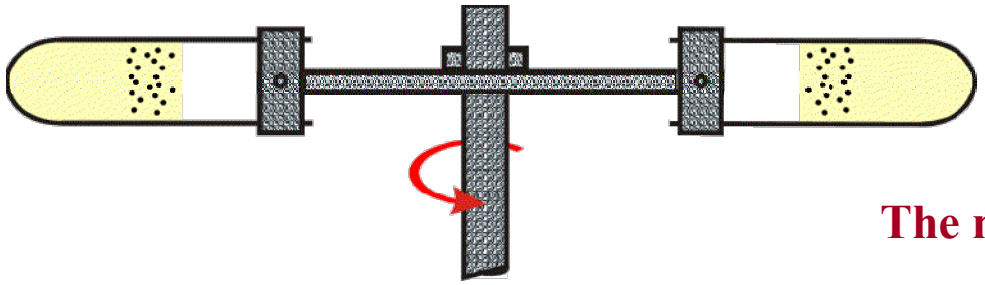


The SUPERNATANT from the first sample is transferred to a second centrifuge tube

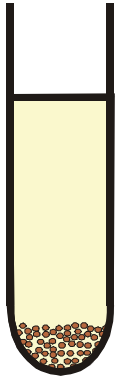
SAMPLE 2

The sediment or pellet from the first sample is removed and examined

THIS SECOND SAMPLE IS THEN CENTRIFUGED AT MODERATE SPEED FOR A LONGER PERIOD OF TIME



The moderately heavy organelles (mitochondria) collect at the bottom of the tube



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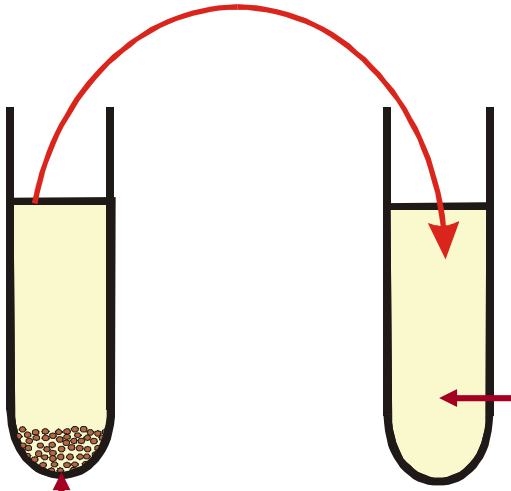
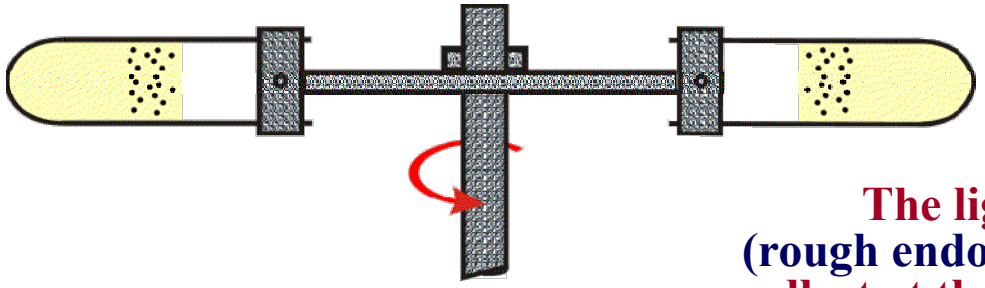


The **SUPERNATANT** from the second sample is transferred to a third centrifuge tube

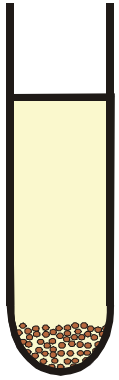
SAMPLE 3

The sediment or pellet from the second sample is removed and examined

THIS THIRD SAMPLE IS THEN CENTRIFUGED AT HIGH SPEED FOR AN EVEN LONGER PERIOD OF TIME



The light organelles (rough endoplasmic reticulum) collect at the bottom of the tube



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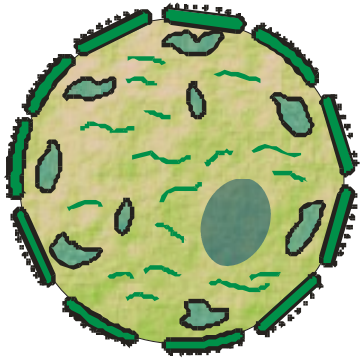
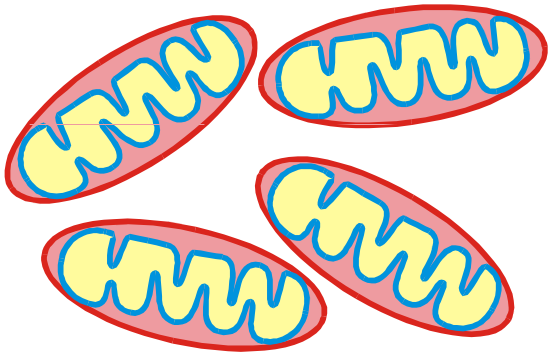
Summary

NUCLEI



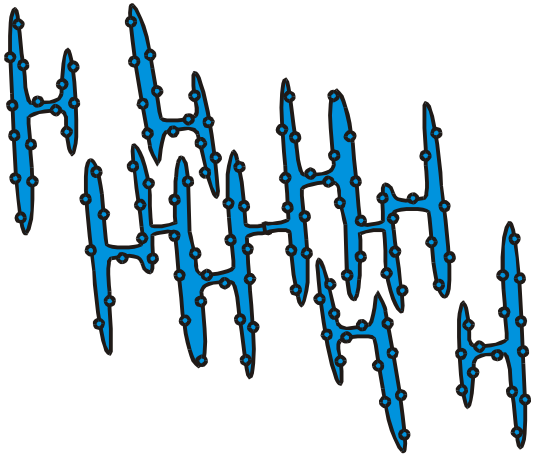
Centrifuge at LOW SPEED for 10 minutes

MITOCHONDRIA



Centrifuge at MODERATE SPEED for 15 minutes

MEMBRANES - ROUGH ENDOPLASMIC RETICULUM



Centrifuge at HIGH SPEED for 30 minutes

NEXT