Organelle Seperation by Centrifugation.

Cell is the basic and fundamental unit of living organisms It is made up of a large no of smaller units called cells organelles having different function The study of different cell organelles and their function is part of modern Cytology it can be done by the chemical characterisation and localisation of enzymes, substances or group of substances in the cells and intracellular materials of a tissue.

centrifugation is a technique which involves the application of centrifugal force to seperate particles from a solution according to their shape, size, density, viscosity of the medium and rotor speed.

cell fractionation - Seperation of subcellular fractions -

cell fractionation method involves homogenization and destruction of cell boundaries by mechanical forces into different fractions according to their mass, surface and specific gravity. A standard cell fractionation can be explained by following example-

The liver of an animal is first perfused with an ice cold saline solution, followed by cold 0.25 sucrose The tissue is then forced through a perforated steel disk and homogenized in 0.25 M sucrose this cell fractionation is directed to subdivision of the cell component into four morphologically distinct fractions (nuclear mitochondrial, microsomal and soluble) and a fifth fraction containing secretory granules may also be obtained.

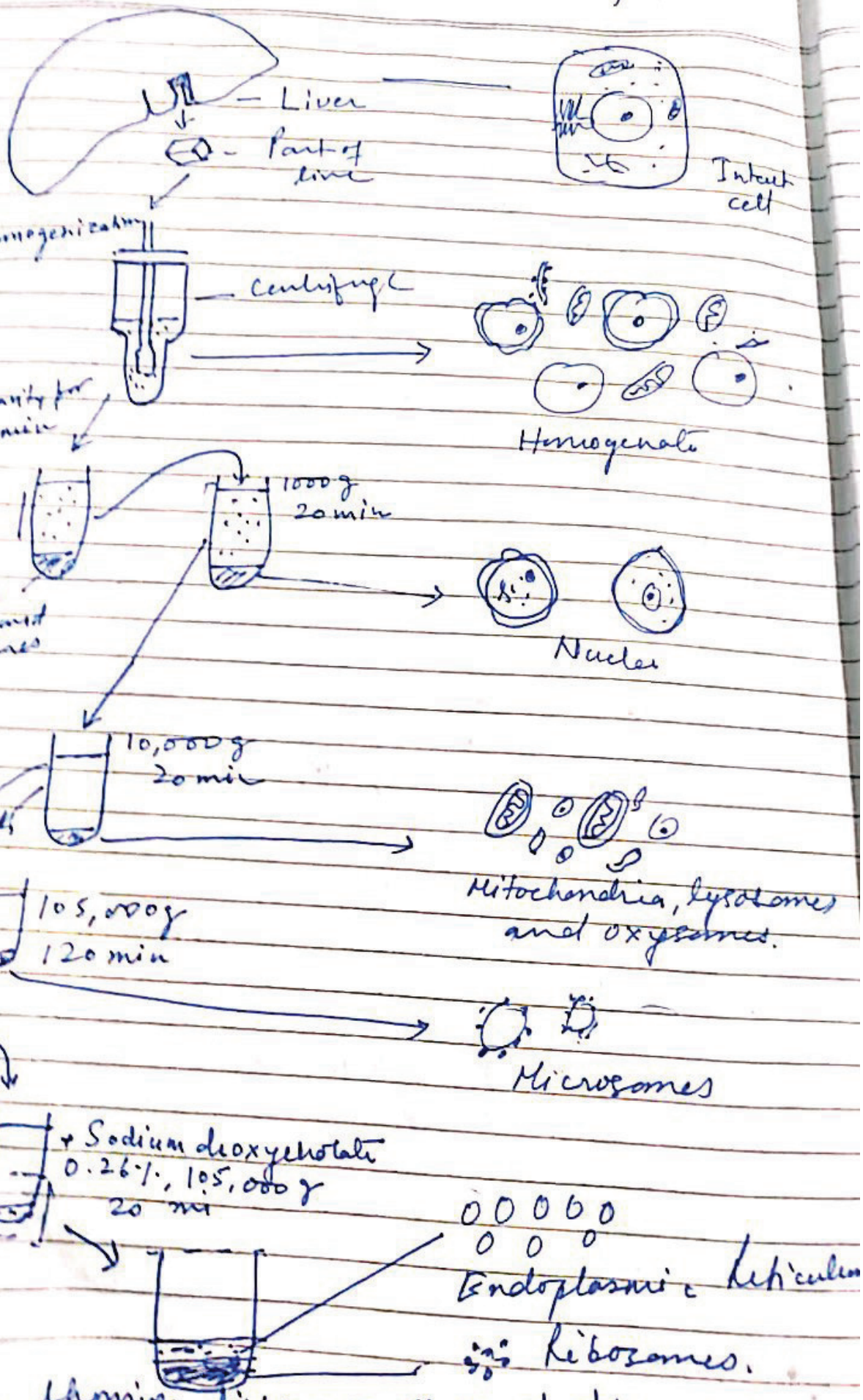

Diagram showing different steps of differential centrifugation.

The method shown in the diagram to separate the subcellular particles particles is called differential centrifugation. according to requirement standard centrifuges or preparative ultracentrifuges are used.

The effect of the centrifugal field on particles of different sizes are as follows

Initially all the particles are distributed homogeneously as centrifugation proceeds the particles settle according to their sedimentation ratios. Complete sedimentation of larger particle is achieved ~~ini~~ initially.

Separation of much smaller particles such as viruses, nucleic acids and proteins is done with the help of analytical ultracentrifuge. This device has a transparent window in the tube and with suitable optical and electronic techniques the moving particle boundries can be visualized and the sedimentation coefficient can be determined This coefficient can be ~~determined~~ expressed in Svedberg (S) units is related to the molecular weight of the particle (for eg. tRNA with 4S has a mol wt of 25,000 daltons)

Application of centrifugation —

1) Production of bulk drugs such as aspirin from its mother liquor.
2) Separation of blood cells.
3) Purification of insulin by selectively ~~precipitation~~ precipetating fractions of proteins.

4) Seperation of bacteria from milk.
5) To determine the purity of sample.
6) To know about the assembly and seperation of sub cellular particles during protein system synthesis and biological manufacturing

Importance of Seperation by Centrifugation in living system-

1) Seperation and purifying mixtures of biological particles in a liquid medium.
2) It is a key technique for isolating and analysing cells, sub cellular fractions supramolecular complexes and isolating macromolecules such as proteins or nucleic acids.
3) It is a critical tool for modern biochemistry and are employed in almost all invasive subcellular studies.
4) Helpful in Biological characterization ~~and~~ of sub cellular particles.
5) Density gradient centrifugation permits the seperation of multicomponent mixtures of macromolecules and the measurement of sedimentation coefficient.

Principles of centrifugation —

1) Sedimentation of cellular particles makes heavier particles sink and lighter particles to float in the solvent or medium.
2) The solution is subjected to high angular velocity measured in rpm, the effect of gravity and thereby the rate of sedimentation greatly increased due to application of an external force called centrifugal force.