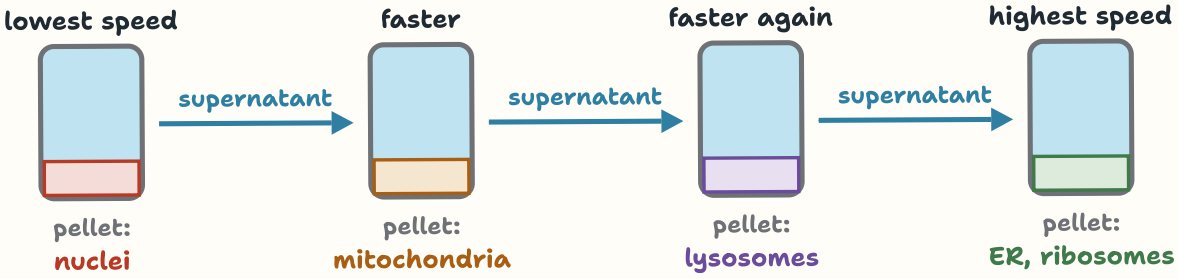
Cell Fractionation and Ultracentrifugation

Homogenise, filter, then spin faster each time to separate organelles by density.

Spin, pour off the supernatant, spin faster: densest pellets first



Each pellet is one fraction; the order is by density

Key terms

homogenisation	the tissue is blended, releasing the organelles.
filtration	removes large debris and unbroken cells; what passes through is the homogenate.
isotonic	the same water potential as the cells, so no net movement of water by osmosis.
buffered	a buffer keeps the pH constant, so enzymes and other proteins are not denatured.
pellet	the heaviest, most dense organelles are pushed to the bottom of the tube.
supernatant	the liquid left above the pellet, holding the lighter organelles.

I can...

- [ ] Principles of cell fractionation and ultracentrifugation to separate cell components
- [ ] Appreciate a long period when the community distinguished between artefacts and organelles

EXAM TRAP

Isotonic, cold and buffered each need a reason. The heaviest organelles pellet first.

5 marks: what earns them

1. Homogenise (blend / break up) the tissue to break open cells and release organelles; filter to remove debris / unbroken cells (either homogenising or filtering earns the mark)
   Not: cut the cells, with no release of organelles
2. Isotonic solution, so no net osmosis / water potential same, so organelles do not burst or shrink
   Not: isotonic alone, with no reason
3. Cold, to reduce enzyme activity so organelles are not digested / damaged
   Not: cold to stop enzymes working, with no link to damage; kills enzymes
4. Buffered, to keep pH constant so enzymes / proteins are not denatured
   Not: buffer stops acid, with no consequence
5. Centrifuge at low speed, heaviest organelles (nuclei) form a pellet; remove supernatant and spin at higher speed, repeating to separate the next organelles (low-speed pellet of heaviest organelles OR repeat at higher speed earns the mark; both not required)
   Not: lightest organelles first; one spin separates all organelles

Remember

Isotonic, cold, buffered: each needs its reason.

Spin faster each time; densest pellets first.

Order: nuclei, mitochondria, lysosomes, ER.

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