

Cell Breakage and Fractionation - Part 1

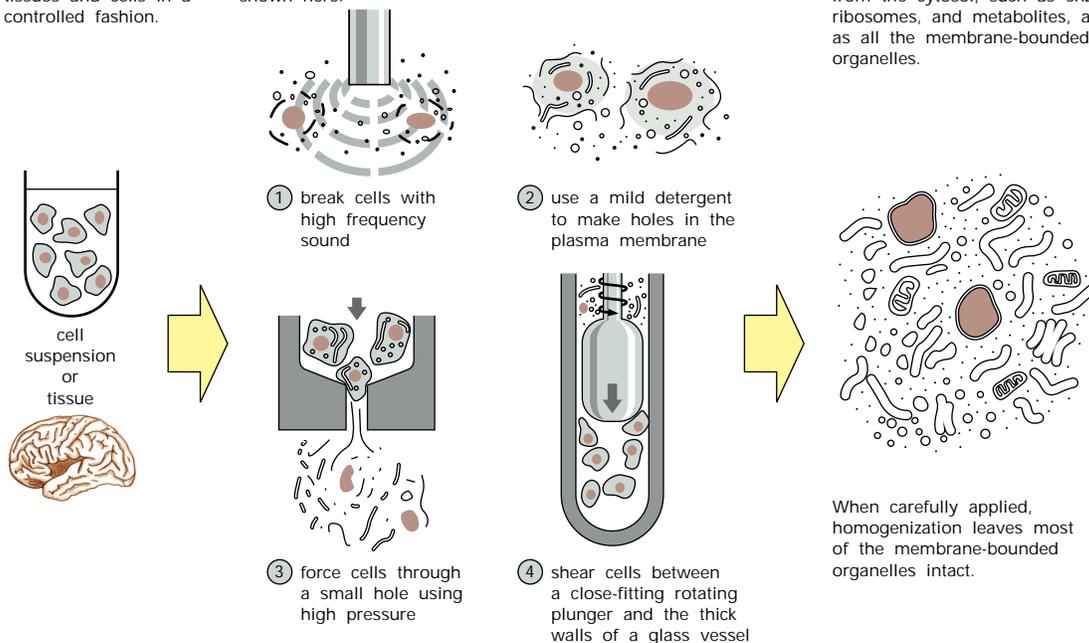
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BREAKING CELLS AND TISSUES

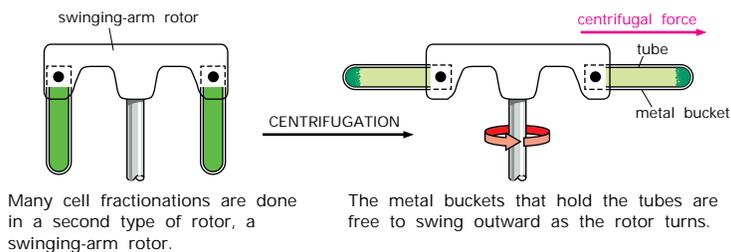
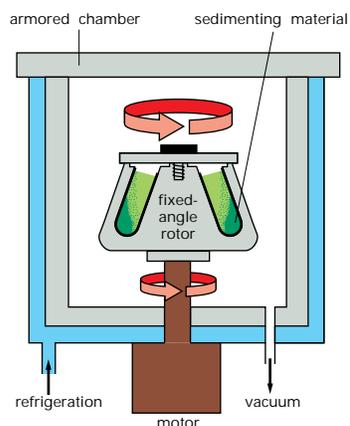
The first step in the purification of most proteins is to disrupt tissues and cells in a controlled fashion.

Using gentle mechanical procedures, called homogenization, the plasma membranes of cells can be ruptured so that the cell contents are released. Four commonly used procedures are shown here.

The resulting thick soup (called a homogenate or an extract) contains large and small molecules from the cytosol, such as enzymes, ribosomes, and metabolites, as well as all the membrane-bounded organelles.



THE CENTRIFUGE



Centrifugation is the most widely used procedure to separate a homogenate into different parts, or fractions. The homogenate is placed in test tubes and rotated at high speed in a centrifuge (sometimes called an ultracentrifuge). Present-day ultracentrifuges rotate at speeds up to 100,000 revolutions per minute and create enormous forces, as high as 600,000 times gravity.

At such speeds, centrifuge chambers must be refrigerated and evacuated so that friction does not heat up the homogenate. The centrifuge is surrounded by thick armor plating, since an unbalanced rotor can shatter with an explosive release of energy. A fixed-angle rotor can hold larger volumes than a swinging-arm rotor, but the pellet forms less evenly.