TABLE 5.1

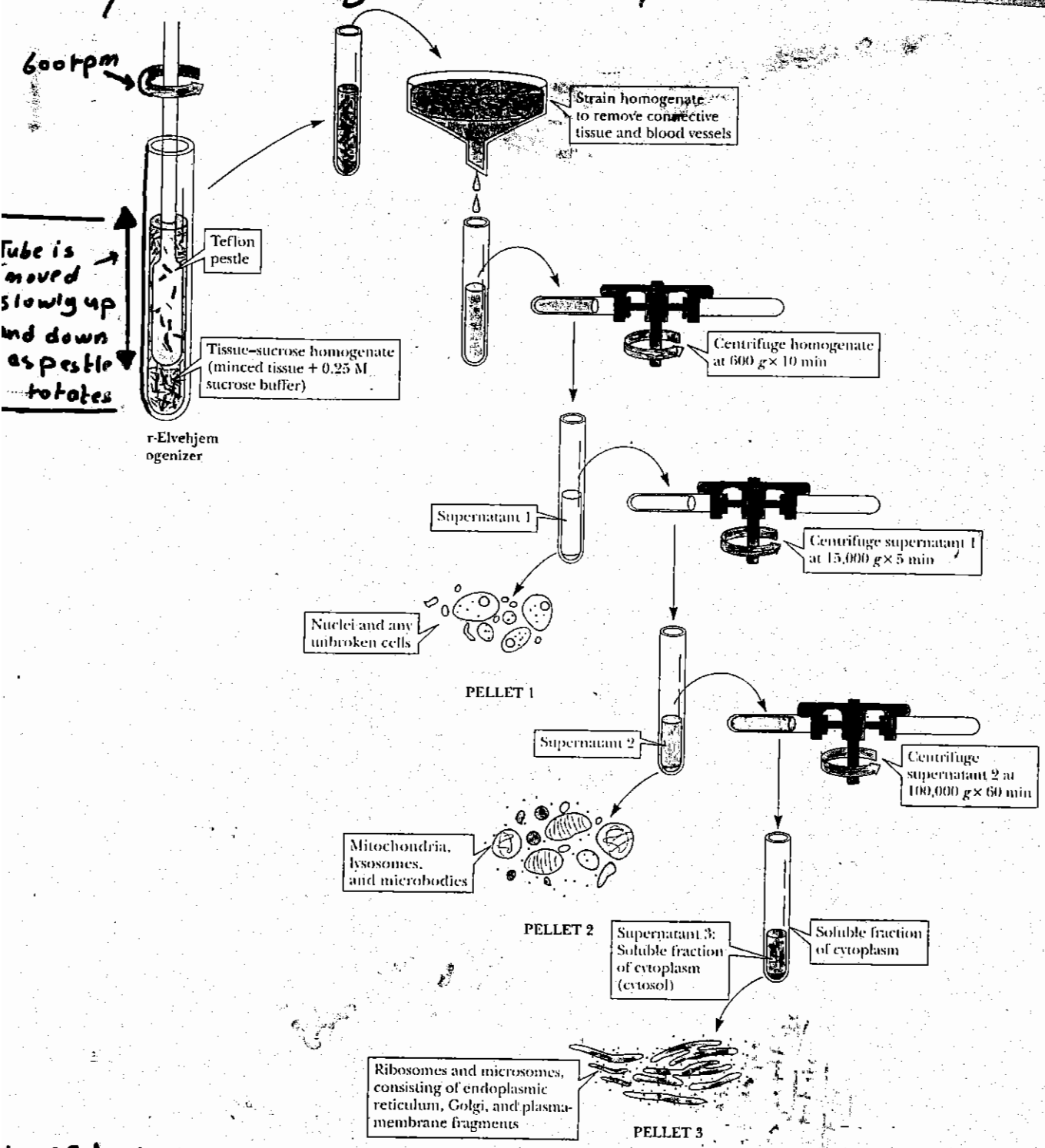
Example of a Protein Purification Scheme: Purification of the Enzyme Xanthine Dehydrogenase from a Fungus

Fraction	Volume (mL)	Total Protein (mg)	Total Activity (units)	Specific Activity (unit/mg prot.)	Percent Recovery
1. Crude extract	3,800	22,800	2,460	0.108	100
2. Salt precipitate	165	2,800	1,190	0.425	48
3. Ion-exchange chromatography	65	100	720	7.2	29
4. Molecular-sieve chromatography	40	14.5	555	38.3	23
5. Immunoaffinity chromatography	6	1.8	275	152.108	11

Separation of Cell Components:-

600rpm

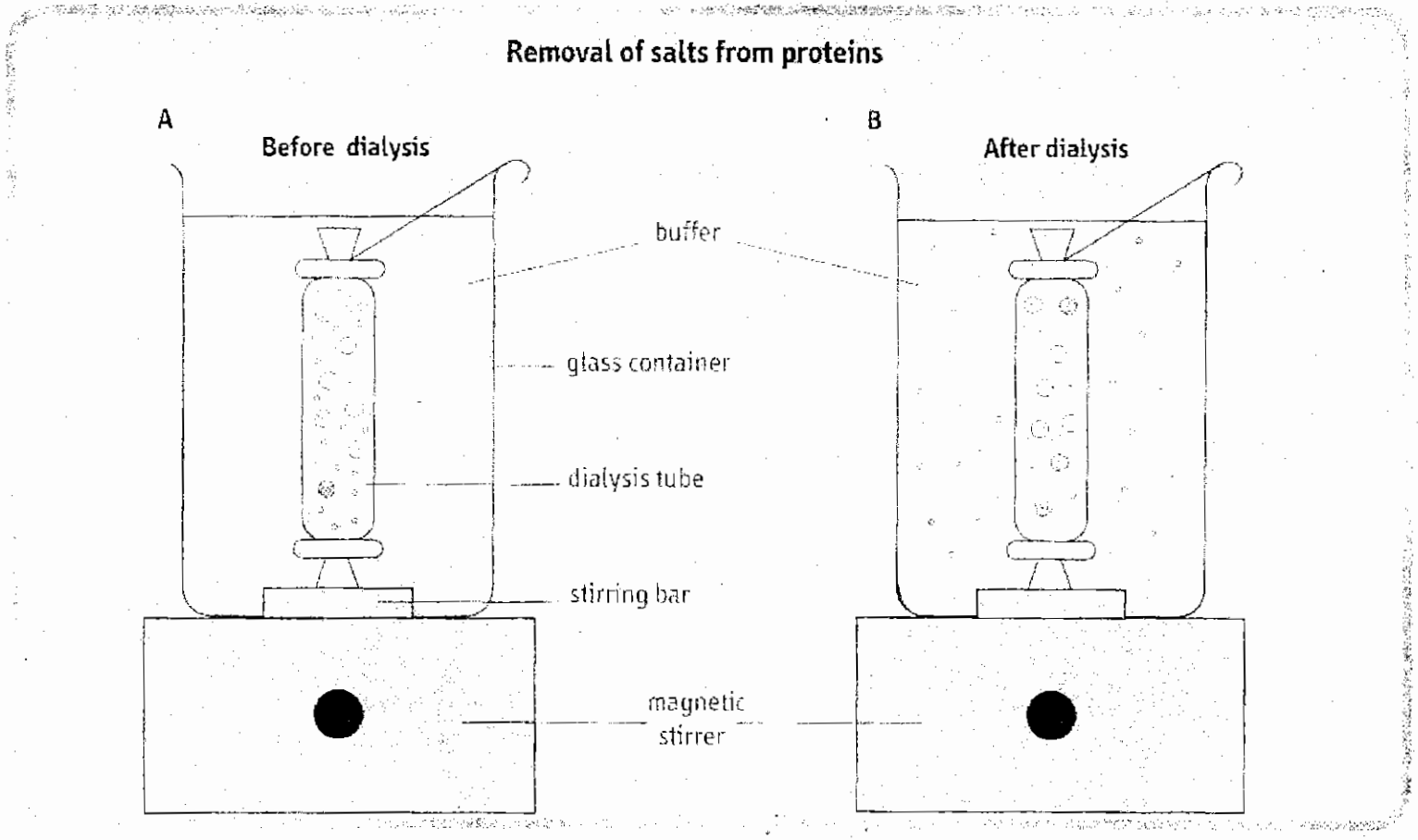
Tube is moved slowly up and down as pestle rotates

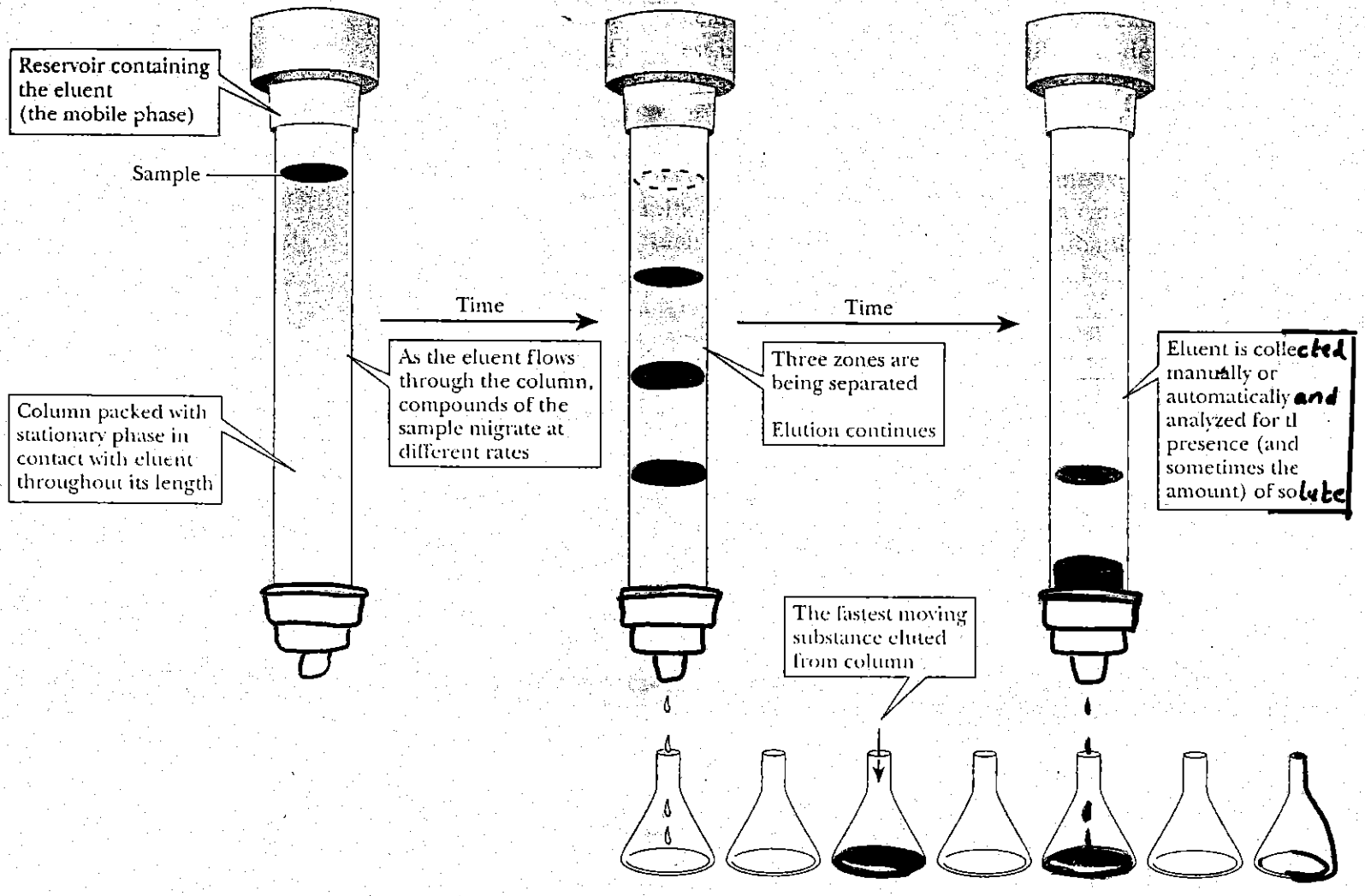


igure 5-1 **Differential centrifugation.** Differential centrifugation is used to separate cell a cell homogenate is subjected to increasing *g* forces, smaller and smaller cell up in the pellet.

Differential Centrifugation

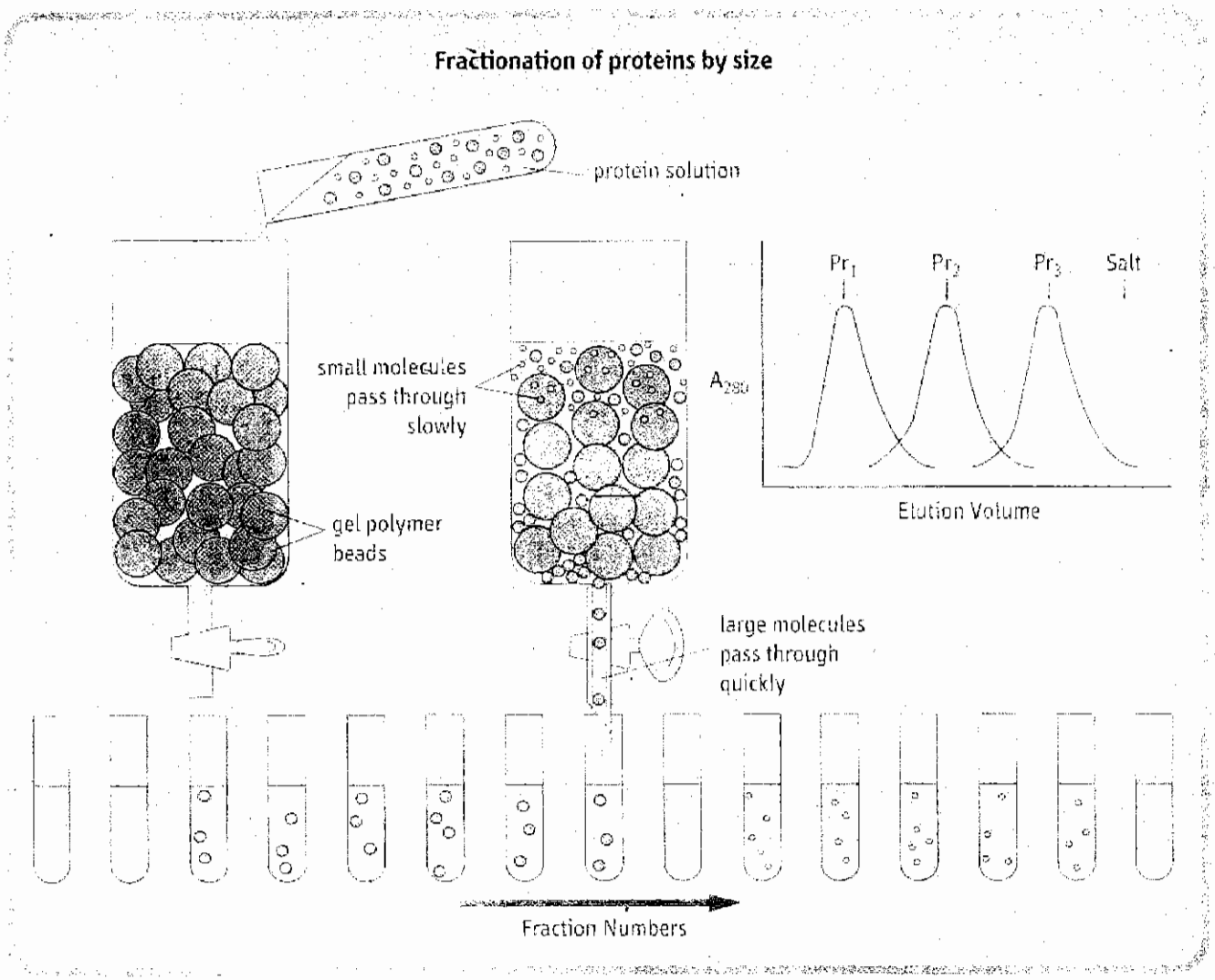
Dialysis


Removal of salts from proteins
A
Before dialysis
B
After dialysis
buffer
glass container
dialysis tube
stirring bar
magnetic stirrer



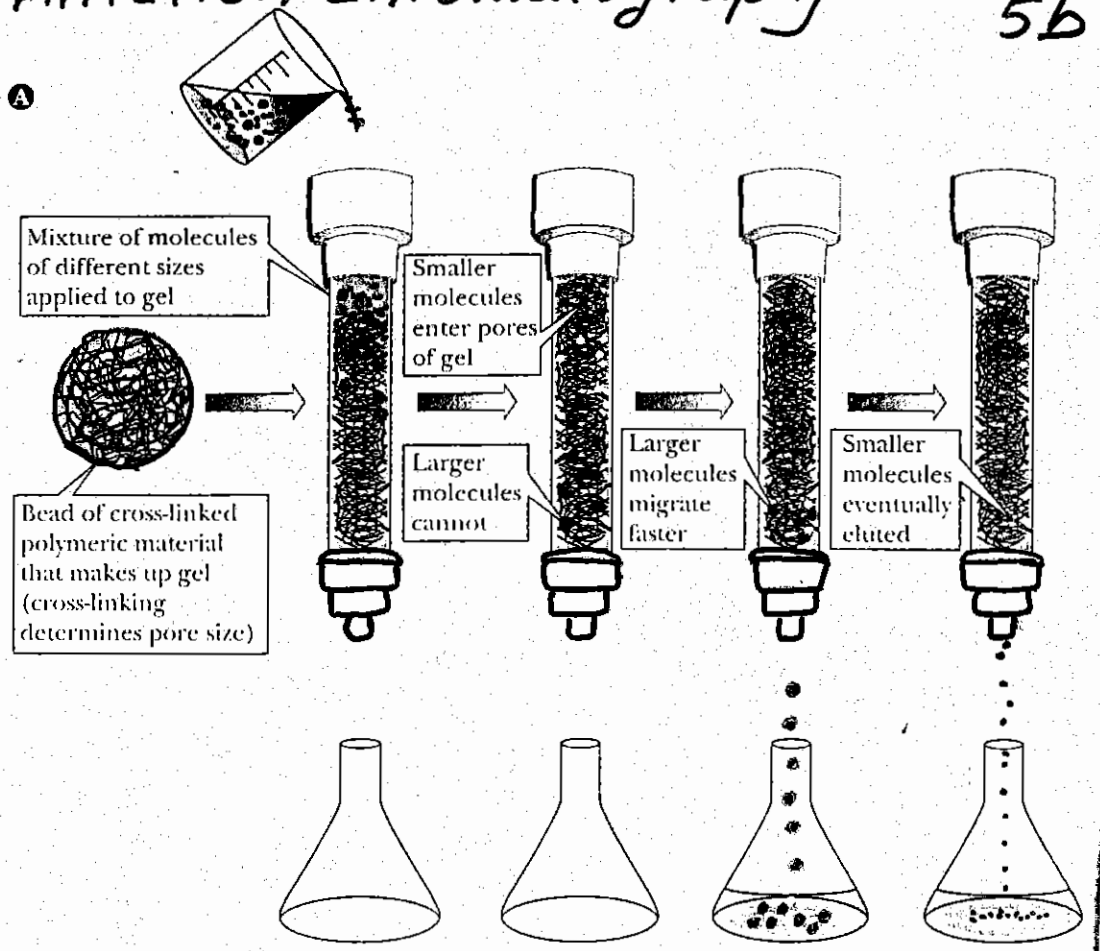
■ FIGURE 5.2 **Column chromatography.** A sample containing several components is app the column. The various components travel at different rates and can be collected indivi

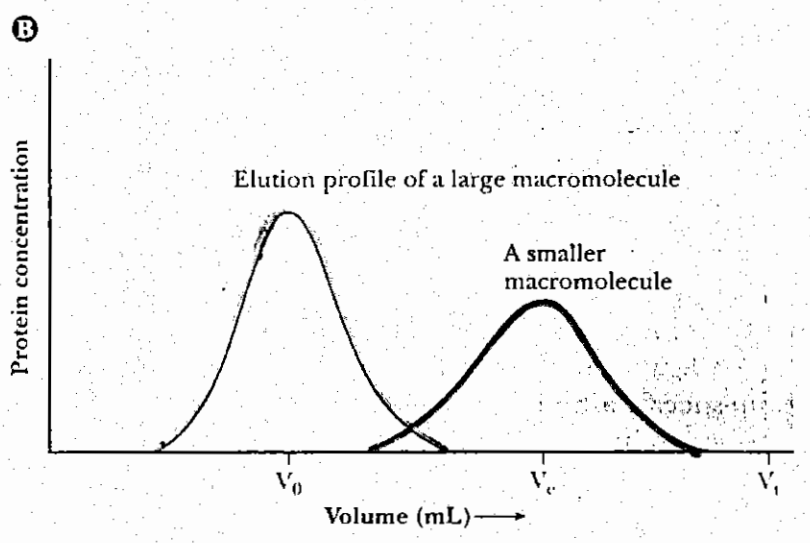
Gel Filtration Chromatography



Gel Filtration Chromatography

5b





Molecular Exclusion Chromatography
(Gel Filtration =)

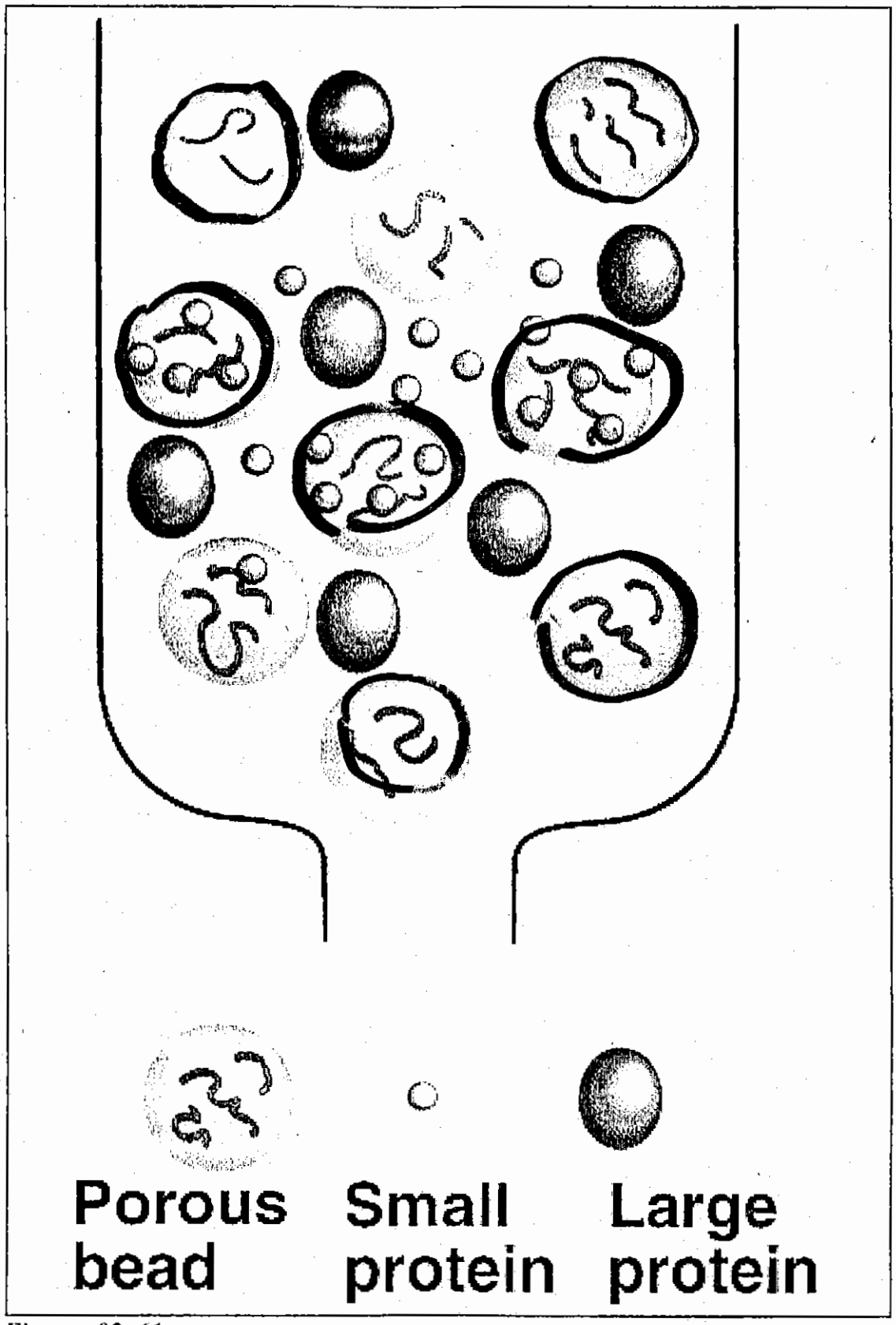
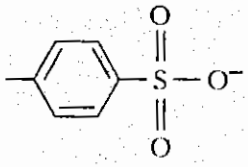
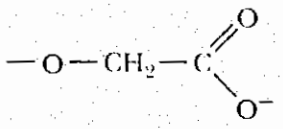
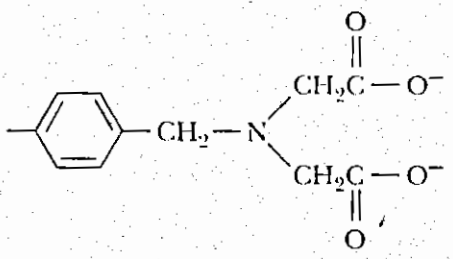
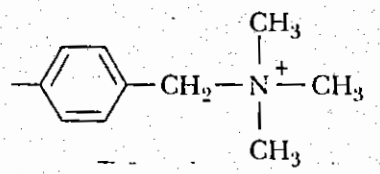
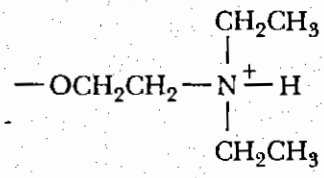


Figure: 02_61
Molecular exclusion chromatography.

Cation-Exchanger

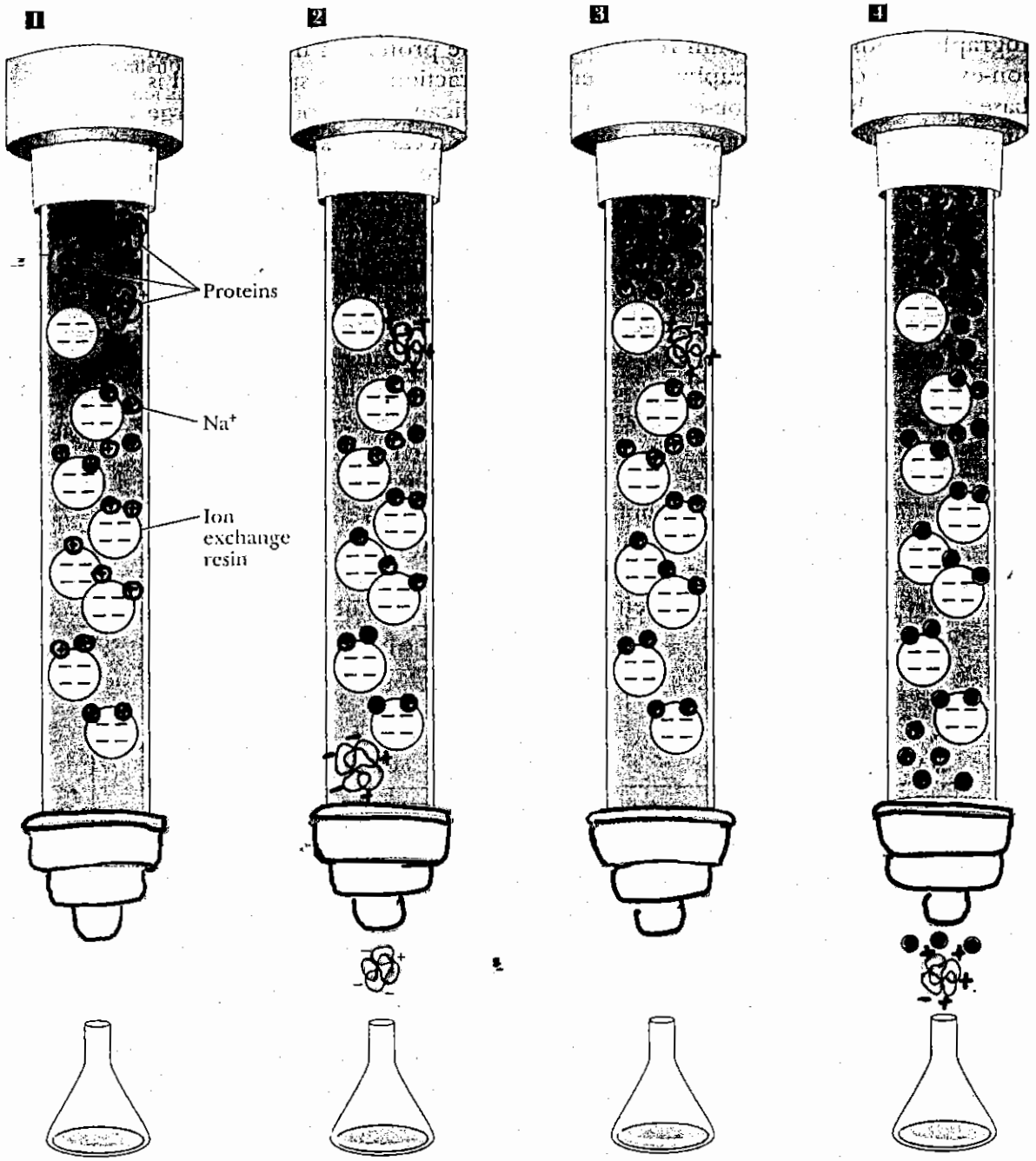
Ⓐ Cation-Exchange Media	Structure
Strongly acidic: polystyrene resin (Dowex–50)	
Weakly acidic: carboxymethyl (CM) cellulose	
Weakly acidic, chelating: polystyrene resin (Chelex–100)	

Anion-Exchanger

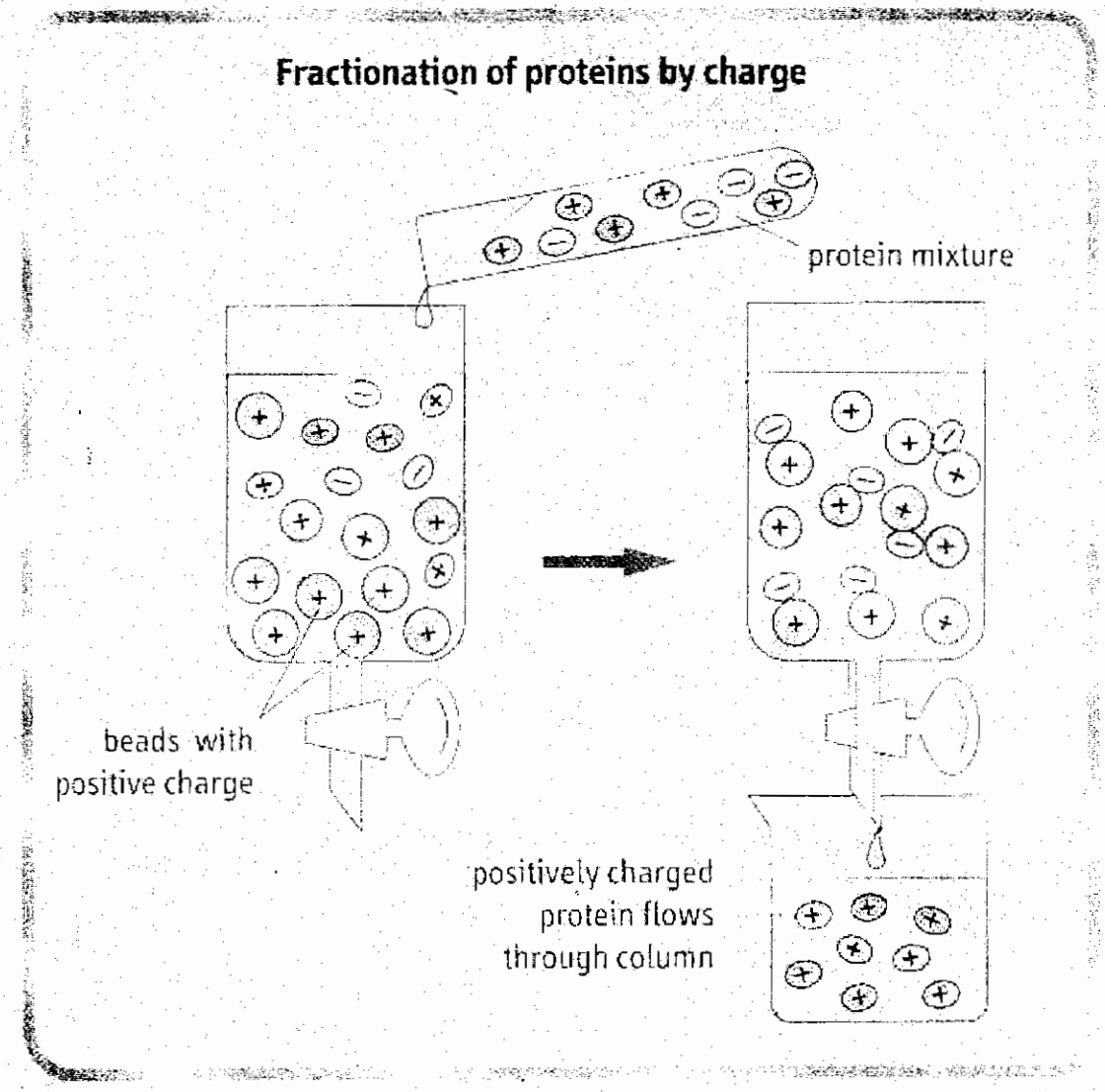
Ⓑ Anion-Exchange Media	Structure
Strongly basic: polystyrene resin (Dowex–1)	
Weakly basic: diethylaminoethyl (DEAE) cellulose	

■ **FIGURE 5.7 Resins used in ion-exchange chromatography.** (a) Cation-exchange resins and (b) anion-exchange resins commonly used for biochemical separations.

Cation exchanger chromatography



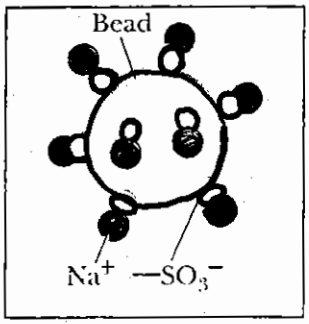
Anion-Exchanger Chromatography



7c

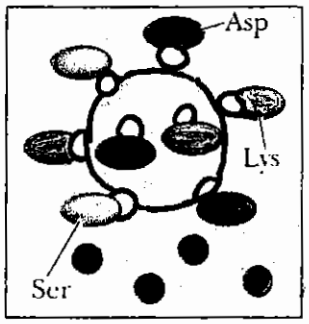
Cation-Exchanger for amino acids

Cation exchange bead before adding sample



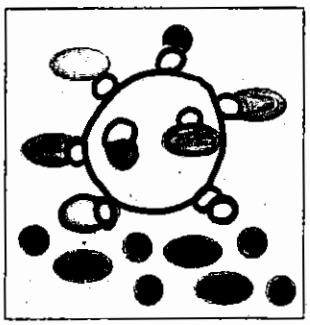
1

Add mixture of Asp, Ser, Lys



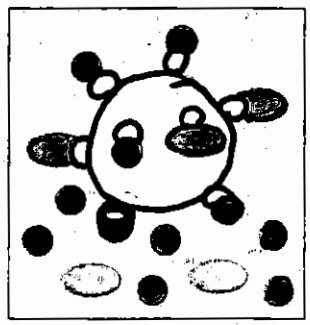
2

Add Na^+ ($NaCl$)



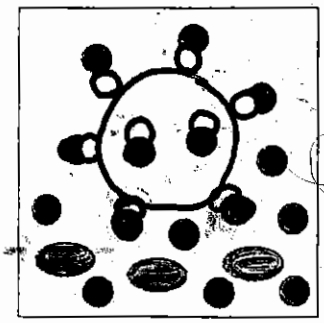
3 Asp, the least positively charged amino acid, is eluted first

Increase $[Na^+]$



4 Serine is eluted next

Increase $[Na^+]$



5 Lysine, the most positively charged amino acid, is eluted last

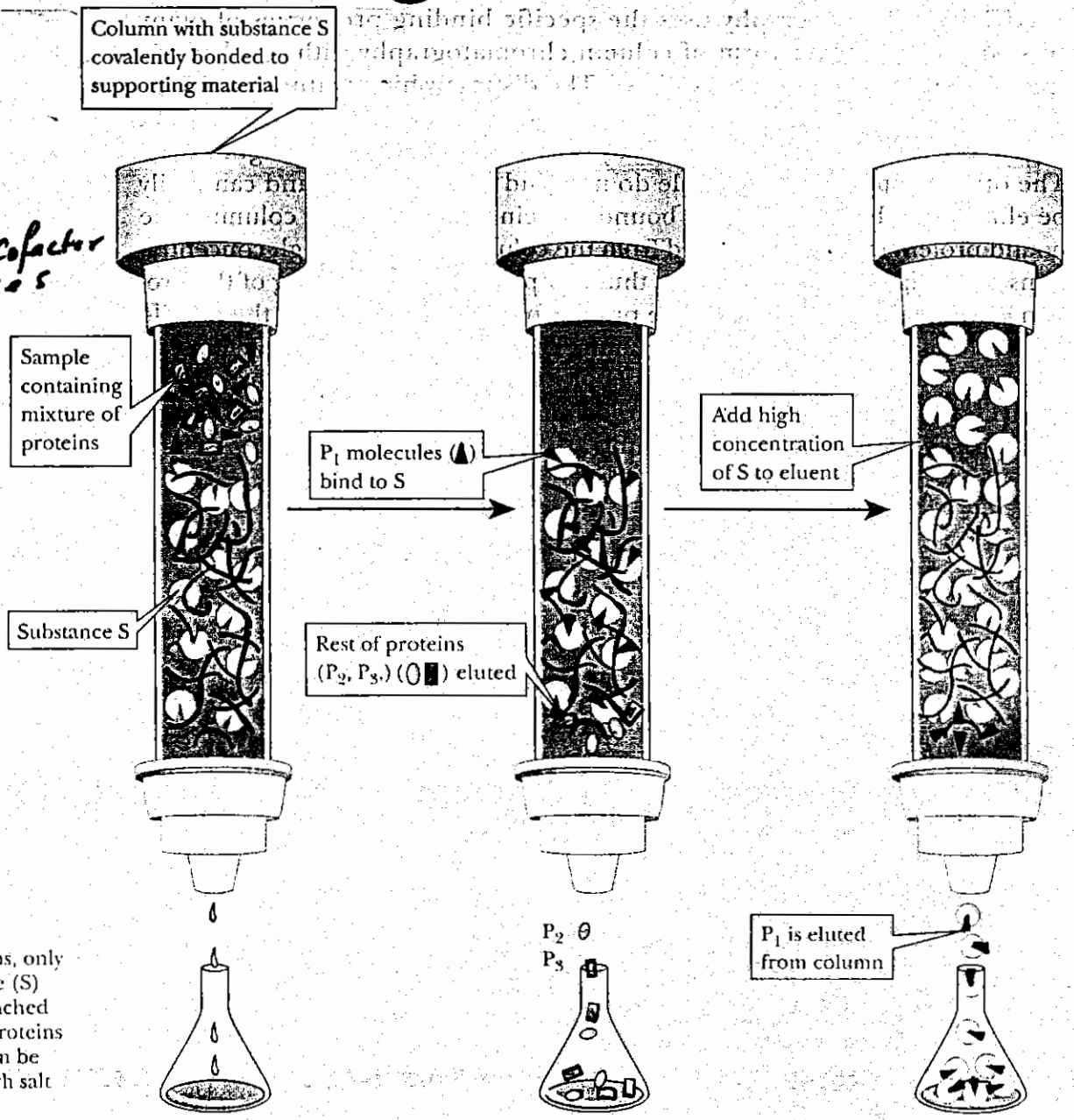
Affinity Chromatography

e.g.
AMP-agarose:

Enzymes with NAD^+ cofactor
ATP-dependent kinases

Poly U-agarose

Nucleic acid containing
Poly (A) sequences



principle of affinity
a mixture of proteins, only
) binds to a substance (S)
. The substrate is attached
rix. Once the other proteins
een washed out, P_1 can be
ding a solution of high salt
y adding free S.

HPLC Chromatography

separation of proteins, peptides and amino acids

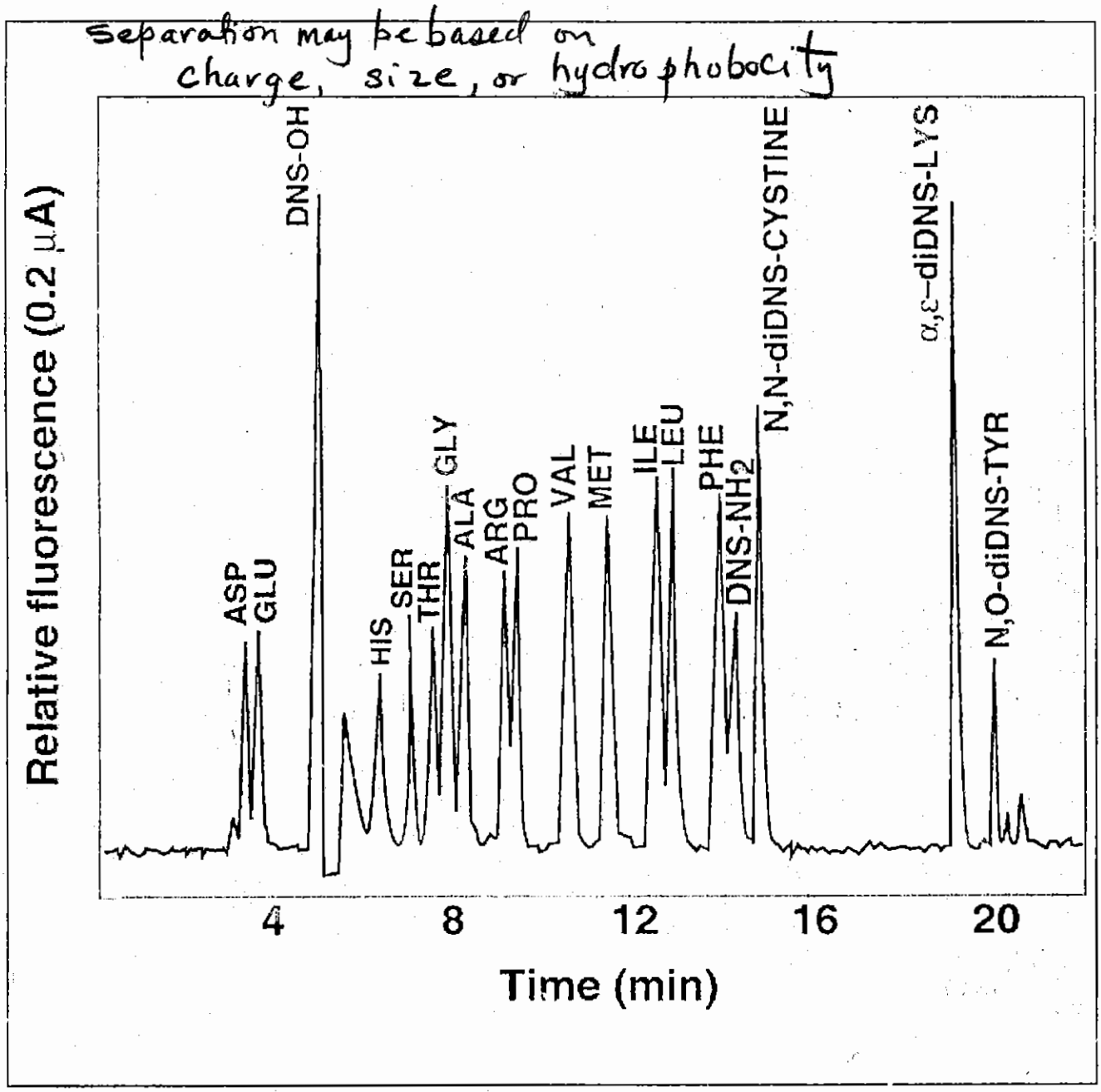
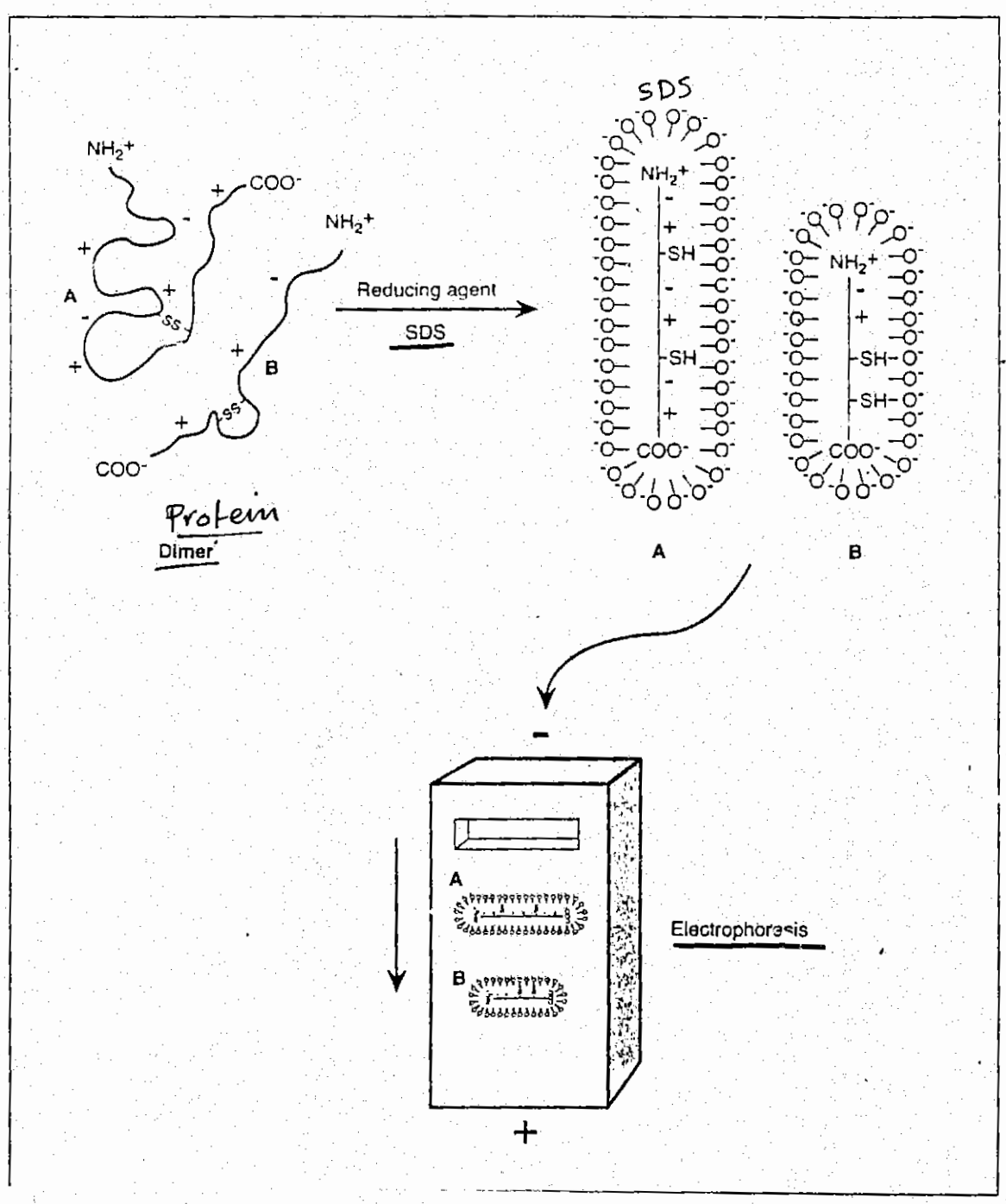


Figure: 02_62
Separation of amino acids utilizing reverse-phase HPLC.

[SDS-PAGE]

SDS-Polyacrylamide Gel Electrophoresis

- determinations of purity of proteins
- " " of molecular weight of proteins



SDS-PAGE

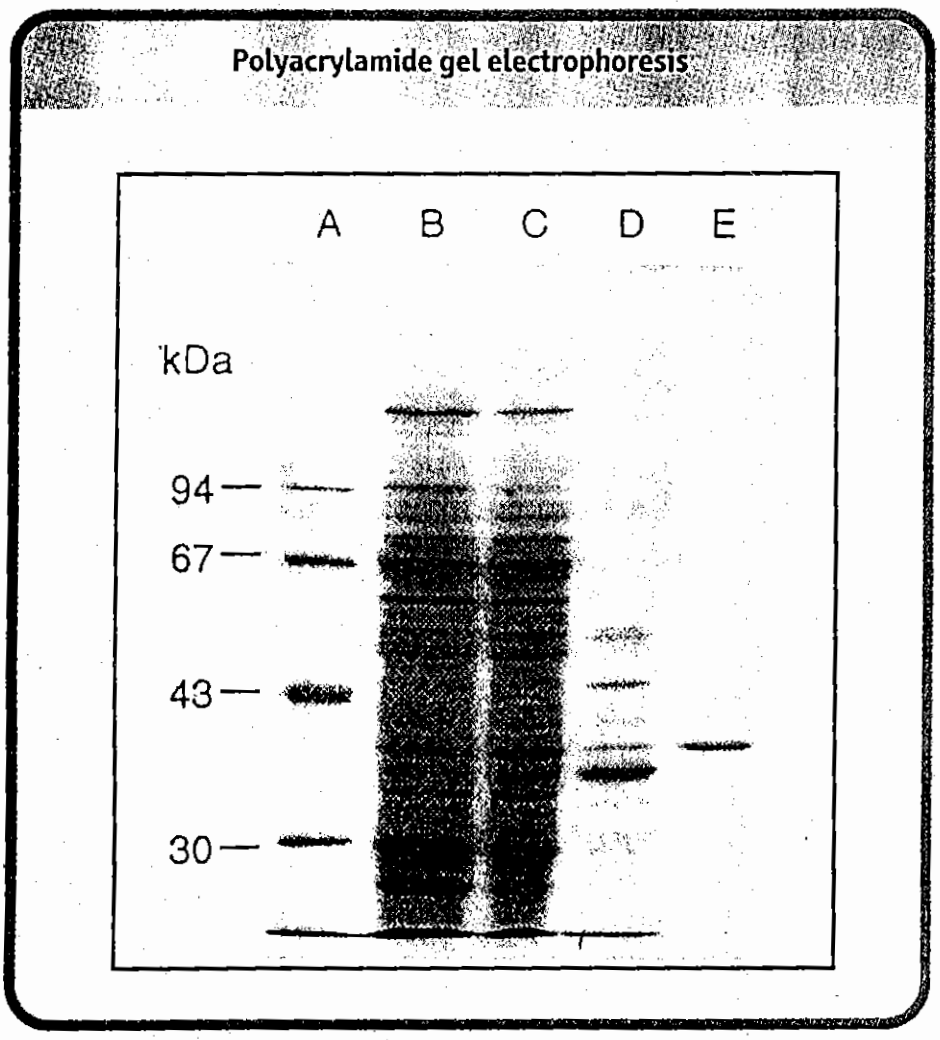
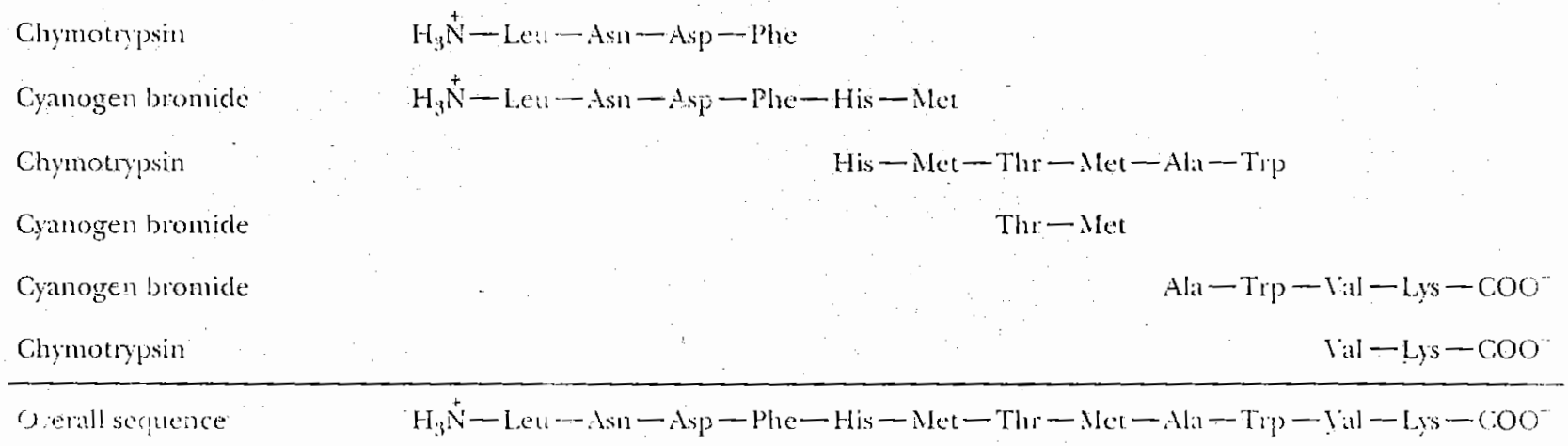


Fig. 2.18 SDS–PAGE. SDS–PAGE is used to separate proteins on the basis of their molecular weights. Larger molecules are retarded in the gel matrix, whereas the smaller ones move more rapidly. Lane A contains standard proteins with known molecular masses (indicated in kDa on the left side). Lanes B, C, D, and E show results of SDS–PAGE analysis of the protein at various stages in purification: B = total protein isolate; C = ammonium sulfate precipitate; D = fraction from gel-permeation chromatography; E = purified protein from ion exchange chromatography.



■ **FIGURE 5.19 Use of overlapping sequences to determine protein sequence.** Partial digestion was effected using chymotrypsin and cyanogen bromide. For clarity, only the original N-terminus and C-terminus of the complete peptide are shown.