ROSE-HULMAN INSTITUTE OF TECHNOLOGY | CHEMISTRY AND BIOCHEMISTRY

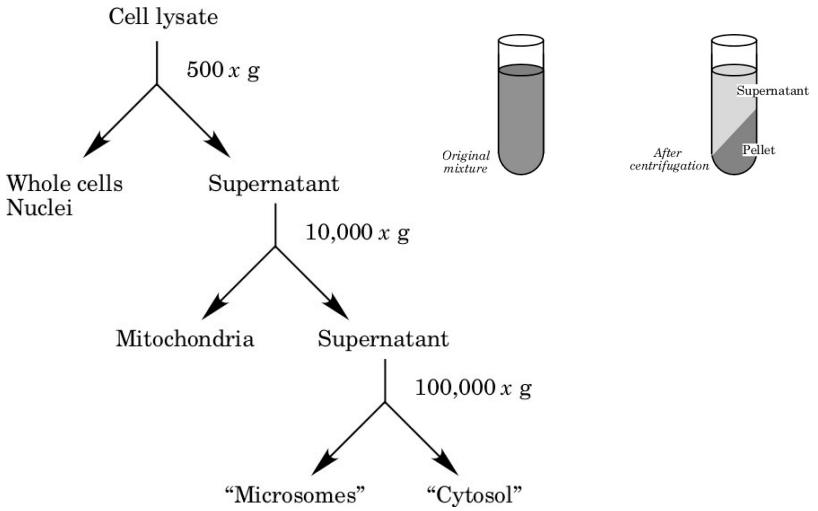
Protein Purification

(Be gentle with me . . . or I will fall apart.)

Topic	Mean	Number of 5
Alzheimers	+	
Biodegradability and Factors that affect this	+	
Biofuels	+	
Drug Delivery techniques	+	
Field trip to Biochemical Lab or Related facility	+	
Food	+	
Immunology	+	
Molecular Modeling	+	
Pharmaceutical Patent Law	+	
Production of alcohol	+	
Tissue Engineering	+	
WMD	+	
Biochemical aspects of drug design	**4.46**	8 x 5
Molecular mechanisms of infectious disease	**4.12**	4 x 5
Molecular mechanisms of carcinogenesis	**3.90**	2 x 5
biochemical issues related to stem cells	**3.69**	4 x 5
Molecular mechanisms of intoxication and anesthesia	**3.57**	1 x 5
Molecular mechanisms of genetic disorders	**3.56**	2 x 5
Gene therapy	**3.54**	3 x 5
Data analysis, experiment design, and project design	**3.46**	4 x 5
Nutrition	**3.46**	5 x 5
Design of nucleic acid-based therapies	3.32	1 x 5
DNA and gene transcriptional manipulation	3.05	1 x 5
Experimental techniques, especially spectroscopy	3.00	2 x 5
Molecular mechanisms of protein transport/targeting	2.92	1 x 5
Protein folding and structure	2.88	2 x 5
DNA repair	2.77	
Protein structure determination and analysis	2.72	1 x 5
Protein purification	2.68	
Endocrine physiology	2.46	1 x 5
RNA Interference	2.46	
Photosynthesis	2.38	2 x 5

Protein Purification

Differential Centrifugation



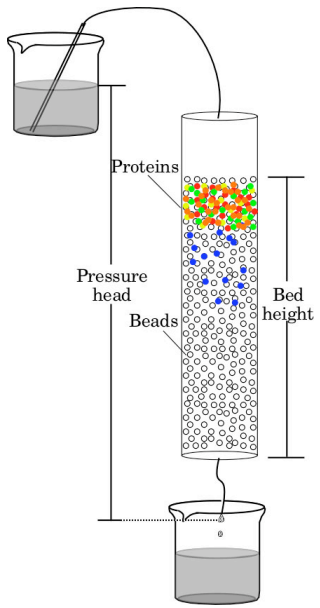
Effects of Solutes

Hofmeister Series

Anions: $PO_4^{3-} > SO_4^{2-} > CH_3COO^- > Cl^- > Br^- > NO_3^- > ClO_4^- > I^- > SCN^-$

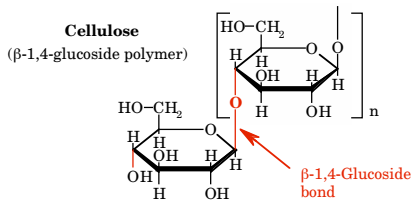
Cations: $NH_4^+ > Rb^+ > K^+ > Na^+ > Cs^+ > Li^+ > Mg^{2+} > Ca^{2+} > Ba^{2+}$

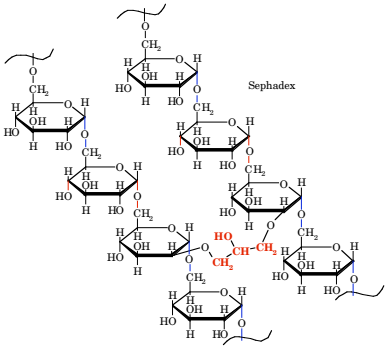
$Na_2SO_4 > K_2SO_4 > (NH_4)_2SO_4 > Na_2HPO_4 > NaCl > LiCl > \text{others} > KSCN$


Proteins
Pressure
head
Beads
Bed
height

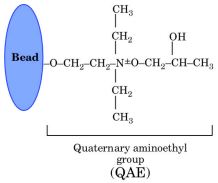
Chromatography Resins

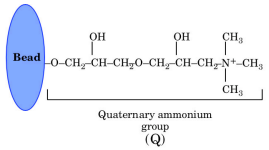
Resin	Carbohydrate	Cross-linking agent
Cellulose	Cellulose	none
Biogel	Polyacrylamide	Bisacrylamide
Sephacel	Cellulose	Epichlorhydrin
Sephacryl	Dextran	Bisacrylamide
Sephadex	Dextran	Epichlorhydrin
Sepharose	Agarose	none
Superdex	Dextran, Agarose	Highly cross-linked
Superose	Agarose	Highly cross-linked

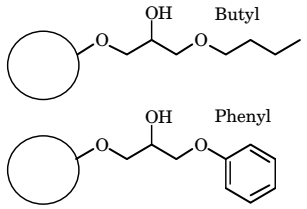


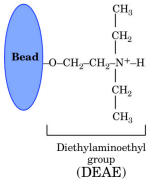


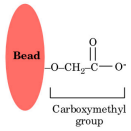
Chromatography Resins







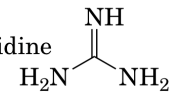




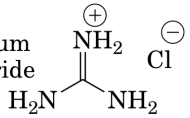
Type	Capacity	Resolution	Expense
Ion exchange			
Hydrophobic interaction			
Gel filtration			
Affinity			

Perturbing Solubility and Structure

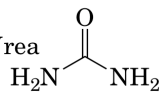
Guanidine



Guanidinium hydrochloride



Urea



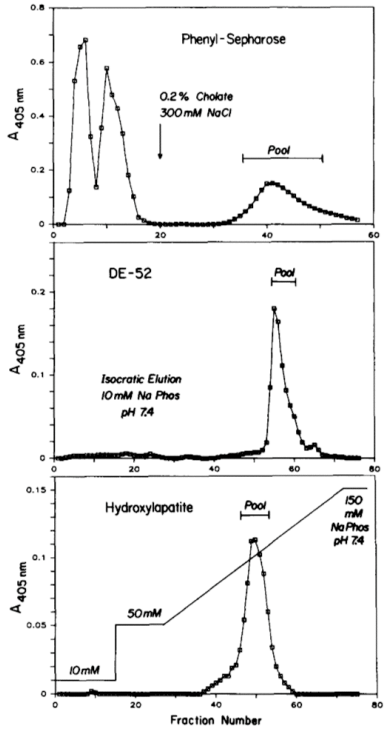


FIG. 2. **Elution profiles of the three column chromatographic steps used in the purification of P-450*arom*.** Fraction volumes: phenyl-Sepharose, 11 ml; DE52, 7 ml; hydroxylapatite, 4 ml.

TABLE I

Purification of human placental aromatase cytochrome P-450

Purification step	Total protein	Total activity[a]	Specific activity	Total P-450*arom*[b]	Specific content	P-450*arom* yield
	mg	*nmol/min*	*nmol/min/mg protein*	*nmol*	*nmol/mg protein*	*%*
Microsomes	4958	915	0.18	255	0.05	100
Cholate extract	3796	626	0.16	251	0.07	98
Ammonium sulfate, 35–55%	934	349	0.37	156	0.17	61
Phenyl-Sepharose	125	304	2.4	107	0.86	42
DE52	7.6	152	20	39	5.1	15
Hydroxylapatite	1.95	111	57	22	11.5	9

[a] Samples were reconstituted with 100 nM rabbit liver P-450 reductase and 0.003% Nonidet P-40 as described under "Experimental Procedures."

[b] P-450*arom* concentration was determined by difference spectra induced by (19*R*)-10-thiiranyl-4-estrene-3,17-dione as described under "Experimental Procedures."

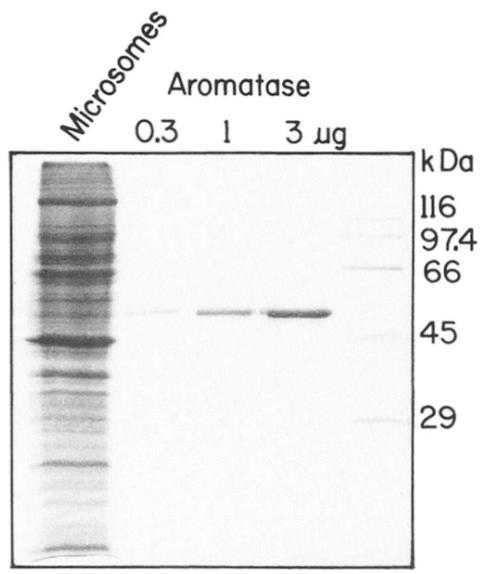
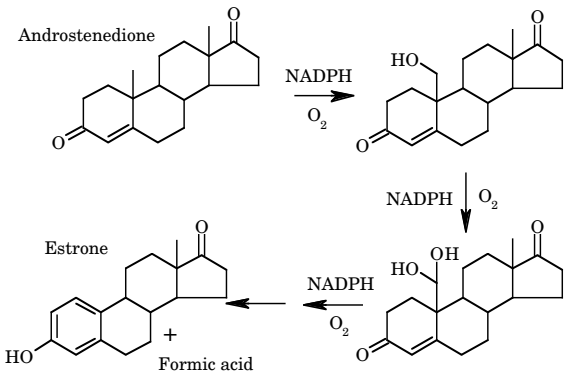


FIG. 3. **SDS-polyacrylamide gel electrophoresis of aromatase.** The *lanes* show human placental microsomes (200 µg of protein), 0.3, 1, and 3 µg of purified P-450*arom* and molecular weight markers.



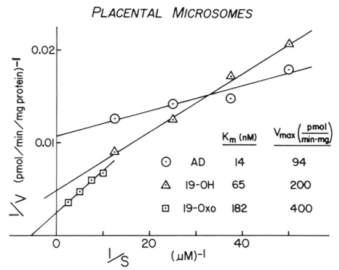


FIG. 8. **Kinetic analysis of aromatization using human placental microsomes.** Substrates: androstenedione (*AD*), 19-hydroxyandrostenedione (*19-OH*), and 19-oxoandrostenedione (*19-Oxo*). Determination of product formation was performed by radioimmunoassay for estrone.

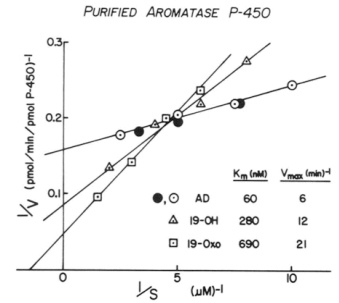


FIG. 9. **Kinetic analysis of aromatization using purified, reconstituted P-450*arom*.** Substrates: androstenedione (*AD*), 19-hydroxyandrostenedione (*19-OH*), and 19-oxoandrostenedione (*19-Oxo*). *Open symbols* indicate that determination of product formation was by radioimmunoassay for estrone. *Closed symbols* indicate a separate experiment in which AD aromatization was measured by tritium release from [1β,2β-^{3}H]androstenedione.