



Introduction

(Let's dig deeper)

CHEMISTRY 430 ADVANCED BIOCHEMISTRY

Spring, 2009-10

MTThF 5th hour in O-203

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TEXTBOOK: *Lehninger Principles of Biochemistry*, 5th edition, David L. Nelson and Michael M. Cox, Freeman & Co., 2008

EXAMS: The exams in this course will be take-home exams. Guidelines for taking the exam will be included on the exam.

HOMEWORK: there will be frequent homework and in-class assignments. While these cannot be performed late, excused missed assignments will not count against your grade.

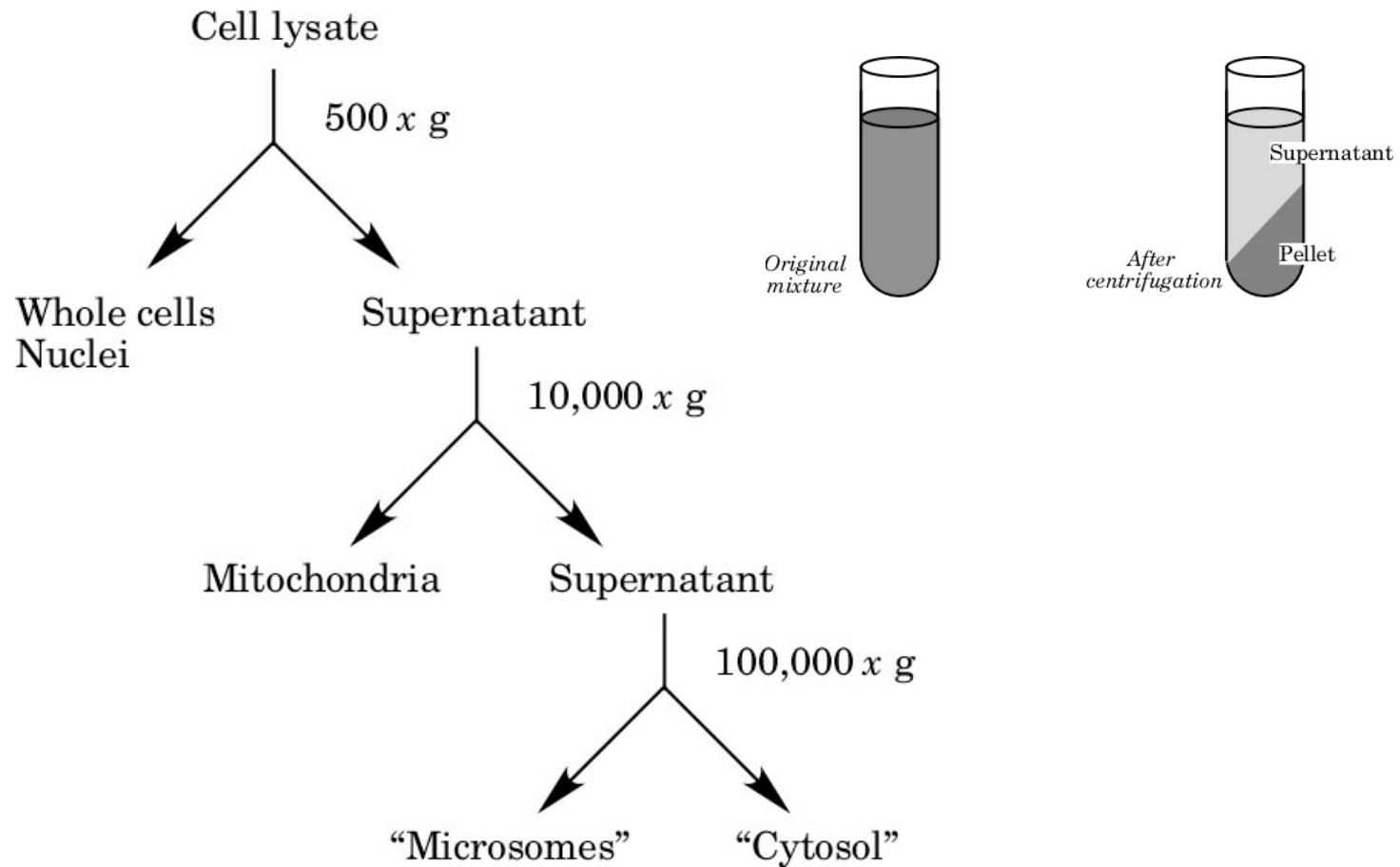
Grade Breakdown:	Exams	60%
	Presentations	20%
	Problem Sets/Quizzes	15%
	Participation	5%

Total Points 100%

Protein purification	2.00
Protein structure determination and analysis	2.20
Protein folding and structure	2.60
Biochemical aspects of drug design	4.60
Experimental techniques, especially spectroscopy	2.60
Data analysis, experiment design, and project design	2.80
DNA repair	2.80
DNA and gene transcriptional manipulation	3.20
RNA Interference	2.40
Molecular mechanisms of intoxication and anesthesia	3.20
Molecular mechanisms of infectious disease	3.80
Molecular mechanisms of genetic disorders	3.60
Molecular mechanisms of carcinogenesis	3.40
Photosynthesis	3.60
Endocrine physiology	2.40
Nutrition	4.20
Nucleotide metabolism	2.00
Molecular mechanisms of protein transport/targeting	2.20
Design of nucleic acid-based therapies	3.00
biochemical issues related to stem cells	3.80
Gene therapy	3.40
Immunology	+
Food	+

Protein Purification

Differential Centrifugation



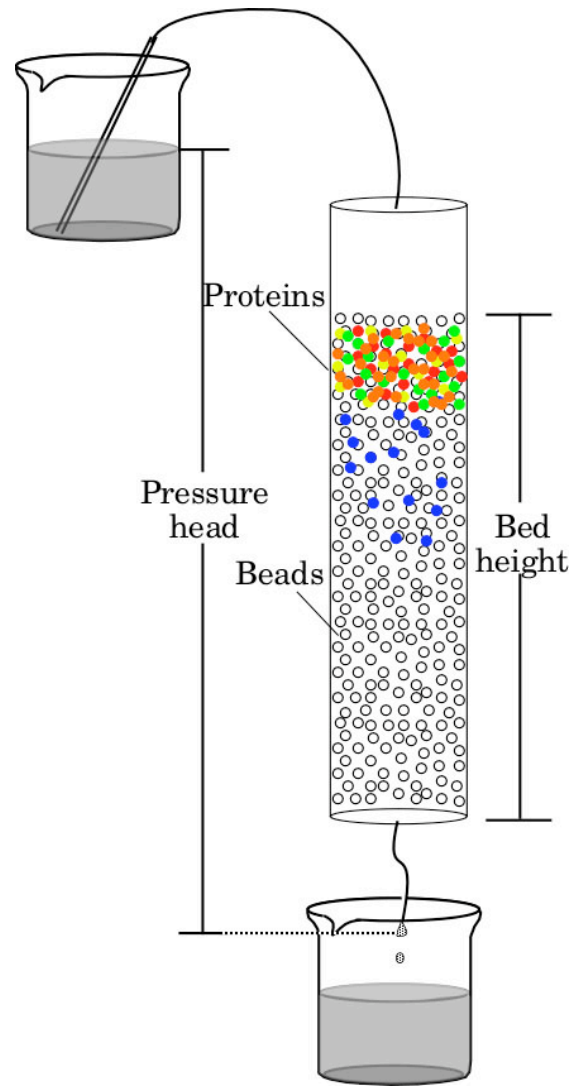
Effects of Solutes

Hofmeister Series

Anions: $\text{PO}_4^{3-} > \text{SO}_4^{2-} > \text{CH}_3\text{COO}^- > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{ClO}_4^- > \text{I}^- > \text{SCN}^-$

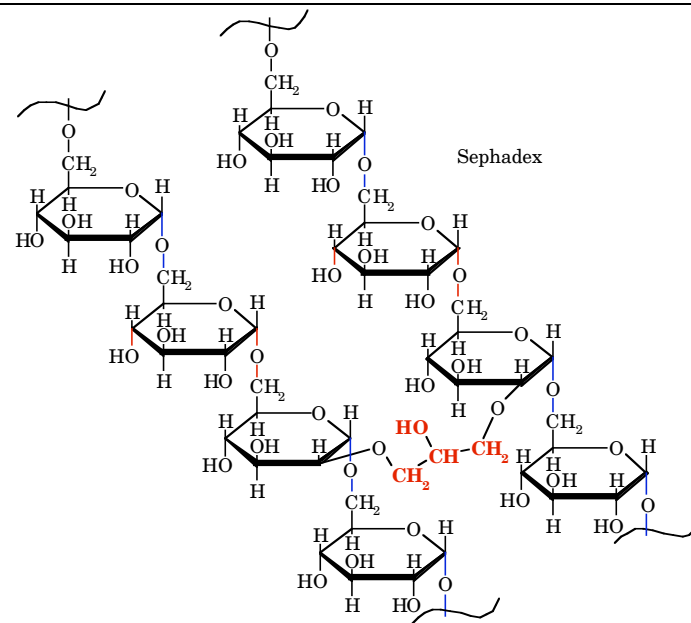
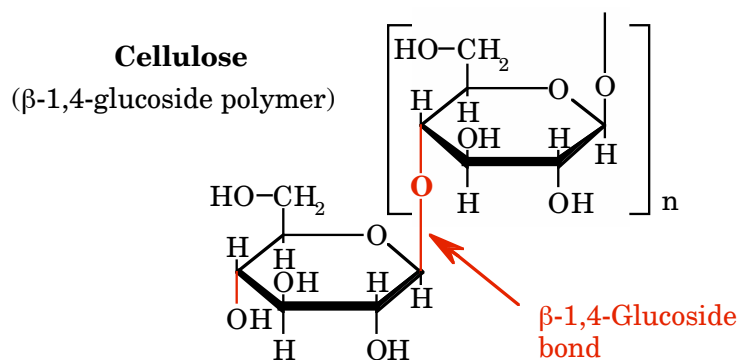
Cations: $\text{NH}_4^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Cs}^+ > \text{Li}^+ > \text{Mg}^{2+} > \text{Ca}^{2+} > \text{Ba}^{2+}$

$\text{Na}_2\text{SO}_4 > \text{K}_2\text{SO}_4 > (\text{NH}_4)_2\text{SO}_4 > \text{Na}_2\text{HPO}_4 > \text{NaCl} > \text{LiCl} > \text{others} > \text{KSCN}$

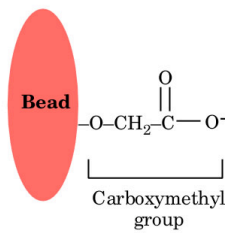
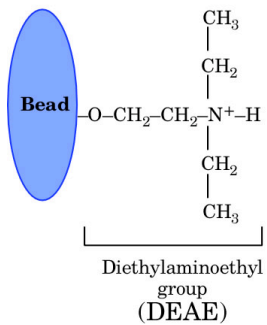
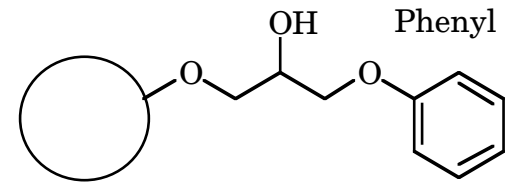
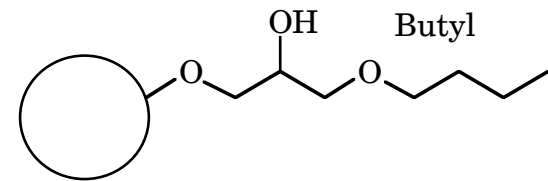
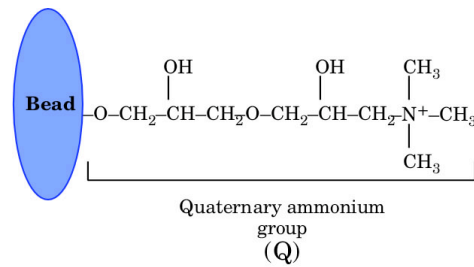
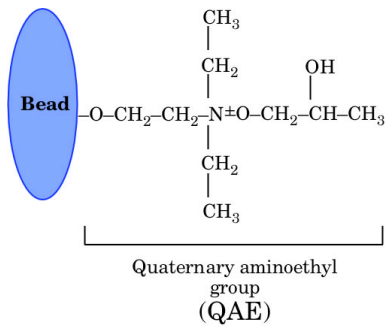


Chromatography Resins

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**

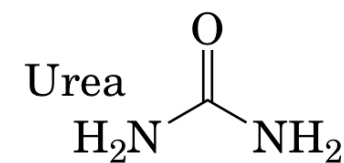
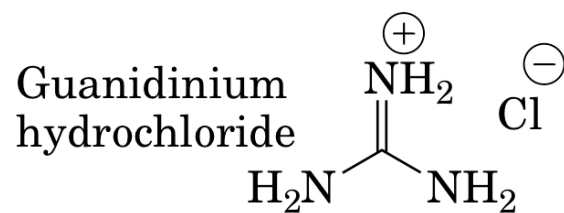
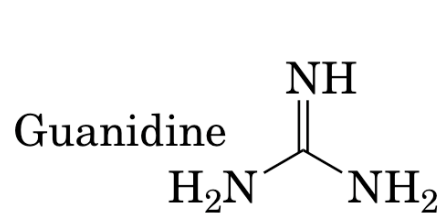
Ion exchange

**Hydrophobic
interaction**

Gel filtration

Affinity

Perturbing Solubility and Structure



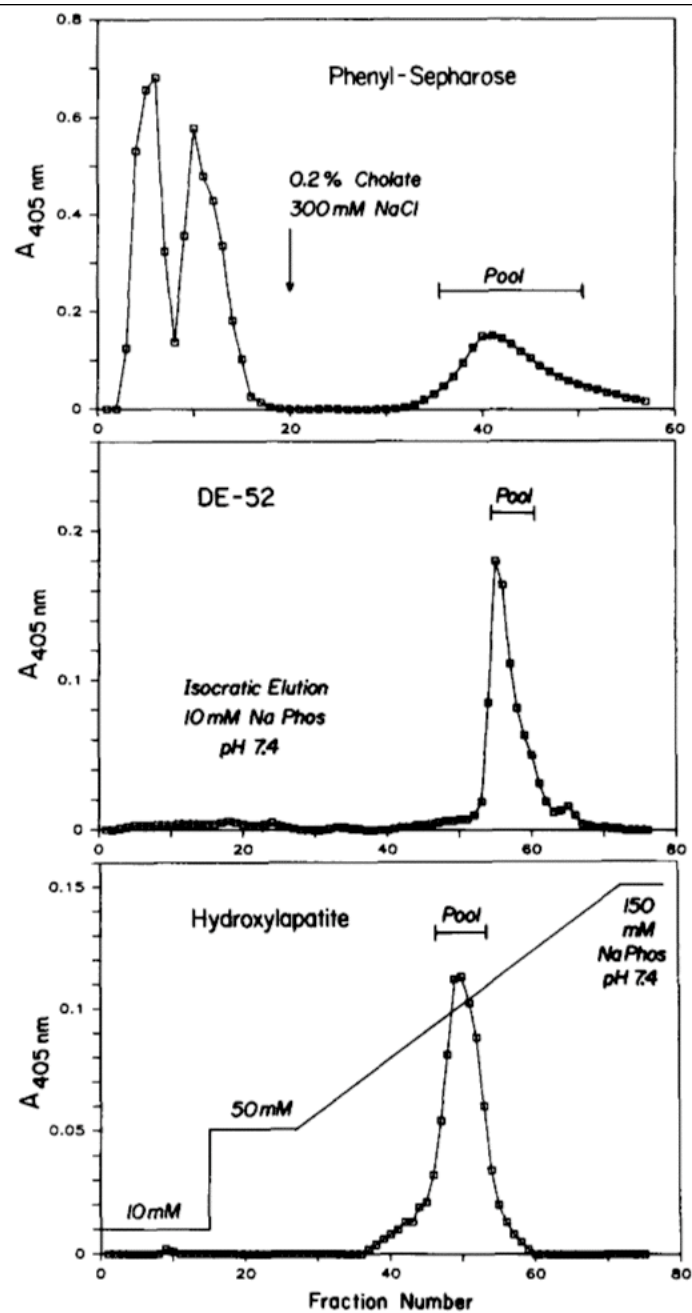


FIG. 2. Elution profiles of the three column chromatographic steps used in the purification of P-450arom. Fraction volumes: phenyl-Sepharose, 11 ml; DE52, 7 ml; hydroxylapatite, 4 ml.

Purification and Characterization of Aromatase

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
	<i>mg</i>	<i>nmol/min</i>	<i>nmol/min/mg protein</i>	<i>nmol</i>	<i>nmol/mg protein</i>	<i>%</i>
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."

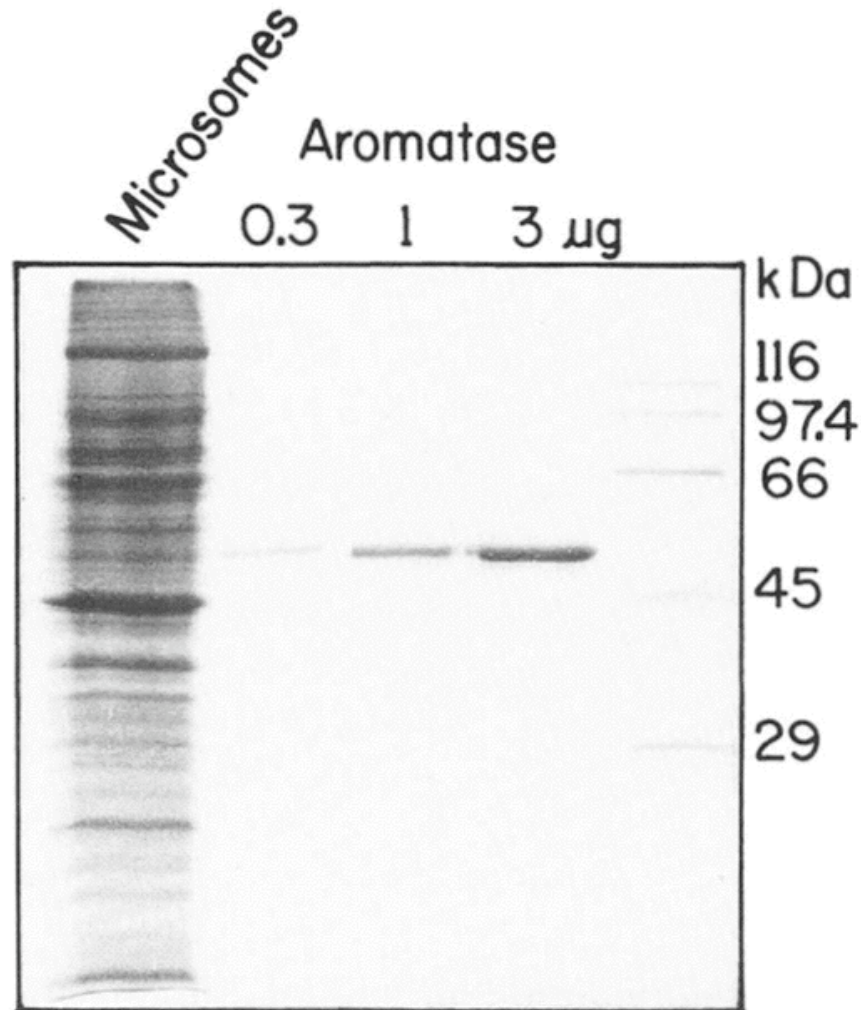


FIG. 3. **SDS-polyacrylamide gel electrophoresis of aromatase.** The *lanes* show human placental microsomes (200 μg of protein), 0.3, 1, and 3 μg of purified P-450 $_{\text{arom}}$ and molecular weight markers.

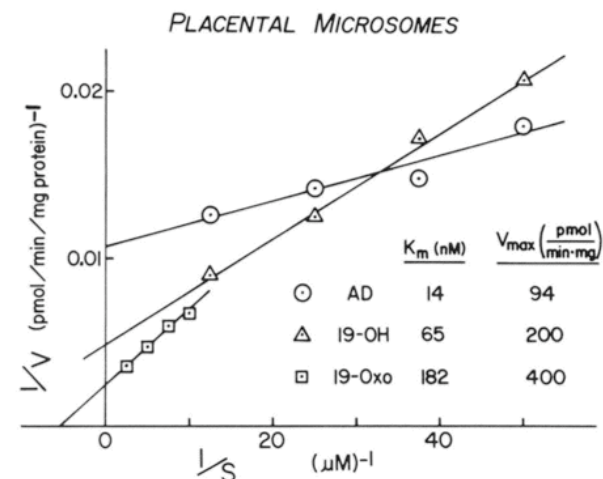
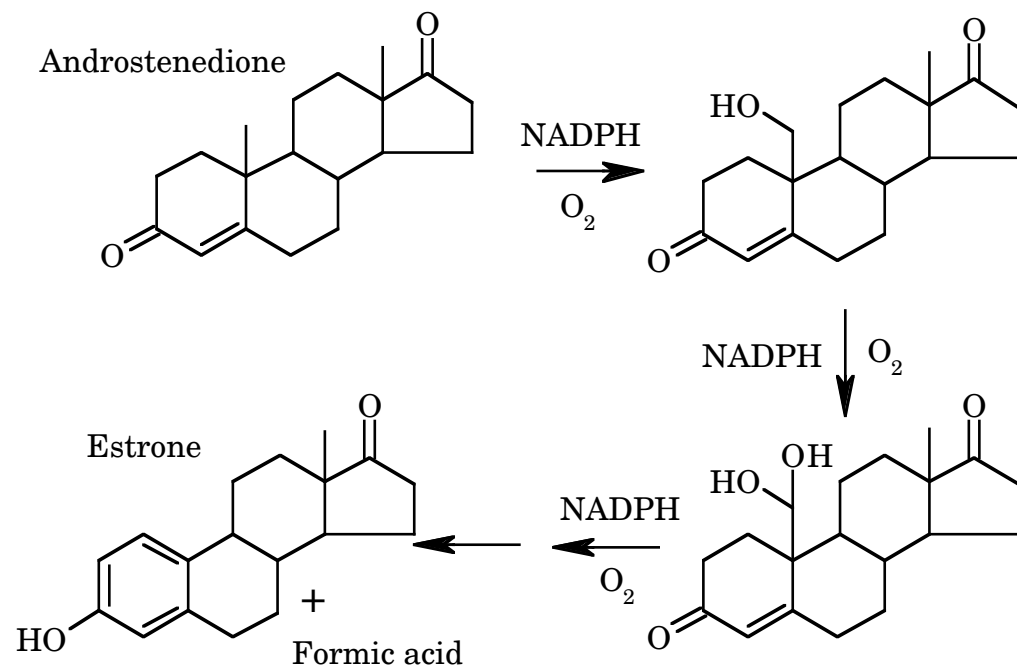


FIG. 8. Kinetic analysis of aromatization using human placental microsomes. Substrates: androstenedione (AD), 19-hydroxyandrostenedione (19-OH), and 19-oxoandrostenedione (19-Oxo). Determination of product formation was performed by radioimmunoassay for estrone.

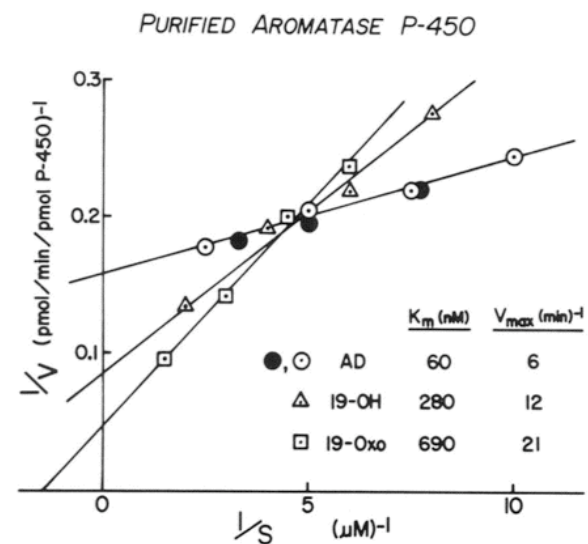


FIG. 9. Kinetic analysis of aromatization using purified, reconstituted P-450_{arom}. Substrates: androstenedione (AD), 19-hydroxyandrostenedione (19-OH), and 19-oxoandrostenedione (19-Oxo). Open symbols indicate that determination of product formation was by radioimmunoassay for estrone. Closed symbols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