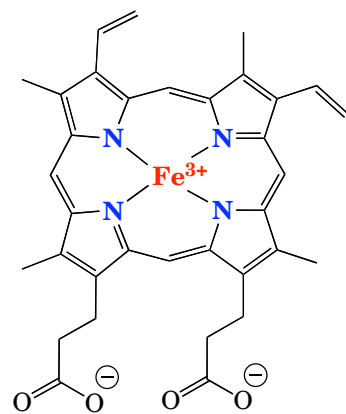


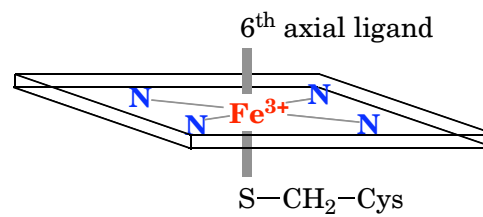
Aromatase & Related Topics

(Turning men into women for millions of
years. . . .)

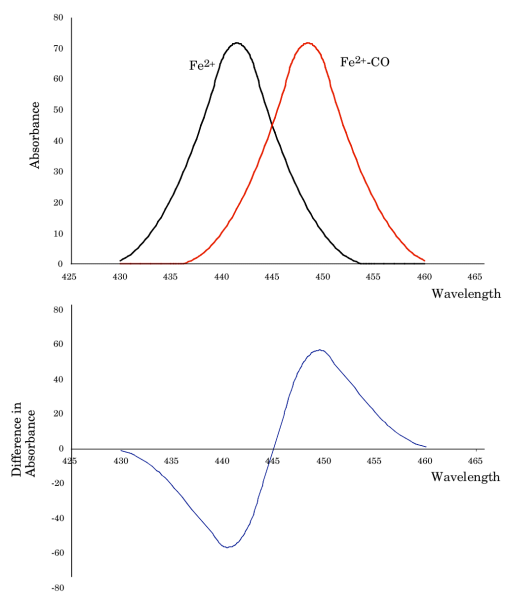
Cytochromes P450



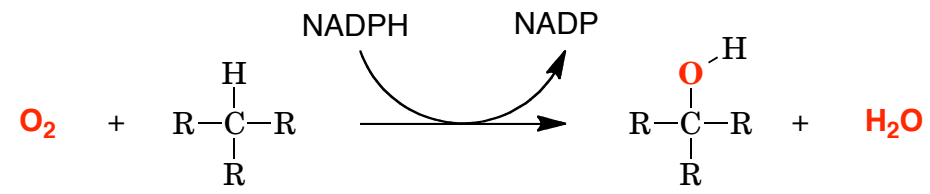
Iron-Protoporphyrin IX



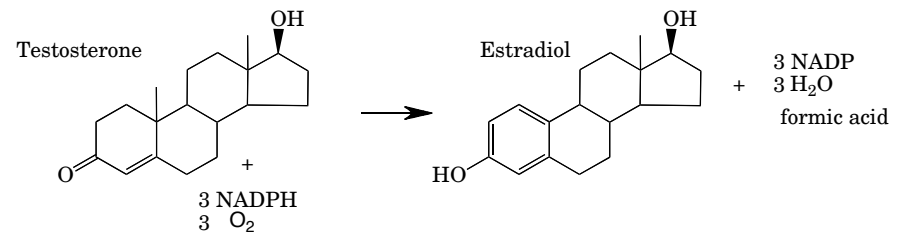
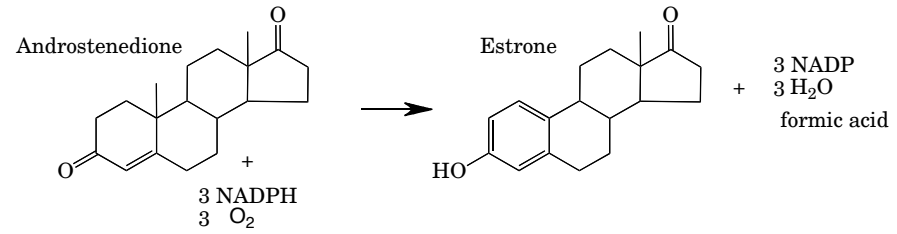
Difference Spectrum



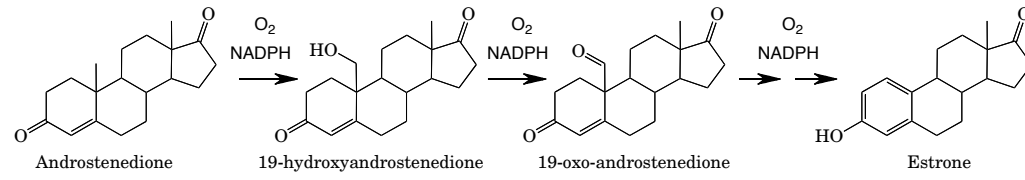
Cytochrome P450



Aromatase



Aromatase



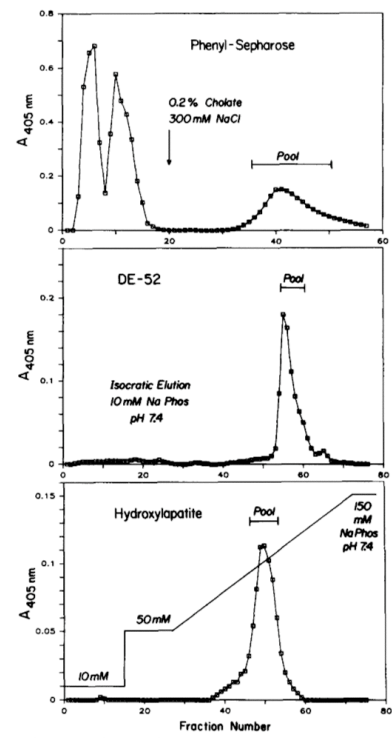


FIG. 2. Elution profiles of the three column chromatographic steps used in the purification of P-450 $arom$. Fraction volumes: phenyl-Sepharose, 11 ml; DE52, 7 ml; hydroxylapatite, 4 ml.

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 (19R)-10-thiiranyl-4-estrene-3,17-dione as described under "Experimental Procedures."

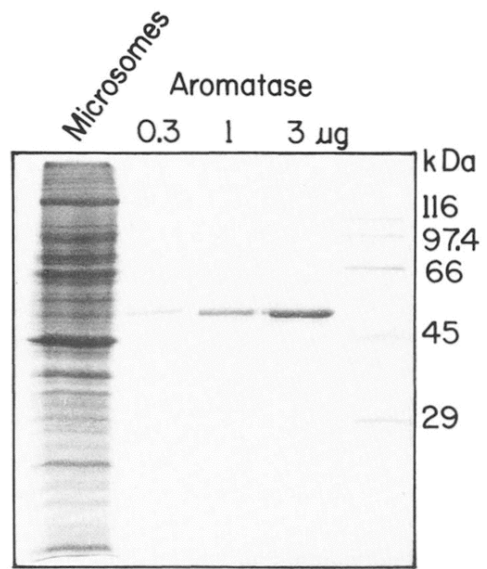
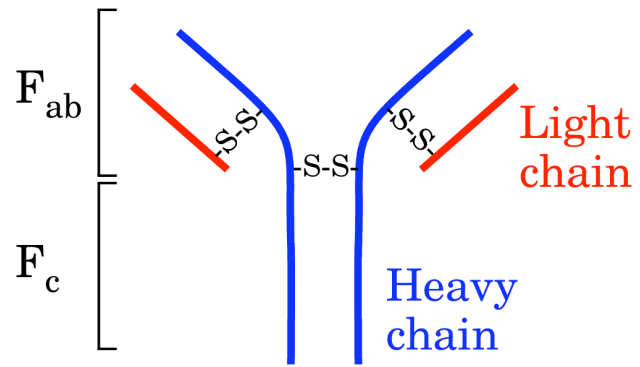
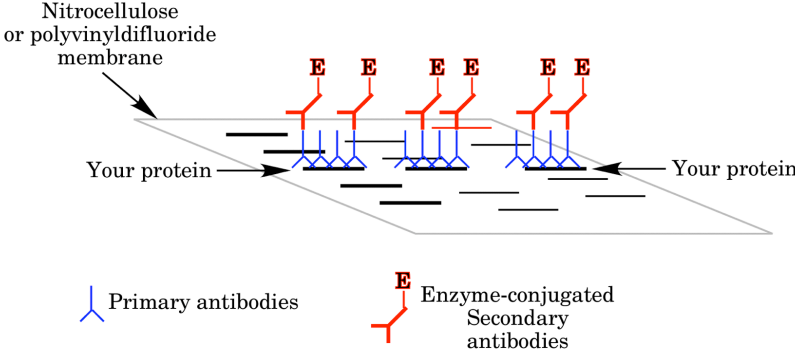


FIG. 3. **SDS-polyacrylamide gel electrophoresis of aromatase.** The lanes show human placental microsomes (200 μg of protein), 0.3, 1, and 3 μg of purified P-450_{arom} and molecular weight markers.

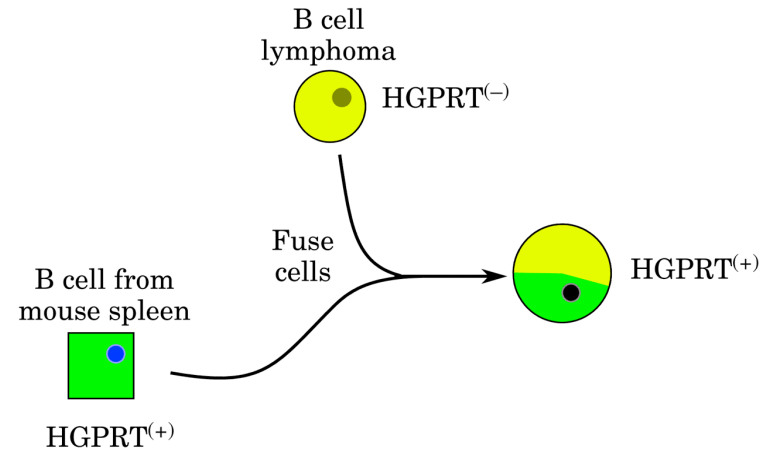
Antibodies



Western Blotting

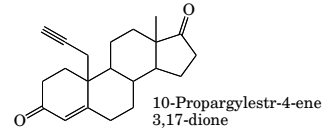
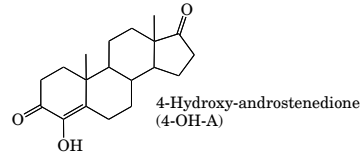
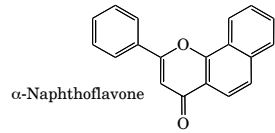
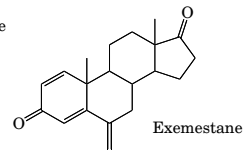
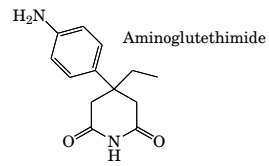
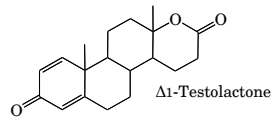


Monoclonal Antibodies



HGPRT = hypoxanthine guanine phosphoribosyltransferase

Aromatase Inhibitors



Protein Expression and Engineering

Native sources

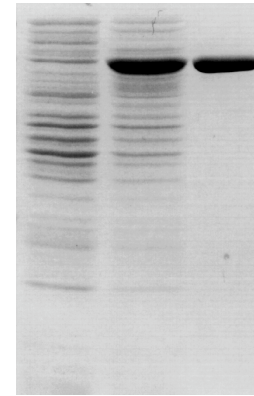
Heterologous Expression

Expression Organisms

Escherichia coli

Advantages

Disadvantages



Expression Organisms

Saccharomyces cerevisiae

Advantages

Disadvantages

Expression Organisms

Pichia pastoris

Advantages

Disadvantages

Expression Organisms

Higher Eukaryotic Cells in Culture

Transformed cells

Xenopus oocytes

Expression Organisms

Cloning