Conversion of androstenedione to estrone by placental microsomes*

The conversion of androgens to estrogens, in relatively low yield, has been conclusively demonstrated with human ovarian and placental slices[1,2,3]. The significance of this enzymic step has not yet been clearly defined, but the conversion has been proposed as an intermediary reaction in the normal biosynthesis of estrogens[4].

The present work has demonstrated the conversion, *in vitro*, of Δ^4-androstene-3,17-dione to estrone in yields of 40% to 60% by a placental-microsome fraction in the presence of reduced triphosphopyridine nucleotide (TPNH). Estrone was not detected by extraction of the complete system at zero time or by incubation without substrate (Table I). The reaction requires oxygen and has a pH optimum of 7. TPN is not active unless a TPNH-generating system is added (Table II).

TABLE I

CONVERSION OF ANDROSTENEDIONE TO ESTRONE

	Estrone yield (μg)
Complete system	45
Complete system at zero time	0
System minus substrate	0

The incubation mixture contained: microsomal fraction (8mg protein/ml) in 0.05 *M* phosphate buffer, pH 7, 100 μg androstenedione and 9 μmoles TPNH in a total volume of 5 ml. Incubation was for 1 h at 37° in air. Estrone production was measured by fluorescence and confirmed by paper chromatography and counter-current distribution. Infra-red spectroscopy of several pooled experiments was used for further identification.

TABLE II

COFACTOR REQUIREMENTS

Additions	*Estrone yield* (μg)
TPN (5 μmoles)	0
TPNH (5 μmoles)	30
TPN (2 μmoles), glucose-6-phosphate and glucose-6-phosphate dehydrogenase	30

Microsome fraction (4 mg protein/ml) in 0.05 *M* phosphate buffer, pH 7, and 200 μg androstenedione. Incubation was for 1 h in air at 37°. Estrone production was measured as in Table I.

Human placentas obtained immediately following delivery were homogenized in buffered 0.25 *M* sucrose and subjected to differential centrifugation procedures described previously[5]. The microsomal fraction was obtained between 10,000 × *g* and 80,000 × *g* and incubated with Δ^4-androstene-3,17-dione, dissolved in propylene glycol, and cofactors in air at 37° for 1 h. The mixtures were extracted three times with 6 vol. chloroform, evaporated and the chloroform residues dissolved in 95% ethanol. Aliquots were used for purification and assay. Production of estrone was measured directly by a modification of the fluorimetric method described by SWEAT[6].

Estrone was identified by the following methods. (1) Paper chromatography was carried out in two systems with modified Bush solvents (ligroine:toluene (2:1)/70% methanol and toluene/75% methanol) and the $FeCl_3$–$K_3Fe(CN)_6$ reagent was used to detect substances on the paper. Mobilities were compared with known standards run on the same chromatograms, and similar results were always obtained whether or not the sample was just separated as a phenolic fraction. (2) After initial chloroform extraction, residues were partitioned between 90% methanol and pentane to remove lipids and then subjected to a highly efficient procedure for separation of phenols[2]. The phenolic fraction was subjected to a 24-transfer counter-current distribution in a 50% methanol/CCl_4 system[7] and good agreement of the experimental and theoretical distribution curves was observed. The partition coefficient was identical with that determined for estrone (3) The peak tubes from the counter-current distribution were pooled, acetylated and estrone acetate identified by infra-red spectroscopy**.

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It is interesting to note that the presence of activity in the microsomal fraction with requirement of a reduced coenzyme is identical with systems described for adrenal hydroxylation[5]. The high yields obtained are consistent with a quantitatively significant role for the reaction. Detailed studies of the mechanisms involved are now in progress.

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1 A. S. MEYER, *Biochim. Biophys. Acta*, 17 (1955) 441.
2 B. BAGGETT, L. L. ENGEL, K. SAVARD AND R. I. DORFMAN, *J. Biol. Chem.*, 221 (1956) 931.
3 H. H. WOTIZ, J. W. DAVIS, H. M. LEMON AND M. GUT, *J. Biol. Chem.*, 222 (1956) 487.
4 S. SOLOMON, R. VANDE WIELE AND S. LIEBERMAN, *J. Am. Chem. Soc.*, 78 (1956) 5453.
5 K. J. RYAN AND L. L. ENGEL, *J. Biol. Chem.*, 225 (1957) 103.
6 M. L. SWEAT, *Anal. Chem.*, 26 (1954) 773.
7 L. L. ENGEL, W. R. SLAUNWHITE, P. CARTER AND I. T. NATHANSON, *J. Biol. Chem.*, 185 (1950) 255.

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Isolation and identification of β-mercaptopyruvate desulfurase*

Earlier experiments of MEISTER *et al.*[1] demonstrated that liver extracts decompose β-mercaptopyruvate to sulfur and pyruvate. More recently SÖRBO[2] reported that homogenates of rat liver and kidney as well as blood cells catalyse the transfer of sulfur from β-mercaptopyruvate to sulfite and sulfinates. These observations suggest that there are at least two types of enzymes which attack β-mercaptopyruvate and result in either "desulfuration" or "transsulfuration".

In the course of our studies dealing with enzymic reactions of cysteine and β-mercaptopyruvate[3,4,5,6] we have isolated a protein from rat liver which in the absence of a sulfur acceptor cleaves β-mercaptopyruvate to sulfur and pyruvate. In agreement with the observations of MEISTER *et al.*[1] we find that β-mercaptoethanol activates this reaction. Sulfite in the absence of mercaptoethanol also increases the rate of enzyme-catalyzed pyruvate formation from β-mercaptopyruvate. In confirmation of SÖRBO's results[2] we find that sulfite is converted to thiosulfate in the course of the latter reaction.

Ultracentrifugal and electrophoretic analyses revealed that our purified enzyme is at least 85 to 95% homogeneous. Thus, it appears that desulfurase and transsulfurase activities are associated with a single protein. The course of reaction is determined by experimental conditions (*e.g* presence of sulfur acceptors). Certain physical properties of this enzyme are: isoelectric point, 7.4; S_{20}, 2.7; D, $5.2 \cdot 10^{-6} cm^2 sec^{-1}$; mol.wt. (from S_{20} and D), 35,000–40,000; absorption maxima, 280 mμ and 415 mμ, $E_{280\ m\mu}/E_{415\ m\mu}$, 15.7; $E_{415\ m\mu}$, 56; S content, 4 atoms/mole. Spectrographic analyses revealed copper as the single metallic constituent of this protein. The protein in its purified form easily loses copper with concomitant loss of enzymic activity. Quantitative copper analyses are for this reason uncertain. It is estimated that two atoms of copper may be associated with one molecule of enzyme. Preliminary analyses indicate that two sulfhydryl groups are present per enzyme molecule as measured by the spectrophotometric method of BOYER[7]. Further biochemical properties of this enzyme as well as methods for its isolation will be published in detail.

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1 A. MEISTER, P. E. FRASER AND S. V. TICE: *J. Biol. Chem.*, 206 (1954) 561.
2 B. SÖRBO, *Biochim. Biophys. Acta*, 24 (1957) 324.
3 E. KUN AND J. L. BRADIN, JR., *Biochim. Biophys. Acta*, 11 (1953) 312.
4 E. KUN, J. L. BRADIN, JR. AND J. M. DECHARY, *Biochim. Biophys. Acta*, 19 (1956) 153.
5 E. KUN, *Biochim. Biophys. Acta*, 25 (1957) 135.
6 W. D. KUMLER AND E. KUN, *Biochim. Biophys. Acta*, 27 (1958) 464.
7 P. D. BOYER, *J. Am. Chem. Soc.*, 76 (1954) 4331.

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