Journal of Steroid Biochemistry. Vol. 10. pp. 653 to 660
Pergamon Press Ltd 1979. Printed in Great Britain

SUBCELLULAR DISTRIBUTION AND PROPERTIES OF 17β-HYDROXYSTEROID DEHYDROGENASE IN PREGNANT RABBIT UTERUS

KUNHARD POLLOW*, WALTER ELGER†, HERMANN HEẞLINGER*
and BARBARA POLLOW*
*Institut für Molekularbiologie und Biochemie, Freie Universität Berlin, Arnimalle 22, 1000 Berlin 33, and †Department Endocrine-Pharmacology of Schering AG, Berlin/Bergkamen, Germany

(*Received* 16 *October* 1978)

SUMMARY

To determine the intracellular distribution of the 17β-hydroxysteroid dehydrogenase, purified microsomes, mitochondria, peroxysomes, lysosomes, nuclei and cytosol were prepared. The purity of each fraction was monitored by marker enzymes. It was found that the 17β-hydroxysteroid dehydrogenase was mainly located in mitochondrial, microsomal and cytoplasmic fractions. Furthermore, it could be demonstrated that the microsomal enzyme was bound tightly to the membranes of the endoplasmic reticulum, while the mitochondrial 17β-hydroxysteroid dehydrogenase was mainly associated with the outer membranes of the organelle. Kinetic parameters of the mitochondrial and microsomal 17β-hydroxysteroid dehydrogenase were compared. K_m-values were nearly identical in both enzyme preparations. NADP was more efficient than NAD as cofactor. For the conversion of estradiol to estrone the optimum temperature was approximately 42°C and the optimum pH 8.0. For the reduction of estrone the optimum pH was 6.0. Sulfhydryl groups were shown to be essential for catalysis.

INTRODUCTION

It has been known for many years that rabbit uterine tissue contains a 17β-hydroxysteroid dehydrogenase which catalyzes the oxidation of the 17β-hydroxyl group of both C_{18} and C_{19} steroids [1, 2].

Since it is well established that uterine tissue contains receptor molecules which are capable of binding estrogenic compounds with a low capacity and high affinity, the metabolism of estrogens in this tissue has been of particular interest, because the estradiol-binding capacity of the receptor can be strongly affected by several possible estradiol metabolites, for instance estrone.

The purpose of this study was to investigate the subcellular distribution of 17β-hydroxysteroid dehydrogenase activity and substrate specificity and reaction kinetics of the membrane-bound enzyme activities of microsomal and mitochondrial fractions.

Studies on a number of mammalian tissues which contain 17β-hydroxysteroid dehydrogenase activity have shown that the enzyme activity is localized in various subcellular fractions [3–8].

EXPERIMENTAL PROCEDURE

Materials

[4-^{14}C]Estradiol (56 mCi/mmol), [4-^{14}C]-estrone (58 mCi/mmol), [4-^{14}C]-testosterone (58.2 mCi/mmol) and [4-^{14}C]-androstenedione (60 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, England and shown by thin-layer chromatography to be without contamination. Non-radioactive steroids were a gift from Schering AG, Berlin, Germany.

Bovine serum albumin was obtained from Behring, Marburg, Germany. DNA (thymus), RNA (yeast), NAD(P), ADP, hexokinase, succinate cytochrome c (bovine), and glucose-6-phosphate dehydrogenase were brought from Boehringer, Mannheim, Germany. The following chemicals were provided by Merck, Darmstadt, Germany: sucrose, CsCl, EDTA, and triethanolamine. Triton X-100 was a product of Serva, Heidelberg, Germany. Reagent grade organic solvents were re-distilled before use.

Buffers included: (A) 10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 12 mM mercaptoethanol, 0.25 M sucrose, 20% glycerol (V/V); (B) 2.4 M sucrose, 1 mM magnesium acetate; (C) 0.25 M sucrose, 10 mM Tris–HCl (pH 7.4), 25 mM KCl, 2 mM $CaCl_2$, 24 mM thioglycerol.

Methods

Preparation of tissue fractions. Pregnant hare rabbits were obtained from Schering AG, Berlin, Germany. The day of mating was designated as day zero of pregnancy. The uterus was excised on day 30 (the activity of the 17β-hydroxysteroid dehydrogenase reaching its highest value during the time of pregnancy), the products of conception were removed, and endometrial tissue was scraped off with a scalpel. The isolated tissue was immediately washed in ice-cold buffer A. Collected tissue was minced finely with scissors and homogenized in 4 vol. (wt./vol.) of buffer A. Homogenization was performed in an ice-bath with an Ultra Turrax homogenizer using three 10-s pulses

at 110 V (a minimum of 2 min was allowed for washing between pulses) followed by homogenization with a glass homogenizer fitted with a Teflon pestle.

The homogenate was filtered through four layers of cheese-cloth and fractionated as follows: nuclei were sedimented at 850 *g* for 15 min (rotor SS-34, Sorvall RC-2), layered on a cushion of buffer B, and re-sedimented at 60,000 *g* for 60 min (rotor SW 25.2, Beckman ultracentrifuge L2-65B). The precipitate was washed twice in buffer A, suspended in buffer C to which 0.25% of Triton X-100 (V/V) had been added, layered on buffer C containing 2.2 M sucrose (but no detergent), and sedimented at 60,000 *g* for 60 min. The resulting precipitate was designated "purified nuclei".

Light mitochondria, peroxysomes, and lysosomes were sedimented from the 850 *g* supernatant at 12,000 *g* for 15 min, washed once in buffer A, and purified according to the method of Brown[9]. Further purification of these fractions was achieved by sucrose gradient centrifugation (20–60%, wt./vol. 65,600 *g* for 4 h, rotor SW 65).

Preparation of submitochondrial fractions was performed by subjecting the washed mitochondrial pellets to controlled osmotic lysis and ultrasonic vibration, followed by differential centrifugation [10]. Microsomes were sedimented from the 12,000 *g* supernatant at 105,000 *g* for 60 min (rotor 30, Beckman), washed twice in buffer A, and separated into rough and smooth particles according to the method of Dallner *et al.*[11].

The purity of each fraction was controlled by marker enzymes. DNA/RNA ratio, or electron microscopy, as described earlier [12]. The 105,000 *g* supernatant was designated as "cytosol" and contained the soluble 17β-hydroxysteroid dehydrogenase.

Assay of 17β-hydroxysteroid dehydrogenase activity. Standard reaction: enzyme activity was calculated from the formation of [^{14}C]-estrone from [^{14}C]-estradiol. 0.42 μmol/l [^{14}C]-estradiol (adjusted with unlabelled estradiol to a final concentration of 100 μmol/l) was incubated for 15 min at 37°C in 10 mmol/l Tris–HCl buffer, pH 7.4, containing 0.1 ml propylene glycol, 500 μmol/l coenzyme, 20% glycerol and enzyme solution (1 mg/ml) in a total volume of 4.1 ml. After incubation, samples were brought into ice-water and extracted three times with 5 ml ether–chloroform (3:1, V/V). The pooled extracts were evaporated under nitrogen and dissolved in 0.5 ml of benzene. An aliquot (50 μl) was removed for liquid scintillation counting in order to estimate the total amount of radioactive steroids present in the extract. The benzene extracts were dried down under nitrogen and the dry residues were transferred with 0.2 ml chloroform–methanol (1:1, V/V) to the thin-layer plates (0.25 mm with fluorescence indicator, Woelm, Eschwege, Germany) in the system benzene–methanol (19:1, V/V). Radioactive steroids were located by fluorescence absorption of unlabelled standards chromatographed simultaneously. Radioactivity of separated steroids was quantitated with a radiochromatogram scanner (Berthold, Wildbad, Germany) equipped with a 2π methane gas counter and count integrator. Counting efficiency for ^{14}C was the same for all assays. Losses of [^{14}C]-estrone were calculated from the recovery of [^{14}C]-estrone. The percentage of radioactivity in each sample was calculated from the initial radioactivity of [^{14}C]-estradiol.

Assays of marker enzyme activities. Glucose-6-phosphate dehydrogenase (cytosol) was determined according to Büchner *et al.*[13], succinate dehydrogenase (mitochondria) according to Arrigoni and Singer[14], urate oxidase (peroxysomes) according to Mahler *et al.*[15], acid phosphatase (lysosomes) according to Leighton *et al.*[16]; monoamine (outer membranes) and adenylate kinase activities (intermembrane fraction) were assayed by the method of Schnaitman *et al.*[17], glutamate dehydrogenase activity (matrix) was determined by the method of Bergmeyer[18], NADPH-cytochrome-*c*-reductase (microsomes) according to the method of Omura and Takesue[19], and cytochrome oxidase (inner membranes) according to Smith[20].

Determination of DNA, RNA and protein. DNA was determined by the method of Burton[21], with calf-thymus DNA as the standard. RNA was determined by the method of Ceriotti[22], with yeast RNA as the standard. Protein was determined according to the method of Lowry *et al.*[23], with bovine serum albumin as the standard.

RESULTS

The degree of purity of various subcellular fractions of pregnant rabbit uterus was determined by the analysis of each fraction for characteristic marker enzymes. The results are presented in Table 1.

The distribution of the enzyme activities indicated that the purity of every subcellular fraction was satisfactory. It should be noted that the purified mitochondrial fraction still had a trace of lysosomal and peroxysomal components and that the microsomal fraction could not be separated completely from mitochondria, lysosomes and peroxysomes. Most of the 17β-hydroxysteroid dehydrogenase activity is located in the cytoplasmic, microsomal and mitochondrial fractions.

From Table 2 it can be seen that the specific activity of 17β-hydroxysteroid dehydrogenase in rough and smooth microsomal particles was similar to that in crude double-washed microsomes. This indicates that the microsomal 17β-hydroxysteroid dehydrogenase is bound tightly to the membranes of the endoplasmic reticulum. The activity of NADPH-cytochrome-*c*-reductase (marker enzyme of microsomes) in the mitochondrial fraction could be reduced considerably by several washings, while in the microsomal fraction the activity stayed nearly the same under the same conditions. The final microsomal contamination of the mitochondrial fraction was 4.4%. (Table 3).

Table 1. Distribution of marker enzymes and 17β-hydroxysteroid dehydrogenase in homogenate and various subcellular fractions of pregnant rabbit uterus

Fraction	Glucose-6-phosphate-dehydrogenase	Succinate dehydrogenase	Acid phosphatase	Urate oxidase	NADPH-cytochrome-c-reductase	17β-Hydroxysteroid dehydrogenase
Homogenate	1.7	0.8	3.6	0.9	1.2	5.8
Cytosol	9.8	n.m.	n.m.	n.m.	n.m.	4.2
Microsomes	n.m.	n.m.	2.7	0.9	18.3	4.8
Mitochondria	n.m.	7.3	13.8	1.7	0.8	1.7
Nuclei	n.m.	n.m.	7.3	0.5	0.3	0.13
Lysosomes	n.m.	0.8	53.8	3.2	0.5	0.14
Peroxysomes	n.m.	1.1	12.7	8.7	0.7	n.m.

All specific activities are expressed as nmol product formed per 15 min per mg protein. nm = not measurable.
The results show the average of duplicate determinations.

Table 2. Contents of RNA and protein and specific 17β-hydroxysteroid dehydrogenase in the submicrosomal fractions of pregnant rabbit uterus

Fraction	RNA (μg/ml)	Protein (μg/ml)	RNA/protein	17β-Hydroxysteroid dehydrogenase (nmol E_1/15 min/mg protein)
Crude microsomes	13.2	321	0.041	20.3
Smooth-surfaced microsomes	18.7	653	0.029	19.7
Rough-surfaced microsomes	76.3	481	0.159	20.7

The results show the average of duplicate determinations.

Table 3. Specific activity of NADPH-cytochrome-c-reductase in mitochondria and microsomes of pregnant rabbit uterus after several washings

Fraction	No. of washes	NADPH-cytochrome-c-reductase	Microsomal contamination (%)
Mitochondria	1	5.3	29
	2	3.2	17.5
	3	1.7	9.3
	4	0.8	4.4
Microsomes	2	18.3	—

Specific activity is expressed as nmol product formed per 15 min per mg protein. The results show the average of duplicate determinations.

Table 4. Distribution of marker enzymes and 17β-hydroxysteroid dehydrogenase in mitochondrial subfractions

Fraction	Cytochrome oxidase	Monoamine oxidase	Adenylate kinase	Glutamate dehydrogenase	17β-Hydroxysteroid dehydrogenase
Inner membrane	124.8	2.7	2.8	n.m.	2.7
Outer membrane	11.3	53.8	1.3	n.m.	18.2
Intermembrane fraction	1.8	n.m.	68.2	2.8	n.m.
Matrix	0.7	n.m.	5.1	38.2	n.m.

All specific activities are expressed as nmol product formed per 15 min per mg protein. The results show the average of duplicate determinations. n.m. = not measurable.

The 17β-hydroxysteroid dehydrogenase of mitochondria was found to be associated with both the outer and inner membranes of the organelle (Table 4), but the activity was seven times higher in the outer membrane. An 11-fold increase in specific activity was achieved with respect to washed mitochondria. The intermembrane fraction and the matrix did not convert estradiol to estrone.

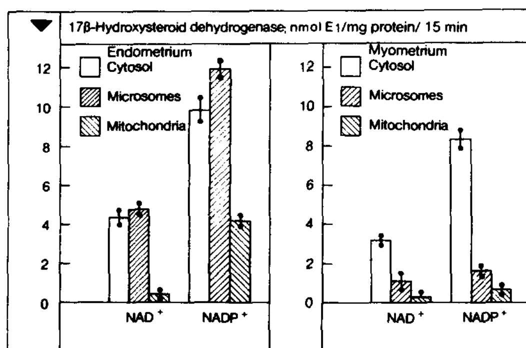


Fig. 1. Specific activity of 17β-hydroxysteroid dehydrogenase in various subcellular fractions of endometrial and myometrial tissue of rabbit pregnant uterus. The results show the average and range of duplicate determinations.

From Fig. 1 it becomes evident that in rabbit endometrium most of the specific activity of 17β-hydroxysteroid dehydrogenase was located in both cytoplasmic and microsomal fractions, whereas in myometrium the highest values were found in the soluble fraction.

The transformation of estradiol to estrone by the microsomal and mitochondrial fractions of pregnant rabbit uterus as a function of enzyme concentration is illustrated in Fig. 2. There is a linear relationship between the amount of estrone formed and the concentration of enzyme.

The transformation of estradiol to estrone in both subcellular fractions as a function of time is shown in Fig. 3. The formation of estrone was found to be linear up to 30 min.

When microsomal and mitochondrial fractions

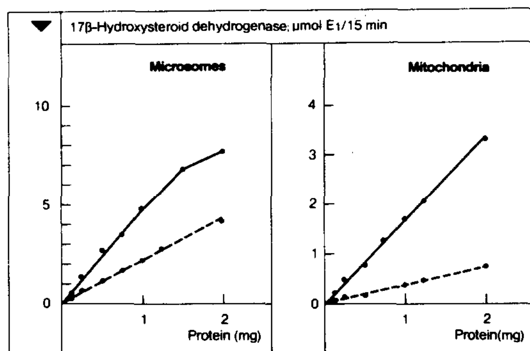


Fig. 2. Effect of enzyme concentration on rate of estradiol-17β oxidation. Microsomal and mitochondrial fractions were prepared from rabbit pregnant uterus. Incubations were carried out as described under "Experimental procedure". Data points shown represent the means of duplicate determinations; O---O: NAD as coenzyme, O——O; NADP as coenzyme.

were incubated with estradiol at various temperatures, the enzyme activity increased up to 42°C and rapidly decreased at temperatures over 50°C.

Rates of estradiol oxidation and estrone reduction in the range of pH 3.0 to 11.0 are shown in Fig. 4. Maximal oxidation of estradiol occurred at pH 8.0 for both microsomal and mitochondrial enzyme activity; and maximal reduction of estrone at pH 6.0. The activity of 17β-hydroxysteroid dehydrogenase varied somewhat with the type of buffer used.

The effect of increasing concentrations of the sulfhydryl reagents *p*-chloromercuribenzoate or iodacetamide on the microsomal and mitochondrial activity of 17β-hydroxysteroid dehydrogenase was examined (Fig. 5). At a concentration of 0.1 mmol/l, both *p*-chloromercuribenzoate and iodacetamide caused more than 50% inhibition of the membrane-bound dehydrogenase activities. When microsomal and mitochondrial enzyme preparations which had been pre-incubated in 1.0 mmol/l *p*-chloromercuribenzoate were exposed to the sulfhydryl-protecting reagent dithiothreitol at concentrations of 1.0 or 10.0 mmol/l, prior to the addition of coenzyme and [^{14}C]-estradiol, the inhibition of the dehydrogenases due to *p*-chloromercuribenzoate could be reversed in part (Table 5).

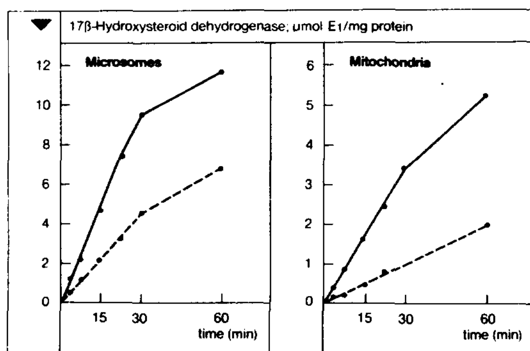
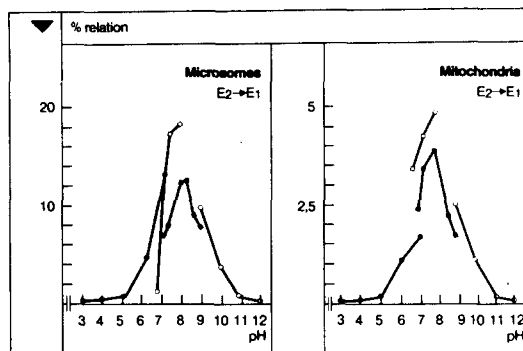


Fig. 3. Time course of estradiol-17β oxidation in microsomal (1 mg/ml) and mitochondrial (1 mg/ml) fractions of rabbit pregnant uterus. Data points shown represent the means of duplicate determinations; O---O: NAD as coenzyme, O——O: NADP as coenzyme.



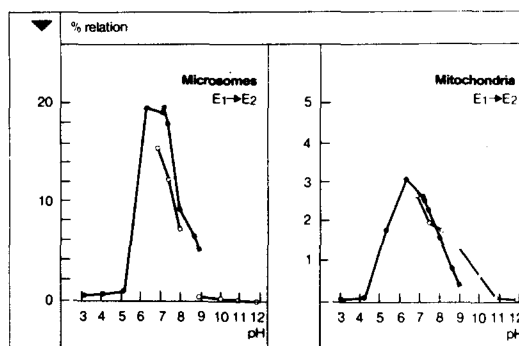


Fig. 4. The effect of pH on 17β-hydroxysteroid dehydrogenase activity. The various pH values of the incubation mixtures were provided by the use of the following buffers: 0.2 M Na_2HPO_4–citrate buffer [●——●], 0.066 M phosphate buffer [□——□], 0.2 M Tris-HCl buffer [■——■], 0.1 M glycine-NaOH buffer [O——O]. E_1 = estrone, E_2 = estradiol-17β.

$MgCl_2$, $ZnCl_2$ and $CuSO_4$ inhibited the 17β-hydroxysteroid dehydrogenase to various extents (Fig. 6).

For further characterization, K_M values for the conversion of estradiol into estrone in the presence of NAD or NADP were estimated. The K_M value was calculated from a double reciprocal plot of concen-

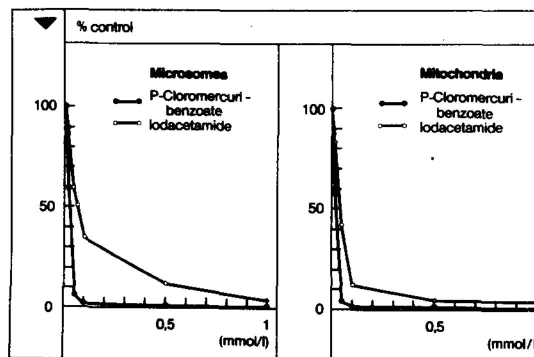


Fig. 5. Effect of increasing concentrations of *p*-chloromercuribenzoate and iodacetamide on microsomal and mitochondrial 17β-hydroxysteroid dehydrogenase of rabbit pregnant uterus.

Table 5. Effect of sulfhydryl and disulfide agents on 17β-hydroxysteroid dehydrogenase activity.

Addition	17β-Hydroxysteroid dehydrogenase	
	Microsomes	Mitochondria
	(% control)	
p-Chloromercuribenzoate (1 mM)	1.12	0.78
p-Chloromercuribenzoate (1 mM) + dithiothreitol (1 mM)	36	42
p-Chloromercuribenzoate (1 mM) + dithiothreitol (10 mM)	77	73
Dithiothreitol (10 mM)	108	98
Cysteine (10 mM)	98	91
Glutathione (10 mM)	96	97

The results show the average of duplicate determinations.

tration of estradiol and reaction velocity to be 7.7 × 10^{-5} mol/l for the NAD-linked enzyme activity of the mitochondrial fraction, and 9.1 × 10^{-5} mol/l when NADP was used as cofactor (Fig. 9). The V for estradiol was determined as 0.17 nmol × min^{-1} × mg $protein^{-1}$ (with NAD as cofactor) and 0.3 nmol × min^{-1} × mg $protein^{-1}$ (with NADP as cofactor). The ratio of the maximal velocity for NADP to that for NAD was 2.3.

The K_M values of the 17β-hydroxysteroid dehydrogenase of the microsomal fraction were in the same range as the mitochondrial enzyme (Fig. 7). The maximal velocity of the dehydrogenase with NAD was 0.52 nmol × min^{-1} × mg $protein^{-1}$, and for the NADP-linked enzyme 2.5 nmol × min^{-1} × mg $protein^{-1}$. The ratio of the maximal velocity for NADP to that for NAD was 4.8.

Aliquots of the microsomal and mitochondrial 17β-hydroxysteroid dehydrogenase preparations were incubated with ^{14}C-labelled estradiol-17β, estrone, testosterone or androstenedione. The relative amounts of oxidation or reduction products of these steroids were estimated (Table 6).

Testosterone was oxidized by all samples at less than 85% of the rate of estradiol-17β. Both mitochondrial and microsomal preparations from pregnant rabbit uterus reduced androstenedione at a lower rate (50% or less) than estrone.

DISCUSSION

The subcellular localization of 17β-hydroxysteroid dehydrogenase has been investigated in many tissues [3–8]. In human placenta the activity (with estradiol as substrate) is mainly located in the soluble fraction, with little activity associated with purified endoplasmic reticulum or inner mitochondrial membranes. In the human endometrium, the activity is distributed about equally in mitochondrial and microsomal frac-

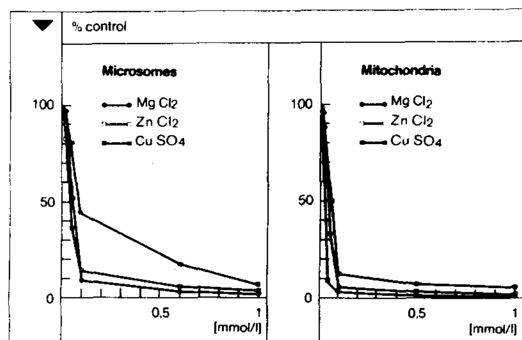
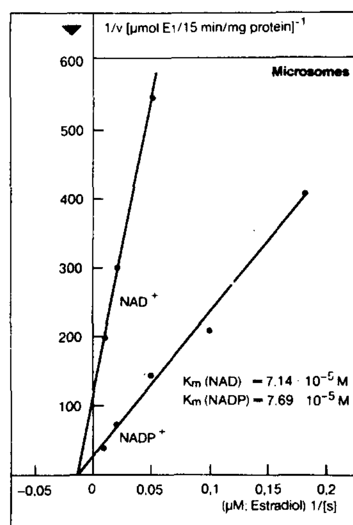


Fig. 6. Effect of increasing concentrations of heavy metals on 17β-hydroxysteroid dehydrogenase.



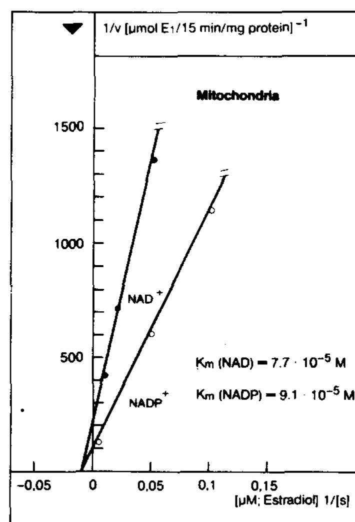


Fig. 7. Reciprocal plot of rate of oxidation of estradiol-17β in the presence of NAD or NADP. For experimental details see text.

Table 6. Substrate and coenzyme specificity of the microsomal and mitochondrial 17β-hydroxysteroid dehydrogenase of pregnant rabbit uterus under standard reaction conditions

Substrate (100 μmol/l)	Product	Coenzyme (500 μmol/l)	17β-Hydroxysteroid dehydrogenase (nmol formed product/15 min/mg protein)	
			Microsomes	Mitochondria
Estradiol-17β	Estrone	NAD	2.3	0.48
		NADP	4.8	1.7
Estrone	Estradiol-17β	NADH	0.85	0.31
		NADPH	2.6	0.8
Testosterone	Androstenedione	NAD	0.21	0.18
		NADP	0.58	0.23
Androstenedione	Testosterone	NADH	0.18	0.08
		NADPH	0.16	0.11

The results show the average of duplicate determinations.

tions with little 17β-hydroxysteroid dehydrogenase activity associated with the cytoplasmic fraction [24].

The data obtained in the present study indicate that there is a 17β-hydroxysteroid dehydrogenase in the microsomal, mitochondrial and cytoplasmic fractions of the pregnant rabbit uterus. This intracellular distribution of the 17β-hydroxysteroid dehydrogenase differs from previous reports of Jütting *et al.*[1, 2], who found that the oxidoreduction between estradiol and estrone was observed only in the soluble fraction from pregnant rabbit uterus. The discrepancy on intracellular distribution of this enzyme has not been clarified.

With regard to submicrosomal distribution of the human placental enzyme, the present findings clearly indicate that in rabbit uterus, 17β-hydroxysteroid dehydrogenase activity was nearly evenly distributed between the smooth and rough-surfaced microsomal subfractions. One can assume that the enzyme is actively synthesized on the surface of the granular endoplasmic reticula and then transferred to agranular endoplasmic reticula. Another possible explanation would be that the enzyme protein is synthesized on the free ribosomes instead of the surface of the granular endoplasmic reticula and that the protein is later incorporated into both types of endoplasmic reticula.

The distribution of 17β-hydroxysteroid dehydrogenase in the sub-mitochondrial membrane fractions suggested that this enzyme is associated with the outer mitochondrial membrane rather than with the inner membrane fraction. This may thus represent a redistribution artifact. To elucidate this problem, quantitative histochemical studies of the activity of this enzyme in whole tissue preparations are planned.

The present investigation revealed that the rabbit uterine 17β-hydroxysteroid dehydrogenase prefers NADP to NAD as a hydrogen donor. In contrast to this, Jütting *et al.*[1, 2] reported that NADP gave a 20% lower activity than an equimolar amount of NAD. As with other 17β-hydroxysteroid dehydrogenase in human placenta [7] and human endometrium [24, 25], broad substrate specificities have been observed for the membrane-bound enzymes isolated from rabbit uterus. Estradiol apparently is more reactive than testosterone, while estrone appears to be more reactive than androstenedione with the 17β-hydroxysteroid dehydrogenase. The substrate specificity of our enzyme preparations thus differs substantially from that described by Jütting *et al.*[1, 2] using a 10,000 *g* supernatant of pregnant rabbit uterus. Jütting *et al.* found that testosterone was dehydrogenated at less than 1% of the rate with estradiol, indicating that an aromatic A ring appears to be characteristic of highly reactive substrates.

The biological function of the 17β-hydroxysteroid dehydrogenase in the target organ, the uterus, is still unknown. It does seem certain than an enzyme which can interconvert biologically active and relatively inactive molecules is a potential candidate for an important physiological regulator role.

It is known that an early step in the action of estradiol in the target organ involves the binding of the hormone to a specific cytoplasmic receptor protein, followed by a change in the molecular properties of the hormone-receptor complex, which is translocated to the nucleus and there associates with one or more specific acceptor regions in the target cell chromatin. The specific binding of the hormone–receptor complex to the chromatin induces the novel synthesis of mRNA, which leads to the synthesis of specific proteins on the cytoplasmic protein synthesis apparatus.

It is possible that the 17β-hydroxysteroid dehydrogenase acts at the target cell level as a regulator enzyme in the complex estradiol effector mechanism, in the sense that a large drop in the actual intracellular estradiol concentration, caused by enzymatic oxidation at the 17β-position, would lead to a decrease in the "sensitivity" of the target organ to estradiol, especially since estrone is an effective competitor for the specific binding region on the receptor protein, and can displace the estradiol from it [26].

Acknowledgements—This work was supported by grant from the Deutsche Forschungsgemeinschaft. We wish to thank Mrs. Wenzel for the skilful technical assistance.

REFERENCES

1. Jütting G.: Hormonale Enzyminduktion im Myometrium—Beispiel einer Oestrogenwirkung am Erfolgsorgan. *Acta endocr. Copenh. Suppl.* **145** (1970) 9–46.

2. Jütting G., Thun K. J. and Kuss E.: Reinigung und Charakterisierung der durch Oestrogene induzierbaren 17β-Hydroxysteroid: NAD[P]-Oxydoreduktase aus dem Myometrium von Kaninchen. *Eur. J. Biochem.* **2** (1967) 146–151.
3. Engel L. L. and Groman E. V.: Human placental 17β-estradiol dehydrogenase: characterization and structural studies. *Rec. Progr. Horm. Res.* **30** (1974) 139–164.
4. Pons M., Nicolas J. C., Boussioux A. M., Descomps B. and Crastes de Paulet A.: Some new developments in the knowledge of human placental estradiol-17β-dehydrogenase. *J. steroid Biochem.* **8** (1977) 345–358.
5. Langer L. J. and Engel L. L.: Human placental estradiol-17β dehydrogenase. I. Concentration, characterization and assay. *J. biol. Chem.* **233** (1958) 583–588.
6. Jarabak J., Seeds A. E. and Talalay P.: Reversible cold inactivation of a 17β-hydroxysteroid dehydrogenase of human placenta: Protective effect of glycerol. *Biochemistry* **5** (1966) 1269–1279.
7. Lehmann W. D. and Breuer H.: Charakterisierung und Kinetik einer mikrosomalen 17β-Hydroxysteroid-Oxydoreduktase der menschlichen Placenta. *Hoppe-Seyler's Z. Physiol. Chem.* **348** (1967) 1633–1639.
8. Pollow K., Sokolowski G., Grunz H. and Pollow B.: Hydroxysteroid-Oxidoreduktasen und ihre Funktion als Katalysatoren des spezifischen Wasserstoff-Transfers zwischen Steroidhormonen in Humanplacenta. I. Morphologische Untersuchungen und Aktivitätsverteilungen der 17β- and 20α-Hydroxysteroid-Oxidoreduktase in subzellulären Fraktionen. *Hoppe-Seyler's Z. Physiol. Chem.* **355** (1974) 501–514.
9. Brown D. H.: Separation of mitochondria, peroxysomes and lysosomes by zonal centrifugation in a Ficoll gradient. *Biochim. biophys. Acta* **162** (1968) 152–153.
10. Parsons D. F., Williams G. R. and Chance B.: Characteristics of isolated and purified preparations of the outer and inner membranes of mitochondria. *Ann. N.Y. Acad. Sci.* **137** (1966) 643–666.
11. Dallner G., Siekevitz P. and Pallade G. E.: Biogenesis of endoplasmic reticulum membranes. I. Structural and chemical differentiation in developing rat hepatocyte. *J. cell. Biol.* **30** (1966) 73–96.
12. Pollow K., Boquoi E., Baumann J., Schmidt-Gollwitzer M. and Pollow B.: Comparison of the *in vitro* conversion of estradiol-17β to estrone of normal and neoplastic human breast tissue. *Molec. Cell Endocr.* **6** (1977) 333–348.
13. Bücher T., Luk W. and Pette D.: Einfache und zusammengesetzte optische Tests mit Pyridin-nucleotiden. In *Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse* (Edited by Hoppe-Seyler and Thierfelder). Springer-Verlag, Berlin-Göttingen-Heidelberg-New York, Vol. VIA (1964) pp. 292–339.
14. Arrigoni O. and Singer T. P.: Limitations of the phenazine methosulfate assay for succinic and related dehydrogenases. *Nature, Lond.* **193** (1962) 1256–1258.
15. Mahler H. R., Hübscher G. and Baum H.: Studies on uricase. I. Preparation, purification and properties of a cuproprotein. *J. biol. Chem.* **216** (1955) 625–641.
16. Leighton F., Poole B., Beaufay A., Boudhuin P., Loffey J. W., Fowler S. and de Duve C.: The large-scale separation of peroxisomes, mitochondria, and lysosomes from the livers of rats injected with Triton WR-1339. Improved isolation procedures, automated analysis, biochemical and morphological properties of fractions. *J. cell Biol.* **37** (1968) 482–513.
17. Schnaitman C. and Greenawalt J. W.: Enzymatic properties of the inner and outer membranes of rat liver mitochondria. *J. cell Biol.* **38** (1968) 158–175.
18. Bergmeyer H. U.: *Methoden der Enzymatischen Analyse.* Verlag Chemie, Weinheim (1962) pp. 752–756, 875.
19. Omura T. and Takesue S.: Solubilization of NADH-cytochrome b_5 reductase from liver microsomes by lysosomal digestion. *J. Biochem.* **67** (1970) 257–266.
20. Smith L.: Cytochromes a, a_1, a_2, and a_3. *Meth. Enzymol.* **2** (1955) 732–740.
21. Burton K.: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonuclei acid. *Biochem. J.* **62** (1956) 315–323.
22. Ceriotti, G.: Determination of nuclei acids in animal tissues. *J. biol. Chem.* **214** (1955) 59–70.
23. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
24. Pollow K., Lübbert H., Boquoi E., Kreuzer G., Jeske R. and Pollow B.: Studies on 17β-hydroxysteroid dehydrogenase in human endometrium and endometrial carcinoma. I. Subcellular distribution and variations of specific enzyme activity. *Acta endocr. Copenh.* **79** (1975) 134–145.
25. Pollow K., Lübbert H. and Pollow B.: Studies on 17β-hydroxysteroid dehydrogenase in human endometrium and endometrial carcinoma. III. Partial purification and characterization of the microsomal enzyme. *Acta endocr. Copenh.* **80** (1975) 355–364.
26. Gurpide E. and Tseng L.: Estrogens in normal human endometrium. In: *Receptors and Mechanism of Action of Steroid Hormones, Part I.* (Edited by Jorge R. Pasqualini). Dekker, New York and Basel, (1976) pp. 109–158.