# IMMUNOLOGICAL MEASUREMENT OF HUMAN 17β-HYDROXYSTEROID DEHYDROGENASE

O. Mäentausta,* H. Peltoketo, V. Isomaa, P. Jouppila[1] and R. Vihko

Biocenter and Department of Clinical Chemistry and [1]Obstetrics and Gynecology, University of Oulu, SF-90220 Oulu, Finland

**Summary**—Human placental 17β-hydroxysteroid dehydrogenase (17-HSD) was purified to apparent homogeneity using ammonium sulfate precipitation and chromatography on Red–Agarose and DEAE–Sepharose columns. Electrophoresis on polyacrylamide gels under denaturing conditions and using silver staining showed a single protein with an apparent molecular weight of 37,800. Antibodies to the purified protein were raised in rabbits and were found by immunoblotting to be specific to 17-HSD.

A sensitive radioimmunoassay was established using $^{125}$I-labeled 17-HSD as a tracer, an appropriate dilution of the antibody, and a kaolin-coupled double antibody for separating the antibody-bound and free fractions. The detection limit of the assay was approximately 150 pg/tube (1.5 μg/l). The cytosol fraction (105,000 g) of term placental tissue contained approximately 0.7 mg of 17-HSD per gram of protein, and the concentrations of 17-HSD measured by immunoassay and enzymatic activity proved to be strictly parallel in different partly purified placental preparations.

The supernatants from centrifugations of human endometrial homogenates at 800 g and 105,000 g (after detergent treatment) displayed cross-reactivity with the antibody. The mean concentration of the cross-reacting substance in the radioimmunoassay was 14.1 μg/g protein (range 2–62.3) in specimens taken on different days in the cycle. These concentrations showed a significant correlation with the 17-HSD activities measured in the endometrial specimens ($r = 0.722$, $P < 0.001$, $n = 21$). Mean concentrations of substance were 8.3 μg/g protein in endometrial specimens taken during the follicular phase (days 4–12, $n = 8$) and 22.9 μg/g protein during the luteal phase (days 16–22, $n = 6$) were obtained using the radioimmuoassay. There was excellent parallelism between the competition curves for [$^{125}$I]iodo-17-HSD with purified 17-HSD standards and placental and endometrial homogenate dilutions. These data strongly suggest that the substance measured in the endometrial specimens was 17-HSD.

## INTRODUCTION

Certain human target tissues for female sex steroid action such as the endometrial and breast epithelia show activity of 17β-hydroxysteroid dehydrogenase (17-HSD, EC 1.1.1.62). The enzyme is thought to play an important role in the regulation of estrogen influence in these tissues by converting estradiol into a less active estrogen, estrone [1–4]. The enzyme activity is present in the endometrium mostly in the glandular cells [5], and is induced by progestins [1, 3, 6, 7], an effect which is nevertheless short-lived [8]. Induction of the enzyme activity by progestins has also been described in breast tissue [9, 10]. In contrast to these findings, 17-HSD activity was not induced by a number of progestins in endometriosis tissue [11]. These data indicate an important role for 17-HSD in the regulation of female sex steroid action.

So far the catalytic activity of this enzyme has been measured, but immunological measurement of its protein has been prevented by the nonavailability

of suitable high affinity antibodies. Immunological measurement of this enzyme in different subcellular fractions requires solubilization of membrane-bound enzyme. Zwitterionic detergents, such as the Zwittergent 312 used here, have been described as being effective for solubilizating a number of enzymes and as being the least detrimental to them [12].

The aim of the present work was to purify the 17-HSD enzyme and develop a radioimmunoassay for quantifying its protein. Placental tissue was chosen as the starting material for purification of the antigen because it has a high activity of this enzyme [13–16].

## EXPERIMENTAL

Tritium-labeled [2,4,6,7-³H]estradiol (85 Ci/mmol), [2,4,6,7-³H]estrone (89 Ci/mmol) and Na[$^{125}$I] (2000 Ci/mmol) were purchased from Amersham International plc. (Amersham, Bucks, England). Non-labeled estradiol and estrone were from Steraloids (Wilton, N.H.), DEAE–Sepharose CL 6B,

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*To whom correspondence should be addressed.

Sephacryl S-300 Superfine and protein molecular weight standards were purchased from Pharmacia (Uppsala, Sweden) and colored molecular weight markers (Rainbow™) from Amersham International plc. (Bucks, England). Kaolin-coupled goat anti-rabbit antibody was from Farmos Diagnostica (Turku, Finland), and Freund's complete and incomplete adjuvants were from Difco Laboratories (Detroit, Mich.). Zwittergent, 3-(*N,N*-dimethyl-palmitylammonio) propane sulfonate, was purchased from Fluka (Buchs, Switzerland). Other reagents were either from Sigma Chemical Co. (St Louis, Mo.) or Merck A.G. (Darmstadt, F.R.G.) and were of the highest purity available.

### Buffer solutions

*Buffer A.* 10 mmol/l potassium phosphate buffer, pH 7.5, 1 mmol/l EDTA, 0.5 mmol/l phenylmethyl-sulfonylfluoride (PMSF), 0.02% $NaN_3$ and 20% glycerol (v/v).

*Buffer B.* 10 mmol/l potassium phosphate buffer, pH 7.5, 1 mmol/l EDTA, 0.5 mmol/l PMSF, 0.02% $NaN_3$ and 50% glycerol (v/v).

*Buffer C.* 10 mmol/l potassium phosphate buffer, pH 7.5, 1 mmol/l EDTA, 0.02% $NaN_3$ and 20% glycerol (v/v).

*Buffer D.* 150 mmol/l potassium phosphate buffer, pH 7.5, 1 mmol/l EDTA, 0.02% $NaN_3$ and 20% glycerol (v/v).

*PBS-BSA.* 10 mmol/l potassium phosphate, pH 7.4, 150 mmol/l NaCl, 0.1% $NaN_3$ and 0.1% bovine serum albumin (BSA).

*TBS buffer.* 50 mmol/l Tris buffer, pH 8.0, 150 mmol/l NaCl.

### Purification of placental 17-HSD

Human term placentae obtained from healthy mothers immediately after delivery were chilled on ice and transported to the laboratory. They were washed with buffer A, trimmed free of connective tissue, fetal membranes and grossly necrotic tissue and stored at −70°C until use. The placental tissue (approx. 500 g) was cut into small pieces with a knife and then homogenized in two volumes (w/v) of buffer A with two 15-s bursts of a Sorvall Omnimixer homogenizer. The homogenate was centrifuged at 9000 g for 30 min. Solid ammonium sulfate was slowly added to the supernatant with continuous stirring to bring it to 50% fraction al saturation. The pH was maintained at 7.5 during the precipitation with 2 mol/l NaOH. The precipitate was collected by centrifugation and was dissolved in a minimal volume of buffer B and dialyzed against buffer A for 48 h. After dialysis, the solution was centrifuged at 9000 g for 15 min and the supernatant applied to a Reactive Red–Agarose column (2.5 × 30 cm) equilibrated with buffer C. The column was washed with buffer C until no protein could be detected in the eluate and the washing was continued with buffer D. The excess phosphate was removed by washing the gel again with buffer C. The

enzyme was eluted with a 0–300 μmol/l $NADP^+$ gradient made up in buffer C. The rate of elution was 50 ml/h. Peak enzyme fractions (eluted at an $NADP^+$ concentration of 60–130 μmol/l) were pooled and applied to a DEAE–Sepharose column (1.3 × 8.0 cm) equilibrated with buffer C. The matrix was washed with 10 column volumes of buffer C and eluted with a linear phosphate gradient in buffer C (10–500 mmol/l). Peak enzyme fractions (eluted between 130 and 210 mmol/l phosphate) were pooled and stored at −20°C.

### Measurement of 17-HSD activity

The enzyme activity was determined using the method described by Tseng and Gurpide [17], with minor modifications. Briefly, 400 μl of sample diluted in buffer C including 0.1% BSA was mixed with [$^3$H]estradiol (about $10^5$ cpm) and non-labeled estradiol to a final concentration of 37 μmol/l. The reaction was started by adding 50 μl $NAD^+$ (1.4 mmol/l). After a 10 min incubation at 37°C the reaction was stopped by adding 5 ml of diethyl ether containing non-labeled estrone as a carrier. The samples were extracted three times with 5 ml of diethyl ether and the extracts pooled and evaporated under a nitrogen flow. The samples were then dissolved in 100 μl of toluene–methanol (92:8). Estrone was separated from estradiol by chromatography on Silica gel thin-layer plates (Merck AG, Darmstadt, F.R.G.) using toluene–methanol (92:8). Appropriate areas of the silica gel were scraped off, the steroid eluted with methanol and the radioactivity measured with a liquid scintillation counter (1210 Ultrobeta, Wallac, Turku, Finland). The enzyme activity is expressed as micromoles of estrone formed per minute.

### Production of antibodies

New Zealand white rabbits were used to raise antibodies against purified 17-HSD. Half of the animals were immunized with reduced antigen prepared by boiling the enzyme solution with 5% β-mercaptoethanol for 5 min prior to use. The other animals were immunized with non-reduced antigen.

About 100 μg of purified enzyme emulsified with Freund's complete adjuvant was used for the initial immunization of each animal. The animals were injected with the antigen subcutaneously at multiple sites. Subsequent booster injections of 100 μg of antigen in incomplete adjuvant were given at 3–4 week intervals. Blood was collected from the ear vein 10 days after each injection, and the serum was separated out by low speed centrifugation and stored at −20°C.

### Iodination of 17-HSD

Purified 17-HSD was labeled with Na[$^{125}$I] by the chloramine T-method, essentially as described by Greenwood *et al.* [18]. 10 μg of 17-HSD in 10 mmol/l $K_2HPO_4$, pH 7.5, were mixed with 1 mCi of Na[$^{125}$I] in 30 μl of 0.5 mol/l potassium phosphate buffer and

$6\,\mu g$ (0.01 ml) of chloramine T. The reaction was allowed to proceed for 20 s at room temperature and terminated by the addition of $6\,\mu g$ (0.01 ml) of sodium metabisulfite. The reaction mixture was applied to a Sephacryl S-300 column (1.6 × 18 cm), which was equilibrated with 10 mmol/l of sodium phosphate buffer, pH 7.4, containing 0.1% BSA. The labeled protein peak fractions were collected, pooled and stored in small aliquots at −20°C until use.

### Radioimmunoassay of 17-HSD

Radioimmunoassay (RIA) of 17-HSD was performed in 11 × 70 mm polystyrene tubes. The standards were prepared in PBS–BSA buffer using purified 17-HSD to give the following concentrations of the protein: 0, 0.3, 1, 3, 10, 30, 100 and 300 $\mu g/l$. Standards (100 $\mu l$) or unknown samples (100 $\mu l$ of diluted placental preparations) mixed with 200 $\mu l$ of the antiserum dilution 1:25000 in PBS–BSA buffer were incubated overnight at 4°C. [$^{125}$I]iodo-17-HSD (20,000 cpm) in 200 $\mu l$ of PBS–BSA buffer was then added and incubated for an additional 2 h at 37°C. After incubation, the antibody bound and free tracer were separated by adding 0.5 ml of goat anti-rabbit antibody coated onto solid phase kaolin particles in the PBS–BSA buffer. After incubation for 30 min at ambient temperature, the mixture was centrifuged at 2000 $g$ for 15 min at +4°C. The supernatants were decanted and the radioactivity remaining in the pellets counted in a gamma counter (Multigamma, Wallac Oy, Turku, Finland). The concentrations of 17-HSD in the samples were calculated from a standard curve run in parallel.

### Polyacrylamide gel electrophoresis and immunoblotting

One dimensional polyacrylamide gel electrophoresis under denaturing conditions (SDS–PAGE) was conducted essentially as described by Laemmli [19] using 10% polyacrylamide gels. After electrophoresis, the gels were stained by silver staining [20]. The proteins were transferred electrophoretically to nitrocellulose paper for immunoblotting [21]. After incubation of the membrane with 20% fetal calf serum in TBS buffer at room temperature for 30 min, the nitrocellulose paper was incubated for 2 h with anti-17-HSD antibody diluted 1:200 in TBS buffer. The filter was washed three times with the buffer and incubated in protein A-peroxidase in TBS buffer containing 5% fetal calf serum. The filter was again washed as above and then developed using 4-chloro-1-naphtol as a substrate.

### Endometrial preparations

Human endometrium was obtained on different days of cycle by Strich currettage from patients operated at the Department of Obstetrics and Gynecology of this university for tubal sterilization. Endometrial specimen was also obtained from seven cases, from which six were in histopathological examination verified as endometrial adenocarcinoma and one as endometrial stromal sarcoma. Part of each specimen was used for diagnostic pathological examinations and part was transported to the laboratory on dry ice and stored at −70°C until analyzed.

20–200 mg of the tissue for analysis was homogenized in 0.5–2.5 ml of buffer A using a glass–Teflon homogenizer. The membrane-bound enzyme was solubilized with the zwitterionic detergent Zwittergent 312. The endometrial homogenate was incubated for 30 min at room temperature gently mixing it with 0.1% Zwittergent. The mixture was centrifuged at 800 $g$ for 10 min and the supernatant further centrifuged at 105,000 $g$ for 1 h. The supernatants were analyzed by RIA. Activities of 17-HSD were measured in the untreated 800 $g$ fraction as described above for placental preparations but using a 60 min incubation of less diluted samples.

### Preparation of subcellular fractions

The homogenate of the endometrial tissue was subjected to centrifugation at 800 $g$ for 10 min to separate out the nuclear fraction. After taking an aliquot for RIA and activity measurements, the supernatants were centrifuged further at 10,000 $g$ to obtain a mitochondrial fraction. The remaining supernatants were centrifuged at 105,000 $g$ to separate the microsomal and cytosolic fractions. The placental tissue was treated in a similar manner to that described for the endometrium, except that the mitochondrial fraction was obtained by centrifugation at 27,000 $g$ for 15 min.

The enzymatic activity was determined from supernatant aliquots as described above, using a 60 min incubation. Each supernatant used for RIA was treated with detergent before the measurement as described above.

### Other methods

The protein concentrations were measured according to Bradford [22] using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.). BSA was used as the standard.

The serum progesterone concentrations were measured by RIA using a commercial kit supplied by Farmos Diagnostica (Turku, Finland), according to the manufacturer's instructions.

### RESULTS

### Purification of 17-HSD

The enzyme was purified from term placental tissue by a 3-step procedure, as shown in Table 1. An approximately 2000-fold purification was required to achieve an apparently homogeneous enzyme preparation with a final sp. act. of 7–8 $\mu$mol of estrone formed/min/mg protein. The apparent sp. act. of the enzyme preparations was higher prior to the chromatography on DEAE–Sepharose (Table 1), although PAGE under denaturing conditions, did

Table 1. Purification of 17β-hydroxysteroid dehydrogenase from human placenta. Two full-term placentae were used as the starting material for enzyme purification as described in the text.

| Purification step | Total protein (mg) | Total act. (U = $\mu$mol $E_1$ formed/min) | Sp. act. (U/mg protein) | Purification ($x$-fold) | Yield (%) |
|---|---|---|---|---|---|
| (1) 9000 $g$ homogenate | 23925 | 105.3 | 0.0044 | 1 | 100 |
| (2) Dialyzed $(NH_4)_2SO_4$ precipitate | 2617 | 73.3 | 0.028 | 6.4 | 70 |
| (3) Red–Agarose eluate | 4.7 | 48.2 | 10.3 | 2336 | 46 |
| (4) DEAE–Sepharose eluate | 3.2 | 24.5 | 7.7 | 1755 | 23 |

show some other protein bands in addition to that for 17-HSD at this stage, whereas the final preparation was homogeneous. It is therefore possible that the activity of the enzyme may have deteriorated slightly during the final purification step. Figure 1 shows a SDS–PAGE analysis of the purified 17-HSD following silver staining. The molecular weight of the protein was 37800 ± 750 ($n = 11$), which corresponds to that of a subunit of 17-HSD [23].

### Characteristics of the antiserum to 17-HSD

Both the native enzyme and the subunits gave rise to antibodies with similar binding and displacement properties. The selected antiserum, obtained after immunization of rabbits with the purified and denaturated 17-HSD from human placental tissue, formed a single band in immunoblotting analysis carried out using either the supernatant from the placental homogenate (Fig. 2, lane 1), or the purified enzyme preparation (lane 2). Since no cross-reacting substances appeared in the analysis of the crude placental cytosol except for the one having the mobility of a subunit of the purified placental 17-HSD, these data indicate a high specificity for the antibody. The

antibody displayed a high affinity for 17-HSD, the apparent equilibrium dissociation constant ($K_d$) being $3.7 \times 10^{-11}$ mol/l as determined by Scatchard-type analysis [24]. The addition of serial dilutions of the antiserum to the enzyme solution prior to the enzymatic activity assay led to a corresponding gradual loss of enzyme activity (data not shown).

### Radioimmunoassay of 17-HSD

A typical standard curve for the radioimmunoassay of 17-HSD is shown in Fig. 3. The detection limit of the assay was approx. 150 pg/tube ($1.5 \mu$g/l) for 17-HSD. Its accuracy was tested by adding known amounts of the purified antigen (25, 50, 100, 150 $\mu$g/l, based on protein analysis) to a human endometrial cytosol preparation apparently containing 2 $\mu$g/l of the enzyme. Corresponding recoveries were 94, 98, 94 and 91%. The precision of the assay was tested by determining intraassay and interassay coefficients of variation. The intraassay coefficients of variation were 6, 6, 4 and 6% at calculated mean amounts of
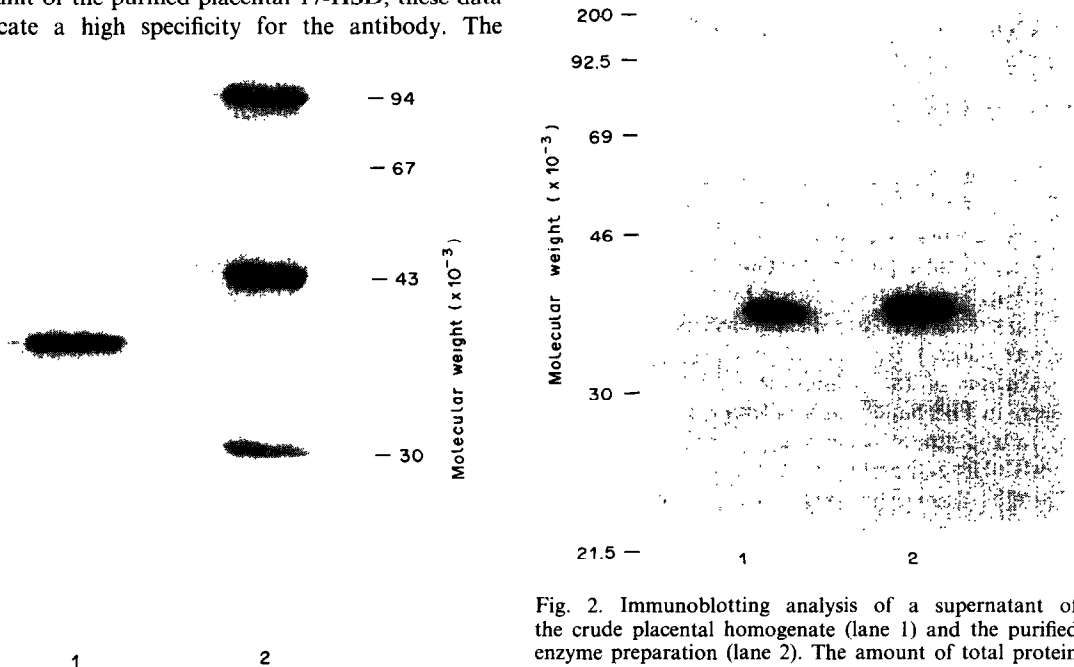


Fig. 1. Electrophoretic analysis of purified human placental 17β-hydroxysteroid dehydrogenase (17-HSD) on polyacrylamide gels containing sodium dodecylsulfate. The purified enzyme (1 $\mu$g, lane 1) was visualized by silver staining. The molecular weight standards (lane 2) were: phosphorylase b (94,000), BSA (67,000, not clearly visible with this staining), ovalbumin (43,000) and carbonic anhydrase (30,000).



Fig. 2. Immunoblotting analysis of a supernatant of the crude placental homogenate (lane 1) and the purified enzyme preparation (lane 2). The amount of total protein applied to lane 1 was 50 $\mu$g, and that of the purified 17-HSD applied to lane 2 was 0.5 $\mu$g. The dilution of the antiserum was 1:200, and the bands were visualized using protein A-peroxidase staining. The molecular weight standards marked but not visible in the photograph were myosin (200,000), phosphorylase b (92,500), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000) and trypsin inhibitor (21,500).
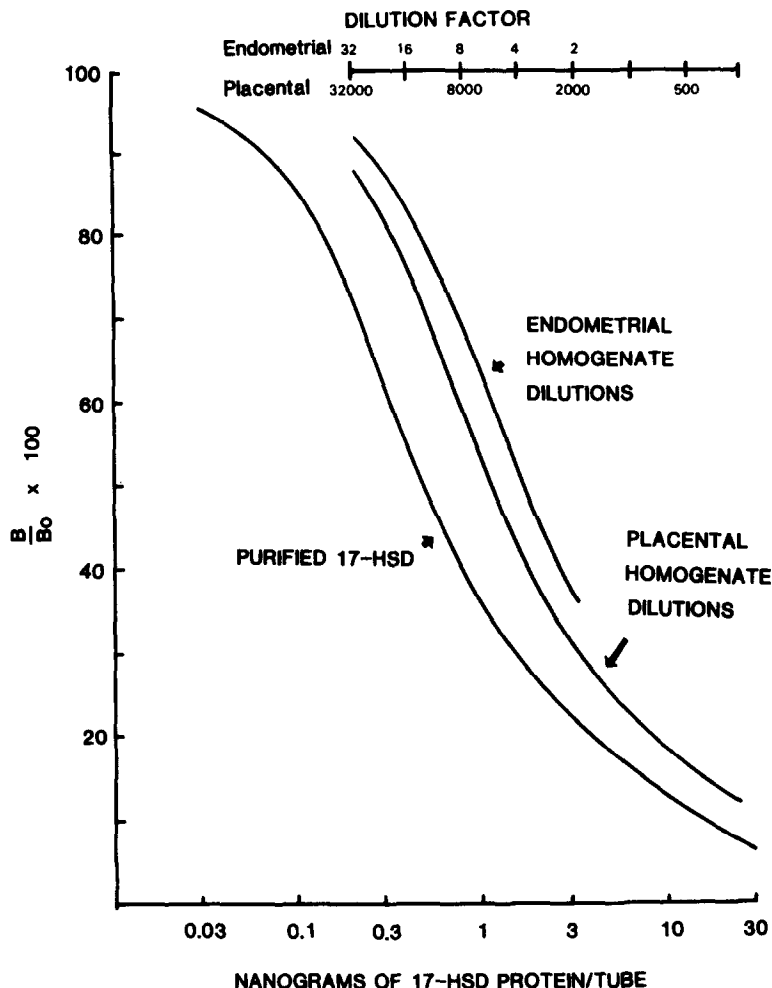
DILUTION FACTOR

Endometrial 32    16    8    4    2

Placental 32000          8000        2000        500

ENDOMETRIAL
HOMOGENATE
DILUTIONS

PURIFIED 17-HSD

PLACENTAL
HOMOGENATE
DILUTIONS

$\frac{B}{B_0} \times 100$

NANOGRAMS OF 17-HSD PROTEIN/TUBE

Fig. 3. Competition of 17β-hydroxysteroid dehydrogenase with [$^{125}$I]iodo-17β-hydroxysteroid dehydrogenase for antibody binding. Labeled enzyme was incubated with the antiserum (final dilution 1:62,500) and with the indicated amounts of purified 17-HSD or of serial dilutions of placental or endometrial homogenate. The antibody-bound and free antigens were separated by a second antibody coated onto kaolin particles.

5, 28, 63 and 121 μg/l of 17-HSD (n = 10 in each case). The interassay coefficients of variation were 7, 7, 5 and 8% at calculated mean amounts of 22, 29, 60 and 118 μg/l of 17-HSD (n = 5 in each case).

There was a close correlation between the 17-HSD levels in the placental tissue preparations following initial homogenization and at different stages of purification when the measurements were based on either radioimmunoassay or catalytic activity of the enzyme (Fig. 4). This indicates that the two analytical approaches performed essentially the same quantification.

### Endometrial 17-HSD

The cytosol fraction following 105,000 g centrifugation after homogenization and detergent treatment of the human endometrial tissue was used in RIA. This fraction displayed cross-reactivity with the antibody raised against human placental 17-HSD. The mean concentration of the cross-reacting substance in the endometrium was 14.1 μg/g protein (range

2.0–62.3) in 21 specimens taken on different days in the cycle. The concentrations of the substance during the follicular phase (days 4–12, n = 8) and luteal phase (days 16–22, n = 6) were 8.3 and 22.9 μg/g protein, respectively. When a radioimmunoassay for 17-HSD was carried out on serial dilutions of endometrial homogenate, the resultant binding curve showed excellent parallelism with those for the purified 17-HSD standards and dilutions of placental homogenate (Fig. 3). There was also a close and significant correlation (r = 0.722, P < 0.001, n = 21) between the endometrial 17-HSD activities and concentrations in the same specimen measured with the 17-HSD radioimmunoassay (Fig. 5). These are strong arguments for the identity of the ligands in the placental and endometrial preparations.

### Subcellular distribution

17-HSD was measured in subcellular fractions obtained by sequential centrifugation of normal human term placentae and endometrium. The
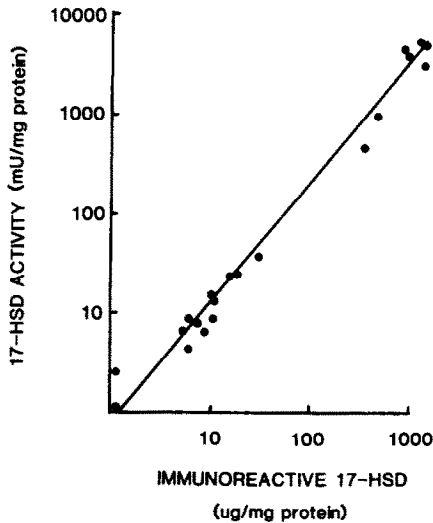
Fig. 4. Correlation between the catalytic activity of 17β-hydroxysteroid dehydrogenase and the immunoreactive enzyme concentration. The specimens analysed by the two techniques were from placental preparations at different stages of purification. The coefficient of correlation between the two values, r = 0.952, was highly significant (P < 0.001, n = 20).
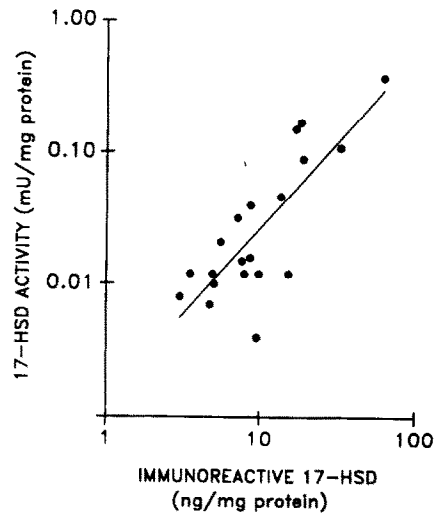


Fig. 5. Correlation between the catalytic activity of 17β-hydroxysteroid dehydrogenase and immunoreactive enzyme concentrations in 21 endometrium specimens. The coefficient of correlation between the two values, r = 0.722, was highly significant (P < 0.001, n = 21).

immunoreactivity in the endometrium (5 samples) was found to be distributed among all the subcellular fractions, the majority being detected in the cytosol (47%) and microsomal fractions (36%) and only a minor part in the mitochondrial fraction (17%). The catalytic activity in the endometrium (8 samples) was distributed mainly as previously described [25], the cytosol fraction containing 9% of the total activity and the microsomal and mitochondrial fractions 40 and 51%, respectively.

The subcellular distribution of immunoreactive enzyme protein in the placental tissue was similar to that of enzymatic activity, the majority of the immunoreactive protein being found in the cytosol fraction (95%) and the rest in mitochondrial fraction (5%). The concentration of 17-HSD in the microsomal fraction was negligible. The catalytic activity in the placenta was distributed in a similar manner to that described previously [25], the cytosol fraction containing 90% of the total activity and both the mitochondrial and microsomal fractions 5% each.

## 17-HSD in the pathological endometrium

The 17-HSD activities and concentrations measured in pathological endometrium samples are shown in Table 2. The activity and concentrations of immunoreactive 17-HSD in cases of endometrial adenocarcinoma were found to be as low as in the proliferative endometrium.

### DISCUSSION

Placental 17β-hydroxysteroid dehydrogenase has been purified and characterized in a number of laboratories, and its cDNA has also been cloned and sequenced recently in this laboratory using the antibody described here [26]. The nucleotide sequence of the cDNA and the deduced amino acid sequence [26] were later confirmed at another laboratory [27]. The enzyme consists of two identical subunits having molecular weights of 34000–37000 in different reports [see Ref. 28]. Our apparently homogeneous subunit of 17-HSD displayed a molecular weight of 37,800 in SDS–PAGE under denaturing conditions.

Table 2. Concentrations of the immunological and enzymatic activities of 17β-hydroxysteroid dehydrogenase in normal and pathological endometrium

| Tissue | Patient's age | RIA (μg/g protein) | Enzymatic act. (E₁ μmol/min/g) | Serum progesterone (nmol/l) |
|---|---|---|---|---|
| Normal endometrium | | | | |
|   Follicular phase (n = 8) | | 8.3 (4.9–15.2) | 0.011 (0.004–0.016) | |
|   Luteal phase (n = 6) | | 22.9 (5.5–63.2) | 0.125 (0.05–0.370) | |
| Endometrial adenocarcinoma | 40 | 2.3 | 0.009 | 1.1 |
| | 40 | 0.6 | 0.003 | 6.4 |
| | 52 | 2.2 | 0.005 | 0.7 |
| | 54 | 0.7 | 0.001 | — |
| | 55 | 6.9 | 0.11 | 2.7 |
| | 56 | 2.3 | 0.002 | 2.8 |
| Endometrial stromal sarcoma | 24 | 0.5 | 0.004 | 11.3 |

The antibody selected for further use had a high apparent binding affinity ($K_d = 3.7 \times 10^{-11}$ mol/l) and a relatively high titer, since a final dilution of $1:62,500$ for the antiserum could be used in the radioimmunoassay which was subsequently developed. The antibody apparently had a high specificity for 17-HSD, because only one reacting zone with the mobility of the subunit of 17-HSD appeared in immunoblotting analysis using placental homogenate preparation. Using this antibody and [$^{125}$I]iodo-17-HSD as the tracer, a radioimmunoassay was developed for placental 17-HSD, and the method was characterized by adequate recovery and precision data. Our method had a sensitivity approximately one order of magnitude higher than a previously published radioimmunoassay intended for the same purpose [29].

The sensitivity of our radioimmunoassay may explain the fact that human endometrial preparations were found to contain an immunoreactive substance in the assay. In a previous immunochemical study [29], the antibody raised against placental 17-HSD did not detect any immunoreactive material in the endometrium. The cross-reacting substance found here was indistinguishable from authentic 17-HSD in parallelism tests based on the radioimmunoassay. The concentrations measured by radioimmunoassay in the endometrial preparations showed a good correlation with the activities of 17-HSD measured in the same specimens. Use of the present antibody, following affinity purification, in order to study the immunohistochemistry of the endometrium displayed staining of the epithelial cells (research still in progress). In addition, a several-fold increase in concentrations was found during the luteal phase as compared with the follicular phase concentrations as evaluated by RIA or from the immunohistochemical appearance of the tissue sections. These data strongly suggest that the substance measured in the endometrial specimens was 17-HSD.

Measurements of 17-HSD using the present technique were also carried out on endometrial specimens from 6 patients with endometrial adenocarcinoma and one with endometrial sarcoma. Values were obtained which were similar to those seen in endometrial specimens during the follicular phase. Variations in the ratio of enzyme concentration to activity of the enzyme in pathological endometrium may be due to the low enzyme activities in these samples. Indeed, the values measured were close to the detection limit of activity assay. The investigations of pathological tissue samples are now being extended, as is the use of immunohistochemical techniques.

The differences in subcellular distribution between the enzymatic and immunoreactive activities in the endometrium cannot yet be explained definitively. They may be due to an apparent immunological non-identity between the mitochondrial enzyme protein and that found in the cytosolic and microsomal fractions, or they may reflect the possibility of insufficient solubilization of the mitochondrial enzyme under these conditions. The subcellular distributions of the enzymatic and immunological reactivities in the placental tissue did display an identical profile, however.

Different detergents were tried for solubilizing the bound enzyme, but only Zwittergent 312 could be used without loss of even immunological activity. The addition of this detergent did not have any effects on the performance of this RIA. Although it was difficult to establish the efficiency of solubilization due to lability of the enzyme during detergent treatment, the increases in immunologically measurable 17-HSD concentrations were 5–10-fold compared with the concentrations in the same homogenates without detergent treatment. In addition, when 17-HSD in the cytosol fraction was measured by RIA after detergent treatment, the concentrations were identical to the values measured in the $800\,g$ fraction. This indicates that enzyme was released from the bound fraction and became available for competitive immunoreaction.

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