THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 234, No. 10, October 1959
Printed in U.S.A.

Human Placental Estradiol-17β Dehydrogenase

II. KINETICS AND SUBSTRATE SPECIFICITIES*

LORNA J. LANGER,† JOYCE A. ALEXANDER, AND LEWIS L. ENGEL‡

From The John Collins Warren Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, and the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts

(Received for publication, June 3, 1959)

A diphosphopyridine nucleotide-linked estradiol-17β dehydrogenase has been partially purified from human term placenta by a process which removes the Δ^5-3β-hydroxysteroid dehydrogenase activity initially present (1). Talalay *et al.* (2, 3) with the use of estradiol-17β dehydrogenase purified by a somewhat different procedure, confirmed several previous observations on its characteristics; namely, the essential sulfhydryl groups, stabilization by estradiol, and almost constant initial reaction velocity from pH 6 to pH 9. The enzyme preparations used in these experiments, as well as those studied by Talalay *et al.* (2, 3), are also active in the presence of triphosphopyridine nucleotide. Hagerman *et al.* (4) have demonstrated that the di- and triphosphopyridine nucleotide-linked dehydrogenation of estradiol are catalyzed by different enzymes, and have succeeded in separating the two activities.

The reaction kinetics and substrate specificity of the DPN-specific estradiol-17β dehydrogenase have now been examined. This enzyme, in the presence of DPN or DPNH, has been found to possess high affinity and structural specificity for its substrates. From the results of these studies, some characteristics of the interactions of the enzyme with steroids could be deduced.

METHODS

The methods used were generally as already described (1). When supplies of a steroid were very limited, concentrations of less than 0.1 μmole per ml were sometimes used. The rates of oxidation or reduction of some steroids measured as DPNH formation or disappearance, were occasionally determined or checked in the Cary recording spectrophotometer. Reaction rates were also measured in an Aminco-Bowman spectrophotofluorometer equipped with a strip chart recorder, with the use of an activating wave length of 360 mμ and a fluorescence wave length of 450 mμ. When the purity and supply of a compound permitted, and its reactivity was sufficient, Michaelis constants and maximal reaction velocities were determined from plots according to Lineweaver and Burk (5). For these studies, the temperature of the reaction mixture was held at 37 ± 1° by means of thermospacers. Human serum albumin was omitted from these assay systems since it also binds steroids (6–9).

Whenever possible, the purity of the steroids studied was investigated chromatographically (1). Estrone appeared to be pure, whereas estradiol was contaminated with very small amounts of dihydroequilenin. Most other steroids used were found to contain little or no contaminating phenolic or ketonic material. Extracts of the reaction mixtures were also chromatographed (1), to provide a check on the spectrophotometric measurements of reactivity and a partial identification of the reaction products of each steroid studied.

RESULTS

Unreactive Positions

The following steroid substituents have been found not to be acted upon by estradiol-17β dehydrogenase: 3α-hydroxy, 3β-hydroxy, 3-keto, 7-keto, 11α-hydroxy, 11β-hydroxy, 16β-hydroxy, 17α-hydroxy, and 21-hydroxy. Estradiol-3,16α was dehydrogenated at about 1% of the rate with estradiol. Because of the very low 16α-hydroxysteroid dehydrogenation rate in all enzyme preparations, it was not possible to determine whether the same enzyme catalyzes 16α- and 17β-hydroxysteroid dehydrogenation. No conclusive results could be obtained by a study of the ratios of reaction rates with different preparations or under different experimental conditions.

Effects of Alterations in Steroid Structure

1. Ring A—The 19-carbon nonaromatic steroids, testosterone, 17β-hydroxy-5α-androstan-3-one, and 17β-hydroxy-5β-androstan-3-one were dehydrogenated at less than 1% of the rate with estradiol. 19-Nortestosterone was oxidized significantly more rapidly, but still at only about 2% of the rate with estradiol. The substrate specificity of estradiol-17β dehydrogenase thus differs substantially from that of the β enzyme from *Pseudomonas testosteroni* (10, 11), which also catalyzes the interconversion of estrone and estradiol (Table I).

The rates of hydrogenation of 4-androstene-3,17-dione, 19-nor-4-androstene-3,17-dione, 1,4-androstadiene-3,17-dione, 10ξ-hydroxy-1,4-estradiene-3,17-dione (12), 3α-hydroxy-5α-androstan-17-one, 3β-hydroxy-5α-androstan-17-one, 3β-hydroxy-5-androsten-17-one, and 3α-hydroxy-5β-androstan-17-one were again roughly 1% or less of the rate with estrone. Paper

* This is Publication No. 969 of the Cancer Commission of Harvard University. This work was supported by grants from the National Cancer Institute, United States Public Health Service, a grant from the American Cancer Society, Inc., and a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

† Predoctoral Fellow of the United States Public Health Service. A part of this work is taken from a thesis submitted in June 1957 to the Radcliffe Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

‡ Permanent Faculty Fellow of the American Cancer Society.

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TABLE I

*Relative rates of steroid dehydrogenation catalyzed by β enzyme and by estradiol-17β dehydrogenase**

Compound	β Enzyme†	Estradiol-17β dehydrogenase
	V_o or V_{max}	V
Estradiol-3,17β	13.3	100
17β-Hydroxy-4-androsten-3-one (testosterone)	100	<1
17β-Hydroxy-5α-androstan-3-one	90	<1
17β-Hydroxy-5β-androstan-3-one	84.7	<1
19-Nor-17β-hydroxy-4-androsten-3-one (19-nortestosterone)	80.0	2

* V_o, relative maximal reaction velocity at optimal substrate concentration; V_{max}, relative maximal reaction velocity from Lineweaver-Burk plots, where Michaelis-Menten kinetics are followed; V, relative reaction velocity at 0.1 mM steroid concentrations.

† Data taken from Talalay and Marcus (11).

TABLE II

Estrone or estradiol derivatives substituted at C-3 or altered in number or location of double bonds, or both

Compound	Reference†	S	V	K_m	S_o	V_{max} or V_o
		× 10^{-5} M		× 10^{-5} M	× 10^{-5} M	
Estrone 3-methyl ether	1				3.0	158
Estradiol 3-methyl ether	1			3.0		175
Estrone 3-oxyacetic acid ether		3.3	45			
6-Dehydroestrone 3-benzoate	14	10.0	0			
Equilin				1.3		165
Equilenin				2.0		155
DL-3-Deoxyequilenin	15				2.0‡	220
DL-6-Methoxy-3-deoxyequilenin	15				4.0‡	287
5,7,9-Estratriene-3β,-17β-diol	16				10.0	635
5,7,9-Estratrien-3β-ol-17-one	17	5.0	200			

* S, steroid substrate concentration; V, % of the reaction velocity with estrone or estradiol at concentration S, determined in the same experiment; K_m, Michaelis constant, calculated from Lineweaver-Burk plots, where Michaelis-Menten kinetics are followed; S_o, optimal substrate concentration, where inhibition occurred at higher concentrations; V_o, reaction velocity at concentration S_o, as % of the reaction velocity with the same concentration of estrone or estradiol, determined in the same experiment; V_{max}, maximal reaction velocity, as % of the maximal reaction velocity with estrone or estradiol, calculated from Lineweaver-Burk plots, where Michaelis-Menten kinetics are followed.

† These references refer to the syntheses or proofs of structure of the compounds used, or both.

‡ Concentration of the D enantiomorph.

chromatograms of extracts of assay mixtures indicated small conversions of the 17β-hydroxysteroids to the corresponding 17-ketosteroids, and of 4-androstene-3,17-dione and 19-nor-4-androstene-3,17-dione to the corresponding 17β-hydroxysteroids.

Dehydrogenation of testosterone by placental extracts has also been noted by Meyer (13). In the present experiments, the ratios of the rates of testosterone or 19-nortestosterone dehydrogenation to estradiol dehydrogenation remained approximately constant with different enzyme preparations at all stages of the purification procedure, and at different pH values. Talalay and Marcus (10, 11) observed inhibition by excess substrate of dehydrogenation of some neutral steroids with the β enzyme. With estradiol dehydrogenase and 19-nortestosterone as substrate, however, no such effect was observed.

Among aromatic steroids, the effect of various substitutions in ring A was also investigated. The phenolic hydroxyl group at carbon 3 was found not essential for reactivity. Methylation or replacement of the hydroxyl group by hydrogen yielded even more reactive substrates (Table II). The considerably bulkier benzoate group, however, completely inhibited hydrogenation of the carbonyl group at carbon 17, whereas the oxyacetic acid or acetyl group inhibited only partially (Tables II and III).

The introduction of substituents in ring A at positions other than C-3 generally depressed reactivity (Tables III and IV). For example, 1-methylestradiol was considerably less reactive than estradiol, and the 1,2-dimethyl derivatives even less reactive (Table IV). The *p*-quinones, 2,5(10)-estradiene-1,4,17-trione and 3-hydroxy-2,5(10)-estradiene-1,4,17-trione are exceptions to the general finding that only aromatic steroids possess high reactivity.

Methyl, nitro, or amino derivatives of estrone or estradiol at C-2 reacted at only about half the rate with the unsubstituted steroid (Table III). 2-Methoxyestrone, however, was almost as reactive as estrone; 2-fluoroestradiol was completely unreactive. The 4-nitro derivatives reacted much like 2-nitroestradiol, but 4-aminoestrone showed significantly higher reactivity. The presence of two nitro groups in ring A further depressed reactivity. 4-Fluoroestradiol, like 2-fluoroestradiol, was totally unreactive.

2. Ring B—Introduction of a double bond between carbons 6 and 7 or 7 and 8, or of an aromatic B ring, increased the re-

TABLE III

C-2 or C-4, or both, substituted derivatives of estrone or estradiol

Compound	Reference	S	V
		× 10^{-5} M	
2-Methylestradiol	18	3.3	53
2-Nitroestradiol		10.0	50
2-Nitroestrone*	19	3.3	43
2-Nitroestrone 3-oxyacetic acid ether	20	10.0	24
2-Aminoestradiol	21	10.0	62
2-Methoxyestrone	21	10.0	84
2-Fluoroestradiol	22	10.0	0
4-Nitroestradiol		10.0	47
4-Nitroestrone	19	3.3	67
4-Aminoestrone	21	10.0	98
4-Aminoestrone 3,4-diacetate		10.0	8
4-Fluoroestradiol	22	10.0	0
2,4-Dinitroestradiol		10.0	27
2,4-Dinitroestrone	19	3.3	19
2,4-Dinitroestrone 3-oxyacetic acid ether	20	10.0	13

* Contaminated with 2,4-dinitroestrone.

activity of the steroid (Tables II and IV). Most unexpected was the observation that 5,7,9-estratriene-3β,17β-diol, with an aromatic B ring and nonaromatic A ring, was dehydrogenated more than 6 times as rapidly as estradiol (Table II).

A carbonyl group at carbon 6 or 7 also increased reactivity of the steroid (Table V). In the 3-deoxyequilenin series, introduction of a 6-methoxy group again increased reactivity (Table II). A 7α-hydroxy group had little or no effect, but a 7β-hydroxy group depressed reactivity about 35% (Table V). The difference in effect of the α and β oriented hydroxyl groups is more pronounced at position 6. 6"α"-Hydroxyestradiol, 6α,7α-dihydroxyestradiol, and 6α,7α-dihydroxyestrone are at least as reactive as estradiol or estrone, but 6β,7α-dihydroxyestrone is hydrogenated only about one-fourth as rapidly as estrone.

3. Ring C—Although introduction of a carbonyl group at carbon 11 in equilenin markedly increased reactivity, both 11α- and 11β-hydroxyestradiol have substantially diminished reactivity (Table VI).

4. Ring D—Substituents at position 16 markedly influence reactivity at position 17. 16-Methylene-estrone, estriol, and 16-ketoestradiol are totally unreactive. 16-Ketoestrone, however, was hydrogenated at about one-fourth the rate with estrone (Table VII), yielding a product with the chromatographic mobility of 16-ketoestradiol. The apparent irreversibility of this reaction may indicate that the enzyme can more readily approach a carbonyl than a hydroxyl group at carbon 17, in the presence of a carbonyl group at carbon 16.

16-Epiestriol was dehydrogenated at about 1% of the rate with estradiol, yielding, after 3 hours of incubation, approximately equal quantities of two α-ketolic products, one of which had the chromatographic mobility of 16-ketoestradiol. The other, less mobile compound, may have been 16β-hydroxyestrone. In view of the observed base-catalyzed rearrangements of 16-hydroxy-17-ketosteroids to 17-hydroxy-16-ketosteroids (37–40), it is probable that 16β-hydroxyestrone was the principal product of the enzymatic reaction.

Table IV

C-1 substituted, C-1, 2 disubstituted, and C-1, 4 disubstituted derivatives of estrone or estradiol

Compound	Reference	S	V	K_m	V_{max}
		× 10^{-5} M		× 10^{-5} M	
1-Methylestradiol	23			1.5	40
1-Methyl-6-dehydroestradiol	23			0.85	80
1-Methyl-6-dehydroestrone	23	3.3	81		
1,2-Dimethylestradiol	18	3.3	18		
1,2-Dimethylestrone	18	3.3	6		
1,2-Dimethyl-6-dehydroestrone	18	3.3	50		
1,3-Dihydroxy-1,3,5(10)-estratrien-17-one (1-hydroxyestrone)	12	10.0	42		
1,4-Dihydroxy-1,3,5(10)-estratrien-17-one	12	10.0	16		
1,4-Diacetoxy-1,3,5(10)-estratrien-17-one	12	10.0	2		
4-Methyl-1,3,5(10)-estratriene-1,17β-diol	24			3.2	35
2,5(10)-Estradiene-1,4,17-trione	12	10.0	72		
3-Hydroxy-2,5(10)-estradiene-1,4,17-trione	12	10.0	15		

Table V

Ring B oxygenated derivatives of estrone or estradiol

Compound	Reference	S	V	K_m	V_{max}
		× 10^{-5} M		× 10^{-5} M	
6-Ketoestrone	25	3.3	130		
7-Ketoestrone	26			4.7	175
7β-Hydroxyestrone	27	10.0	65		
7α-Hydroxyestrone	27	10.0	90		
7α-Hydroxyestradiol	27	10.0	100		
6"α"-Hydroxyestradiol	28	10.0	157		
6β,7α-Dihydroxyestrone	27	10.0	23		
6α,7α-Dihydroxyestradiol	27	10.0	100		
6α,7α-Dihydroxyestrone	27	10.0	123		

Table VI

Ring C substituted derivatives of estrone or estradiol

Compound	Reference	S	V	K_m	V_{max}
		× 10^{-5} M		× 10^{-5} M	
11α-Hydroxyestradiol	29	3.3	29		
11β-Hydroxyestradiol	29	3.3	20		
11-Ketoequilenin acetate	30			1.2	525

Table VII

Ring D substituted derivatives of estrone or estradiol

Compound	Reference	S	V
		× 10^{-5} M	
Estriol		10.0	0
16-Epiestriol	31	10.0	1
16-Ketoestradiol	32	10.0	0
16-Ketoestrone	32	3.3	23
16-Methylene-estrone		10.0	0
Estradiol-3,16β	33	10.0	0
Estradiol-3,16α	34	10.0	1
Estradiol-3,17α		10.0	0
Dihydroequilenin-3,17α	35	10.0	0
Dihydroequilin-3,17α	35	10.0	0
18-Nor-D-homoestrone 3-methyl ether	36	3.3	30

5. Stereoisomers—The reactivities of some steroids differing from the natural estrogens in configuration at the B:C or C:D ring fusions, or both, were also measured. Most of these synthetic compounds were available only as racemates. Interpretation of rate data in such situations is complicated by the possibilities of inhibition by one enantiomorph of the reaction of the other, or of different rates of reaction of the two enantiomorphs. Little or no hydrogenation of the following steroids with *cis* C:D ring fusions was detectable:[1] L-8-iso-9-iso-14-isoestrone 3-methyl ether (lumiestrone methyl ether), DL-iso-equilenin 3-methyl ether, DL-3-deoxyisoequilenin, or DL-6-methoxy-3-

[1] The assignments of configuration at the ring fusions are based primarily on the conclusions of Johnson *et al.* (41). In this paper carbon 13 is considered the reference position in naming the isomers.

TABLE VIII

Estrone or estradiol isomers

Compound	Anner and Miescher name*	Johnson name†	S‡	V
			$\times 10^{-5}$ M	
DL-8-Iso-9-isoestrone	Estrone e		5.0	48
D-8-Isoestradiol			10.0	260
L-8-Iso-9-iso-14-isoestrone 3-methyl ether (lumiestrone 3-methyl ether)			10.0	0
DL-14-Isoestrone	Estrone a		3.3	<1
DL-9-Isoestrone	Estrone d	Estrone α_2	3.3	<1
DL-8-Iso-14-isoestrone		Estrone α_1	3.3	<1

* Anner and Miescher (42, 43).

† Johnson *et al.* (36).

‡ In the case of racemates, *S* is the concentration of the D enantiomorph.

TABLE IX

Natural estrogens and their racemates

Compound	Reference	S	V
		$\times 10^{-5}$ M	
D-Estrone		2.5	100
DL-Estrone	42	5.0	74
D-Equilenin		10.0	160
DL-Equilenin	15	20.0	139

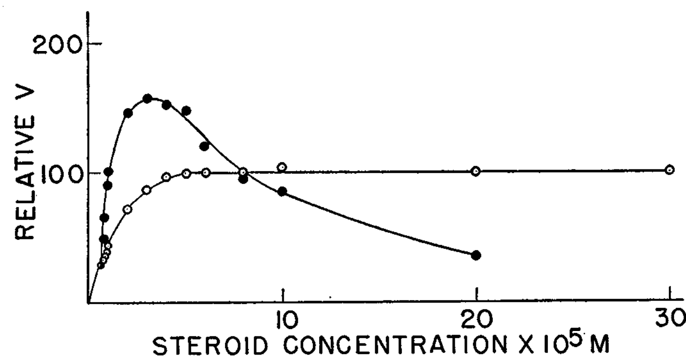

FIG. 1. Relation between the initial rate of hydrogenation and the substrate concentration. ⊙——⊙, estrone; ●——●, estrone 3-methyl ether. The ordinate is the relative initial reaction velocity. V_{max} for estrone = 100.

deoxyisoequilenin. DL-14-Isoestrone and DL-8-iso-14-isoestrone were reduced very slowly (Table VIII). DL-9-Isoestrone also exhibited very low reactivity, but the D-8-isoestradiol obtained on hydrogenation of equilenin (16) reacted even more rapidly than estradiol, and DL-8-iso-9-isoestrone also showed substantial reactivity.

A comparison of the initial rates of dehydrogenation for D-estrone and D-equilenin to the rates for their racemates reveals that the L-enantiomorph significantly inhibits estradiol-17β dehydrogenase (Table IX), whereas the stoichiometry of the reaction with DL-estrone, DL-8-iso-9-isoestrone, and DL-equilenin indicates that only the natural D form is attacked. Estradiol-17β dehydrogenase may therefore be used for the resolution of racemic modifications of suitable steroids. A Saccharomyces has been

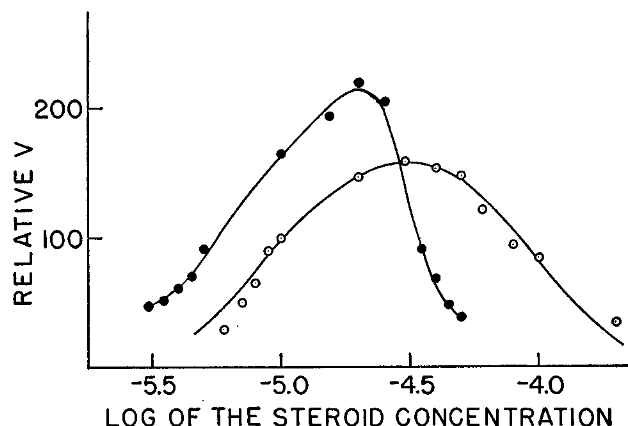

FIG. 2. Relation between the initial rate of hydrogenation and the log of the substrate concentration. ⊙——⊙, estrone 3-methyl ether; ●——●, DL-3-deoxyequilenin (the concentration of the D enantiomorph is used).

The ordinate is the relative initial reaction velocity. V_{max} for estrone = 100.

similarly employed to resolve racemic estrone into the readily separable L-estrone and D-estradiol (44).

Kinetics

The reaction velocities of most of the aromatic steroids so studied followed Michaelis-Menten kinetics within the concentration range investigated (Fig. 1). The Michaelis constants determined are all quite low (Tables II, III, V, and VI), indicating high affinity of the enzyme for these steroids. The average Michaelis constant for both estrone and estradiol at 37° is 2.2×10^{-5} M. At about 23°, determined in the Cary instrument, the even lower values for estradiol of 3.0, 3.4, and 3.5×10^{-6} M were obtained.[2] The Michaelis constant for estradiol was evaluated as 4.5×10^{-6} M, with the use of the Aminco-Bowman spectrophotofluorometer at this same temperature.

Estrone 3-methyl ether, 5,7,9-estratriene-3β,17β-diol, DL-3-deoxyequilenin, and DL-6-methoxy-3-deoxyequilenin were found to inhibit estradiol-17β dehydrogenase at high substrate concentrations (Fig. 1). Similar inhibitions have been observed with some neutral, but not phenolic, steroids and the β-enzyme from *P. testosteroni* (10, 11), and have been attributed to formation of inactive complexes involving two molecules of substrate and one of enzyme (45, 46). Such complex formation may account for the results obtained with estrone 3-methyl ether and 5,7,9-estratriene-3β,17β-diol. Plots of the initial reaction rates against the logarithm of the substrate concentrations for these two steroids yield fairly symmetrical bell-shaped curves (Fig. 2), as predicted by the theory of such inactive complex formation (46). The even more marked inhibition produced by DL-3-deoxyequilenin (Fig. 2) and DL-6-methoxy-3-deoxyequilenin may reflect a combination of nonactivated complex formation and other factors, such as inhibition by enantiomorphs (see above).

DISCUSSION

The DPN-specific estradiol-17β dehydrogenase shows absolute stereochemical specificity for the 17β-hydroxy group. 17α-Hy-

[2] The authors are indebted to Mr. Dale Cowan for these determinations.

droxysteroids are not dehydrogenated, and only the 17β-hydroxy compound is formed on hydrogenation of the 17-ketosteroids.

Although some nonaromatic steroids are very slowly attacked, an aromatic A or B ring, or both, appears generally to be an essential characteristic of highly reactive substrates. The low reactivity of 19-nortestosterone indicates that the angular methyl group (C-19) is responsible for only a small portion of the depression of reactivity with nonaromatic steroids.

The phenolic hydroxyl group is not required for steroid reactivity, and ionization of this group has essentially no effect on the reaction velocity (1). The inhibition of estradiol-17β dehydrogenase by neutral aromatic steroids at high concentrations suggests that the phenolic hydroxyl group may influence orientation of the steroid on the enzyme surface. In the absence of a phenolic hydroxyl group to provide a specific point of interaction, some steroids may be able to approach the enzyme in more than one way, leading to non-activated complex formation. The hydrogenation of 18-nor-D-homoestrone 3-methyl ether (Table VII) demonstrates that the angular methyl group (C-18) is not required for reactivity, and also that expanding the D ring to a 6-membered ring does not completely destroy reactivity.

There appears to be a correlation between the degree of planarity of the steroid and its reactivity and affinity for estradiol-17β dehydrogenase. The planarity of the aromatic ring may be an important factor contributing to the much greater reactivity of aromatic steroids. The only significantly reactive nonaromatic steroids, 2,5(10)-estradien-1,4,17-trione and 3-hydroxy-2,5(10)-estradien-1,4,17-trione, possess a planar *p*-quinonoid A ring. Additional double bonds in the B ring increase planarity. Examination of models suggests that carbonyl groups at carbons 6, 7, or 11 further increase planarity, and all also increase the reactivity of the steroid. The more unsaturated steroids exhibit greater affinity for the enzyme, whereas the influence of the carbonyl groups at different positions is not consistent.

The high affinity for substrates, the steric specificity, and the often pronounced effect of distant modifications on steroid reactivity indicate multiple sites of interaction with the enzyme over the whole steroid surface. Cumulative forces of the van der Waals-London dispersion type are presumably responsible for the interaction of the nonpolar steroid groups with the enzyme surface. The interaction of the additional methyl groups of 1-methylestradiol and 1-methyl-6-dehydroestradiol with the enzyme (Table IV) is thus probably responsible for the stronger binding of these steroids.

Inhibition of reactivity by an α-oriented angular methyl group (C-18), and more complete inhibition by a 16α-hydroxyl than by a 16β-hydroxyl (estriol *versus* 16-epiestriol, Table VII) suggest that it is the rear or α surface of steroids that binds to estradiol-17β dehydrogenase.

Comparison of the reactivities of several estrone diastereomers emphasizes the importance of the contour of the α surface for substrate reactivity. In the D series, it is apparent from molecular models that the α surfaces of 14-isoestrone, 8-iso-14-isoestrone, and 9-isoestrone, none of which possess significant reactivity, are markedly distorted from the slightly convex shape of the α surface of natural estrone. These molecules possess concave α surfaces, so that they fold back upon themselves to varying degrees. The α surfaces of the significantly more reactive 8-isoestradiol and 8-iso-9-isoestrone are instead somewhat more convex than the α surface of estradiol or estrone.

It is difficult, however, to reconcile this picture of interaction of the enzyme with the α surface of the steroid with the observation that the 6β-, 7β-, and 11β-hydroxy groups all depress reactivity of the steroid substrate. The greater depression exhibited by the axial 6β- and 11β-hydroxy than by the equatorial 7β-hydroxy strongly suggests interaction also of the β surface of the steroid with the enzyme.

Multiple sites of interaction, involving the whole steroid, have also been suggested by several studies on the nature of steroid interactions with other substances. Distant functional groups have been observed to induce marked alterations in the reactivity of steroids in several chemical reactions. It has been emphasized that the entire steroid molecule should thus be considered to be the reactive entity (47). Multiple sites for steroid interactions with serum albumin have been suggested by the work of Schellman *et al.* (48) and of Westphal and Ashley (9). Binding over the whole α surface of steroids to the β enzyme was postulated by Talalay and Marcus (10, 11). Purines and purine derivatives have been demonstrated by Munck *et al.* (49) also to interact with a variety of steroids. In this study, too, the α surface of the C and D rings, and possibly of part of the B ring, was thought to bind to the purine.

Villee and Gordon (50–52) have demonstrated that human placenta also possesses an enzyme system capable of stimulating α-ketoglutarate production in the presence of minute quantities of estrone or estradiol. The studies of Talalay *et al.* (2, 3) revealed that the estrogen-sensitive reaction involved pyridine nucleotide transhydrogenation. Evidence for the nonidentity of this transhydrogenase system and the estradiol-17β dehydrogenases of human placenta has been reported by Hagerman *et al.* (4).

The DPN-specific estradiol-17β dehydrogenase and the steroid-sensitive transhydrogenase, however, exhibit remarkably similar steroid specificities. Villee and Gordon (53) reported that equilin and equilenin were as effective as estrone or estradiol in their steroid-sensitive system, and more recently 6-ketoestradiol and estriol were added to the list of activators (54). In more extensive specificity studies, Hollander *et al.* (55) reported that estrone and estradiol 3-methyl ethers, 3-deoxy-equilenin, 6- or 7-hydroxylated estrone, or both, or estradiol derivatives, 7-ketoestrone, and 11β-hydroxyestradiol, among others, were effective stimulators of α-ketoglutarate production. Among estrone isomers, only DL-8-isoestrone was found to possess significant stimulatory activity (56).

The weak stimulatory activities of estriol (54), 17α-ethynylestradiol, and 16-ketoestradiol, and lack of activity of 16-ketoestrone (55) represent the only reported exceptions to the correlation of specificities of the two enzyme systems. This marked similarity of steroid specificities suggests a corresponding similarity of the reactive sites of the two enzymes.

SUMMARY

The effects of substitution at various positions and of stereoisomerism of the steroid substrates upon the kinetics of the reactions catalyzed by the diphosphopyridine nucleotide-specific human placental estradiol-17β dehydrogenase have been investigated. These studies have shown that (*a*) this enzyme preparation has absolute steric specificity for the 17β-hydroxy group, (*b*) the steroid substrate must possess a highly planar A or B ring, or both, for significant reactivity, (*c*) the enzyme interacts with the entire steroid surface. The steroid specificities

of this enzyme and of the steroid-activated pyridine nucleotide transhydrogenase of human placenta have been compared.

Acknowledgments—The authors wish to express their gratitude to the following sources for steroids used in these experiments: Dr. W. G. Dauben, University of California; Dr. M. N. Huffman, Oklahoma Medical Research Foundation; Dr. W. S. Johnson, University of Wisconsin; Dr. N. L. McNiven, Worcester Foundation for Experimental Biology; Dr. G. C. Mueller, McArdle Memorial Laboratory, University of Wisconsin Medical School; Dr. G. Pincus, Worcester Foundation for Experimental Biology; Dr. E. Schwenck, Worcester Foundation for Experimental Biology; Ayerst, McKenna, and Harrison, Ltd.; Ciba Pharmaceutical Products, Inc.; Ciba Ltd., Basel; The Squibb Institute for Medical Research; Syntex, S. A.; and The Upjohn Company.

Dr. Anne Stoffyn of this laboratory prepared the following steroids used in these studies: 2-nitroestradiol, 2-nitroestrone, 2-nitroestrone 3-oxyacetic acid ether, 2-aminoestradiol, 2-methoxyestrone, 4-nitroestradiol, 4-nitroestrone, 4-aminoestrone, 4-aminoestrone 3,4-diacetate, 2,4-dinitroestradiol, 2,4-dinitroestrone, and 2,4-dinitroestrone 3-oxyacetic acid ether.

The authors are indebted to Miss Mary Ellen Collins for skillful assistance in the operation of the Cary recording spectrophotometer and Aminco-Bowman spectrophotofluorometer.

The generous cooperation of C. A. Villee, Boston Lying-In Hospital, in making available placental tissue is gratefully acknowledged.

REFERENCES

1. Langer, L. J., and Engel, L. L., *J. Biol. Chem.*, **233**, 583 (1958).
2. Talalay, P., and Williams-Ashman, H. G., *Proc. Natl. Acad. Sci. U. S.*, **44**, 15 (1958).
3. Talalay, P., Hurlock, B., and Williams-Ashman, H. G., *Proc. Natl. Acad. Sci. U. S.*, **44**, 862 (1958).
4. Hagerman, D. D., Villee, C. A., and Wellington, F. M., *J. Biol. Chem.*, **234**, 2031 (1959).
5. Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.*, **56**, 658 (1934).
6. Eik-Nes, K., Schellman, J. A., Lumry, R., and Samuels, L. T., *J. Biol. Chem.*, **206**, 411 (1954).
7. Rothchild, I., *Endocrinology*, **50**, 583 (1952).
8. Westphal, U., *Arch. Biochem. Biophys.*, **66**, 71 (1957).
9. Westphal, U., and Ashley, B. D., *Federation Proc.*, **16**, 269 (1957).
10. Marcus, P. I., and Talalay, P., *Proc. Roy. Soc. London, B*, **144**, 116 (1955).
11. Talalay, P., and Marcus, P. I., *J. Biol. Chem.*, **218**, 675 (1956).
12. Gold, A. M., and Schwenck, E., *J. Am. Chem. Soc.*, **80**, 5683 (1958).
13. Meyer, A. S., *Biochim. et Biophys. Acta*, **17**, 441 (1955).
14. Pearlman, W. H., and Wintersteiner, O., *J. Biol. Chem.*, **132**, 605 (1940).
15. Johnson, W. S., Petersen, J. W., and Gutsche, C. D., *J. Am. Chem. Soc.*, **69**, 2942 (1947).
16. Dauben, W., and Ahramjian, L., *J. Am. Chem. Soc.*, **78**, 633 (1956).
17. Heard, R. D. H., and Hoffman, M. M., *J. Biol. Chem.*, **138**, 651 (1941).
18. Iriarte, J., and Ringold, H. J., *Tetrahedron*, **3**, 28 (1958).
19. Werbin, H., and Holoway, C., *J. Biol. Chem.*, **223**, 651 (1956).
20. Ercoli, A., and Mamoli, L., *Gazz. chim. ital.*, **68**, 142 (1958).
21. Kraychy, S., and Gallagher, T. F., *J. Biol. Chem.*, **229**, 519 (1957).
22. Zillig, W., and Mueller, G. C., *Federation Proc.*, **15**, 503 (1956).
23. Djerassi, C., Rosenkranz, G., Romo, J., Pataki, J., and Kaufmann, S., *J. Am. Chem. Soc.*, **72**, 4540 (1950).
24. Woodward, R. B., and Singh, T., *J. Am. Chem. Soc.*, **72**, 494 (1950).
25. Longwell, B., and Wintersteiner, O., *J. Biol. Chem.*, **133**, 219 (1940).
26. Pearlman, W. H., and Wintersteiner, O., *J. Biol. Chem.*, **130**, 35 (1939).
27. Iriarte, J., Ringold, H. J., and Djerassi, C., *J. Am. Chem. Soc.*, **80**, 6105 (1958).
28. Wintersteiner, O., and Moore, M., *J. Am. Chem. Soc.*, **81**, 422 (1959).
29. Magerlein, B. J., and Hogg, J. A., *J. Am. Chem. Soc.*, **80**, 2220 (1958).
30. McNiven, N. L., *J. Am. Chem. Soc.*, **76**, 1725 (1954).
31. Huffman, M. N., and Darby, H. H., *J. Am. Chem. Soc.*, **66**, 150 (1944).
32. Huffman, M. N., and Lott, M. H., *J. Biol. Chem.*, **172**, 325 (1948).
33. Huffman, M. N., and Lott, M. H., *J. Biol. Chem.*, **213**, 343 (1955).
34. Huffman, M. N., and Lott, M. H., *J. Biol. Chem.*, **215**, 627 (1955).
35. Shoppee, C. W., *Chem. & Ind. London*, 810 (1950).
36. Johnson, W. S., Banerjee, D. K., Schneider, W. P., Gutsche, C. D., Shelberg, W. E., and Chinn, L. L., *J. Am. Chem. Soc.*, **74**, 2832 (1952).
37. Leeds, N. S., Fukushima, D. K., and Gallagher, T. F., *J. Am. Chem. Soc.*, **76**, 2943 (1954).
38. Cooley, G., Ellis, B., Hartley, F., and Petrow, V., *J. Chem. Soc.*, 4373 (1955).
39. Marrian, G. F., Watson, E. J. D., and Panattoni, M., *Biochem. J.*, **65**, 12 (1957).
40. Marrian, G. F., Loke, K. H., Watson, E. J. D., and Panattoni, M., *Biochem. J.*, **66**, 60 (1957).
41. Johnson, W. S., David, I. A., Dehm, H. C., Highet, R. J., Warnhoff, E. W., Wood, W. D., and Jones, E. T., *J. Am. Chem. Soc.*, **80**, 661 (1958).
42. Anner, G., and Miescher, K., *Helv. Chim. Acta*, **32**, 1957 (1949).
43. Anner, G., and Miescher, K., *Helv. Chim. Acta*, **33**, 1379 (1950).
44. Vischer, E., Schmidlin, J., and Wettstein, A., *Experientia*, **12**, 50 (1956).
45. Alberty, R. A., *Advances in Enzymol.*, **17**, 1 (1956).
46. Friedenwald, J. S., and Maengwyn-Davies, G. K., in W. D. McElroy and B. Glass (Editors), *The mechanism of enzyme action*, Johns Hopkins Press, Baltimore, 1954.
47. Engel, L. L., in S. Graff (Editor), *Essays in biochemistry*, John Wiley and Sons, Inc., New York, 1956, p. 85.
48. Schellman, J. A., Lumry, R., and Samuels, L. T., *J. Am. Chem. Soc.*, **76**, 2808 (1954).
49. Munck, A., Scott, J. F., and Engel, L. L., *Biochim. et Biophys. Acta*, **26**, 397 (1957).
50. Villee, C. A., *J. Biol. Chem.*, **215**, 171 (1955).
51. Gordon, E. E., and Villee, C. A., *J. Biol. Chem.*, **216**, 215 (1955).
52. Villee, C. A., and Gordon, E. E., *J. Biol. Chem.*, **216**, 203 (1955).
53. Villee, C. A., and Gordon, E. E., *Bull. soc. chim. Belges*, **65**, 186 (1956).
54. Villee, C. A., and Hagerman, D. D., *J. Biol. Chem.*, **233**, 42 (1958).
55. Hollander, V. P., Nolan, H., and Hollander, N., *J. Biol. Chem.*, **233**, 580 (1958).
56. Hollander, V. P., Hollander, N., and Brown, J. D., *Endocrinology*, **64**, 621 (1959).