The Comparative Specificity of Three Oestradiol-Binding Proteins

RAT α -FOETOPROTEIN, RAT LIVER 17 β -HYDROXY STEROID DEHYDROGENASE AND ANTI-(OESTRADIOL-6-CARBOXYMETHYLOXIME-BOVINE SERUM ALBUMIN) ANTISERUM

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1. The specificity of three oestradiol-binding proteins was studied. Two of these proteins are naturally occurring (rat α -foetoprotein and rat liver microsomal 17 β -hydroxy steroid dehydrogenase) and the third is an artificially induced model, anti-(oestradiol-6-carboxymethyloxime-bovine serum albumin) γ -globulins. 2. A specific binding procedure for each protein model permitted a determination of its affinity for oestradiol and for 30 other steroids. 3. The results obtained have brought to light the different areas of the steroid molecule that are important for its recognition by each of the three proteins. The two naturally occurring proteins (α -foetoprotein and 17 β -hydroxy steroid dehydrogenase) recognize the edge of the steroid defined by C-4, C-6, C-8 and C-15. On the other hand, the γ -globulins recognize the opposite edge, i.e. that defined by C-2, C-10, C-11 and C-17. 4. Diethylstilboestrol, whose structure is analogous to that of a steroid, is only recognized by the two naturally occurring proteins.

In the course of their metabolism, steroids interact with different types of proteins. These proteins may either transport the steroids, participate in their transformation or act as cellular receptors. Numerous studies have shown the importance of these different interactions (Westphal, 1971; King & Mainwaring, 1974*b*). In these studies some authors have attempted to show the existence of a specific steroid-binding protein in serum or in a tissue and to determine its binding parameters (Jensen *et al.*, 1967; Savu *et al.*, 1973), others have compared the affinities of the binding protein for a series of steroids (Mulder *et al.*, 1972; de Lauzon *et al.*, 1973; Goertz *et al.*, 1974).

It was decided, with regard to oestradiol-specific proteins, to investigate three points: first, to identify the positions of attachment of the steroid to the protein, secondly, to discover the nature or the orientation of the functional groups implicated in such binding, and, finally, to determine certain factors of steric hindrance.

In this study, we made a comparison of the positions of attachment of oestradiol to three protein models, two naturally occurring and one artificially induced, either by measurements of the competition between each steroid and oestradiol (17 β -hydroxy steroid dehydrogenase, γ -globulins) or by direct measurement of affinity (α -foetoprotein). An idea of the topography of the binding site of each of the protein models may be deduced from this study.

For this work, 30 steroids have been used, including oestrogens, androgens, progestins and glucocorticosteroids.

Materials and Methods

Proteins

The anti-oestradiol immune serum was obtained by immunization of rabbits against oestradiol-6-*O*-carboxymethyloxime coupled with bovine serum albumin (weekly subcutaneous injections for 20 weeks with 1 mg of the antigen emulsified in 0.1 ml of saline and 0.1 ml of complete Freund's adjuvant) (Dray *et al.*, 1971). The α -foetoprotein was purified by affinity chromatography (Cittanova *et al.*, 1974*a*). The 17 β -hydroxy steroid dehydrogenase was prepared from rat liver microsomal fractions: the fractionation was effected at 4°C in 10 mM-sodium phosphate buffer, pH 7.4, containing 0.25 M-saccharose, by differential ultracentrifugation (Ch. Laurent, S. de Lauzon, N. Christeff, N. Cittanova & M.-F. Jayle, unpublished work). Some 40% of the enzymic activity was found in the microsomal fraction (105 000 g pellet) and 16% in the other fractions (mitochondria + cytosol). Mitochondrial contamination of the microsomal fraction was less than 3%. The specific activity of the microsomal enzyme was 5.5 munits/mg (1 munit corresponds to the transformation of 1 nmol of oestradiol/min per mg of protein at 37°C under conditions of saturating oestradiol and NAD⁺ concentrations and at initial velocity). The proteins were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Antibody cross-reaction test

To measure the competition of the different steroids with respect to oestradiol, we used the 'CR 1 ng' test

developed in this laboratory (de Lauzon *et al.*, 1973). The degree of cross-reaction CR 1ng is given by the equation:

$$\text{CR 1ng} = \frac{X}{Y} \times 100$$

where X is the percentage displacement of radioactive tracer (10pg of [2,4,6,7-³H]oestradiol, 100Ci/mmol) by 1ng of competing steroid and Y is the percentage displacement of this same tracer by 1ng of oestradiol.

α-Foetoprotein fluorescence test

Cittanova *et al.* (1974b) showed that rat *α*-foetoprotein bound 8-anilidonaphthalene-1-sulphonate (stoichiometry: 0.5mol of 8-anilidonaphthalene-1-sulphonate/mol of *α*-foetoprotein, $K_a = 2 \times 10^6 \text{ M}^{-1}$). The interfering interaction of oestradiol or of oestrone with *α*-foetoprotein is manifested by a diminution in the fluorescence of the 8-anilidonaphthalene-1-sulphonate bound to the protein. Cittanova *et al.* (1974b) also showed that competition exists between 8-anilidonaphthalene-1-sulphonate and the steroid for the same site.

Fluorescence experiments were made in a final volume of 1ml of 0.1 M-borate buffer, pH 8.7, containing 1–5 μM -*α*-foetoprotein (molecular weight 70000) and 0.2–1.0 μM -8-anilidonaphthalene-1-sulphonate, by use of an absolute and differential FICA 55 Spectrofluorimeter. The excitation maximum is at 370nm and the emission maximum is at 465nm.

The quenching of 8-anilidonaphthalene-1-sulphonate fluorescence obtained by the addition of 1 μg of oestradiol (20% extinction) is considered as 100% interfering interaction (Y). To determine the interfering interaction of each steroid, the quenching of the 8-anilidonaphthalene-1-sulphonate fluorescence was measured in the presence of 1 μg of that steroid (X). The degree of interfering interaction is given by:

$$\frac{Y-X}{Y} \times 100$$

17β-Hydroxy steroid dehydrogenase assay

This assay was done at 37°C in 10mM-sodium phosphate buffer, pH 7.4, with EDTA (2mM) and gelatin (1g/litre). The incubation medium, final vol. 0.25ml, contained: 0.88 μM -oestradiol, 60000 d.p.m. of [2,4,6,7-³H]oestradiol (specific radioactivity 100Ci/mmol), 75 μM -NAD⁺ and 50 μl of the microsomal suspension (44 μg of protein per tube).

The reaction was stopped after 6min (within initial velocity range) by precipitating the proteins with 2ml of a cyclohexane-ethyl acetate (1:1, v/v) mixture, which permitted a simultaneous extraction of the steroids. The radioactive oestrone formed was

separated from the other steroids on a Sephadex LH-20 microcolumn [1ml of gel was equilibrated in benzene-methanol (19:1, v/v), the steroid extract was applied in 0.2ml of the same solvent and the oestrone eluted with 2.3ml of solvent; the oestradiol peak did not start to appear until a further 1ml of solvent had been passed through the column]. The radioactivity was counted in Bray's (1960) scintillator [1 litre of dioxan contained naphthalene (100g), 2,5-diphenyl-oxazole (7.5g) and 1,4-bis-(5-phenyloxazol-2-yl)-benzene (300mg)] with an Inter technique SL 30 liquid-scintillation counter. In the absence of any interfering steroid, the percentage of oestrone formed (Y) was considered as 100% for the reaction. The interference by a given steroid was measured in the conditions described above but with the presence of 100, 500 and 1000ng of that steroid in the reaction medium. If the quantity of oestrone formed under these conditions was X, the percentage interference was defined as:

$$\frac{Y-X}{Y} \times 100$$

Results and Discussion

Table 1 shows the degrees of interference of the 30steroids studied with respect to oestradiol for the three protein models. The numbers given for the enzyme correspond to the inhibition obtained with 100ng of each steroid, those obtained with 500 and 1000ng of steroid being identical except for four steroids, which are discussed below.

A classification of these steroids according to the structural modifications in the A, B, C or D rings allows the identification of the key positions in the steroid molecule with respect to each of the three proteins (Table 2). Fig. 1 illustrates the structure of the basic nuclei submitted to modifications. In this classification we have attempted to show up successive modifications at a single position in the structure of the steroids. For this, we have analysed each pair of steroids between which there is only one point of difference in their structure, indicating the degree of interference for these two steroids. The number of + or – signs alongside these numbers allows an appreciation of the relative decrease or increase in interaction due to this modification. We have thus been able to establish the determinant regions of the steroid molecule for its recognition by one or other of the three proteins.

Antibody

The factors intervening in the interaction of steroids with the anti-oestradiol γ -globulins are, in decreasing order of importance: (i) the integrity of the aromatic A ring; (ii) the presence of a single function at C-17, with a β -orientation; (iii) the absence of hindrance on

Table 1. Degree of competition of 30 steroids with respect to oestradiol for α -foetoprotein, 17 β -hydroxy steroid dehydrogenase (enzyme) and anti-(oestradiol-6-carboxymethylloxime-bovine serum albumin) γ -globulins (γ -globulins)

For details, see the text.

Steroids	Competition (%)		
	α -Foetoprotein	Enzyme	γ -Globulins
Oestradiol [1,3,5(10)-oestratriene-3,17 β -diol]	100	100	100
6-Oxo-oestradiol [3,17 β -dihydroxy-1,3,5(10)-oestratrien-6-one]	70	25.7	109
7 α -Hydroxy-oestradiol [1,3,5(10)-oestratriene-3,7 α ,17 β -triol]	20	27.4	54.3
Oestrone [3-hydroxy-1,3,5(10)-oestratrien-17-one]	90	0	46.3
6-Oxo-oestriol [3,16 α -17 β -trihydroxy-1,3,5(10)-oestratrien-6-one]	—	19.0	41.7
6 α -Hydroxyoestrone [3,6 α -dihydroxy-1,3,5(10)-oestratrien-17-one]	105	16.2	36.2
Oestriol [1,3,5(10)-oestratriene-3,16 α ,17 β -triol]	42	3.8	31.2
Oestratrien-17-one [1,3,5(10)-oestratrien-17-one]	95.0	4.9	25.1
Nortestosterone [17 β -hydroxy-19-nor-4-androsten-3-one]	65.0	55.0	21.4
15 α -Hydroxyoestriol [1,3,5(10)-oestratriene-3,15 α ,16 α ,17 β -tetrol]	0	18.5	17.6
Oestradiol-17 α [1,3,5(10)-oestratriene-3,17 α -diol]	70.0	9.4	16.1
Oestradiol-16 α [1,3,5(10)-oestratriene-3,16 α -diol]	—	5.4	15.2
17-Desoxo-oestrone [1,3,5(10)-oestratrien-3-ol]	125	21.8	14.2
16 α -Hydroxyoestrone [3,16 α -dihydroxy-1,3,5(10)-oestratrien-17-one]	35.0	17.7	11.6
2-Hydroxyoestrone [2,3-dihydroxy-1,3,5(10)-oestratrien-17-one]	50.0	14.1	8.8
17 α -Ethinyloestradiol [17 α -ethinyl-1,3,5(10)-oestratrien-3,17 β -diol]	70.0	21.9	7.9
Oestrone-16 [3-hydroxy-1,3,5(10)-oestratrien-16-one]	90.0	13.8	6.5
1,3,5(10)-Oestratriene	110	9.7	5.3
Testosterone [17 β -hydroxy-4-androsten-3-one]	0	49.6	4.2
16-Oxo-oestrone [3-hydroxy-1,3,5(10)-oestratriene-16,17-dione]	15	15.8	4.0
Cortol [5 β -pregnane-3 α ,11 β ,17 α ,20 α ,21-pentol]	5	23.9	3.8
Dehydroepiandrosterone [3 β -hydroxy-5-androsten-17-one]	0	16.3	3.6
Cholesterol [5-cholesten-3 β -ol]	0	36.8	3.5
Androsterone [3 α -hydroxy-5 α -androstan-17-one]	35	28.6	2.3
11 β -Hydroxyoestrone [3,11 β -dihydroxy-1,3,5(10)-oestratrien-17-one]	50	18.8	1.0
Pregnanetriol [5 β -pregnane-3 α ,17 α ,20 α -triol]	0	44.4	0.7
Diethylstilboestrol [<i>trans</i> -3,4-bis-(<i>p</i> -hydroxyphenyl)-3-hexene]	80	32.5	0
Corticosterone [11 β ,21-dihydroxy-4-pregnene-3,20-dione]	15	29.5	0
Cortisol [11 β ,17 α ,21-trihydroxy-4-pregnene-3,20-dione]	0	24.0	0
Pregnanediol [5 β -pregnane-3 α ,20 α -diol]	15	29.9	0
Progesterone [4-pregnene-3,20-dione]	20	26.6	0
Androstanediol [5 α -androstan-3 β ,17 β -diol]	15	76.8	0
Pregnanolone [3 β -hydroxy-5 β -pregnan-20-one]	0	32.0	0

the edge of the molecule opposite to that of the 6-carboxymethylloxime-bovine serum albumin link (cf. steroids substituted in C-2, C-11 or C-19). On the other hand, a substitution in positions C-6 or C-7, i.e. in the region of the hapten-bovine serum albumin coupling (used for antigen synthesis), does not modify the affinity of the antibody, as has been shown by a number of authors (Exley *et al.*, 1971; Jeffcoate & Searle, 1972). (iv) The total absence of functional groups on the oestratriene skeleton appears to cause a dramatic fall in recognition by the antibody (Kuss *et al.*, 1973). (v) Diethylstilboestrol, a non-steroidal oestrogen with certain structural analogies, is not recognized by the anti-oestradiol γ -globulins.

α -Foetoprotein

Among the factors intervening in steroid recognition by the α -foetoprotein, the absence of hindrance at

C-19 and the presence of an aromatic A ring play an essential role. Thus testosterone, which does not possess an aromatic ring and which has a methyl group at C-19, has no interaction with the α -foetoprotein. On the other hand, 19-nortestosterone, whose only difference from the oestradiol molecule is the presence of a non-aromatic A ring, has a significant interaction (65% of that of oestradiol) with this protein.

In terms of the oestrogenic compounds, the affinity of α -foetoprotein is greater the fewer substituents at C-7, C-15 and C-16. On the other hand, the affinity of α -foetoprotein is influenced very little, or not at all, by a modification of the hindrance at C-17 (addition of a function, change in orientation of a function). It is noteworthy that α -foetoprotein has the greatest affinity for oestratriene, which has no functional group.

Table 2. *Variations, for the three protein models, in the degrees of inhibition corresponding to structural modifications of the steroid molecule*

For each pair of steroids joined by an arrow the only structural modification involved is that indicated in *italics*. The degrees of inhibition of these two steroids for the three models are indicated each time and joined by an arrow. The number of $\oplus$ or $\ominus$ signs indicates the importance of the variation in the degree of inhibition: $\circ$ variation less than 5%; $\oplus$ variation of 10–20%; $\oplus\oplus$ variation of 20–50%; $\oplus\oplus\oplus$ variation of 50–90%; $\oplus\oplus\oplus\oplus$ variation greater than 90%. For the different basic rings, see Fig. 1. Enzyme denotes 17 β -hydroxy steroid dehydrogenase and γ -globulins, anti-oestradiol γ -globulins.

Modification in relation to the basic ring	Basic ring	Degree of inhibition for each pair of steroids					
		α -Foetoprotein		Enzyme		γ -Globulins	
Ring A							
Absence of —OH at C-3							
Oestrone $\rightarrow$ oestratrien-17-one	I	90 $\rightarrow$ 95	○	0 $\rightarrow$ 5		46 $\rightarrow$ 25	⊖⊖
Addition of —OH at C-2							
Oestrone $\rightarrow$ 2 α -OH-oestrone	I	90 $\rightarrow$ 50	⊖⊖	0 $\rightarrow$ 14		46 $\rightarrow$ 9	⊖⊖
Substitution of —OH by =O at C-3 and non-aromatic A ring							
Oestradiol $\rightarrow$ nortestosterone	I	100 $\rightarrow$ 65	⊖⊖	100 $\rightarrow$ 55	⊖⊖	100 $\rightarrow$ 24	⊖⊖⊖
Addition of —CH ₃ at C-10							
Nortestosterone $\rightarrow$ testosterone	II	65 $\rightarrow$ 0	⊖⊖⊖	55 $\rightarrow$ 50	○	21 $\rightarrow$ 4	⊖
Addition of —CH ₃ at C-10 and non-aromatic A ring							
Oestradiol $\rightarrow$ androstanediol	I	100 $\rightarrow$ 15	⊖⊖⊖	100 $\rightarrow$ 76	⊖⊖	100 $\rightarrow$ 0	⊖⊖⊖⊖
Ring B							
Addition of —OH at C-7							
Oestradiol $\rightarrow$ 7 α -OH-oestradiol	III	100 $\rightarrow$ 20	⊖⊖⊖	100 $\rightarrow$ 27	⊖⊖⊖	100 $\rightarrow$ 54	⊖⊖
Addition of —OH at C-6							
Oestrone $\rightarrow$ 6 α -OH-oestrone	III	90 $\rightarrow$ 105	⊕	0 $\rightarrow$ 16		46 $\rightarrow$ 36	⊖
Addition of C=O at C-6							
Oestradiol $\rightarrow$ 6-oxo-oestradiol	III	100 $\rightarrow$ 70	⊖⊖	100 $\rightarrow$ 26	⊖⊖⊖	100 $\rightarrow$ 109	⊕
Oestriol $\rightarrow$ 6-oxo-oestriol	III			4 $\rightarrow$ 19	⊕	31 $\rightarrow$ 42	⊕
Ring C							
Addition of —OH at C-11							
Oestrone $\rightarrow$ 11-OH-oestrone	IV	90 $\rightarrow$ 50	⊖⊖	0 $\rightarrow$ 19		46 $\rightarrow$ 1	⊖⊖
Ring D							
Absence of function at C-17							
Oestradiol $\rightarrow$ 17-desoxo-oestrone	V	100 $\rightarrow$ 125	⊕⊕	100 $\rightarrow$ 22	⊖⊖⊖	100 $\rightarrow$ 14	⊖⊖⊖
Oestrone $\rightarrow$ 17-desoxo-oestrone	V	90 $\rightarrow$ 125	⊕⊕	0 $\rightarrow$ 22		46 $\rightarrow$ 14	⊖⊖
Oestratrien-17-one $\rightarrow$ oestratriene	V	95 $\rightarrow$ 110	⊕	5 $\rightarrow$ 10	○	25 $\rightarrow$ 5	⊖⊖
Function transferred from C-17 to C-16							
Oestrone $\rightarrow$ 16-oestrone	V	90 $\rightarrow$ 90	○	0 $\rightarrow$ 14		46 $\rightarrow$ 6.5	⊖⊖
17 α -Oestradiol $\rightarrow$ 16 α -oestradiol	V			9 $\rightarrow$ 5	⊖	16 $\rightarrow$ 15	○
Function transferred from C-17 β to C-17 α							
17 β -Oestradiol $\rightarrow$ 17 α -oestradiol	V	100 $\rightarrow$ 70	⊖⊖	100 $\rightarrow$ 9	⊖⊖⊖⊖	100 $\rightarrow$ 16	⊖⊖⊖
Substitution at C-17 of —OH by =O							
Oestradiol $\rightarrow$ oestrone	V	100 $\rightarrow$ 90	⊖	100 $\rightarrow$ 0	⊖⊖⊖⊖	100 $\rightarrow$ 46	⊖⊖⊖
Androstanediol $\rightarrow$ androsterone	V	15 $\rightarrow$ 35	⊕⊕	77 $\rightarrow$ 29	⊖⊖	0 $\rightarrow$ 2	○
Testosterone $\rightarrow$ dehydroepiandrosterone	V	0 $\rightarrow$ 0	○	50 $\rightarrow$ 16	⊖⊖	4 $\rightarrow$ 4	○
Addition at C-17 α of —OH							
Pregnanediol $\rightarrow$ pregnanetriol	VI	15 $\rightarrow$ 0	⊖	30 $\rightarrow$ 44	⊕	0 $\rightarrow$ 0	○
Corticosterone $\rightarrow$ cortisol	VII	15 $\rightarrow$ 0	⊖	29 $\rightarrow$ 24	○	0 $\rightarrow$ 0	○
Addition at C-17 α of C=CH							
Oestradiol $\rightarrow$ 17 α -ethinyloestradiol	V	100 $\rightarrow$ 70	⊖⊖	100 $\rightarrow$ 22	⊖⊖⊖	100 $\rightarrow$ 8	⊖⊖⊖⊖
Addition at C-16 of —OH or =O							
Oestradiol $\rightarrow$ oestriol	V	100 $\rightarrow$ 42	⊖⊖⊖	100 $\rightarrow$ 4	⊖⊖⊖⊖	100 $\rightarrow$ 31	⊖⊖⊖
6-Oxo-oestradiol $\rightarrow$ 6-oxo-oestriol	V			26 $\rightarrow$ 19	○	109 $\rightarrow$ 42	⊖⊖⊖
Oestrone $\rightarrow$ 16 α -OH-oestrone	V	90 $\rightarrow$ 35	⊖⊖⊖	0 $\rightarrow$ 18		46 $\rightarrow$ 12	⊖⊖
Oestrone $\rightarrow$ 16-oxo-oestrone	V	90 $\rightarrow$ 15	⊖⊖⊖	0 $\rightarrow$ 16		46 $\rightarrow$ 4	⊖⊖
Addition at C-15 of —OH							
Oestriol $\rightarrow$ 15 α -OH-oestriol	V	42 $\rightarrow$ 0	⊖⊖	4 $\rightarrow$ 18	⊕	31 $\rightarrow$ 18	⊖

Fig. 1. Basic nuclei illustrating the structure of the steroids mentioned in Table 2

For details see the text and Table 2.

It may also be noted, as opposed to what has been found for the γ -globulins, diethylstilboestrol interacts fairly strongly with the α -foetoprotein.

17 β -Hydroxy steroid dehydrogenase

A certain amount of non-specific binding of steroid hormones, including oestradiol, by rat liver microsomal preparations, has been reported (King & Mainwaring, 1974a). However, in the present study of 17 β -hydroxy steroid dehydrogenase activity, it was the formation of oestrone by the enzyme that was measured, a function of the enzyme alone, and thus non-specific binding does not enter the results.

The degrees of interference measured for the enzyme confirm the particular importance of the 17 β -hydroxyl function at C-17 of a steroid molecule for this to be recognized (Mulder *et al.*, 1972). Any steroid modified in this position (by a transfer, an addition, a substitution or the disappearance of the hydroxyl function) no longer interacts with the enzyme. Apart from this essential factor, other modifications intervene in the enzyme-steroid interaction; in particular the addition of functions at positions C-6 or C-7 results in a decrease in affinity. On the other hand, the addition of a methyl group at C-19, or the presence of a non-aromatic A ring, intervenes little in this interaction.

Like α -foetoprotein, the enzyme recognizes diethylstilboestrol to a significant extent.

Among the 30 steroids studied, some of them may be transformed and compete with the oestradiol: these are the steroids possessing a hydroxyl function at the 17 β position. It seems in particular that the four steroids for which growing degrees of inhibition have been obtained with increasing steroid concentration are competitive substrates (Table 3). This hypothesis is confirmed by the work of Mulder *et al.* (1972), who have shown competition between substrates with human erythrocyte 17 β -hydroxy steroid dehydro-

Table 3. Influence of steroid concentration on the degrees of inhibition measured for 17 β -hydroxy steroid dehydrogenase

For details see the text.

Steroid	Inhibition (%)		
	Steroid (100ng)	Steroid (500ng)	Steroid (1000ng)
6-Oxo-oestradiol	25.7	46.4	62.7
Testosterone	49.6	75.2	88.7
Nortestosterone	55.0	76.5	86.1
Androstenediol	76.8	89.0	100.0
Oestradiol	100.0	100.0	100.0

genase. According to these authors, the reaction rate for conversion of two of the substrates, androstenediol and oestradiol, when added simultaneously, was smaller than the sum of the individual reaction rates, and each substrate competitively inhibited the conversion of the other substrate.

For all steroids other than those indicated above, several hypotheses may be proposed, taking into account the fact that there is no significant increase in the degree of inhibition as a function of their concentration. (i) If one supposes that certain steroids occupy the enzymic site (oestriol, oestratriene, derivatives of oestrone), this absence of change with concentration would show that the value of K_i is already largely surpassed at the lowest concentration (100ng per incubation). (ii) If they are not competitive inhibitors the hypothesis could be formulated that their concentration is much greater than the K_D of the binding equilibrium.

Although oestrone derivatives inhibit the enzyme, this is not found for oestrone itself, for since this steroid is a reaction product, it is rejected by the enzyme in the presence of oestradiol.

These interpretations may be incomplete, for working with a microsomal suspension containing numerous NAD⁺-dependent enzymic systems and not a pure enzyme, one may speculate that the metabolism of the steroids studied could influence the extent of steroid binding.

The comparison with the three protein models allows several important conclusions to be discussed: (i) The α -foetoprotein essentially recognizes the oestrogen skeleton. The more one removes the functional groups from the oestrogen molecule, the greater the affinity of the α -foetoprotein for that molecule. (ii) For the enzyme 17 β -hydroxy steroid dehydrogenase, it is the C-17 of the steroid molecule which constitutes the essential characteristic; the oestrogen skeleton intervenes secondarily. (iii) Diethylstilboestrol, which is an analogue of the oestrogens but which is a smaller molecule, acts on the two naturally occurring models. (iv) With the anti-oestradiol γ -globulins it seems, on the contrary, that the integrity of the oestradiol molecule is necessary for interaction with the antibody protein. With diminution of the hindrance of the molecule there is a decrease in the recognition of the protein by the steroid, the most striking case being that of diethylstilboestrol, which the antibody does not recognize at all.

In conclusion, the study reported may orientate research on the following points. (i) The choice of steroids for a more detailed study of the enzyme-inhibitor reactions. (ii) If it appears that the antibody essentially attaches itself to the C-2-C-10-C-11-C-17 edge of the steroid, leaving the opposite C-4-C-6-C-8-C-15 accessible and the latter being precisely the edge interacting with the naturally occurring models, it would be of interest to devise methods for purifying the functional proteins by using a steroid- γ -globulin complex linked to an insoluble matrix. (iii) The study of the action of membrane-bound enzymes on a steroid bound to soluble proteins (α -foetoprotein, γ -globulins).

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