COMPARATIVE STUDY ON THE BINDING OF ESTROGENS BY HUMAN AND RAT SERUM PROTEINS IN DEVELOPMENT.

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SUMMARY -
The binding of estrone,17β-estradiol and testosterone on rat and human sera as well as on pure serum proteins has been studied comparatively. It is known that the rat embryo serum contains an α_1-fetoprotein with high binding affinity for estrogens. By contrast, no estrogen fixation has been found with human sera containing α_1-fetoprotein (fetus, pregnant women, cord blood and hepatoma). However, the adult human and particularly the female at the end of pregnancy, display a serum sex binding globulin with high affinity for both 17β-estradiol and testosterone. The amount of this protein is extremely low in fetuses up to the 5th month of pregnancy and in the cord blood.

The α_1-fetoprotein is a specific fetal protein appearing in the human embryo as early as the first weeks of pregnancy. It disappears almost completely after birth (1) but is found again during several hepatic, digestive, ovarian and testicular diseases (2,3,4,5,6,7). The mechanism and the biological meaning of this re-appearance is at present the object of many hypotheses and researches(1).

It is known that the embryo rat serum contains an α_1-fetoprotein (8) which reaches maximum concentration before birth; this protein disappears almost completely between the 21th and 28th day of post-natal life (9). This α_1-fetoprotein binds specifically with great affinity estrone and 17β-estradiol (10,11,12). The adult rat serum binds neither estrogens nor testosterone to any significant extent (10,11).

On the contrary, in the adult human, and particularly in the female during late pregnancy, the estradiol and the testosterone are fixed on a protein that has been identified and isolated (13,14), i.e. the Sex-Binding-Globulin. At our best knowledge, this protein has never been studied in the human fetus.

Some authors (12), in somewhat different experimental conditions have shown that the α_1-fetoprotein from a patient bearing a hepatocellular

carcinoma presents an affinity for 17β-estradiol. In the present work, we have compared the binding of estrogens and testosterone during the rat and human development. We have particularly sought to establish if the human α_1-fetoprotein presents the binding affinity for estrogens observed in the case of rat α_1-fetoprotein.

MATERIAL AND METHODS -

I - BIOLOGICAL MATERIAL AND RADIOACTIVE COMPOUNDS

Animals : the rats were Charles Rivers strain C.D.Each experience has been performed 2-3 times on pooled sera from mixed male and female embryos and on pooled sera from adult females only. The sera have been obtained as previously described (10).

Purified rat α_1-fetoprotein : two preparations of pure α_1-fetoprotein have been used, one has been prepared in our laboratory (15), the other was a gift from Professors Nishi and Hiraï, Sapporo, (Japan).

Human sera : fetal blood was collected from fetuses of different ages after therapeutic abortions.

- The sera of pregnant women and of ombilical cord were provided by Pr. Philippe Engelmann (Gynecology and Obstetrics Department Lariboisière Hospital, Paris). Every experience was repeated 2-3 times on pooled sera collected from 3-5 pregnant women, for each of the pregnancy terms.

- Hepatoma sera were obtained from patients hospitalized with a diagnosis of hepatoma.

- Control sera were obtained from normal male and female apparently healthy subjects.

Purified human α_1-fetoprotein preparations were kind gifts from Professors Nishi and Hiraï (Sapporo, Japan) and Pr. Masseyeff (Nice, France).

Human antisera :

- specific anti α_1-fetoprotein antisera (CIRC, Lyon, Dr. Sizaret).

- total human antiserum prepared in our laboratory as described previously (16).

Radioactive steroids :

[6 - 7 ^{3}H]	17β-estradiol	CEN	46 Ci/mM
[4 - ^{14}C]	17β- estradiol	CEN	40 mCi/mM
[6 - 7 ^{3}H]	estrone	CEA	34 Ci/mM

[4 - ^{14}C]	estrone	CEA	51.3 mCi/mM
[1 - 2 ^{3}H]	testosterone	CEA	42 Ci/mM

These steroids were all purified on celite columns prior to use.

II - METHODS

- Assay of the protein binding of steroids

- gel filtration on Sephadex G 100 : (0.7g, 285 mm height, 10 mm diameter) : the protein sample was incubated 30 mn at 37°C with the labelled steroid ($8.8\ 10^{-8}$ M/mg Protein) in a phosphate buffer 0.15 M, PH : 7.3, then applied to the gel column, which allowed separation of bound from unbound steroid. The results are given as picomoles of bound steroid per mg of protein.

- Equilibrium dialysis according to Pearlman and Crepy (14).

- Immunoautoradiography as described previously (16).

- Counting of radioactivity in the conditions already described (17).

- Protein assay according to Lowry and al (18)

- Determination of the amount of α_1-fetoprotein by the immunodiffusion method of Mancini (19) on M-Partigen α_1-fetoprotein plates (Berhing). The calibration was done with a purified α_1-fetoprotein (Berhing).

RESULTS -

Correlations between the binding of estrone, 17β-estradiol and testosterone and the α_1-fetoprotein concentrations in different human sera i.e. human fetuses of different age, cord blood, pregnant women in the three terms of pregnancy, primary fetoproducing hepatoma (table I).

The binding of 17β-estradiol by sera of human fetuses of different ages and by cord blood is of the same order of magnitude as the binding in the adult normal sera of both sexes. In the pregnant woman, the binding is significantly higher during the 3rd trimester of pregnancy.

The binding of testosterone is always lower in the embryos than in the normal adult or in the pregnant woman. It does not vary significantly throughout fetal development; it is always higher than the fixation of 17β-estradiol.

The binding levels of all the hormones studied in the sera of the hepatoma patients are similar to those found in controls.

These results show that in man, during fetal development, there is no significant binding of estrogens although α_1-fetoprotein levels are

	Steroid binding (pmoles/mg Protein)			% α_1-fetoprotein	Range of α_1-fetoprotein concentration mg/ml
	17β-estradiol	estrone	testosterone		
6 - 8 week embryos	0.36 ± 0.16	0.45 ± 0.06	0.55 ± 0.06	28 % (1)	2.8 mg/ml
13 - 16 week embryos	0.14 ± 0.06	0.50 ± 0.07	0.47 ± 0.08	6.8 % ± 2.7	0.16 - 3.3 mg/ml
19 - 21 week embryos	0.31 ± 0.15	0.50 ± 0.20	0.51 ± 0.1	2.85 % ± 0.49	0.6 - 3.4 mg/ml
cord blood	0.24 ± 0.18	0.54 ± 0.15	0.50 ± 0.15	1.4 % (2)	0.5 mg/ml
Primary hepatoma	0.26 ± 0.13	0.50 ± 0.08	1.92 ± 0.73	0.012 % ± 0.003	0.01 - 0.015 mg/ml
Pregnant women 1st trimester	0.45 ± 0.08	-	3.14 ± 0.5	(3)	(3)
Pregnant women 2nd trimester	0.31 ± 0.1	-	3.80 ± 0.65	(3)	(3)
Pregnant women 3rd trimester	1.40 ± 0.14	-	5.09 ± 0.92	(3)	(3)
Adult female control	0.22 ± 0.04	-	1.01 ± 0.17	(3)	(3)
Adult male control	0.32 ± 0.08	0.91 ± 0.31	1.22 ± 0.17	(3)	(3)

Pmoles of steroid bound per milligram of serum proteins. Gel filtration assay.

(1) α_1-fetoprotein concentration in the 8 weeks old embryo.

(2) α_1-fetoprotein concentration in only one cord blood serum.

(3) Not detectable by our methods.

TABLE II

Serum	Steroid binding pmoles/mg Protein	
	17β-estradiol	Testosterone
19 day embryo	66	0.09
New born rat	49.1	0.06
21 day immature rat	5.5	0.03
Hepatoma	35	0.17
Adult rat	0.3	0.2

Pmoles of steroid (17β-estradiol and testosterone) bound per milligram of protein. Gel filtration assay of different ages rat sera and sera form rats treated with hepatocarcinogens (16).

high. This is in contrast to the results obtained with rat embryo sera (10,11) and with sera from rats bearing experimental hepatoma (16) where the levels of estrogen binding, measured by the same methods are very high (Table II).

On the other hand, the low levels of testosterone and of 17β-estradiol binding in the embryos, by comparison with controls and pregnant women, suggest that there is little or no Sex Binding Globulin in the serum during fetal life.

Comparison of the binding of 17β-estradiol by human and rat purified α_1-fetoprotein (Table III).

These results show that there is almost no binding of 17β-estradiol on pure human α_1-fetoprotein.

These results have been further substantiated by immunoautoradiography of human α_1-fetoprotein against a specific immune serum. No image of $[^{14}C]$ 17β-estradiol fixation was obtained on the specific precipitation lines, neither after immunodiffusion nor after immunoelectrophoresis; the same negative results were obtained with both immune sera (M -Partigen, CIRC).

TABLE III

		Pure rat α_1-fetoprotein		Pure human α_1-fetoprotein	
Steroid	Method	Purified in our laboratory	Purified by Pr. Nishi	Purified by Pr. Masseyeff	Purified by Pr. Nishi
17β-estradiol	gel filtration pmoles/mg α_1-fetoprotein.	88	-	0.12	0.10
	Affinity index (1) 1/P	150	205	-	∿ 0

17β-estradiol binding by human and rat pure α_1-fetoprotein

(1) Determined by equilibrium dialysis, according to Pearlmann and Crepy (14).

We remind that when a steroid is effectively bound by a protein, it is possible to localize this fixation on the specific immunoprecipitation line, by using the steroid labelled with $[^{14}C]$. (12,16).

DISCUSSION AND CONCLUSION -

In our experimental conditions, the human α_1-fetoprotein, either of fetal origin or from neoplasic sera does not bind the estrogens, thus being different from rat α_1-fetoprotein (10,11,12) and from an α_1-fetoprotein found in a case of human hepatoma (12). It is possible that in this last particular case the physiopathological situation was entirely different from that of the human hepatomas studied by us.

On the other hand, it seems that human Sex Binding Globulin, which has been detected in the adult and especially during the 3rd term of pregnancy is absent or extremely scarce in the fetus.

There are consequently two utterly different biological situations in Man and in rat during pregnancy : the estrogen binding protein is found in high amounts in the human mother while in the case of the rat, this binding protein appears in the embryo. The biological significance of these facts must be elucidated.

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