Testosterone-Binding Globulins in Human Plasma: Studies on Sex Distribution and Specificity

WILLIAM ROSNER and SUSAN M. DEAKINS

From the Department of Medicine, Roosevelt Hospital, New York 10019

ABSTRACT When human plasma is mixed with testosterone-^{3}H and subjected to electrophoresis on paper in glycine acetate buffer at pH 8.6, at least two proteins other than albumin bind the testosterone. In normal women 80.5 ± 1.9% (SEM) of the recovered radioactivity migrates with the β-globulins, 7.3 ± 0.80% with the inter-α-globulins, and 4.3 ± 0.40% with albumin. In normal men the percentages are 69.3 ± 3.0%, 14.3 ± 1.6%, and 6.2 ± 1.1%, respectively. These differences between men and women in binding among the β-globulins and inter-α-globulins are statistically significant ($P < 0.001$). The highest percentages of radioactivity associated with the β-globulins are seen in infants of both sexes, men receiving diethylstilbestrol, and pregnant women. These same subjects have the lowest percentages of radioactivity associated with the inter-α-globulins. Experiments with carrier testosterone indicate that at least some of the differences between the normal men and women and infants can be explained by differences in the concentration of endogenous testosterone. This factor alone, however, cannot explain the increased binding among the β-globulins in the men receiving diethylstilbestrol or in the pregnant females. In this system estrone, estradiol, dehydroisoandrosterone, androsterone, 17α-hydroxyprogesterone, and 19-nortestosterone compete with testosterone for binding sites on the proteins. None is as potent as testosterone itself.

Received for publication 23 March 1968 and in revised form 3 May 1968.

INTRODUCTION

Evidence from the laboratories of Pearlman and Crepy (1) and Rosner, Kelly, Deakins, and Christy (2) has demonstrated the existence in human plasma of a macromolecular binding factor for testosterone which is not albumin. To date little is known about this binding factor. It seems to be a protein with a low capacity and high affinity for testosterone,[1] which is present in higher concentration in females than in males, and is increased in concentration in women in late pregnancy. The present study was designed to elucidate further the nature of this protein.

METHODS

Subjects. Laboratory and hospital personnel, aged 18–65, served as normal adult subjects. All the females were premenopausal. The three pregnant subjects were in the third trimester of normal pregnancies, and the four pediatric subjects came from a well baby clinic. The two men receiving diethylstilbestrol both had carcinoma of the prostate; one had been castrated.

[1] The chemical names for the steroids used in this paper are as follows: 17β-hydroxyandrost-4-en-3-one (testosterone); estra-1,3,5(10)-triene-3,16α,17β-triol (estriol); pregn-4-ene-3,20-dione (progesterone); 11β,21-dihydroxypregn-4-ene-3,20-dione (corticosterone); 11β,-17α,21-trihydroxypregnene-3,20-dione (cortisol); 21-hydroxypregn-4-ene-3,20-dione (deoxycorticosterone, DOC); 5β-pregnane-3α,20α-diol (pregnanediol); 11β-hydroxyandrost-4-ene-3, 17-dione (11β-hydroxyandrostenedione); 17α-hydroxyandrost-4-en-3-one (epitestosterone); 3-hydroxyestra-1,3,5 (10)-trien-17-one (estrone); 3β-hydroxyandrost-5-en-17-one (dehydroepiandrosterone, DHEA); 3α-hydroxy-5α-androstan-17-one (androsterone); 17α-hydroxypregn-4-ene-3,20-dione (17α-hydroxyprogesterone); estra-1,3,5 (10)-triene-3, 17β-diol (estradiol-17β); and 19-nor-17β-hydroxyandrost-4-en-3-one (19-nortestosterone).

Steroids. Testosterone-1,2-^{3}H,[2] SA 41.8 c/mmole, was purified by partition chromatography on Celite, and its radiochemical homogeneity established by reverse isotope dilution as specified in a previous publication (3). We diluted it with recrystallized carrier testosterone to a specific activity of 30 c/mmole and used this preparation throughout the experiments reported below. All non-radioactive steroids were recrystallized before use.

Paper electrophoresis. Testosterone-^{3}H (11,300 cpm, 0.11 ng), dissolved in 0.1 ml of methanol, was placed in a small test tube and the methanol evaporated under nitrogen. When used, carrier testosterone and other steroids were dried simultaneously with the radioactive material. We added 0.2 ml of the test plasma or 0.2 ml of a solution of human serum albumin (5 g/100 ml) to the dried steroid, which resulted in a final concentration of 0.56 ng of testosterone per ml, and let the mixture stand at room temperature for approximately 30 min. We then applied 50μl of the mixture (five applications of 10μl) to strips of Whatman No. 3MM filter paper 3.8 × 30 cm). Electrophoresis was carried out at room temperature in Durrum tanks[3] for 20 hr in 0.055 M glycine acetate buffer, pH 8.6, with an applied voltage of 5.3 v/cm. We used the same technique to determine the migration of testosterone-^{3}H applied in 0.01 M sodium phosphate buffer, pH 7.4, made 0.15 M in sodium by the addition of NaCl.

Determination of radioactivity. At the end of the electrophoresis the paper strips were dried in air at room temperature and the protein bands located and marked under ultraviolet light (254 mμ). We cut the strips into 0.5-cm portions at right angles to the direction of migration of the proteins, placed each 0.5 cm portion into a glass counting vial of low potassium content, and added 0.2 ml of methanol and 10 ml of a toluene-based phosphor (3). This amount of methanol is not enough to cause quenching and leads to a 2–3-fold improvement in recovery of the radioactivity from the paper strip (4). Radioactivity was determined in a Tri-Carb liquid scintillation spectrometer,[4] model 3003, with an efficiency of 55% for a sealed tritium standard.

RESULTS

When testosterone-^{3}H in buffer is used in the electrophoretic system, it migrates so that the peak of radioactivity is 1 cm cathodal to the point of application (Fig. 1). Movement of uncharged particles toward the cathode is to be expected in the system used and is explained by electro-osmotic flow (5). Fig. 1 also shows the pattern of radioactivity evolved when a mixture of albumin (5 g/100 ml) and tritiated testosterone is submitted to electrophoresis. There is no distinct peak at, or cathodal to, the origin; rather a symmetrical peak of radioactivity migrates with albumin preceded by a trail of radioactivity extending to the point where one would expect free testosterone. There is no change in this pattern when 100 ng of carrier testosterone is added to the mixture of albumin and labeled testosterone. Fig. 1 also illustrates the coincident electrophoretic migration of testosterone-^{3}H and plasma from a normal male. Four peaks of radioactivity are seen. The first peak migrates 1 cm cathodal to the origin, as does testosterone alone; the second peak migrates with the β-globulins, 0.5–1 cm anodal to the origin; the third peak moves with the inter-α-globulins, 3–3.5 anodal to the origin; and the fourth peak migrates with albumin, 5.5–6.0 cm anodal to the origin. The four peaks have been observed in all the plasmas examined. Freezing and defrosting of portions of the same plasma sample five times over a period of 5 wk did not alter these patterns.

Fractional distribution of testosterone-^{3}H among the plasma proteins of normal men and women. The percentage of radioactivity in a given peak was determined by totalling all the radioactivity recovered in a given strip and dividing by the counts recovered in a given peak. The mean recovery ± 1 SD in 50 strips was 82.7 ± 5.0% (6).

Table I shows the fractional distribution of the testosterone-^{3}H among the four peaks in normal men and women. The mean percentage of radioactivity in peak II is significantly higher in women than in men ($P < 0.001$), whereas peak III is significantly lower in women than in men ($P < 0.001$). There is no statistically significant difference between peaks I and IV between the sexes. Within each group, as peak II decreases, peak III and, to a more variable degree, peaks IV and I increase. The correlation coefficients for these variables are as follows:

	Female	Male
II:III	−0.94	−0.96
II:IV	−0.58	−0.56
III:IV	+0.63	+0.73
II:I	−0.87	−0.48

The values for peaks II, III, and IV in the last two male subjects, F.G. and E.M., fall almost 2 SD away from the mean of the normal males. The values for these two subjects were used in calculating the means and standard deviations.

[2] Nuclear-Chicago Corporation, Des Plaines, Ill.
[3] Beckman Instruments, Inc., Mountainside, N. J.
[4] Packard Instrument Co., Inc., Downer's Grove, Ill.

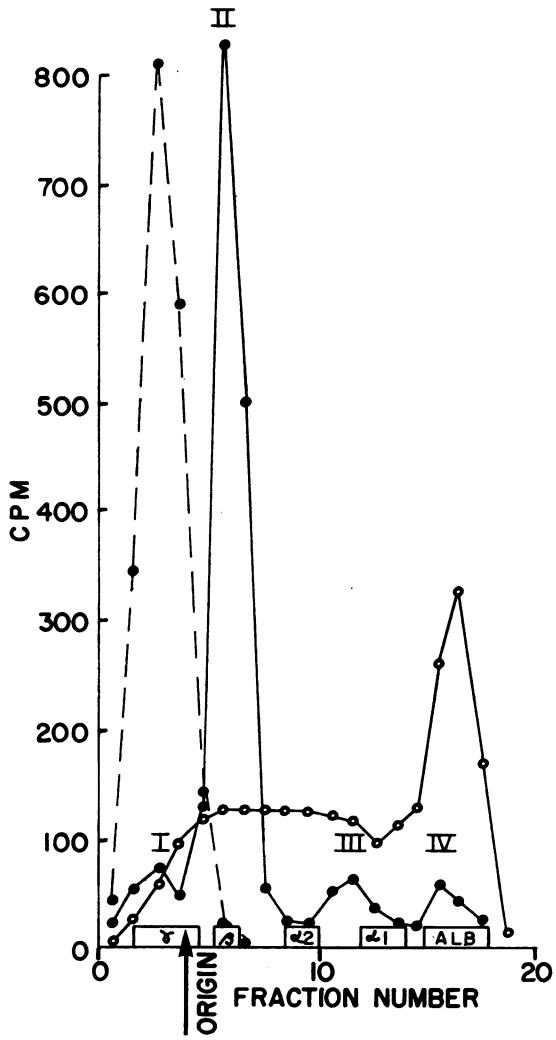

FIGURE 1 The electrophoretic migration of testosterone-^{3}H in buffer, with human serum albumin, and with plasma from a normal male. The anode is on the right. The origin indicates the point of application. Each fraction number is a 0.5 cm portion of the paper cut at right angles to the direction of migration of the proteins, starting 2.25 cm cathodal to the origin. The amount of radioactivity recovered in a single cut is represented by a point in the center of the fraction. The dashed line represents the migration of testosterone-^{3}H in pH 7.4 phosphosaline buffer. The solid line with open circles represents the migration of testosterone-^{3}H when mixed before application with human serum albumin, 5 g/100 ml. The solid line with closed circles represents the migration of testosterone-^{3}H with plasma. *I* indicates the peak of radioactivity which migrates cathodal to the origin as does testosterone in buffer; *II* indicates the peak of radioactivity associated with the β-globulins; *III*, that with the inter-α-globulins; and *IV*, that with albumin. Electrophoresis was carried out at room temperature in Durrum tanks for 20 hr in 0.055 M glycine acetate buffer, pH 8.6, with an applied voltage of 5.3 v/cm.

Table II shows the effect of several variables on the fractional distribution of testosterone-^{3}H among the plasma proteins after electrophoresis. The highest values for peak II and the lowest values for peak III are seen in the two females in the third trimester of normal pregnancies. These values are more than 2 SD away from the mean value for normal females. Two men receiving diethylstilbestrol, one of whom had also been castrated, show a similar pattern. The four infants, two males and two females, aged 6–36 months, all show values for peak II well above the mean for the normal women and values for peak III well below the mean for normal women.

Addition of carrier testosterone. We added carrier testosterone to the plasma of a normal female, S.D., and a normal male. Duplicate or triplicate determinations were done at each concentration of carrier, and the mean percentages were compared to the base line values for these subjects (Table III). With the addition of 10 ng/ml of carrier testosetrone to the plasma of the normal female peak II fell to 75% of its base line value at the same time that peaks III and IV doubled. At this concentration of testosterone the values for these peaks were more than 2 SD away from their base line values. At a concentration of 100 ng/ml peak II fell to 40% of its original value, and peaks III and IV were four to five times their base line values. With the addition of 250 and 500 ng/ml no further significant changes were noted.

The only concentrations of testosterone at which the electrophoretic patterns of the normal female and the normal male differed were 0.56 and 5.0 ng/ml. With the addition of 5.0 ng/ml to the plasma of B.M. the values for peaks II, III, and IV were increased or decreased more than 2 SD away from their base line values. To produce changes of the same significance in the plasma of the normal female it was necessary to add 10 ng/ml. At concentrations of 10 ng/ml or more of carrier testosterone the electrophoretic patterns of S.D. and B.M. were indistinguishable.

Competition experiments. Table IV shows the effect of the addition in vitro of various steroids on the distribution of testosterone-^{3}H between its two major binding proteins. Competition was carried out with the plasma of a normal female, S.D., at two levels, 1.7×10^{-7} mmoles/ml and $4.9 \times$

TABLE I

The Fractional Distribution of Testosterone-^3H among Serum Proteins after Electrophoresis

Subjects	I	II	III	IV
Females				
M. C.	2.6	90.6	3.7	3.0
L. T.	2.9	90.3	3.9	2.9
C. J.	2.9	89.7	4.1	3.3
P. G.	3.6	86.6	5.5	4.4
S. D. ± SD*	7.7 ± 2.2	82.8 ± 3.4	5.8 ± 1.3	3.8 ± 1.0
M. L.	6.5	82.7	7.3	3.5
B. L.	5.6	82.4	5.7	6.0
L. M.	12.2	80.1	7.9	3.3
R. D.	9.5	79.9	5.7	4.9
C. P.	9.3	79.5	8.5	4.4
A. G.	7.7	79.2	8.0	5.1
E. E.	12.3	75.1	9.5	3.3
L. R.	16.0	74.8	7.7	1.5
K. C.	11.1	67.6	14.4	7.7
M. B.	14.3	66.4	12.0	7.4
Mean (15) ± SEM	8.3 ± 1.1	80.5 ± 1.9	7.3 ± 0.8	4.3 ± 0.4
Males				
J. H.	5.8	81.2	8.0	6.8
K. W.	6.7	80.5	9.0	3.7
J. D.	11.7	79.5	8.0	1.0
E. H.	10.2	76.3	10.8	2.8
T. D.	3.6	76.2	13.5	6.7
E. G.	11.9	75.9	10.1	2.1
B. M. ± SD‡	7.3 ± 1.8	74.5 ± 0.5	9.9 ± 0.5	8.3 ± 1.9
H. H.	7.0	73.2	13.6	6.1
O. L.	11.5	68.3	16.4	3.8
B. R.	17.4	67.0	11.5	5.5
D. M.	16.0	64.5	17.6	1.9
J. K. ± SD§	15.2	55.4	19.5	10.1
F. G.	11.8	49.5	26.5	12.2
E. M. ± SD‖	10.0 ± 1.7	48.4 ± 3.4	25.6 ± 2.6	15.9 ± 4.3
Mean (14) ± SEM	10.4 ± 1.1	69.3 ± 3.0	14.3 ± 1.6	6.2 ± 1.1

I indicates the peak of radioactivity which migrates cathodal to the origin as did testosterone in buffer; II indicates the peak of radioactivity associated with the β-globulins; III, that with the inter-α-globulins; and IV, that with albumin.
* These percentages represent the means of 12 separate determinations made on the same plasma sample over 7 wk.
‡ These percentages represent the means of six separate determinations made on the same plasma sample over 2 wk.
§ These percentages represent the means of six separate determinations on the same plasma sample over 5 wk.
‖ These percentages represent the means of five separate determinations made on the same plasma sample over 3 months.
The rest of the percentages represent the results of single determinations except for one female, L. T., and one male, J. H., which represent the means of two determinations. The mean percentage of recovered radioactivity for the normal males and females ±SEM 83.0 ± 5.0%.

10^{-7} mmoles/ml. These concentrations are equivalent to 50 and 140 ng/ml of testosterone respectively. Duplicate or triplicate determinations were performed at both concentrations and the mean values compared to the base line mean values for S.D. Progesterone, corticosterone, cortisol, DOC, pregnenediol, 11β-hydroxyandrostenedione, *l*-thyroxine, and epitestosterone did not compete with testosterone in this system. Estrone at the higher concentration and DHEA, androsterone, 17α-hydroxyprogesterone, estradiol, and 19-nortestosterone at both concentrations successfully did compete. None were as potent as testosterone itself. At the higher concentration of androsterone

TABLE II

The Effect of Various Physiologic Conditions on the Fractional Distribution of Testosterone-^3H among Serum Proteins after Electrophoresis

Subjects	I	II	III	IV
Females (15)	8.3 ± 1.1*	80.5 ± 1.9	7.3 ± 0.8	4.3 ± 0.4
Males (14)	10.4 ± 1.1	69.3 ± 3.0	14.3 ± 1.6	6.2 ± 1.1
Pregnant females				
M. P.	1.2	96.7	1.3	0.7
O. E.	1.8	95.5	1.6	1.0
A. P.	0.1	95.4	2.0	1.6
Males receiving diethystilbestrol				
A. H.	2.8	92.1	3.9	1.2
C. W.‡	3.2	91.6	2.9	2.4
Infants				
Males				
W. M.	3.2	92.0	2.0	2.8
D. R.	3.6	89.9	4.6	1.8
Females				
M. R.	3.7	89.6	3.8	2.9
L. R.	5.3	89.0	3.5	2.3

I–IV are as in Table I. The percentages represent the results of single determinations except for the two males receiving diethylstilbestrol which are the means of two determinations.

* The mean percentages ±SEM for the normal males and females are given at the top for comparison.

‡ This male had also been castrated.

and 17α-hydroxyprogesterone there was no further decrease in peak II or increase in peak III as was seen with estradiol and 19-nortestosterone.

DISCUSSION

The interpretation of part of the results presented in this communication offers some theoretical difficulties. Ideally one would like to study binding under equilibrium conditions in which both the macromolecule and its ligand are present in pure form. The state of knowledge of the binding of testosterone to plasma proteins does not yet allow such an approach. Despite the fact that we are studying a mixture of proteins under nonequilibrium conditions it is still possible to make reasonable qualitative statements.

Let us first consider the difference in patterns observed between the sexes. It has been shown

TABLE III

Effect of the Addition in Vitro of Increasing Amounts of Carrier Testosterone on the Distribution of Testosterone-^3H between its Two Major Binding Proteins in the Plasma of a Normal Male and Female

Weight of testosterone, *ng/ml*.....		0.56	5	10	50	100	250	500
Female (S. D.)	II	83	82	63	52	41	41	36
	III	5.8	6.8	14	24	30	32	30
Male (B. M.)	II	75	69	63	52	37	32	35
	III	9.9	13	17	24	30	29	27

II and III refer to the peaks of radioactivity associated with the β-globulins and inter-α-globulins respectively. The numbers represent the percentage of recovered radioactivity associated with each peak at the stated concentration of testosterone.

TABLE IV

Effect of the Addition in Vitro of Various Steroids on the Distribution of Testosterone-^3H between its Two Major Binding Proteins

Steroid	1.7×10^{-7} mmoles/ml		4.9×10^{-7} mmoles/ml	
	II	III	II	III
Testosterone	51.5‡	24.2‡	40.0‡	30.8‡
Estriol	83.7	5.6	82.0	7.8
Progesterone	84.6	5.7	83.4	6.2
Corticosterone	82.9	5.6	81.5	5.3
Cortisol	81.8	6.4	80.0	7.8
DOC	84.8	5.0	82.6	7.0
Pregnenediol	80.5	7.0	83.2	6.9
11β-hydroxyandrostenedione	81.5	7.3	82.9	7.8
L-thyroxine	84.8	5.7	78.4	8.5
Epitestosterone	81.0	6.9	78.2	8.0
Estrone	85.1	7.4	75.6*	11.9*
DHEA	76.5*	9.1*	72.4‡	10.6‡
Androsterone	66.6‡	12.2‡	69.3‡	9.8‡
17α-hydroxyprogesterone	64.1‡	10.4‡	68.8‡	9.5*
Estradiol	71.0‡	11.4‡	61.9‡	22.7‡
19-nortestosterone	72.2‡	14.6‡	61.0‡	23.8‡

DOC, deoxycorticosterone; DHEA, dehydroepiandrosterone.
Duplicate or triplicate determinations were performed at both concentrations, and the mean values were compared to the base line values for S.D. as reported in Table I.
* Indicates that the values are more than 2 SD outside these values.
‡ More than 3 SD.

that normal women have a greater percentage of recovered radioactive testosterone associated with β-globulins and a lesser percentage associated with inter-α-globulins than do normal men. This piece of information can be interpreted in several ways. The proper analysis is dependent on a knowledge of the relationship between the concentration of ligand and the concentration of binding sites. The fraction of steroid bound to protein is a function of three factors: the association constant, the concentration of binding sites, and the relative concentrations of ligand and protein. However, in the special situation in which there is a large excess of binding sites (i.e. protein), the *fraction* of steroid bound is dependent only on the association constant and the concentration of binding sites and is independent of the concentration of ligand. In other words we have defined a set of conditions in which variations in the concentration of steroid would not lead to a change in the fraction of bound material. If the concentration of steroid is increased sufficiently then, of course, there will be a decrease in the fraction bound. A more rigorous delineation of the foregoing follows from the discussion in the chapter on binding in reference 7.

With these factors in mind let us return to the possible explanations for the differences observed between the sexes in the binding of testosterone. For simplicity we will discuss only binding in the β-globulin region. The inverse of all the arguments holds for binding in the inter-α region. It is possible that women have a greater association constant than men. This would imply a difference in the protein between the sexes which does not alter the electrophoretic mobility. This is possible, and we cannot prove that such a difference does not exist. It is also possible that the same protein is present in both sexes but is present in higher concentration in women. We have no direct evidence on this point. Finally, the differences may be explained by the fact that men have a greater concentration of testosterone in their plasma than women. If the association constant(s) and the concentration of binding sites were the same in both sexes, the percentage of testosterone bound would be a function only of the relative concentration of protein and steroid. This is true only if the relative concentrations of steroid and protein are such that increasing the steroid concentration would lead to a decrease in the fraction bound in at least one of the sexes. If there were a large excess of protein in both sexes, and the aforementioned assumptions true, the binding in both sexes would be equal. The average concentration of testosterone in plasma is 6.6 ng/ml for men and 0.5 ng/ml for women.[5] This difference may be the main factor responsible for the differences observed in binding. Table III shows that when 10 ng/ml of testosterone are added to the plasma of a normal female, the pattern of binding is indistinguishable from that of the normal male who has had no exogenous steroid added to his plasma.

It is reasonable to conclude, therefore, that the differences in binding between normal males and females may in large part be attributed to differences in testosterone concentration. A low testosterone concentration may also explain the "female pattern" seen in the two male infants examined. The differences observed among individual males and females (Table I) are no doubt due to the simultaneous physiologic variation of the concen-

[5] These values were obtained by taking the weighted average for the concentration in blood reported from three different laboratories (8–10). A total of 47 male and 37 premenopausal females was reported.

tration of both steroid and proteins. The last two male subjects, F.G. and E.M. (Table I), must certainly have a lower concentration of binding sites among the β-globulins and a higher concentration of binding sites among the inter-α-globulins, since to explain their values for peaks II–IV solely by a difference in the concentration of endogenous testosterone, one would have to postulate a concentration in plasma of 50 ng/ml. These two subjects may be drawn from a different population than the remainder of the group or may represent one end of the physiologic spectrum.

In C.W., the intact male receiving diethylstilbesterol, the value for peak II is higher than that of all the normal females, and the value for peak III is among the lowest values seen in the normal females. To explain these findings in view of the factors discussed above one would postulate either a decrease in the concentration of testosterone, and (or) an increase in the concentration of binding sites among the β-globulins, and (or) a decrease in the concentration of binding sites among the inter-α-globulins. The daily administration by mouth of 1 mg of ethinylestradiol to normal men has been shown to decrease the mean plasma testosterone concentration from 8.4 to 2.0 ng/ml (11). Since this concentration of testosterone is still four times that of the normal female, i.e., 2.0 ng/ml compared to 0.50 ng/ml, one would expect the value of peak II in C.W. to be higher than that of the normal females and the value of peak III to be among the lowest of the normal females, only if there were also an increase in the concentration of binding sites among the β-globulins and (or) a decrease in the concentration of binding sites among inter-α-globulins.

The values for peak II in the three pregnant females were the highest we observed, and the values for peak III, the lowest. To explain these findings one would again postulate either a decreased concentration of testosterone, and (or) an increased concentration of binding sites among the β-globulins, and (or) a decreased concentration of binding sites among the inter-α-globulins. Rivarola, Forest, and Migeon have recently shown that the concentration of testosterone in the pregnant female is more than double that the nonpregnant female, i.e., 1.1 ng/ml compared to 0.50 ng/ml (12). If this increase in the concentration of testosterone were not accompanied by an increase in the concentration of binding sites, there would be either no change in the electrophoretic pattern or a slight decrease in binding among the β-globulin and an increase in the binding among the inter-α-globulins. Since one sees instead increased binding among β-globulins and decreased binding among the inter-α-globulins, there must be an increase in the concentration of binding sites among the β-globulins and (or) a decrease in the concentration of binding sites among the inter-α-globulins. Both pregnancy and exogenous estrogens seem to affect the concentration of binding sites for testosterone in plasma. This situation is not unique since both pregnancy and the administration of estrogens change the concentration of other binding proteins (13–15).

Although we have obtained data which indicate that certain steroids do or do not compete with testosterone for binding sites on the proteins, it would seem prudent not to draw firm conclusions as to the steroid configuration necessary for binding. For instance, in this system it is possible that there are steroids which are capable of competing with testosterone for binding sites on the proteins but which are strongly bound to the paper and therefore have their binding to the proteins partially or totally obscured. Conclusions regarding the steroid configuration necessary for binding should be made from data obtained under conditions of equilibrium.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Mr. John Kasch for the excellent technical assistance rendered during the course of this work.

This study was supported in part by research grant AM11852 from the National Institutes of Health.

Dr. Deakins is a fellow in medicine, The Roosevelt Hospital, supported in part by training grant TIAM-5531-02 from the National Institutes of Health.

REFERENCES

1. Pearlman, W. H., and O. Crepy. 1967. Steroid-protein interactions with particular reference to testosterone binding by human serum. *J. Biol. Chem.* **242**: 182.
2. Rosner, W., W. G. Kelly, S. M. Deakins, and N. P. Christy. 1967. The binding of estrogens and testosterone in human plasma. Proceedings of Symposium of Radioisotopes in Medicine—*In Vitro* Studies. A.E.C. Symposium Series (CONF-671111). In press.

3. Tavernetti, R. R., W. Rosenbaum, W. G. Kelly, N. P. Christy, and M. S. Roginsky. 1967. Evidence for the presence in human plasma of an estrogen-binding factor other than albumin: abnormal binding of estradiol in men with hepatic cirrhosis. *J. Clin. Endocrinol.* **27**: 920.
4. Cayen, M. N., and P. A. Anastassiadis. 1966. A simplified technique for the liquid scintillation measurement of radioactivity on paper chromatograms containing toluene-insoluble C^{14}- and H^3-labeled compounds. *Analyt. Biochem.* **15**: 84.
5. Smith, I. 1960. Chromatographic and Electrophoretic Techniques. Interscience Publishers, Inc., New York. 2nd edition.
6. Dixon, W. J., and F. J. Massey, Jr. 1959. Introduction to Statistical Analysis. McGraw-Hill Book Company, New York. 2nd edition.
7. Edsall, J. T., and J. Wyman. 1958. Some general aspects of molecular interactions. *In* Biophysical Chemistry. Academic Press, Inc., New York. 1st edition. 617.
8. Coppage, W. S., Jr., and A. E. Cooner. 1965. Testosterone in human plasma. *New Engl. J. Med.* **273**: 902.
9. Rivarola, M. A., and C. J. Migeon. 1966. Determination of testosterone and androst-4-ene-3, 17-dione concentration in human plasma. *Steroids.* **7**: 103.
10. Bardin, G. W., and M. B. Lipsett. 1967. Estimation of testosterone and androstenedione in human peripheral plasma. *Steroids.* **9**: 71.
11. Forchielli, E., G. S. Rao, I. R. Sarda, N. B. Gibree, P. E. Pochi, J. S. Strauss, and R. I. Dorfman. 1965. Effect of ethinyloestradiol on plasma testosterone levels and urinary testosterone excretion in man. *Acta Endocrinol.* **50**: 51.
12. Rivarola, M. A., M. G. Forest, and C. J. Migeon. 1968. Testosterone, androstenedione and dehydroepiandrosterone in plasma during pregnancy and at delivery: concentration and protein binding. *J. Clin. Endocrinol. Metab.* **28**: 34.
13. Russ, E. M., and J. Raymunt. 1956. Influence of estrogens on total serum copper and caeruloplasmin. *Proc. Soc. Exptl. Biol. Med.* **92**: 465.
14. Dowling, J. T., N. Freinkel, and S. H. Ingbar. 1956. Effect of diethylstilbesterol on the binding of thyroxine in serum. *J. Clin. Endocrinol. Metab.* **16**: 1491.
15. Slaunwhite, W. R., Jr., and A. A. Sandberg. 1959. Transcortin: a corticosteroid-binding protein of plasma. *J. Clin. Invest.* **38**: 384.