Studies of Plasma β-Globulin: Sex Difference and Effect of Ethinyl Estradiol and Testosterone[1] (34864)

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(Introduced by K. Lange)

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The binding of testosterone in plasma has been shown to reside in the β-globulin fraction (1–3). Doe *et al.* (4) found that estrogens increase the ceruloplasmin and plasminogen components of the β-globulin fraction. Dickinson *et al.* (5) however, were not able to demonstrate a significant effect of testosterone on these components or on the total β-globulins.

This study, in normal men and women, reports the presence of a distinct sex difference in the protein content of a β-globulin band, separated from plasma by starch gel electrophoresis. In addition, the administration of ethinyl estradiol and testosterone was found to significantly increase and decrease, respectively, this protein moiety.

Materials and Methods. Normal adult volunteers were used in this study. They consisted of 17 men, aged 21 to 32 years, and 19 women, aged 19 to 36 years. Three of the men received ethinyl estradiol and 5 of the women were given testosterone. The details of hormonal administration are given in Tables I and II.

Starch gel electrophoresis was carried out on plasma from 17 normal adult males and 19 normal adult females. Each plasma was diluted 1:2 with distilled water. Fifty μl of each diluted plasma were applied to slots in starch gel in a vertical electrophoresis apparatus. Electrophoresis was carried out at 4° for 20 hr using a constant voltage of 190. The electrode and starch gel buffers consisted of 0.3 *M* boric acid, 0.06 *M* sodium hydroxide, respectively. Aniline blue-black dye was added to one of the slots to identify the albumin band. When the dye traveled 12 cm the electrophoresis was discontinued. The strip containing the dye was cut from the rest of the starch gel and stained to identify the proteins. The β-globulin band was usually found 4.6 cm from the origin. A 15 mm segment containing only the β-globulin band was cut from each strip and the protein content was eluted. Each segment was cut into smaller pieces, transferred to a gooch-type crucible with medium fritted disc and frozen at −10° overnight. The next morning the contents of each crucible was centrifuged at 1300*g* for 10 min, eluted three times with 2 ml of water and the eluates were combined. Centrifugation was repeated after each addition of water. The protein concentration of the sample was determined by Lowry's method (6) using crystalline, A grade, bovine serum albumin as the standard. The coefficient of variation in 2 samples repeated five times was 4.5%.

Three normal male subjects were studied before and during the oral administration of ethinyl estradiol. Five normal women were studied before and during the intramuscular administration of testosterone. The medication and dosage schedules are detailed in Tables I and II.

Plasma testosterone was determined by competitive protein binding using a modification of the method of Mayes and Nugent (7). The percentage binding of testosterone in plasma was measured using the equilibrium dialysis method of Forest *et al.* (8) with

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TABLE I. The Effect of Ethinyl Estradiol (E-E_2) Administration on the Concentration of Protein in the β-Globulin Band and on the Percentage Binding of Testosterone in Plasma of Normal Men.

		Pretreatment			During treatment			
Subject	Medication (mg po daily)	β-Globulin (mg/100 ml)	PC^T[a] (μg/100 ml)	% Binding	Day	β-Globulin (mg/100 ml)	PC^T (μg/100 ml)	% Binding
RD	E-E_2, 0.2	268	0.652	89.7	21	410	0.102	98.3
KB	E-E_2, 0.2	261	0.726	86.6	21	441	0.079	96.1
MT	E-E_2, 0.2	286	0.532	90.8	21	389	0.036	98.5

[a] PC^T = plasma concentration of testosterone.

TABLE II. The Effect of Testosterone Administration on the Concentration of Protein in the β-Globulin Band and on the Percentage Binding of Testosterone in Plasma of Normal Women.

		Pretreatment			During treatment			
Subject	Medication[a] (mg im) Frequency	β-Globulin (mg/100 ml)	PC^T[b] (μg/100 ml)	% Binding	Day	β-Globulin (mg/100 ml)	PC^T (μg/100 ml)	% Binding
MG	TP, 5 Daily	321	0.08	93.1	10	185	0.76	89.1
MS	TP, 2.5 Daily	372	0.06	94.8	17	276	0.36	94.8
MA	TE, 200 q 5 d × 3 doses	418	0.04	94.2	13	309	1.30	87.1
AM	TE, 200 q 5 d × 3 doses	400	0.05	92.6	13	327	1.62	85.9
DH	TE, 100 q 5 d × 3 doses	449	0.06	94.8	11	343	1.71	90.3

[a] TP = testosterone propionate; TE = testosterone enanthate.

[b] PC^T = plasma concentration of testosterone.

the exception that the cellulose dialysis casings were pretreated with 0.1 *M* nitric acid for 24 hr followed by 0.01 *M* nitric acid for 48 hr and then washed with distilled water until neutral. In addition, 300 dpm of 4-^{14}C-testosterone (sp act 40 mCi/mmole) was used as a recovery indicator during the ether extraction of both the internal and external aliquots.

Results. The mean concentration of protein in the β-globulin band in 17 normal men and 19 normal women was 315 ± 53 (SD) and 391 ± 49 mg/100 ml, respectively. The difference between the 2 groups was highly significant ($p < 0.001$). The recovery of the protein in the β-globulin band after centrifugation and in each of the elutions was 53, 28, 15, and 4%, respectively. Additional elutions failed to yield any further significant amounts of protein. The amount of protein in the β-globulin band was corrected for a starch blank of 31 mg/100 ml. The protein values reported were due to the β-globulins uncontaminated by the αβ fraction.

The corresponding percentage binding of testosterone in plasma in the men (90.1 ± 1.1(SD)% and women(93.6 ± 0.8%) were also significantly different ($p < 0.001$).

The administration of ethinyl estradiol produced a significant increase in the concentration of protein in the β-globulin band and in the percentage binding of testosterone in plasma. The plasma testosterone concentration decreased from male to or towards female levels during administration of the hormone (Table I).

The adminisration of testosterone to 5 normal women produced a significant decrease in the concentration of protein in the β-globulin band and in the percentage binding of testosterone in plasma. The plasma concentration of testosterone increased to normal male and supraphysiologic male levels of the steroid during administration of the hormone (Table II).

Discussion. A sex related difference in the

concentration of protein in the β-globulin band was found, with normal women having significantly higher levels than normal men. Administration of the sex steroids markedly altered the concentration of protein in the β-globulin band. Ethinyl estradiol significantly increased this measurement in normal men while the administration of testosterone to normal women produced the opposite effect. These data suggest that the sex steroids are important determinants of the sex difference in the protein content of the β-globulins. Since the β-globulin band consists of a number of proteins including transferrin, hemopexin, fibrinogen, plasminogen, and testosterone binding protein it is not possible, using the present data, to ascribe the changes demonstrated to any particular component. However, it is well documented that women have a higher percentage binding of testosterone in plasma than men (8–10) and that the specific testosterone binding protein is a β-globulin. Moreover, administration of ethinyl estradiol and testosterone markedly increases and decreases, respectively, the percentage binding of testosterone in plasma. Thus, it might be speculated that the sex difference in the amount of protein in the β-globulin band may be related, in part, to differences in its content of testosterone binding protein.

The discrepancy between the finding of Dickinson *et al.* (5) and the present results may be related to differences both in the technique used for separation and quantitation of the protein moiety and to the sex of the study subject. Dickinson *et al.* (5) used paper electrophoresis to separate the β-globulin fraction which was then quantitated by densitometry. Starch gel electrophoresis provides a considerably better separation of the β-globulin band from the other proteins than does paper electrophoresis. Moreover, the elution of the protein band, in the present study, was stoichiometric and the quantitation of the protein was chemically precise. Densitometry, as used by Dickinson's group, can provide only relative measurements of the various protein moieties. In addition, normal men are perhaps not as suitable as women for the demonstration of the effects of testosterone since they are already exposed to large amounts of the circulating steroid.

Summary. A sex related difference in the concentration of protein in a β-globulin band, separated from plasma by starch gel electrophoresis, was found, with normal women (391 ± 49 (SD) mg/100 ml) having significantly higher levels than normal men (315 ± 53 mg/100 ml). Administration of estrogens to normal men significantly increased both the concentration of protein in the β-globulin band and the percentage binding of testosterone in plasma while the administration of testosterone to normal women produced the opposite effect. The findings suggest that the concentration of protein in the β-globulin band may be related, in part, to the effect of the sex steroids.

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