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ISOLATION, CHARACTERIZATION AND RADIOIMMUNOASSAY OF CORTICOSTEROID-BINDING GLOBULIN (CBG) IN HUMAN SERUM – CLINICAL SIGNIFICANCE AND COMPARISON TO THYROXINE-BINDING GLOBULIN (TBG)

By

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ABSTRACT

Isolation of the corticosteroid-binding globulin CBG was achieved by 5 chromatographical steps on cortisol Sepharose, QAE-Sephadex A-50, Con A-Sepharose and hydroxylapatite. The purity of the isolated CBG was demonstrated in polyacrylamide gel electrophoresis, SDS electrophoresis, immunodiffusion and ultracentrifugation. Microheterogeneity was shown in isoelectric focusing by 5 bands in the pH range of 3.7–4.2, which could be reduced to one major band after neuraminidase treatment. The equimolar binding of cortisol to CBG was demonstrated by binding studies. The association constant for cortisol was $2.8 \times 10^8\,\text{M}^{-1}$, for progesterone

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Abbreviations used in this paper:
CBG, corticosteroid-binding globulin; TBG, thyroxine-binding globulin.

$1.7 \times 10^6 M^{-1}$. From analytical ultracentrifugation, the molecular weight was calculated on 50 700; the sedimentation coefficient was 3.6 S, the partial specific volume 0.690 ml/g, the Stokes radius 38 Å and the frictional coefficient ratio 1.5.
A specific radioimmunoassay for CBG was established using the purified CBG for immunization, radioiodination and for calibration standards. The normal range of CBG levels in human serum was 2.4–4.4 mg/100 ml (mean ± 2 SD). Studies were performed to compare the levels of CBG and thyroxine-binding globulin (TBG). No sex differences but a significant biphasic age dependence were observed for both proteins. In pregnancy and under oestrogen treatment of women and men, CBG was demonstrated to be the more distinct indicator of oestrogenic activity as compared with TBG, whereas the sensitivity of TBG was more pronounced to supposedly antioestrogenic substances like Danazol, and in severe disease. No coincidence of genetic CBG and TBG deficiencies have been found so far.

In human serum, cortisol is bound to a specific transport protein, corticosteroid-binding globulin (CBG) or transcortin, with high affinity and low capacity which migrates on paper electrophoresis as an alpha$_1$-globulin. In addition cortisol is bound to albumin which has low affinity, but high capacity (*Daughaday* 1956*a,b; Slaunwhite & Sandberg* 1959; *Slaunwhite et al.* 1966; *Muldoon & Westphal* 1967).

The concentration of CBG was until now estimated by measuring the total binding capacity of serum for cortisol using gel filtration and equilibrium dialysis (*De Moor et al.* 1962; *Murphy & Pattee* 1963; *Westphal* 1971; *Schwartz & Hammerstein* 1975; *Angeli et al.* 1977), and recently more specifically by radial immunodiffusion (*Rosner et al.* 1973; *Racadot et al.* 1974; *Van Baelen & De Moor* 1974). There is major evidence that CBG has a buffer function for the biologically active free hormone fraction in blood (*Slaunwhite et al.* 1962; *De Moor et al.* 1963; *Sandberg & Slaunwhite* 1963), rather than an active transport function for steroid hormones to the target organ cell. But a possible role of proteins in hormone-receptor interactions is also discussed (*Westphal* 1971; *Werthamer et al.* 1973; *Wong et al.* 1973). The CBG concentrations in human serum have been estimated mainly under the aspect of oestrogen influence (*Sandberg & Slaunwhite* 1959; *De Moor et al.* 1962; *Doe et al.* 1964; *Schwartz & Hammerstein* 1975) and genetic deficiency variations (*Rosner et al.* 1973).

Recently, characteristic changes of the thyroxine-binding globulin (TBG) have been demonstrated under the influence of different metabolic conditions (*Horn et al.* 1977; *Horn & Gärtner* 1979). Therefore, it was investigated, whether parallelism in changes of these two different transport proteins, CBG and TBG could be detected.

For this purpose CBG had to be isolated from human serum and a specific method for its determination had to be established.

Reagents. – Pure cortisol was purchased from the Merck AG, Darmstadt, FRG, [^{3}H] cortisol (110 mCi/mg cortisol) from the Radiochemical Center in Amersham, England, and cortisol hemisuccinate from Sigma Chemical Company, St. Louis, USA. Sodium^{125} iodide (10 Ci/mg I) came from the Hoechst AG, Frankfurt, FRG. AH-Sepharose 4B for affinity chromatography, Con A-Sepharose, QAE-Sephadex A-50 for ion exchange chromatography and Sephadex G-10 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. The hydroxylapatite Biogel HTP was purchased from Bio-Rad Laboratories, Richmond, USA. Florisil, acrylamide and bovine gamma globulin were obtained from the Serva Biochemica, Heidelberg, FRG. Sodium Lauryl Sulphate (SDS) from Sigma Chemical Company, St. Louis, USA. Bovine albumin and alpha-methyl-D-mannoside from Roth, Karlsruhe, FRG, neuraminidase (clostridium perfringens) from Boehringer Mannheim GmbH. The following reagents came from the Merck AG, Darmstadt, FRG: N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide-hydrochlorid, polyethylenglycol 6000, complete Freund's adjuvant and all reagents (pro-analysis grade) for the preparation of the buffer solutions. Human transferrin and pertussis vaccine were obtained from the Behringwerke, Marburg, FRG. The human plasma anticoagulated with acid-citrate-dextrose (ACD) was freshly obtained from the blood bank and immediately used for preparation.

Isoelectric focusing. – Isoelectric focusing studies (*Radola & Graesslin* 1977) of isolated CBG were performed in slab gels (16 x 0.4 cm), using LKB equipment (LKB produkter, Bromma, Sweden). The concentrations of polyacrylamide and Ampholine® solutions pH 3.5–5.0 and pH 3.5–10 respectively (LKB produkter) were each 5 % (v/v). After isoelectric focusing (500 V, 20 W, 6 hours) gels were either stained using bromophenol blue or cut in slices of 0.5 cm. After elution with destilled water by diffusion pH-values were measured with a microelectrode, the CBG-concentrations by radioimmunoassay and [^{3}H]cortisol in a β-scintillation counter.

Isolation of CBG from human plasma

Affinity chromatography. – Using a modification of the method described by *Cuatrecasas* (1970), 2 g cortisol hemisuccinate (= 4.1 mmol) dissolved in 50 % dimethylformamide were covalently bound to 15 g AH-Sepharose 4B (= 0.48 mmol amino groups) by activation with 2.17 g carbodiimide (= 10 mmol) for 20 h at room temperature. The cortisol Sepharose was then washed alternatively with 1 M glycine and 0.2 M Tris-HCl buffer pH 8.6. From 5 liters human plasma the endogenous steroids were removed by 50 g florisil. Then, plasma and cortisol Sepharose were stirred for 30 min at room temperature and for 60 min at 4°C. The plasma was then filtered, the gel was washed with 1000 ml cold 0.2 M Tris-HCl buffer pH 8.0 and packed in a water-jacketed column. From this column CBG was eluted by elevation of the temperature up to 30°C with 20 mg cortisol hemisuccinate in 100 ml 0.16 M NaCl in 0.05 M Tris-HCl buffer pH 8.6.

Purification of CBG by column chromatography techniques

The following purification steps were performed at 4°C. The CBG peak from cortisol Sepharose was given on a QAE-Sephadex A-50 column (4.2 x 24 cm) which was equilibrated with 0.18 M NaCl in 0.05 M Tris-HCl buffer pH 8.6. After washing the gel with one column volume of starting buffer, CBG was eluted by the elevation of the NaCl concentration up to 0.22 M in 0.05 M Tris-HCl buffer pH 7.4 in a volume of

90 ml. This CBG peak was transferred on a Con A-Sepharose column (3.2×22 cm). The column was washed with one column volume of 0.05 M sodium phosphate buffer pH 7.4 and CBG eluted with 0.06 M alpha-methyl-D-mannoside in the same buffer. This CBG peak of 70 ml was dialyzed and concentrated in a collodion bag. The following chromatography was performed on a hydroxylapatite column (1.2×45 cm) equilibrated with 0.001 M sodium phosphate buffer pH 6.8. The same buffer was used for the elution step. The CBG peak was concentrated and transferred to a QAE-Sephadex A-50 column (2.2×45 cm), equilibrated with 0.19 M NaCl in 0.05 M Tris-HCl buffer pH 8.6. The final elution was performed with a linear gradient (400 ml) from 0.19 to 0.24 M NaCl in 0.05 M Tris-HCl buffer 8.6 in a volume of 75 ml. The CBG solution was repeatedly dialyzed in a collodion bag against aqua bidest., frozen, lyophilized and stored at $-20°C$. The CBG peaks in the eluates from each column were identified using a modification of the [^{3}H]cortisol-uptake-test on 5 ml Sephadex G-50 (*Horn et al.* 1975).

Radioimmunoassay of CBG

Immunization. – Rabbits were immunized with 120 μg CBG in complete Freund's adjuvant and 0.5 ml pertussis vaccine antigen using a multiple intradermal injection technique (50 sites) on the back flanks. They were boosted every three weeks with the same technique.

Radioiodination of CBG was done with the chloramine-T-method (*Greenwood et al.* 1963). The separation of the CBG tracer was performed only on a 1 ml Con A-Sepharose column. The CBG tracer was eluted with 0.06 M alpha-methyl-D-mannoside and diluted in 2 g/l bovine albumin in 0.05 M sodium phosphate buffer pH 7.4. The tracer could be used for 8 weeks without further purification.

For the radioimmunoassay 100 μl CBG standard solution or 1:100 diluted serum were incubated with 100 μl CBG tracer and 100 μl diluted CBG antiserum. The bound/free-separation could be performed by the polyethylene glycol precipitation (*Desbuquois & Aurbach* 1971) or the double antibody technique.

RESULTS

Criteria of purity and characterization

Electrophoretical methods. – The isolated CBG migrated on polyacrylamide gel electrophoresis and SDS electrophoresis (*Maurer* 1971) each performed in two different buffer systems pH 8.9 and pH 7.0 and three different gel concentrations (10, 7.5 and 5%) in one homogeneous band. Immunoelectrophoresis and Ouchterlony double diffusion test demonstrated a single precipitation line between CBG antiserum and CBG solution and normal human serum, respectively. The overlapping of both precipitation lines indicated identity between endogenous and isolated CBG.

Isoelectric focusing studies revealed a microheterogeneity of 5 different bands in the pH range of pH 3.7–4.2. In the same pH area CBG was detected in the radioimmunoassay as well as the radioactivity of [^{3}H]cortisol pre-incubated with CBG. Neuraminidase treatment of CBG reduced the micro-

heterogeneity to one major and one minor band in the pH range of pH 6.0 (Fig. 1).

Analytical ultracentrifugation. – The purity and the homogeneity of the isolated CBG could be demonstrated by a homogeneous curve in sedimentation velocity runs and by a straight line in the high speed sedimentation equilibrium runs. The sedimentation coefficient corrected to 20° C and water (*Schachmann* 1957; *Schachmann & Edelstein* 1966) was calculated to be 3.6 S. Considering a partial specific volume of 0.690 ml/g derived from the amino acid and carbohydrate composition (Table 1) a molecular weight (*Yphantis* 1964) of 50 700 ± 2500 (n = 4) for CBG was determined. The diffusion coefficient was calculated to be 5.6 D, the Stokes radius 38 Å and the frictional coefficient ratio 1.5.

Quantitative amino acid analysis in two different CBG preparations is summarized in Table 1. The high content of aspartic acid and glutamic acid explains the low isoelectric point of CBG. The carbohydrate composition is shown in the lower part of Table 1.

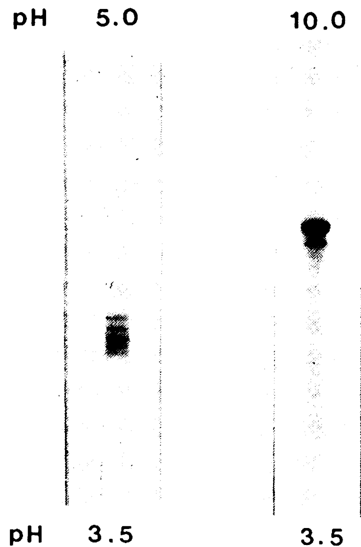

Fig. 1.
Isoelectric focusing of isolated CBG. Left: untreated CBG, pH gradient 3.5–5.0. Right: Desialylated CBG (0.03 U/ml neuraminidase, acetate buffer pH 5.6, 30 min) pH gradient 3.5–10.

Table 1.

Amino acid and carbohydrate composition of CBG. The amino acid analysis was performed with two different preparations of CBG, the carbohydrate analysis only with one preparation. Assuming a molecular weight of 50 700 the left column indicates the numbers of amino acid residues per mol CBG, the right column the percentage of the single amino acids of the polypeptid residue (molecular weight 32 950). The lower part of the table indicates the carbohydrate composition of the second preparation of CBG.

amino acids:	No. of residues/mol CBG		g of residue/100 g of polypeptid	
preparation	I	II	I	II
Lysine	15	15	6.7	6.7
Histidine	11	12	5.2	5.7
Arginine	10	10	5.3	5.3
Aspartic acid	35	37	14.1	14.9
Threonine	18	17	6.5	6.1
Serine	21	16	6.7	5.1
Glutamic acid	33	34	14.7	15.2
Proline	–	8	–	2.7
Glycine	11	10	2.5	2.2
Alanine	14	15	3.7	4.0
Cystine	–	–	–	–
Valine	18	21	6.4	7.4
Methionine	12	11	5.4	5.0
Isoleucine	15	18	5.9	7.1
Leucine	35	39	13.9	15.5
Tyrosine	13	9	7.1	4.9
Phenylalanine	23	25	11.8	12.8

carbohydrate composition in % by weight	II
Mannose	9.5
Galactose	5.3
Glucosamine	10.3
Sialic acid	10.0

Binding of steroid hormones to the isolated CBG

The binding of several steroids to CBG was investigated by two different methods, firstly equilibrium dialysis in micro-cells (Dianorm Apparatus, Diachemica AG, Switzerland) as the reference method and secondly, gel filtration on small columns with 2 ml Sephadex G–10 as a very simple method for estimation of the relative affinity constants of several steroids. The binding studies were performed in phosphate buffer pH 7.4 at 4° C. The incubation time for gel filtration was 15 min, the dialyzing time 8 h. Equilibrium dialysis was performed for four different steroids using firstly the corresponding tracer and secondly [^{3}H]cortisol in order to test for cross reactivity between the different steroids and cortisol tracer in CBG binding. There was a large measure of agreement between the calculated binding constants of each method (Table 2). Gel filtration resulted in lower association constants than equilibrium dialysis. This was due to the disturbance of equilibrium during the filtration on Sephadex G–10. The binding constant for cortisol was found to be approximately 8 times lower in gel filtration than in equilibrium dialysis. Utilizing this factor for the correction of the association constants of 17α-hydroxy-progesterone, 11-deoxycortisol, progesterone and testosterone, each calculated by displacement of [^{3}H]cortisol using gel filtration, the corrected

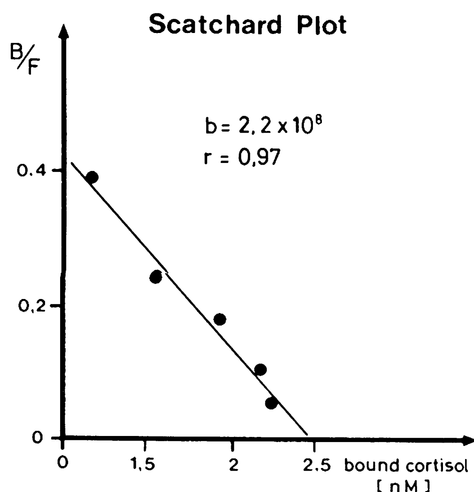

Fig. 2.

Determination of the association constant of cortisol and isolated CBG by means of Scatchard plot. Ordinate: ratio of CBG bound and free cortisol tracer. Abscissa: amount of bound cortisol, calculated as product of the percentage of [^{3}H]cortisol (B/T) and the molecular concentration.

Table 2.

Association constants of different steroids, determined by equilibrium dialysis using the corresponding tracer for each steroid (first column), equilibrium dialysis using [^{3}H]cortisol tracer only (second column), and gel filtration on Sephadex G-10 using [^{3}H]cortisol tracer only (third column).

For methodological details see text. In the right part of the table the molecular differences of the investigated steroids compared with cortisol are indicated.

affinity	steroid	association constants [M⁻¹]: equilibrium dialysis, Corresponding Tracer	equilibrium dialysis, Cortisol Tracer	gel filtration	molecular differences compared with Cortisol (pregnane structure): Δ1 dehydro	C_{17}	C_{11}	C_{21}	Esterified in C_{21}	Oxo in C_{11}	Methyl in C_6	Aldehyde in C_{18}	Δ4 Androstene structure	Fluorine in 9α	Estrogene Structure
	Cortisol	2.2×10^8	2.2×10^8	2.2×10^8											
high affinity	Prednisolone			5.4×10^7	•										
high affinity	Corticosterone			5.0×10^7		•									
high affinity	17α-Hydroxyprogesterone	5.3×10^7	4.9×10^7	2.8×10^7			•	•							
high affinity	11-Deoxycortisol	1.3×10^7	1.6×10^7	2.8×10^7			•								
high affinity	Deoxycorticosterone			2.2×10^7		•	•								
high affinity	Cortisol-21-succinate			1.6×10^7					•						
moderate affinity	Cortisone			7.2×10^6						•					
moderate affinity	Methylprednisolone			4.8×10^6	•						•				
moderate affinity	Progesterone	1.7×10^6	5.7×10^6	4.1×10^6		•	•	•							
moderate affinity	Prednisone			3.4×10^6	•					•					
moderate affinity	Aldosterone			2.6×10^6		•						•			
moderate affinity	Testosterone	2.5×10^6	2.9×10^6	1.6×10^6									•		
low affinity	9α-Fluorohydrocortisone			5.6×10^4										•	
low affinity	Estriol			2.4×10^4											•
low affinity	Estradiol			2.3×10^4											•
low affinity	Triamcinolone-acetonid			1.6×10^4										•	

no affinity: Dexamethasone, Ethinylestradiol, Carbenoxolone

values were in the same order of magnitude as found in the equilibrium dialysis (Table 2). The maximal binding capacity calculated by Scatchard analysis was found to be 715 μg cortisol per 100 mg CBG indicating an equimolar binding of CBG and cortisol (Fig. 2). By comparing the association constants with the molecular differences of several steroids to cortisol (Table 2) it might be supposed that the binding affinity decreased in dependence on the electron attraction and the size of the substitute.

Radioimmunological determination of CBG. – The antiserum with the highest titer of 1:160 000, determined by 50% tracer binding, was obtained after the third booster. The maximal tracer binding (specific activity: 31 μCi/μg CBG) was nearly 100%. The nonspecific binding without antiserum in the reaction mixture was in the range between 5 to 10%. Using an antiserum dilution of 1:3000 the limit of detection (3 SD from the zero standard) was 2 ng CBG per tube. The 50% intercept was 24 ng CBG per tube, the recovery of added CBG standard in serum was 100%, dilution curves of normal and pregnancy sera were found to be exactly on the calibration curve. The interassay variation coefficient was 7.4% (mean 3.3 mg/100 ml; n = 23).

There was no evidence for cross reactivity of CBG antiserum with alpha$_2$-macroglobulin, alpha$_2$-haptoglobin, alpha$_1$-antitrypsin and albumin in the Ouchterlony double diffusion test, cross reactivity of TBG was excluded in the radioimmunoassay. Cortisol in serum had no effect on the results of CBG determination.

Comparison of CBG and TBG levels in human serum

CBG levels in human serum. – In 40 control persons between the ages of 15–50 years the range of serum CBG was 2.4–4.4 mg/100 ml. CBG levels of patients with Cushing's syndrome (n = 4) and Addison's disease (n = 4) were

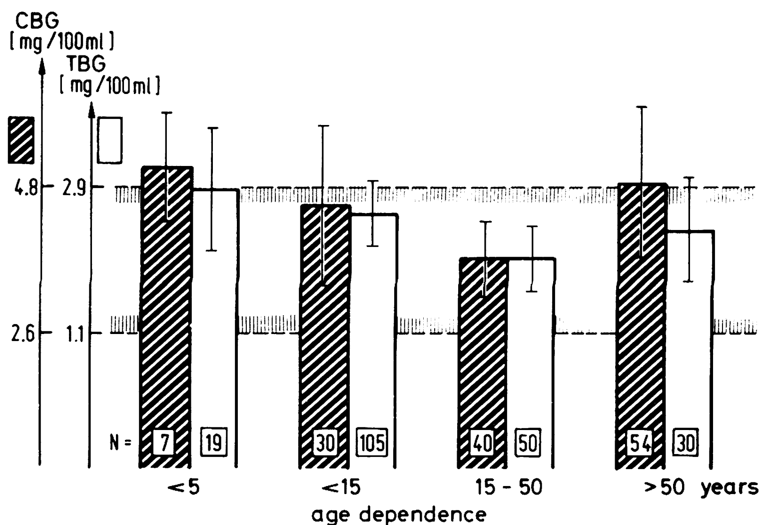

Fig. 3.
Age dependence of CBG and TBG levels. Hatched columns: CBG levels in mg/100 ml. Open columns: TBG levels in mg/100 ml. Hatched lines indicate the normal range of CBG and TBG in controls in the age of 15 to 50 years. I mean ± SD.

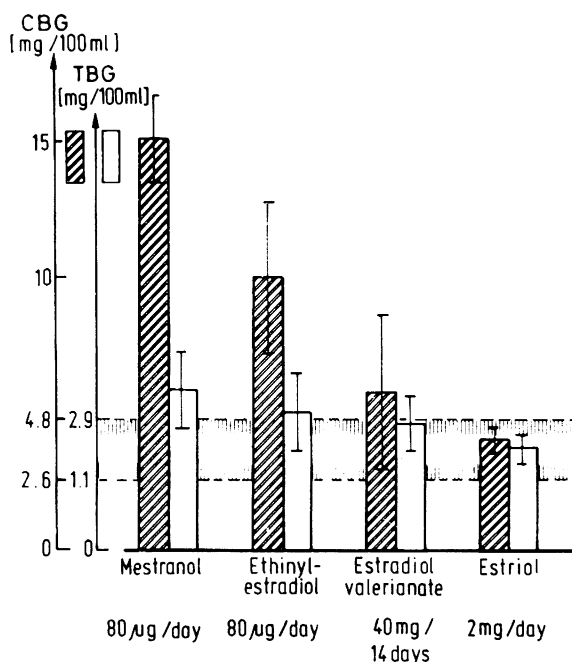

Fig. 4.

CBG and TBG levels of 4 women subsequently treated with 4 different oestrogen preparations. The hatched columns: CBG levels, the open columns: TBG levels. The hatched lines represent the normal ranges of CBG and TBG for the age from 15 to 50 years.

found in this range. In 134 healthy controls the CBG levels showed a biphasic age dependence of CBG levels (Fig. 3). Significant sex differences of CBG and TBG levels could not be ascertained in any period of life.

Oestrogen dependence of CBG and TBG. – In pregnancy CBG as well as TBG levels increased continuously and reached a plateau during the third trimenon (6.9 ± 0.24 mg/100 ml, $n = 16$).

The effect of exogenous oestrogens on CBG and TBG levels was investigated in four ovarectomized women (Fig. 4). The patients were subsequently treated with mestranol (80 µg/day), ethinyl oestradiol (80 µg/day), oestradiol valerianate (40 mg/14 days), and oestriol (2 mg/day), each preparation for four weeks.

Between the phases of oestrogen therapy, a period of placebo administration was inserted for 4 weeks. The response of CBG to oestrogens was more pronounced than the TBG increase. The maximal increase for both was observed after mestranol, whereas oestriol had no effect (Fig. 4). In 10 male

patients undergoing fosfestrol treatment (Honvan®) for a prostatic carcinoma, a dose-related elevation of CBG levels was observed. Both, CBG and TBG levels reached values of females treated with oestrogens, and again CBG was shown to be the more sensitive indicator of oestrogenic activity.

CBG levels in TBG deficiency states. – TBG deficiency was induced by treatment with Danazol (2,3-isoxazol-derivative of ethinyl-testosterone, Winthrop, Gießen, FRG). Fifteen women were treated with 400 mg Danazol/day for endometriosis, since Danazol is known to induce endometrium atrophy, and to suppress ovulation and midcycle peaks of gonadotrophins and oestradiol, whereas the mean basal values of LH and oestradiol remain constant (*Goebel & Rjosk* 1978). The TBG levels were decreased to 50 % of the initial values after four weeks of therapy, whereas the CBG levels did not change significantly even after 12 weeks (Fig. 5).

In 7 patients with severe chronic diseases such as decompensated liver cirrhosis and chronic heart failure, TBG levels were found to be decreased to 0.72 ± 0.23 mg/100 ml. This was interpreted as symptomatic TBG deficiency. In these patients the mean CBG levels were decreased as well to 2.6 ± 1.0 mg/100 ml. But the decrease of CBG was less pronounced than that of TBG.

In nine patients (8 men and 1 female) with genetic TBG deficiency (0.4 ± 0.3 mg/100 ml, ± SD), CBG levels were found to be in the normal range.

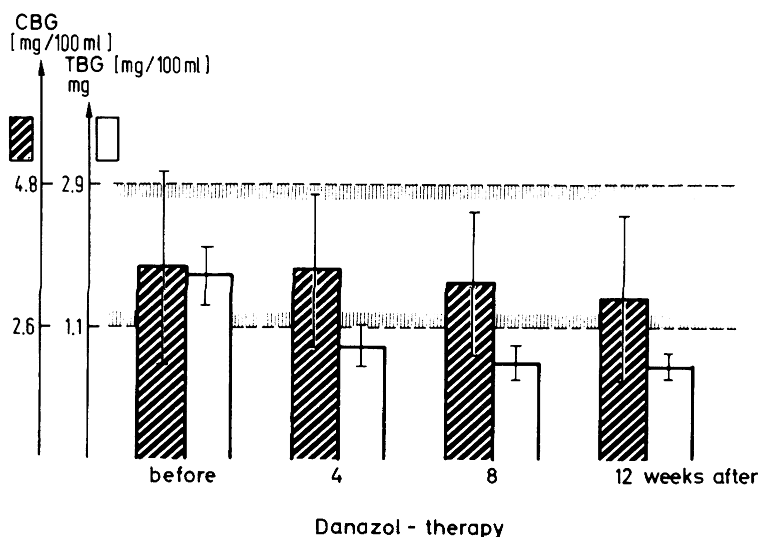

Fig. 5.

CBG and TBG levels during Danazol therapy. Hatched columns: CBG levels, open columns: TBG levels. The hatched lines represent the normal range of CBG and TBG for the age from 15 to 50 years.

DISCUSSION

The principle of affinity chromatography as described by *Cuatrecasas* (1970) and used at first by *Rosner & Bradlow* (1971) for the isolation of CBG was modified in our study.

Although *Rosner & Bradlow* (1971) performed the coupling of cortisol hemisuccinate in pure dioxane and *Le Gaillard et al.* (1974) thought the coupling in 50 % dimethylformamide not to be practicable, we found that the best results were obtained with the latter method. For displacement of CBG from the cortisol Sepharose, cortisol hemisuccinate addition to the elution buffer was preferred in order to avoid possible denaturation of CBG which may occur if more aggressive eluents are used.

The observation of *Rosner & Bradlow* (1971) who found only CBG and gamma globulins in the eluate of the affinity chromatography column could not be confirmed. Therefore, several additional chromatographic purification steps were required. The overall yield of 20% after 5 different preparatory steps was satisfactory. The purity of the isolated CBG was shown by a single band in overloaded polyacrylamide and SDS electrophoreses and by ultracentrifugation studies.

The properties of CBG ascertained in our laboratory agreed for the most part with the results of other authors. The molecular weight is reported to be in the range of 49 500 to 58 500 (*Slaunwhite et al.* 1966; *Muldoon & Westphal* 1967; *Le Gaillard et al.* 1975). By ultracentrifugation, we determined the value of 50 700, the sedimentation coefficient of 3.6 S and the partial specific volume of 0.690 ml/g, which corresponds to the values published by *Westphal* (1971). The hydrodynamic parameters particularly the frictional coefficient ratio suggest that CBG can still be regarded as a globular protein. The Stokes radius of the molecule is about 38 Å which corresponded well with other proteins with a molecular weight in this range. The association constants for cortisol and for other steroids partially obtained by two different methods agreed well with the literature (*Westphal* 1977; *Stroupe et al.* 1978). Only the affinity of progesterone to CBG was found to be lower (*Westphal* 1971).

The carbohydrate content of 35% by weight and the mean N-acetylneuraminic acid content of approximately 16 residues per mol isolated CBG was surprisingly high as compared with the literature (*Slaunwhite et al.* 1966; *Le Gaillard et al.* 1975; *Rosner* 1976).

After treatment with neuraminidase the microheterogeneity of CBG consisting of 5 single bands was focused to one major band into the alkaline direction. The residual more acidic minor band may be due to incomplete desialylation. Therefore the microheterogeneity of CBG may be due only to the different N-acetylneuraminic acid content as is already established for other glycoproteins and as recently shown for TBG (*Horn & Gärtner* 1979).

Radioimmunological quantitation of CBG in serum. – Although *Rosner et al.* (1973) and *Van Baelen & De Moor* (1974) preferred the subcutaneous or intramuscular application technique using a ten-times higher amount of CBG, a monospecific antiserum was obtained by the intracutaneous injection of approximately 120 μg CBG. As the precision and practicability of the radioimmunoassay was satisfactory, the method was preferable to the radial immunodiffusion technique (*Rosner et al.* 1973; *Racadot et al.* 1974; *Van Baelen & De Moor* 1974) for measuring CBG concentrations.

The CBG levels in serum of healthy adults corresponded well with the values estimated by the earlier published methods (*Westphal* 1971; *Rosner et al.* 1973; *Racadot et al.* 1974; *Van Baelen & De Moor* 1974; *Rosner* 1976).

CBG levels in normal controls. – Elevated binding capacities of CBG in serum have been observed in newborns and infants by several authors (*De Moor et al.* 1962; *Angeli et al.* 1977; *Wagner* 1978). These findings could now be ascertained by the direct CBG radioimmunoassay and in addition a further increase of CBG was seen in elder subjects. This biphasic age dependence was parallel with the TBG levels (*Horn et al.* 1977). Sex differences of CBG or TBG levels could not be ascertained in any period of life. These results were surprising with regard to the known oestrogen influence on the levels of both proteins, therefore apart from the well known oestrogen induced increase of both transport proteins supposedly other factors have an influence on CBG and TBG.

Oestrogen dependence of CBG and TBG levels. – The oestrogen induced increase of CBG and TBG levels, well known from earlier investigations (*Doe et al.* 1964; *Sandberg & Slaunwhite* 1959; *Ingbar* 1971; *Horn et al.* 1977; *Wagner* 1978), was now ascertained by the quantitative and specific radioimmunological determination. The investigation of sera in pregnancy and during oestrogen therapy of women and men demonstrated a more pronounced increase of CBG as compared with TBG. The increase is probably due to an augmented synthesis of this protein as the half-life is identical in controls and oestrogen treated persons (*Sandberg et al.* 1964). After therapy of four women with supposedly equivalent doses of four different oestrogen preparations, no increase of CBG and TBG was found after oestriol. As expected, the increase was significantly higher after ethinyloestradiol as compared with oestradiol valerianate. Surprisingly the most pronounced increase was induced by mestranol, which may be due to its hepatic metabolism (*Bird & Clark* 1973).

CBG levels in TBG deficiency states. – During Danazol therapy, only a decrease of the TBG levels was observed, while the CBG levels did not change. During the Danazol therapy, clinical signs of peripheral oestrogen deficiency were observed despite of normal oestradiol levels. There is possibly an inter-

ference of Danazol and oestrogens on liver cell receptors, which effects only TBG decreases. Likewise, the decrease of TBG levels in catabolic states was more pronounced than that of CBG. These differences in the behaviour of CBG and TBG under various hormonal and metabolic influences cannot yet be explained.

No coincident genetic defects of the two different transport proteins have been observed.

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