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5 The epidemiology and function of sex hormone-binding globulin

JOHN W. MOORE AND RICHARD D. BULBROOK

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I Introduction

Our interest in sex-hormone-binding globulin[1] (SHBG) arose because we were unable to demonstrate a convincing relationship between plasma oestradiol concentations and any aspect of the biology of human breast cancer. This was puzzling because indirect evidence from epidemiological studies strongly implicated ovarian function as a prime determinant of risk of the disease. In 1981, Siiteri, Hammond and Nisker claimed that women with breast cancer had abnormally high proportions of non-protein-bound oestradiol in their blood. Their concept was that an increase in the fraction of the blood oestradiol available for biological activity was of importance in the aetiology of the disease and since this fraction was largely controlled by the concentration of SHBG, the epidemiology of this protein became important.

It rapidly became apparent that the variation of SHBG concentrations between women was very large and it was obviously desirable to attempt to identify some of the causes of this variability. This was partly because of an

1. It was decided, by vote, at the First International Symposium on Steroid Hormone Binding Proteins, Lyons, France, April 1986, that this protein should be called either sex hormone-binding globulin (SHBG) or sex steroid-binding protein (SBP). It was also agreed that other names such as testosterone-estradiol binding globulin (TeBG) or steroid binding β-globulin (SBβG) should no longer be used.

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interest in the biology of the protein but mainly because it had been suggested that assays of SHBG might be a useful means of identifying women at risk of breast cancer who could then be treated with anti-oestrogenic drugs in a trial of prevention (Cuzick, Wang, and Bulbrook 1986). These considerations led to a massive epidemiological study in which serum SHBG was measured in a population of nearly 5000 women.

In order to interpret data from such a study, many factors had to be taken into account including the effects of drug administration, disease and physiological conditions on SHBG concentration as well as fundamental questions concerning the role of blood steroid-binding proteins and the availability of sex-steroids to target organs. Some of these questions have been considered in the two extensive monographs by Westphal (1971; 1986) and in reviews by Anderson (1974), Heyns (1977), Wagner (1978) and Lobl (1981). From our reading of the recent literature it became clear that there continues to be a keen interest in sex-steroid binding proteins and exciting developments have occurred in many areas. For this reason, in our review, we have tried to encompass a fairly broad spectrum of the literature on SHBG. Areas to be covered include an examination of current concepts of how blood steroid-binding proteins determine cellular availability and metabolic clearance of sex steroids, recent advances in the characterization and measurement of SHBG, physiological and pharmacological factors affecting SHBG activity and finally an examination of SHBG in disease states in the human.

The study of binding of sex steroids to blood proteins began in the early 1950s with the extensive investigation of the interactions of steroids with albumin. In 1958, Daughaday and Kozak discovered an α-globulin, subsequently called corticosteroid-binding-globulin (CBG), with a high affinity for steroids bearing the C-21 side chain such as cortisol and progesterone and a lower affinity for testosterone. Direct evidence for a testosterone-binding protein, distinct from CBG and albumin was obtained by Mercier, Alfsen, and Baulieu (1966) and Pearlman and Crepy (1967). It was shown shortly after this that oestradiol binds to the protein at the same site as testosterone (van Baelen, Heyns, Schonne, and De Moor 1968; Murphy 1968). For extensive reviews of these early studies the reader is referred to Westphal (1971), Anderson (1974) and King and Mainwaring (1974).

The site of synthesis of SHBG is assumed to be the liver and there is indirect evidence to support this. Thus hepatoma cells (Hep G2) in culture secrete a protein which is indistinguishable from SHBG (Kahn, Knowles, Aden, and Rosner 1981) and immunofluorescent studies have shown that monkey and human hepatocytes are specifically labelled after incubation with anti-human SHBG antiserum (Bordin and Petra 1980; Mercier-Bodard and Baulieu 1986). Plasma proteins to which sex steroids bind with high affinity have been found in most species. In human and other primates, SHBG binds naturally occurring 17β-hydroxy-androgens and oestradiol whereas in other animals, for example, the cat, dog and rabbit, they are

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mainly androgen binding proteins. Adult rats, mice and guinea pigs do not have SHBG. For a summary of the literature on SHBG in non-human mammals and non-mammalian vertebrates see Westphal (1986).

II Blood binding proteins and compartmentalization of sex steroids

1 SHBG AND ALBUMIN

In human blood, the most biologically active sex steroids, 5α-dihydrotestosterone, testosterone and oestradiol circulate in the non-protein-bound form or in association with SHBG, albumin and possibly other proteins (see **II**, 2). Albumin binding of steroids is of low affinity, with association constants (Ka) in the region of 10^4 M^{-1}, but of enormous capacity because of the high concentration of the protein (0.6–0.8 mmol/l) and the large number of binding sites on the molecule for some steroids. Steroid binding to albumin usually follows the polarity rule with less polar steroids, such as oestradiol, binding with higher affinity than those of greater polarity such as dihydrotestosterone and testosterone.

SHBG, on the other hand, is present in the blood at much lower concentrations than albumin and has one binding site of high affinity and specificity for 17β-hydroxy steroids (section **III**). The association constants for the binding of sex steroids to SHBG are in the region of $10^9 M^{-1}$ and there is general agreement that the Ka for dihydrotestosterone binding to SHBG is approximately three times greater than that of testosterone which, in turn, is about twice that of oestradiol. For extensive details of steroid-protein binding see the monographs by Westphal (1971, 1986).

The factors which determine the blood distribution of steroids between the free compartment and the various binding proteins are very complex and involve the association and dissociation constants for steroid-protein binding as well as the concentrations of interacting molecules. From some of these variables, Dunn, Nisula, and Rodbard (1981) have calculated the theoretical distribution of most of the naturally occurring steroids between the free and protein-bound compartments.

Table 5.1 shows the distribution of oestradiol, testosterone and 5α-dihydrotestosterone, experimentally determined in our laboratory. It can be seen that testosterone and 5α-dihydrotestosterone are bound more to SHBG than to albumin whereas in the case of oestradiol, there is greater binding to albumin. This reflects the greater affinity of oestradiol for albumin and its lower affinity for SHBG compared with testosterone and 5α-dihydrotestosterone.

The percentages of free (non-protein-bound) oestradiol shown in Table 5.1 are lower than those calculated by Dunn *et al.* (1981) for men and women. They are also lower than the experimentally determined results of Burke and Anderson (1972) and our own previous data for normal women which were

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Table 5.1

Mean (± SD) percentages of free, SHBG-bound and albumin-bound 17β-OH steroids in normal male and female sera

	Male				Female			
Steroid	n	Free (%)[1]	SHBG bound (%)[2]	Albumin bound (%)[2]	n	Free (%)[1]	SHBG bound (%)[2]	Albumin bound (%)[2]
Testosterone	10	1.73 ± 0.32	59.9 ± 9.3	38.3 ± 9.0	10	1.18 ± 0.20	71.9 ± 4.6	27.0 ± 4.4
Oestradiol	10	1.21 ± 0.11	33.6 ± 5.6	65.2 ± 5.5	10	0.96 ± 0.14	39.8 ± 9.7	59.2 ± 9.5
Dihydro-testosterone	10	1.42 ± 0.26	74.3 ± 5.2	24.3 ± 4.9	10	1.18 ± 0.21	80.2 ± 4.7	18.6 ± 4.5

SHBG concentrations: Male = 28.4 ± 9.3 nmol/l, Female = 50.4 ± 21.6 nmol/l.
[1] Measured by centrifugal-ultrafiltration-dialysis (Hammond, Nisker, Siiteri, and Jones 1980) as modified (Moore, Hoare, Quinlan, Clark, and Wang 1987). [2] Calculated from the percentage of free steroid in serum, heat-treated (60°C/60 min) to denature SHBG (see Siiteri, Murai, Hammond, Nisker, Raymoure, and Kuhn 1982; Hammond, Lähteenmäki, Lähteenmäki and Lukkainen 1982).

obtained using centrifugal-ultrafiltration-dialysis as originally described (Moore, Clark, Bulbrook, Hayward, Murai, Hammond, and Siiteri 1982). The disagreement with our previous data is because we have now improved the methodology and the interested reader is referred to our recent publication for technical details (Moore, Hoare, Quinlan, Clark, and Wang 1987).

Our results in serum from non-pregnant females are very similar to those of Dowsett, Mansfield, Griggs, and Jeffcoate (1984) who also found percentages of free oestradiol in the region of 1 per cent. The very low levels of steroid hormones in saliva are thought to be equal in concentration to the non-protein-bound steroid levels in serum (Riad-Fahmy, Read, Walker, and Griffiths 1982) and it has recently been shown, by direct radioimmunoassay, that oestradiol concentrations in samples of saliva from premenopausal women are about 1 per cent of those of serum (Wang, Fantl, Habibollahi, Clark, Fentiman, Hayward, and Bulbrook 1986) which lends support to our findings.

Within the physiological range of SHBG values found in male, female and pregnancy sera, highly significant negative correlations between the percentages of free testosterone and oestradiol and the concentration of SHBG are observed. Our non-protein-bound testosterone and oestradiol data from Table 5.1 are plotted against the concentrations of SHBG (see Fig. 5.1). As shown by Burke and Anderson (1972) changes in the concentration of SHBG have a much greater effect on the percentage of free testosterone than they have on free oestradiol. When the relationship between SHBG and the percentages of albumin-bound oestradiol and testosterone are considered, however, it is apparent that the calculated regression lines, though different in elevation, are similar in shape over the SHBG concentration range studied

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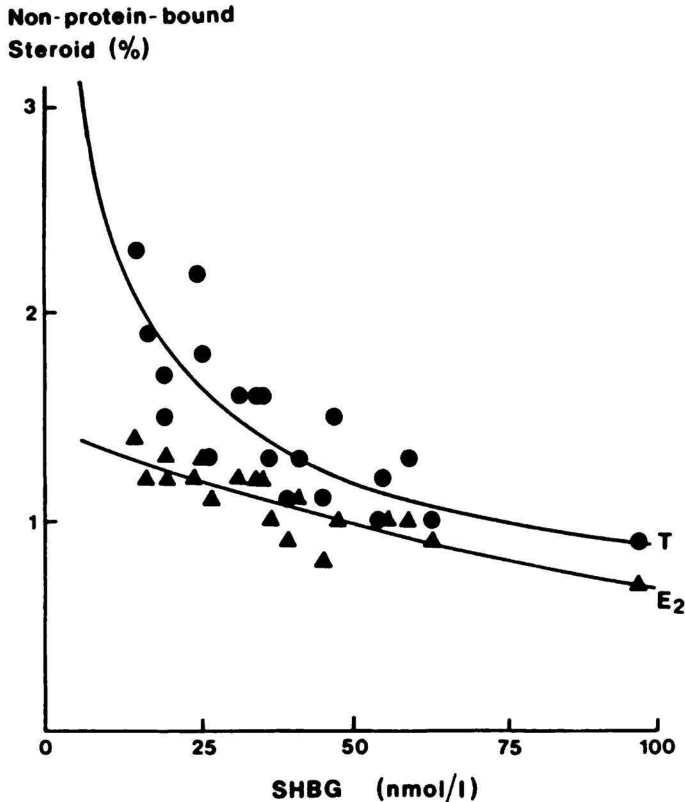

Fig. 5.1. Relationship between SHBG and the percentages of non-protein bound oestradiol (E2) and testosterone (T) in human serum. Calculated linear regression equations: log non-protein bound T (%) = 1.89–0.436 log SHBG (nmol/l); log non-protein bound E2 (%) = 0.358–0.00746 SHBG (nmol/l).

(Fig. 5.2). Changes in SHBG, therefore, result in similar percentage changes in the albumin-bound fractions of oestradiol and testosterone. Thus, in this study group, the percentages of albumin-bound oestradiol and testosterone are about 25 per cent higher at the low end of the SHBG range compared with those at the high end.

The relationship between the percentage of SHBG-bound steroids and the amount of SHBG present in the sample is the converse of that seen for albumin-bound steroids and SHBG concentration. The small changes in concentration of albumin, within the physiological range, do not affect the distribution of steroids because of the low affinities of the steroid-albumin interactions (Moore *et al.* 1982).

2 OTHER SEX STEROID-BINDING PROTEINS IN HUMAN BLOOD

SHBG and albumin are not the only binding proteins for sex-steroids in the

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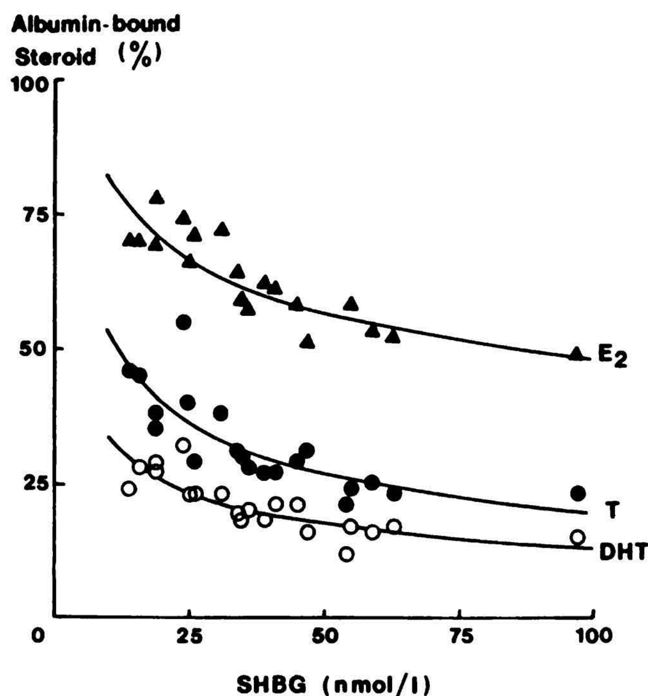

Fig. 5.2. Relationship between SHBG and the percentages of albumin-bound (Alb-Bound) oestradiol (E2), testosterone (T) and 5α-dihydrotestosterone (DHT) in human serum. Calculated linear regression equations: log Alb-Bound E2 (%) = 4.96–0.24 log SHBG (nmol/l); log Alb-Bound T (%) = 4.99–0.44 log SHBG (nmol/l); log Alb-Bound DHT (%) = 4.42–0.41 log SHBG (nmol/l).

human circulation. Others include orosomucoid (α_1-acid glycoprotein), oestradiol-binding protein, corticosteroid-binding globulin (CBG), fetal steroid-binding protein and fetal steroid-binding glycoprotein.

Orosomucoid binds progesterone, testosterone and androstenedione with affinities about ten times higher than albumin, whereas oestradiol binds with an affinity similar to that of its binding to albumin. The protein has only one binding site for most of the steroids studied. The concentration of orosomucoid in serum, less than 25 μmol/l, is very much lower than that of albumin and therefore its importance in determining the distribution of steroids in the blood is small (see Englebienne 1984; Westphal 1986).

Oestradiol-binding protein, another protein distinct from SHBG, which has a high affinity and low capacity for oestradiol but which does not bind 5α-dihydrotestosterone or testosterone significantly, was identified in human serum by O'Brien, Higashi, Kanasugi, Gibbons and Morrow (1982). It is suggested that there is a relationship between oestrogen status and synthesis of this protein since concentrations range from 0.26 nM in male blood to 2 nM in blood from females and it is reported that in pregnancy the levels are higher. Concentrations of oestradiol-binding protein are very much less than those of SHBG (see **III**, 3) and its importance remains to be determined.

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CBG in the human and several other species shows some affinity for testosterone ($Ka = 1.4 \times 10^6\ M^{-1}$ in the human) but no significant binding to oestradiol. Although in the adult mouse and rat, where SHBG is absent, it may be an important testosterone-binding protein, it is unlikely that binding of testosterone to CBG is particularly extensive in the human (see Dunn *et al.* 1981; Westphal 1986).

Fetal steroid-binding protein (FSBP) was recently discovered in cytosolic preparations of human fetal liver because, unlike SHBG, it is immobilized by the cibachrome blue-agarose (blue gel) portion of a two-tier column described by Iqbal and Johnson (1977). 5α-Dihydrotestosterone, testosterone and oestradiol bind to FSBP with high affinity and in this regard the protein shows similarities to SHBG. However, unlike SHBG, the protein does not bind 5α-androstane-3β, 17β-diol specifically and appears to differ from SHBG immunologically and in molecular weight (Wilkinson, Iqbal, and Williams 1983; Iqbal, Wilkinson, and Williams 1983; Iqbal, Forbes, Wilkinson, Moore, Williams, and Bulbrook 1987).

Another binding protein distinct from SHBG, with high affinities for sex-steroids was identified in cord blood and amniotic fluid by Dalton (1984) and termed fetal steroid binding globulin (FiSBOG). It shares some steroid binding characteristics with FSBP and is found in human blood only in late pregnancy where the reported mean level was 11.15 nmol 5α-dihydrotestosterone bound/l. Further investigation of FSBP and FiSBOG is required. It is possible that they may be related proteins and could also be variants of SHBG.

III Purification, characterization and measurement of SHBG

1 PURIFICATION OF SHBG

Attempts at purification of SHBG were first undertaken by Mercier-Bodard, Alfsen, and Baulieu (1970) who were able to partially purify the protein. SHBG was first purified to homogeneity by Mickelson and Petra (1975) using an affinity matrix in which 5α-dihydrotestosterone was linked to agarose through the 17β position. Yields were very low, however, and the introduction of affinity matrices in which the steroidal 17β-hydroxyl group were exposed (Rosner and Smith 1975; Iqbal and Johnson 1979) did not much alter the situation.

The most dramatic improvement in yield came with the introduction of affinity adsorbents in which derivatives of 5α-dihydrotestosterone were coupled to the supporting media through the 17α position leaving both the 3-oxo function and the 17β-hydroxyl group free to interact with SHBG (Suzuki, Itagaki, Mori, and Hosoya 1977; Petra and Lewis 1980; Cheng, Musto, Gunsalus, and Bardin 1983). Further refinements in technique have made it possible to isolate milligram quantities of SHBG from serum within 48 hours on a routine basis by incorporating these types of chromatographic

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methods into the purification procedures (Khan, Ehrlich, Birken, and Rosner 1985; Hammond, Robinson, Sugino, Ward, and Finne 1986; Petra, Namkung, Senear, McCrae, Rousslang, Teller, and Ross 1986*a*).

2 CHARACTERIZATION AND STRUCTURE

Native SHBG is considered to be a homodimeric glycoprotein with one steroid binding site (Petra, Stanczyk, Senear, Namkung, Novy, Ross, Turner, and Brown 1983; Hammond *et al.* 1986; Petra, Kumar, Hayes, Ericsson, and Titani 1986). A comparison of the physical characteristics of SHBG from the rabbit, baboon, macaque and human reveal that the protein in each species has similar molecular weights (ca 85,000 Daltons) and the same dimeric molecular organization although they differ in carbohydrate content (Petra *et al.* 1986*a*; Petra, Namkung, Titani, and Walsh 1986*b*).

The amino acid sequence of the purified protein has been largely elucidated (Walsh, Titani, Takio, Kumar, Hayes, and Petra, 1986). Hammond *et al.* (1986) have sequenced the amino (N)-terminal of the protein and obtained the same sequence as Walsh and colleagues but observed an additional N-terminal leucine residue. Walsh *et al.* (1986) suggest that their failure to detect this residue was due to its proteolytic removal during storage of serum prior to purification. This probably also accounts for previously revealed heterogeneity at the N-terminus (Fernlund and Laurell 1981; Petra *et al.* 1983). Although affinity labelling and X-ray diffraction analysis will be needed to provide proof, Petra *et al.* (1986*b*) have identified an internal amino acid sequence repeat within a hydrophobic region which they suggest may be the steroid binding site of the protein.

There is no sequence similarity between human SHBG and either the human oestradiol and glucocorticoid receptors or with the oncogene product of the erythroblastosis virus (v-*erb*A) with which the receptor proteins share some homology (Hollenberg, Weinberger, Ong, Cerelli, Oro, Lebo, Thompson, Rosenfeld, and Evans 1985; Weinberger, Hollenberg, Rosenfeld, and Evans 1985; Green, Gilna, Waterfield, Baker, Hort, and Shine 1986). The amino acid sequences of the rat androgen-binding protein (Joseph, Hall, and French, 1986; 1987) show many regions of homology including the putative binding site (Petra, Titani, Walsh, Joseph, Hall, and French 1986).

3 MEASUREMENT OF SHBG

Until the late 1970s, the measurement of SHBG in serum or plasma has relied upon the determination of the binding capacity of the protein by saturation of high affinity binding sites with tritium or ^{14}C labelled 17β-hydroxy steroids followed by the separation of specifically bound from non-specifically bound and free label. Many methods of separation have been employed and these include equilibrium dialysis (Vermeulen, Stoica, and Verdonck 1971), ammonium sulphate precipitation (Rosner, 1972; Anderson, Lasley, Fisher,

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Shepherd, Newman, and Hendrickx 1976), DEAE filter paper adsorption (Mickelson and Petra 1974), agar gel electrophoresis (Wagner 1978), charcoal separation (Hammond and Lähteenmäki 1983) and affinity chromatography (Iqbal and Johnson 1977; Nisula, Loriaux, and Wilson 1978; Bruning, Bonfrer and Nooyen 1985). This methodology has recently been reviewed (Englebienne 1984; Rosner 1986).

Following the isolation of purified preparations of SHBG and the production of specific antibodies (Bordin, Lewis, and Petra 1978), several antibody-based assays have been established which allow quantitation of the concentration (in contrast to the binding capacity) of the protein. These include "rocket" immunoelectrophoresis (Laurell and Rannevik 1979), radioimmunoassay (Mercier-Bodard, Renoir, and Baulieu 1979; Kahn, Ewan, and Rosner 1982; Cheng, Bardin, Musto, Gunsalus, Cheng, and Ganguly 1983; Maruyama, Aoki, Suzuki, Sinohara, and Yamamoto 1984; Lapidus, Lindstedt, Lunberg, Bengtsson, and Gredmark 1986), enzyme-linked immunoassay (Bordin, Torres, and Petra 1982), immunoprecipitation (Degrelle 1986) and immunoradiometric assay (Hammond, Langley, and Robinson 1985). Since it is established that there is one steroid binding site per mole of SHBG, binding capacity measurements are equivalent to concentrations of the protein measured by immunological techniques so for the sake of simplicity, in this review, we have not distinguished between binding capacities and amounts of SHBG except where such a distinction would be instructive.

With so many methods of analysis available it is inevitable that some variation in the ranges of SHBG in comparable physiological situations occurs when different methods are used or when the same method is employed in different laboratories. As discussed by Rosner (1986) this disagreement has many reasons, a major one being the lack of a standard preparation of SHBG with which to calibrate individual assays. Table 5.2 shows "normal ranges" obtained with some of the more widely used methods.

One major advantage of the immuno-type of assay compared with the binding capacity assay is that sensitivity is much greater and this has allowed the detection of SHBG, with ease, in various body fluids where concentrations of the protein are much lower than those found in serum or plasma. SHBG has been found in amniotic fluid, ovarian follicular fluid, breast cyst fluid and saliva (Hammond, Leinonen, Bolton, and Vihko 1983; Rosner, Kahn, Breed, Fleisher, and Bradlow 1985; Ben-Raphael, Mastroianni, Meloni, Lee, and Flickinger 1986; Hammond and Langley 1986). Another advantage is that larger numbers of samples can be assayed easily.

IV Significance of SHBG

Although SHBG has been called a transport protein, sex steroids, at the concentration found in the blood are fully soluble and do not require a high

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Table 5.2

SHBG binding capacities and concentrations (nmol/l ± S.E.M.) in male, female, and pregnant female blood

Reference	Male	Female	Pregnancy	Assay types	Separation method
Rosner (1972)	32 ± 2 (27)	64 ± 4 (16)	427 ± 23 (19) (stage not specified)	Binding capacity (^{3}H-DHT)	
Anderson, Lasley, Fisher, Shepherd, Newman, and Hendrickx (1976)	35 ± 2 (23)	74 ± 9 (40)	367 ± 21 (24) (at term)	Binding capacity (^{3}H-DHT	Ammonium sulphate precipitation
Rudd, Duignan, and London (1974)	50 ± 4 (10)	78 ± 12 (12)	290 ± 38 (10) (third trimester)	Binding capacity (^{14}C-T)	
Iqbal and Johnson (1977)	26 ± 6 (13)	60 ± 6 (18)	—	Binding capacity (^{3}H-DHT)	Affinity chromatography
Hammond, Langley, and Robinson (1985)	23 ± 2 (20)	53 ± 4 (32)	402 ± 77 (5) (third trimester)	Immunoradiometric assay	—
Cheng, Bardin, Musto, Gunsalus, Cheng, and Ganguly (1983)	18 ± 3 (12)	54 ± 5 (8)	115 ± 24 (4) (weeks 10–15) 212 ± 17 (4) (weeks 20–25) 374 ± 22 (6) (weeks 35–40)	Radioimmunoassay	—

^{3}H-DHT = [^{3}H]-5α-dihydrotestosterone, ^{14}C-T = [^{14}C]-testosterone, Numbers of subjects shown in parenthesis.

affinity binding protein for this purpose (Anderson 1974). The conventional view of steroid uptake by cells enunciated by Tait and Burstein (1964) is that only the free fraction of steroid is transported across the cell membrane. Protein-bound steroid in a capillary must first dissociate from the protein before it becomes available for uptake. In recent years, Pardridge and his colleagues have intensively investigated factors such as cell membrane permeability, capillary transit time and steroid-protein dissociation rates which affect the availability of steroids to organs such as the brain, liver and uterus. They have concluded that, in general, the free and albumin-bound fractions of testosterone and oestradiol are available for uptake by most tissues and that in the liver even some of the SHBG bound fractions may be available (see Pardridge 1981 and also this volume).

It is apparent that since the amount of SHBG in the blood undoubtedly controls the proportion of free oestradiol and testosterone and also the proportions which are bound to albumin, it is of profound importance in controlling the hormonal stimulus to responsive cells, if the conventional dogma of steroid uptake is correct. Evidence from studies in the human and non-human primates that the SHBG-bound steroid is generally not available for uptake comes from metabolic and *in vitro* studies which will be briefly reviewed.

1 INFLUENCE OF SHBG BINDING ON THE METABOLIC CLEARANCE RATE OF SEX STEROIDS

Indirect evidence that specific binding to proteins protects steroid hormones from metabolism came from early studies on the metabolic clearance rate (MCR) of testosterone. Serum SHBG is higher in adult females than in adult males and the MCR of testosterone in normal women is lower than in normal men. In pregnancy, when SHBG levels are high, the MCR of testosterone is diminished compared to that of non-pregnant women. Hirsute women, who often have low SHBG capacities, tend to have higher MCR for testosterone than do normal women (Bardin and Lipsett, 1967; Vermeulen, Verdonck, Van der Straeten, and Orlie 1969; Vermeulen and Ando 1979).

Highly significant correlations between the percentages of free and non-SHBG bound testosterone and also 5α-dihydrotestosterone and the metabolic clearance rates of these steroids were demonstrated in normal males and postmenopausal females (Vermeulen and Ando 1979). Since the percentages of free and non-SHBG bound steroids in the blood are dependent on the SHBG activity, negative correlations between the MCRs of sex-steroids and SHBG binding capacity would be expected. This has been confirmed for testosterone and oestradiol in postmenopausal and perimenopausal women (Siiteri, Murai, Hammond, Nisker, Raymoure, and Kuhn 1982; Longcope, Hui, and Johnston 1987).

In hyperthyroid patients, where SHBG capacities are usually elevated

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(Anderson 1974; Ridgway, Longcope, and Maloof 1975), the metabolic clearance rates of testosterone and oestradiol are diminished (Gordon, Southren, Tochimoto, Rand, and Olivo 1969; Gordon, Olivo, Rafii, and Southren 1975; Ridgway *et al.* 1975) whereas those of oestrone and androstenedione, which do not bind to SHBG, are normal (Ridgway, Maloof, and Longcope 1982). This implies an important role for SHBG in controlling the metabolic clearance of the two former steroids.

Direct experimental evidence demonstrating a role for SHBG in regulating the metabolic clearance rate of testosterone in non-human primates was provided by Petra and his colleagues (Petra, Stanczyk, Namkung, Fritz, and Novy 1985; Stanczyk, Namkumg, Fritz, Novy, and Petra 1986) who infused pure human or rhesus SHBG into female rhesus macaques and observed increases of between 150 and 300 per cent in the levels of SHBG which was accompanied by a decrease of about 10 per cent in the metabolic clearance rates. In further experiments they infused purified antibodies to human SHBG, which cross-reacted with macaque SHBG, and observed a steady increase in metabolic clearance with decreasing SHBG levels. Using equations derived from the law of mass action they calculated the distribution of testosterone between SHBG and albumin and were able to show that as long as the percent of testosterone bound to SHBG was equal to or higher than that bound to albumin, the effect on the clearance rate was small. A dramatic increase in metabolic clearance of testosterone occurred, however, if SHBG levels were reduced to the extent that testosterone was mostly bound to albumin.

It must be said that the small fall in MCR of testosterone which accompanied quite a large rise in SHBG in the monkey does not accord with the situation in the human where in males the SHBG capacity is about half but the MCR of testosterone is twice that of females (Vermeulen and Ando 1979). Neither does the further finding that the effect on the clearance rate of testosterone is small if the percentage bound to SHBG is greater or equal to 50 per cent since, as shown in Table 5.1, the percentage of testosterone bound to SHBG in the male and female are both above 50 per cent and yet the clearance rates of testosterone are very different. This may reflect differences between the calculated and experimentally determined binding of testosterone to SHGB, or that the regulation of the MCR of testosterone in monkeys by SHBG may be slightly different from that in humans.

In contrast to the unanimity in the literature that SHBG and MCR of oestradiol and testosterone are negatively related, Hotchkiss (1985) found that in the prepubertal rhesus monkey (*Macacca mulatta*) both the MCR of oestradiol and SHBG binding capacities were high when compared to the adult. The author points out that, in addition to SHBG levels, the mature and immature animals differ in a great many respects and speculates that other factors which affect clearance (see Pardridge 1981) such as hepatic blood flow, may change during development.

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2 INFLUENCE OF SHBG ON THE PERIPHERAL CONVERSION OF STEROIDS

Interconversions between androstenedione, testosterone and 5α-dihydrotestosterone occur widely in peripheral tissues (see Vermeulen and Ando 1979); indeed, as shown by Horton and Tait (1966), approximately 60 per cent of testosterone in female plasma arises from extraglandular conversion from androstenedione. Vermeulen and Ando (1979) observed that, in addition to metabolic clearance rates, the blood conversion ratios of testosterone to androstenedione and to a lesser extent those of testosterone to 5α-dihydrotestosterone were significantly correlated with both the free and the percentage of non-SHBG bound testosterone but not with the total amount of the hormone. Decreased testosterone—androstenedione conversion ratios in hyperthyroid patients are fully consistent with elevated SHBG causing a decrease in the fraction of circulating testosterone available for extraction by peripheral tissues (Ridgway *et al.* 1982). *In vitro* studies in various cell systems have arrived at similar conclusions (Anderson 1974; Egloff, Savoure, Tardival-Lacombe, Massart, Nicol, and Degrelle 1981).

3 POSSIBLE BIOLOGICAL AVAILABILITY OF SHBG-BOUND SEX STEROIDS

Bordin and Petra (1980) have advanced the hypothesis that steroid hormones bound to their specific binding proteins may be available for transfer across cell membranes in some circumstances, possibly by a receptor mediated process (Stanczyk *et al.* 1986). Subsequently, others have also proposed the same idea (Siiteri *et al.* 1982; Siiteri 1986). Evidence in support of this concept include the immunocytochemical localisation of SHBG in the prostate, testis and epididymis of the monkey (Bordin and Petra, 1980) and human (Egloff, Vendrely, Tardival-Lacombe, Dadoune, and Degrelle 1982), and in human breast cancer tissue (Tardival-Lacombe, Egloff, Mazabraud, and Degrelle 1984). Using ^{125}Iodine labelled SHBG, Kahn, and Rosner (1985) have found two binding sites on human prostate membranes, one of high affinity and one of low affinity. Strel'chyonok, Avvakumov, and Survilo (1984) showed that ^{125}Iodine labelled SHBG binds to decidual endometrial membranes only when complexed with oestradiol. SHBG devoid of steroid or complexed with testosterone does not bind. They propose that in plasma membranes of oestrogen target tissues, there is a recognition system for SHBG-oestradiol which may allow these cells to take up from the blood, not only free oestradiol but also oestradiol complexed with the binding proteins.

Baulieu (1986) defines three categories of steroid binding proteins; those originating in the liver and circulating in the blood; those found in a restricted system such as seminiferous tubules; and those which are found within target cells. In the latter case, he suggests that the binding proteins are

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synthesized *in situ*. The possible function of SHBG (or SHBG like molecules) is discussed extensively by Baulieu (1986). He advances the delightful hypothesis that "we are seeing the situation upside down", that is to say that the binding proteins were originally intracellular molecules which controlled the access to various cellular compartments. The binding proteins in the blood would then be secretory products playing a secondary role in controlling the availability of steroids to other cells.

4 TISSUE CONCENTRATIONS OF STEROIDS

During the last few years, some extraordinary data have appeared concerning the concentrations of steroids within tissues. If oestradiol is considered briefly as an example, amounts of this steroid within normal and malignant breast tissue and fat are some twenty fold greater than those in the peripheral blood (Santen 1986; Vermeulen 1986). What is more, no correlation is found between blood and tissue oestradiol concentrations.

How this concentration gradient is achieved is not clear. Some of the oestrogen is synthesized locally via the aromatization of androstenedione to oestrone. Some comes from the action of sulphatase on oestrone sulphate but it seems doubtful if the activity of the enzymes involved would account for extreme cases where the concentration gradient may be 200-fold. Santen (1986) points out that while the plasma levels of oestradiol in postmenopausal women are 4 to 40 per cent of those found in premenopausal women, the tumour tissue concentrations are similar. He goes on to argue that the blood/tissue gradient is not wholly dependent on the classical oestrogen receptor proteins because a substantial gradient is still found in receptor-negative breast tumours. Also, the receptor binds oestrone with about one third of the affinity of oestradiol but the two steroids are present in equal concentrations within the tissue. If tissue concentrations are determined by receptor protein, one would expect lower concentrations of oestrone. Finally, no correlation has been found between receptor levels and tissue concentrations of oestradiol (for review see Vermeulen 1986).

The question has to be asked whether extracellular (or the hypothesized intracellular) SHBG or other blood binding proteins play any part in the determination of tissue concentrations? This is an area which has been pursued vigorously by Pardridge and is fully discussed in his chapter (this volume).

V SHBG in physiological conditions

In vivo evidence suggests that SHBG synthesis by the liver is stimulated by oestrogens and thyroid hormones and inhibited by androgens (see Anderson 1974 and Section VI). Although, in some physiological situations the impact of the net androgen/oestrogen/thyroid balance on SHBG synthesis is apparent, in others it is not so obvious. There follows, therefore, a review of rele-

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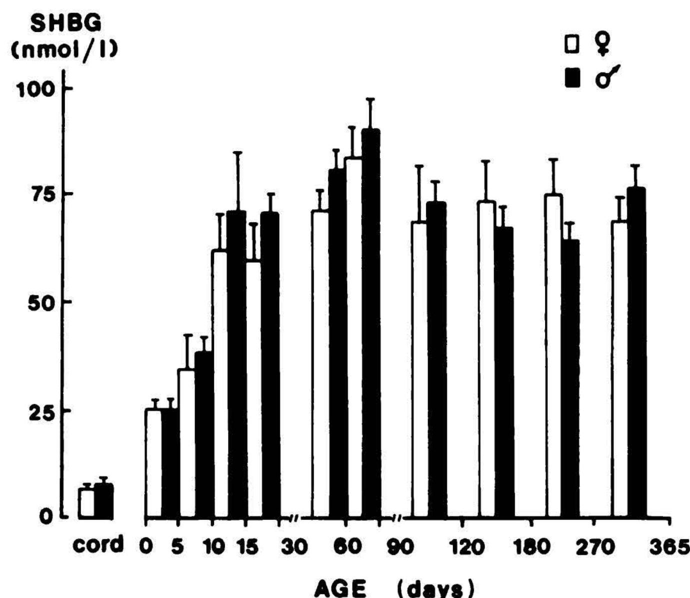

Fig. 5.3. Change in plasma levels of SHBG (mean + S.E.M.) in infants. Reproduced with permission from Forest, Bonneton, Lecoq, Brebant, and Pugeat (1986).

vant literature on changes in SHBG in various physiological situations and an attempt has been made to relate these to the prevailing endocrine environment.

1 SHBG LEVELS IN CHILDREN

i SHBG in children and during puberty

At birth, SHBG levels in cord plasma and in the plasma of new-born infants of both sexes are less than 10 per cent of the mother at term and similar to those of the adult male (August, Tkachuk, and Grumbach 1969; Forest, Ances, Tapper, and Migeon 1971; Anderson *et al.* 1976). This gradient is independent of fetal sex and weight (Forest *et al.* 1971; Anderson *et al.* 1976). After delivery SHBG rises rapidly within 30 days to achieve levels approximating to those found in non-pregnant adult females (August *et al.* 1969; Wenn, Kamberi, Vossough, Kariminejad, Torabee, Ayoughi, Keyvanjah, and Sarberi 1977; Forest, Bonneton, Lecoq, Brebant, and Pugeat 1986). The data from Forest *et al.* (1986) are shown in Fig. 5.3.

Most studies suggest that the high levels of SHBG achieved during the weeks after birth are maintained until 8 to 10 years of age (August *et al.* 1969; Horst, Bartsch, and Dirksen-Thedens 1977; Wenn *et al.* 1977; Bartsch, Horst, and Derwahl 1980; Lee, Lawder, Townend, Wetherall, and Hahnel 1985; Forest *et al.* 1986). The literature is not entirely unanimous on this point, however, since Belgorosky and Rivarola (1986) in their study of 91 boys demonstrated a steady decline in SHBG from the age of 3 months

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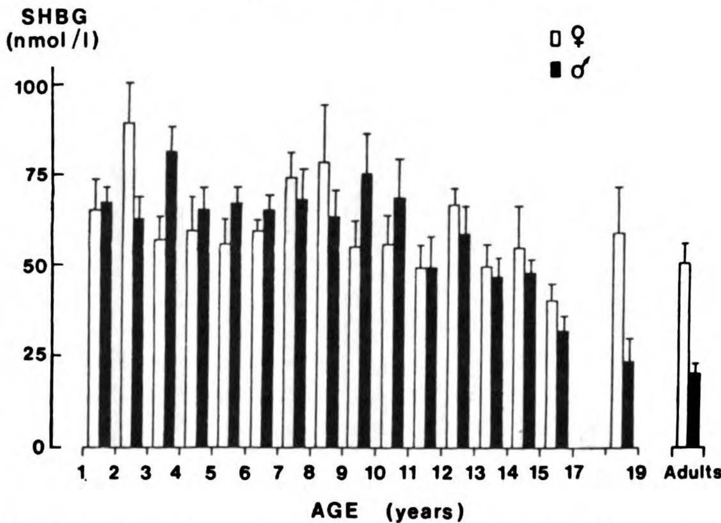

Fig. 5.4. Changes in SHBG (mean + S.E.M.) in males and females from childhood to adulthood. Reproduced with permission from Forest, Bonneton, Lecoq, Brebant, and Pugeat (1986).

onward. SHBG levels were not found to differ with the sex of prepubertal children (Wenn *et al.* 1977; Lee *et al.* 1985) although an earlier report suggested there was a difference (August *et al.* 1969).

There is general agreement that from around the ages of 8 to 10 in boys, blood levels of SHBG decline steadily and stabilize by about the age of 18 at levels approximately four-fold lower than those found before puberty (Horst *et al.* 1977; Blank, Attanasio, and Gupta 1978; Forest *et al.* 1986; Belgorosky and Rivarola 1986).

In a cross sectional examination of puberty in girls, Bartsch *et al.* (1980) demonstrated that by the age of about 15 (approximately mid-puberty), SHBG levels were about half the prepubertal levels and in a longitudinal study (Apter, Bolton, Hammond, and Vihko 1984), the concentration of SHBG, measured by an immunoradiometric assay, declined gradually (30 per cent) during puberty. This study also revealed that girls with menarche before 13 years of age had lower SHBG concentrations at 10–15.9 years than girls with later menarche.

In late female puberty, SHBG levels higher than those found at mid-puberty have been reported (Odlind, Carlstrom, Michaelsson, Vahlquist, Victor, and Mellbin 1982; Forest *et al.* 1986), and normal young adult females have only slightly lower SHBG capacities than those found before puberty (Bartsch *et al.* 1980; Forest *et al.* 1986). The changes in SHBG levels before and during puberty in boys and girls found by Forest *et al.* (1986) are shown in Fig. 5.4.

In a small study of pubertal changes in boys and girls, Gaidano, Berta,

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Rovero, Valenzano, and Rosatti (1980) showed that the binding capacities for testosterone and 5α-dihydrotestosterone diminished. They also observed relative changes in capacity according to the ligand used in the assay and suggested that the apparent changes in SHBG were in fact changes in the binding characteristics of the protein. This appears to be unlikely, however, since Belgorosky and Rivarola (1986) found no differences in association constant (Ka) of SHBG binding to 5α-dihydrotestosterone between boys of different ages.

ii Hormonal control of SHBG before and during puberty

The hormonal control of SHBG synthesis in the fetus and neonate is not clear. Levels of oestrogen in fetal blood at term are high, falling abruptly after birth (Tulchinsky and Chopra 1973) so the oestrogens do not appear to induce synthesis of SHBG by the neonatal liver. A stimulatory role for thyroid hormones in the neonatal rise in SHBG synthesis has been suggested (Anderson *et al.* 1976). Forest, Cathiard, and Bertrand (1973) and Wenn *et al.* (1977) showed that although newborn males have on average five times more testosterone than newborn females there were no differences in SHBG.

In children before puberty, no correlations were observed between the major sex steroids (testosterone, 5α-dihydrotestosterone or oestradiol) and SHBG (Horst *et al.* 1977; Bartsch *et al.* 1980; Belgarosky and Rivarola 1986).

During male puberty, the decline in SHBG is thought to be due to increasing adrenal and gonadal androgen production (but see below). The increase in testosterone is about 10 fold during puberty (Blank *et al.* 1978).

In girls, the 50 per cent fall in SHBG described during puberty by Bartsch *et al.* (1980) was accompanied by a 10-fold increase in oestradiol and a 5-fold increase in testosterone. Multivariate analysis showed that only the androgens were significantly related (negatively) with SHBG, suggesting a predominant role of these hormones in the control of the synthesis of the protein in these subjects. Apter *et al.* (1984) observed weakly significant negative correlations between SHBG and blood androgens (testosterone and androstenedione). The closest relationships shown, however, were between SHBG and bodyweight (and body fat percentage) suggesting that factors other than steroids have to be considered in the regulation of SHBG during puberty in girls. Among factors known in the human to regulate SHBG synthesis, thyroid hormones are unlikely candidates since they do not appear to change during the course of puberty (Lamberg, Kantero, Saarinen, and Widholm 1973; Bartsch *et al.* 1980). Growth hormone is a possibility since it decreases SHBG levels (De Moor, Heyns, and Bouillon 1972) and an increase in secretion at mid-puberty has been reported (Kantero, Wide, and Widholm 1975; Bierich 1983).

Some doubt has been cast on the importance of androgens in the pubertal decline in SHBG values in both boys and girls by the finding that in patients

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with untreated isolated gonadotrophin deficiency there was a highly significant inverse correlation between SHBG levels and age during the second decade of life even though testosterone levels did not rise (Cunningham, Loughlin, Culliton, and McKenna 1984). The numbers of patients in this study were small (n = 4) and the maximum period over which individuals were studied was 24 months so confirmation of these findings in a larger study would be helpful to our understanding. Cunningham *et al.* (1984) also observed a decline in SHBG during puberty in two 46 XY siblings, phenotypically female with complete androgen insensitivity.

In summary, factors controlling SHBG synthesis in children before and during puberty are poorly understood. The high levels in prepubertal children may stem from a lack of endocrine suppression of synthesis. This becomes manifest as androgens increase towards puberty. The marked fall during male puberty is probably due to increasing androgens, and the gradual decline in SHBG in girls may be due to increasing androgens opposed by oestrogens. The greater increase in oestrogens compared with that of androgens would indicate a higher potency for the androgens in controlling SHBG synthesis during puberty in the female. It is evident, however, that the role of androgens is becoming increasingly controversial and bodyweight appears to be much more important than steroid secretion (cf. Adams and Steiner, this volume).

2 EFFECT OF AGEING ON SHBG IN THE ADULT

i Men

Many studies have demonstrated that SHBG capacities in men older than 60–65 are higher than those of younger men (Vermeulen, Rubens, and Verdonk 1972; Pirke and Doerr 1973; Bartsch 1980; Winters and Troen, 1982). Recently, significantly positive correlations between age and SHBG capacities (Purifoy, Koopmans and Mayes 1981) and concentrations (Maruyama *et al.* 1984) have been observed between the third and ninth decades of life. Using multivariate analysis a similarly significant relationship was observed between the third and fifth decades when corrections were made for obesity and endurance fitness measured on a bicycle ergometer (Semmens, Rouse, Beilin, and Masarei 1983*a*). These increases in SHBG activity with age probably reflect the androgen-oestrogen balance brought about by the decreasing androgen output by the Leydig cells (Vermeulen *et al.* 1972; Pirke and Doerr 1973; Bartsch 1980) and increasing levels of oestradiol found by many but not all workers in the field (see Winters and Troen 1982).

ii Women

The situation in women is less clear. We have measured SHBG concentra-

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tions, by the immunoradiometric method of Hammond *et al.* 1985, in serum samples from 1200 women between the ages of 35 and 75 who had never used oral contraceptives or hormone replacement therapy, had no history of endocrine diseases or cancer, and were not taking drugs likely to affect SHBG. The data were analysed by multivariate analysis. There was little variation in weight-adjusted SHBG levels with age during the reproductive years as also found by Semmens *et al.* (1983*a*). In postmenopausal subjects the mean SHBG concentration were very significantly lower than those of premenopausal women, a finding which is consistent with the cessation of ovarian function. Curiously, when the postmenopausal SHBG levels were considered there was a statistically significant, approximately linear relationship with the number years past the menopause (Moore, Key, Bulbrook, Clark, Allen, Wang, and Pike 1987). Reasons for this increase are not clear and require further investigation.

The diminution in SHBG following the menopause has previously been observed (Murayama Sakuma, Udagawa, Utsonomiya, Okamoto, and Asano 1978; Moore *et al.* 1982; Moore, Clark, Takatani, Wakabayashi, Hayward, and Bulbrook 1983).

Longcope *et al.* (1987) studied SHBG, free oestradiol and free testosterone in 78 perimenopausal women and concluded that the menopause is not associated with changes in SHBG or percent free steroids. Compared to our population, however, the age range of women in this study (42–58 years) was considerably narrower and the numbers were very much smaller and this may have obscured an effect of age on SHBG.

In a study of 168 normal non-pregnant Japanese females between the ages of 20 and 90 years, a highly significant correlation between age and SHBG concentration was observed (Maruyama *et al.* 1984), the postmenopausal group clearly showed higher concentrations than the younger women. No account was taken of body weight in this study, however.

3 THE RELATIONSHIP BETWEEN SHBG AND BODYWEIGHT

Of all the variables affecting SHBG levels in the blood, the inverse relationship between bodyweight or some index of body size is the most consistently reported. Low SHBG levels have been found in obese men, women, and children. Higher than normal levels were found in male and female patients with anorexia nervosa (see Table 5.3 for references). Diminished SHBG in obese women is reversible with weight loss (Enriori, Orsini, Cremona, Etkin, Cardillo, and Reforzo-Membrives 1986) and in female anorectics weight gain is associated with a decline in SHBG binding capacity (Estour *et al.* 1986). In the study by Wheeler *et al.* (1983) weight gain in male anorectics was not associated with a decrease in SHBG despite an increase in levels of testosterone. Among possible reasons, they suggest that this could be due to compensatory changes in oestradiol production which was not measured.

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Table 5.3

References to SHBG in obese men, women and children and in patients with anorexia nervosa

Conditions studied	Blood SHBG	Reference
Obesity in men	Low	Glass, Swerdloff, Bray, Dahms, and Atkinson (1977) Amatruda, Harman, Pourmo, and Lockwood (1978) Schneider, Kirschner, Berkowitz, and Ertel (1979)
Obesity in women	Low	Hosseinan, Kim, and Rosenfield (1976) Kopelman, Pilkington, White, and Jeffcoate (1980) Plymate, Fariss, Bassett, and Matej (1981) Cunningham, Loughlin, Culliton, and McKenna (1985) Lapidus, Lindstedt, Lunberg, Bengtsson, and Gredmark (1986)
Obesity in children	Low	Dunkel, Sorva, and Voutilainen (1984) Apter, Bolton, Hammond, and Vihko (1984)
Anorexia nervosa	High	Wheeler, Crisp, Hsu, and Chen (1983) Estour, Pugeat, Lang, Dechaud, Pellet, and Rousset (1986)

In men and women within the normal weight range, statistically significant negative correlations between SHBG activity and weight have usually been observed (Moore *et al.* 1982; Semmens *et al.* 1983*a*; Moore, Clark, Hoare, Millis, Hayward, Quinlan, Wang, and Bulbrook 1986). In our study of women in Guernsey (Moore *et al.* 1987), we observed a highly significant, inverse correlation between SHBG and Quetelet's index $\left(\frac{\text{weight, kg}}{\text{height, m}^2}\right)$ in both premenopausal and postmenopausal women. The average SHBG concentration in the serum of women with Quetelet's index of less than 20 are almost twice that of those with the index of greater than 32 (see Fig. 5.5). It was noted that in premenopausal women there was little decrease in SHBG until a Quetelet's index of greater than 26 was reached.

Obesity is often associated with anovulation and amenorrhoea (Rogers and Mitchell 1952) and most of the studies which have attempted to explain why obese women have diminished SHBG have been part of wider investigations and have often included only small numbers of obese but otherwise normal women (Hosseinian *et al.* 1976; Plymate *et al.* 1981). In obese, oligomenorrhoeic patients with or without hirsutism, strikingly lower SHBG capacities were associated with significantly increased serum testosterone

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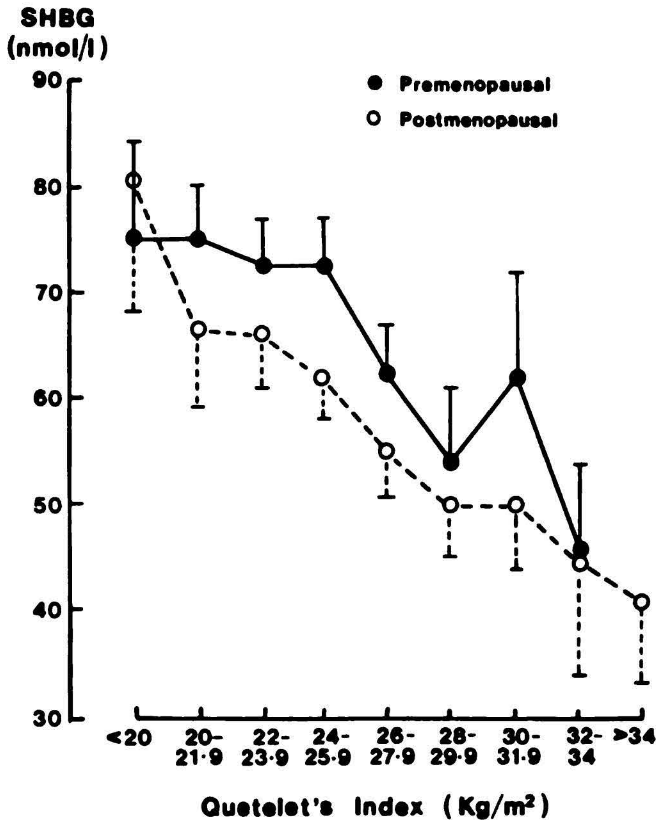

Fig. 5.5. Relationship between Quetelet's index and SHBG in a population of normal women. Calculated from data of Moore, Key, Bulbrook, Clark, Allen, Wang, and Pike (1987).

(Hosseinian *et al.* 1976). However, in a small group (n = 6) who were obese but without signs of hirsutism or ovulatory disturbances, no significant differences were observed in either SHBG or testosterone compared with women of normal weight. Other workers, though consistently reporting diminished SHBG, have failed to find any abnormalities in testosterone concentrations in obese women (Kopelman *et al.* 1980; Plymate *et al.* 1981; Cunningham *et al.* 1985). The same applies to oestradiol and FSH (Plymate *et al.* 1981; Cunningham *et al.* 1985). In this latter study, oestrone was elevated and Kopelman *et al.* (1980) found a non-significant increase in the oestrone/oestradiol ratio and androstenedione levels.

A simple hormonal mechanism, therefore, does not explain the changes in SHBG associated with altered weight in women. This is even more apparent in men where morbid obesity is associated with low SHBG, low levels of serum testosterone (Glass, Swerdloff, Bray, Dahms, and Atkinson 1977; Amatruda, Harman, Pourmo, and Lockwood, 1978; Schneider, Kirschner, Berkowitz and Ertel 1979) and increased oestradiol (Schneider *et al.* 1979). In children no differences in testosterone or oestradiol were observed in obese

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boys and girls compared to children of normal weight (Dunkel, Sorva, and Voutilainen 1984). In their study, however, Apter *et al.* (1984) found that in premenarchial girls, low SHBG correlated with high oestradiol levels although androgens (testosteròne and DHEA) were normal.

A consequence of lower SHBG and normal or elevated testosterone levels found in obesity is, of course, that the calculated free testosterone index is usually higher than normal (Hosseinian *et al.* 1976; Schneider *et al.* 1979; Cunningham *et al.* 1985). Cunningham *et al.* (1985) have suggested that the increased free testosterone resulting from diminished SHBG contributes to further diminution of SHBG production by the liver. Although this is a possibility, it is probable that other factors cause the observed changes in SHBG with weight.

Obesity is associated with increased conversion of androgens to oestrogens and a two-fold increase in serum oestradiol levels compared to controls was found in obese men by Schneider *et al.* (1979). They suggested, but no evidence was presented, that the failure of the liver to produce more SHBG under this greater oestrogenic stimulus was due to weight-related alterations in the activity of oestrogen receptors on the hepatocyte.

In summary, there is no question that SHBG concentrations are negatively related to weight and while this is a strong correlation, it should be borne in mind that in our population study, weight only accounts for 20 per cent of the variance in SHBG levels. The hormone related changes in obesity remain to be clarified and do not appear to be closely associated with variations in SHBG.

4 CHANGES IN SHBG DURING THE MENSTRUAL CYCLE

The postulated relationship between SHBG synthesis and oestrogen secretion suggest that changes in blood levels of the protein ought to be seen over the menstrual cycle. Some workers found no differences in the SHBG binding capacities during the cycle in normally ovulating women (Wu, Motohasi, Abdel-Rahman, Flickinger, and Mikhail 1976; Motohashi, Wu, Abdel-Ramen, Marymor, and Mikhail 1979; Odlind, Elamsson, Englund, Victor, and Johansson 1982; Bolufer, Antonio, Garcia, Munoz, Rodriguez, and Romeu 1983; Cerutti, Gibin, Fede, Mozzanega, and Marchesoni 1984). In contrast, Solomon, Iqbal, Dalton, Jeffcoate, and Ginsburg (1979) demonstrated a marginally significant increase in binding capacity in the luteal phase compared to the follicular phase in a small group of spontaneously ovulating women. In a more extensive investigation, Dowsett, Attree, Virdee, and Jeffcoate (1985) observed significant increases of 15 per cent in the mean SHBG binding capacity two days after ovulation which were maintained for the first 10 days of the luteal phase. The initial increase was found to correlate with levels of oestradiol in the follicular phase. It was presumed that the

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elevated levels in the luteal phase were maintained by the secondary rise in oestradiol.

Several other groups who have examined this question have reported that, compared to the follicular phase, SHBG does increase significantly during the luteal phase (Mattsson, Silfverstolphe, and Samsioe 1984; Plymate, Moore, Cheng, Bardin, Southworth, and Levinski 1985; Apter *et al.* 1984) and this does not occur in non-ovulatory cycles (Apter *et al.* 1984). The ability to detect the small changes which occur over the cycle is undoubtedly due to the high precision of the methods used (Dowsett *et al.* 1985; Plymate *et al.* 1985) and also to the study of large populations (for example, Apter *et al.* 1984, studied 145 adolescent cycles). In our cross-sectional study of over 600 premenopausal women SHBG was higher in the first 12 days of the luteal phase compared with the rest of the cycle (Moore *et al.* 1987).

In their study, Apter *et al.* (1984) found a positive correlation between SHBG and progesterone concentration in late luteal phase blood. This was not confirmed in the mid-luteal phase by Dowsett *et al.* (1985): however, Dalton (1984*a*) reported increases in SHBG binding capacity in women with premenstrual syndrome given therapeutic dosages of oral progesterone (see Section **VI, 2**).

In summary, the bulk of recent evidence now supports an increase in SHBG in the luteal phase of the cycle and this is probably due to an effect of oestradiol but the role of progesterone is not certain.

5 EFFECT OF AGE AT MENARCHE ON SHBG

In our population study in premenopausal women there was an approximately linear relationship between SHBG and age at menarche. This relationship was reduced in magnitude by adjusting for Quetelet's index because there is a marked inverse relationship between Quetelets index and age at menarche. In postmenopausal women, the unadjusted values showed a tendency for SHBG to be higher in women who had a late menarche, but this relationship disappeared after adjustment for Quetelet's index (Moore *et al.* 1987).

6 SHBG IN PREGNANCY

i Changes during pregnancy

Between weeks five and seven of pregnancy, SHBG in the maternal circulation starts to rise, achieving levels five to ten times higher than those found in the non-pregnant female by week 30. The most dramatic rise occurs during the first half of pregnancy, presumably under the influence of the rapidly increasing oestrogen concentration (de Hertogh, Thomas, and Vanderheyden 1976; Uriel, Dupiers, Rimbout, and Buffe 1981; Hertz and Johnsen

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1983). During the second half of pregnancy, despite a further three- to four-fold rise in oestradiol concentrations, there is only a small (30 per cent) further increase in SHBG (de Hertogh *et al.* 1976; Uriel *et al.* 1981). It is not known whether this indicates maximal synthesis by the end of the first half of pregnancy or antagonism by other hormones during the later stages of gestation. The maternal levels of the binding protein fall after delivery with a half life of about 7 days (Anderson *et al.* 1976).

Although the increase in SHBG is thought to be stimulated by the increase in oestradiol from the non-pregnant luteal phase levels of 0.2–0.5 ng/ml to 20–30 ng/ml in late pregnancy (see Reed and Murray 1979), there is no evidence for this, apart from what is inferred from the administration of exogenous oestrogens. Indeed in the pregnant rhesus macaque, although there is a slight rise in SHBG in early gestation, the levels later actually fall in spite of increasing oestradiol concentrations, so that before delivery the SHBG activity is lower than that of the non-pregnant animal, whereas the total oestradiol levels are increased 4–5 fold (Anderson *et al.* 1976; Stanczyk, Hess, Namkung, Senner, Petra, and Novy 1986).

Bernstein and her colleagues reported that the SHBG binding capacity was approximately 10 per cent higher, and the percentage free and total oestradiol levels lower, in the early part of a woman's second pregnancy compared to that found early in her first pregnancy (after adjustments were made for stage of pregnancy and weight at the beginning of pregnancy). They argue that this may result in increased exposure of the germ cells to oestrogen and account for the increased risk of cryptorchidism and testicular cancer in first-born males (Bernstein, Depue, Ross, Judd, Pike, and Henderson 1986).

ii SHBG in abnormal pregnancies

In a study of SHBG capacities in women who were admitted for vaginal bleeding between the sixth and eighteenth gestational week, those whose pregnancies ended in spontaneous abortion had lower SHBG capacities compared to those whose pregnancies went to term. This was especially true after the 13th week. There were, however, many women with high SHBG who subsequently aborted and it was concluded that measurements of SHBG would not be as reliable a marker for threatened abortion as other hormonal parameters such as chorionic gonadotrophin, placental lactogen, progesterone and pregnancy specific β_1-glycoprotein (Hertz and Johnsen 1983).

Uriel *et al.* (1981) measured SHBG concentration by an immunodiffusion technique and also the binding capacity for 5α-dihydrotestosterone using the filter disc method of Mickelson and Petra (1974). The correlation between these methods in non-pregnant and healthy pregnant women was highly significant ($r = 0.85$). Six patients who had abnormal pregnancies had normal SHBG concentrations, but in five of these the ratios of the binding capacity to the SHBG concentrations were much lower than normal. This would

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appear to indicate defective binding by SHBG and needs to be confirmed in a larger study.

iii SHBG in amniotic fluid

SHBG has been detected in amniotic fluid and levels fifty fold lower than in the maternal circulation do not appear to vary much between early (weeks 13–20) and late pregnancy (weeks 36–37) (Caputo and Hosty 1972; Hammond *et al.* 1983; Dalton 1984; Forest *et al.* 1986). The physicochemical characteristics of SHBG derived from the amniotic fluid do not differ from those of pregnancy serum and it is probable that the source of SHBG and other serum proteins is largely the maternal circulation via the amniochorion (Hammond *et al.* 1983).

iv Possible role for SHBG in pregnancy

It is often suggested that the purpose of SHBG in the maternal circulation is to protect the mother from the high levels of testosterone and other 17β-hydroxy androgens found in pregnancy by reducing the free, biologically active steroid (see Anderson 1974). While it is true that in pregnancy the free testosterone concentrations are below those of non-pregnant women in the presence of an approximate doubling in concentration of total testosterone (Vermeulen 1979), the testosterone levels are elevated because of the diminished clearance due to increased peripheral SHBG. Therefore, if SHBG did not rise, since there is no evidence that the production rate of testosterone is increased in pregnancy, it is probable that total and free testosterone concentrations would remain at the levels found before pregnancy which in the normal female do not cause virilization.

SHBG may act by sequestering androgens from the fetal into the maternal circulation, preventing virilization of the female fetus (Anderson 1974). Hammond *et al.* (1983) suggest that SHBG in amniotic fluid may also play a role in buffering any virilizing effect of androgens on the fetus since the small amounts of SHBG in amniotic fluid are well in excess of the testosterone concentrations. As pointed out by Forest *et al.* (1986), however, the percentage of free testosterone is higher in amniotic fluid than it is in plasma. This is presumably a consequence of the lower albumin and total protein concentrations in amniotic fluid compared with plasma (cf. Hammond *et al.* 1983).

vi Long-term effects of pregnancy on SHBG

As part of a wider study aimed at elucidating the hormonal basis for the association between reproductive factors and risk of breast cancer (see Yu, Gerkins, Henderson, Brown, and Pike 1981), it was shown that young parous women had a 12 per cent greater mean SHBG binding capacity and

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lower urinary and plasma oestrogen levels than nulliparous women of similar age and cycle length (Bernstein, Pike, Ross, Judd, Brown, and Henderson 1985). This was confirmed in our study. However, further examination of our data showed that this was due solely to the lower concentration of SHBG in the unmarried, nulliparous group. We found a similar pattern postmenopausally, in that unmarried nulliparous women had lower SHBG levels than married nulliparous women. The reason for this is not clear (Moore *et al.* 1987).

7 NYCHTHEMERAL VARIATION

Plasma SHBG binding capacity for testosterone shows a significant nychthemeral rhythm in healthy adult men with the lowest capacities between midnight and 6 a.m. (Clair, Claustrat, Jordan, Dechaud, and Sassolas 1985). We have recently investigated nychthemeral variation of SHBG concentration in blood samples taken every 2 hours in a group of 28 normal women and found essentially the same variation over the 24 hours (Clark, Moore, Fentiman, and Wang unpublished).

These findings are consistent with previous investigations which have shown that total serum protein is lower by 5 to 10 per cent in supine subjects compared with subjects who are erect. The minimum is obtained within 2 to 4 hours after assuming the horizontal position and the maximum within 2 hours after rising. This phenomenon is usually ascribed to haemoconcentration in the erect position (Fawcett and Wynn 1956; Henry 1968).

To summarize, while we are prepared to accept that a wide variety of factors such as the stage of the menstrual cycle, pregnancy, menopausal status and nychthemeral rhythms affect SHBG concentrations, it has to be admitted that the evidence for a precise hormonal control of this protein is equivocal and unconvincing.

VI The effect of drugs on SHBG

1 OESTROGENS

The oestrogenic components of oral contraceptives (ethinyloestradiol or its 3-methyl ether, mestranol) are potent inducers of SHBG synthesis in men (van Look and Frolich 1981) and women (van Kammen, Thijssen, Rademaker, and Schwartz 1975; Briggs 1975; Helgason, Damber, Damber, von Schoultz, Selstam, and Sodergard 1982). Significant dose dependent increases in SHBG are also observed after the oral administration of conjugated equine oestrogens to postmenopausal women (Pogmore and Jequier 1979; Geola, Frumar, Tataryn, Lu, Hershman, Eggena, Sambhi, and Judd 1980; Mathur, Landgrebe, Moody, Semmens, and Williamson 1985). Other

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oestrogen inducible liver proteins include CBG, TBG and pregnancy zone protein. SHBG synthesis is more sensitive to oestrogen administration than either CBG or TBG (Geola *et al.* 1980) but less sensitive than pregnancy zone protein (Ottosson, Damber, Damber, Selstam, Solheim, Stigbrand, Sodergard, and von Schoultz 1981). In terms of potency, ethinyloestradiol is much more oestrogenic as an inducer of SHBG synthesis than oestradiol or oestrone sulphate when administered orally (see Helgason *et al.* 1982).

2 PROGESTATIONAL AGENTS

The progestational agents commonly used in contraceptive medications are usually derivatives of 19-nortestosterone, 17αOH-progesterone or 19-norprogesterone. In addition to their progestational activity the 19-nortestosterone derivatives also have anti-oestrogenic activity and this is evidenced by the depressive effect on hepatic synthesis of oestrogen-sensitive proteins such as SHBG. They appear to operate through androgen receptors rather than the oestrogen receptors in the liver (Bergink, Hamburger, de Jager, and van der Vies, 1981; Hammond, Langley, Robinson, Numi, and Lund 1984).

Levonorgestrel administered orally (Crona, Silfverstolpe, and Samsioe 1984; Ruokonen and Kaar 1985) or slowly released from a vaginal ring (Cekan, Jia, Landgren, and Diczfalusy 1985) reduced blood levels of SHBG significantly. Oral desogestrel and lynestrenol (Ruokonen and Kaar 1985) and dl-norgestrel (El Makhzangy, Wynn, and Lawrence 1979) had similar effects. In this latter study, women using norethisterone followed the trend towards lower SHBG although the decrease was not significant (see Table 5.4).

Of the 17α-hydroxy-progesterone derivatives, medroxyprogesterone acetate (MPA) (250 mg i.m. weekly) significantly decreased serum binding of tritiated 5α-dihydrotestosterone in girls with precocious puberty, the changes being related to the duration of treatment (Forest and Bertrand 1972). Reductions in the SHBG binding capacity were also observed in women given MPA (150 mg every third month) by van Kammen *et al.* 1975. Victor and Johansson (1977) argue that a direct effect of MPA on liver synthesis of SHBG is unlikely because of its close similarity to megestrol acetate which actually increased SHBG slightly as shown by van Kammen *et al.* (1975) and subsequently by El Makhzangy *et al.* (1979). Rather they suggest that SHBG diminution may be associated with the significant reduction in oestradiol levels induced by MPA (Jeppsson and Johansson 1976).

No change in SHBG binding capacity occurred when MPA was administered by means of impregnated silastic intravaginal rings (Victor and Johansson 1977). The reason is probably dose related since the intramuscular dosage used by van Kammen *et al.* (1974) gives rise to 3–8 times higher

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Table 5.4

Effect of some progestagen only and progestogen/ethinyloestradiol (EO) combination oral contraceptives (OC) on blood SHBG (mean ± S.E.M.) in women

OC composition	Duration of treatment[1]	Baseline SHBG (nmol/l)	Treatment SHBG (nmol/l)	P	Reference
dl-Norgestrel (75 μg)	3–24 months	44 ± 5	24 ± 3	<0.05	El Makhzangy Wynn and Lawrence (1979)
Norethisterone (350 μg)	3–16 months	38 ± 6	27 ± 4	NS	
Levonorgestrel (125 μg)	30 days	65 ± 5	43 ± 4[2]	<0.001	Ruokonen and Kaar (1985)
Desogestrel (125 μg)	30 days	65 ± 5	31 ± 3[2]	<0.001	
Lynestrenol (5 mg)	30 days	65 ± 5	38 ± 3[2]	<0.001	
EO (50 μg) Megestrol (4 mg)	3–18 months	48 ± 4	205 ± 8	<0.001	El Makhzangy Wynn and Lawrence (1979)
EO (50 μg) Norethisterone (1 mg)	3–24 months	39 ± 4	81 ± 9	<0.001	
EO (30 μg) Desonorgestrel (150 μg)	80 days[3]	48 ± 3	150 ± 10	<0.001	Hammond, Robinson, Nummi, and Lund (1984)
EO (30 μg) Levonorgestrel (150 μg)	80 days[3]	50 ± 4	60 ± 4	NS	

[1] Indicates duration of treatment before blood samples taken; [2] Estimated from graphical data; [3] Approximation: samples were taken between days 18 and 21 of the third cycle during treatment.

plasma MPA concentrations than the vaginal rings (Jeppsson and Johansson 1976).

The 19-norprogesterone derivative ST-1435 (Merck) did not affect SHBG or CBG when administered subcutaneously (Lahteenmaki, Hammond, and Luukkainen 1983). As previously mentioned, there is one report that dose dependent increases in SHBG binding capacity were observed with therapeutic administration of progesterone to women with premenstrual syndrome (Dalton 1984*a*).

3 COMBINED OESTROGEN-PROGESTOGEN PREPARATIONS

The relative effect of the synthetic progestogens in relation to liver stimulation of SHBG can be seen when they are combined with synthetic oestrogens in oral contraceptives. As shown in Table 5.4, when ethinyloestradiol was accompanied by megestrol acetate, there was no inhibition of SHBG and

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levels rose dramatically to a mean of 205 nmol/l during treatment from a basal level of 48 nmol/l. Norethisterone acetate combined with ethinyloestradiol produced a far less significant surge in SHBG, indicating some antagonism by the progestogen (El Makhzangy *et al.* 1979). The reduction induced by norethisterone was shown to be dose dependent (Ottosson *et al.* 1981).

Similar diminutions in the ethinyloestradiol-stimulated synthesis of SHBG were observed with combinations containing norethisterone (Ottosson *et al.* 1981; Granger, Roy, and Mishell 1982) and desogestrel (Crona *et al.* 1984; Hammond *et al.* 1984; Cullberg, Hamberger, Mattsson, Mobacken, and Samsioe 1985). Levonorgestrel (150 µg) in combination with ethinyloestradiol (30 µg) resulted in no significant changes in SHBG capacity or concentration (El Makhzangy *et al.* 1979; Crona *et al.* 1984; Hammond *et al.* 1984).

4 BINDING OF CONTRACEPTIVE STEROIDS TO SHBG

In contrast to the naturally occurring oestrogens, ethinyloestradiol in human serum is bound only to serum albumin with an apparent affinity constant about three times that of oestradiol-17β (Akpoviroro and Fotherby 1980). This has recently been confirmed for undiluted serum at physiological temperatures and it has been further shown that the percentage of free steroid is between 1 and 2 per cent of the total (Hammond, Lähteenmäki, Lähteenmäki, and Luukkainen 1982).

Progestogens synthesized from either 17-hydroxyprogesterone or 19-norprogesterone do not bind specifically to SHBG (Lähteenmäki *et al.* 1983). In contrast 19-nortestosterone derivatives display significant but varying affinities for the binding protein. Levonorgestrel has the highest affinity for SHBG, the association constant behind midway between those of testosterone and oestradiol (Victor, Weiner, and Johansson 1976; Jenkins and Fotherby 1980; Bergink *et al.* 1981; Pugeat, Dunn, and Nisula 1981). Cekan, *et al.* (1985) have shown that blood levels of levonorgestrel in young women who had undergone vaginal implantation with silastic rings containing the steroid, were positively and significantly correlated with SHBG levels obtained before and during treatment. This is of course entirely consistent with the role of SHBG as an important binding protein for levonorgestrel. Pre-treatment levels of SHBG were also related to their potency as anti-ovulatory agents.

The serum distribution between SHBG and albumin of several commonly used contraceptive steroids has recently been determined in undiluted serum at 37°C (Lähteenmäki *et al.* 1983; Hammond *et al.* 1984). For more detailed discussion of the biological importance of the interactions between oral contraceptives and their metabolites and blood and cellular binding proteins the interested reader is referred to these papers and also to those of Bergink *et al.* (1981), Bergink, Holma and Pyorola (1981) and Fotherby (1984).

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5 ANDROGENS

i Anabolic steroid and testosterone administration

The antagonistic effect of the administration of androgens on SHBG synthesis in the normal androgen-responsive human is indisputable. With self-administration, by athletes, of extreme pharmacological doses of testosterone and other anabolic steroids, a 90 per cent fall in basal SHBG levels was observed and they remained low for 16 weeks after withdrawal of the drug (Ruokonen, Alen, Bolton, and Vihko 1985). Men with testicular insufficiency often show increased levels of SHBG (Anderson 1974) and administration of testosterone (200 mg i.m. every two weeks) to 5 normal men and 5 men with Klinefelter's syndrome resulted in significant increases in total and free testosterone concentrations and decreased SHBG binding capacity after 3 months (Plymate, Leonard, Paulsen, Fariss, and Karpas 1983).

The administration of depot dehydroepiandrosterone enanthate to oophorectomized women resulted in small but significant decreases in SHBG and high density lipoprotein cholesterol (HDL-C) (Mattson, Cullberg, Tangkeo, Zador, and Samsioe 1980). In terms of clinical usefulness, Belgorosky and Rivarola (1985) were able to distinguish abnormalities in androgen secretion from abnormal target cell responses in prepubertal patients with male pseudohermaphroditism by measuring SHBG after administration of testosterone.

ii Danazol and Gestrinone

Danazol is a derivative of ethisterone widely used in the treatment of endometriosis presumably acting by reducing peripheral oestrogen levels (Meldrum, Pardridge, Karow, Rivier, Vale, and Judd 1983). Androgenic side-effects (Potts 1977; Wynn 1977) are probably due to the rise in levels of free, biologically active, testosterone (Nilsson, Sodergard, Damber, Damber, and von Schoultz 1982; Dowsett, Forbes, Rose, Mudge, and Jeffcoate 1986). Part of this increase is due to the rapid fall in the SHBG binding capacity (Schwarz, Tappeiner, and Hintner 1981; Nilsson *et al.* 1982; Gershagen, Doberl, and Rannevik 1984; Dowsett *et al.* 1986) and also to competitive displacement of testosterone from SHBG by danazol and its metabolites (Nilsson *et al.* 1982; Dowsett *et al.* 1986).

Gestrinone is another effective agent in the treatment of endometriosis which also suppresses SHBG synthesis. In a recent study, Dowsett *et al.* (1986) showed that in patients with endometriosis both danazol and gestrinone reduced blood levels of SHBG to similar extents after one week of treatment. There were, however, significantly greater increases in the percentage of free testosterone in plasma samples of patients treated with danazol. Further, *in vitro* studies suggested that this additional increase in free testo-

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sterone was largely due to the competitive effect of ethisterone which is a major metabolite of danazol with a greater affinity for SHBG (Pugeat *et al.* 1981; Pugeat, Nicolas, Tourniaire and Forest 1984).

6 GONADOTROPHINS

In adult men, Leydig cell stimulation by long or short term administration of human chorionic gonadotrophin (hCG) does not result in a decrease in SHBG (Plymate *et al.* 1983; Willemse, Sleijfer, Pratt, Sluiter, and Doorenbos 1984). This is contrary to the situation which obtains in prepubertal boys where significant decreases have been observed (Belgorosky and Rivarola 1982; Dunkel 1985). The probable reasons for this difference are twofold. First, in boys, the increase in testosterone following a single dose of hCG can be 100 fold (Dunkel 1985) whereas in adults the increase is only 2.4 times base line levels (Saez and Forest 1979). Second, as well as testosterone stimulation, there is significant oestradiol stimulation after acute administration of hCG in adult males which probably counteracts the rise in testosterone with regard to SHBG induction (Forest, Lecoq, and Saez 1979; Plymate *et al.* 1983). In the pre-pubertal male, oestradiol concentrations remain low following hCG (Dunkel 1985).

Odlind *et al.* (1982) studied eight amenorrhoeic and previously anovulatory women who were undergoing treatment with gonadotrophins and ovulation induction with hCG. They observed a pronounced increase in SHBG capacity, from a mean of 31 nmol/l at the beginning of treatment to a mean of 64 nmol/l 14 days after a very large, hCG induced, ovulatory peak of serum oestradiol (a mean of three times the normal concentration). Similar findings were reported by Dowsett *et al.* (1985), and also by Clair, Claustrat, Brun, Dechaud and Thoulon (1985).

7 GLUCOCORTICOIDS

The literature is conflicting. In a variety of clinical conditions, glucocorticoid administration has been reported to have no effect on SHBG (Anderson 1974; Kim, Rosenfield, and Dupon 1976; Lachelin, Judd, Swanson, Hanck, Parker, and Yen 1982; Lobo, Paul, March, Granger, and Kletzky 1982; Pugeat, Forest, Nisula, Corniau, de Peretti, and Tourniaire 1982; Darley, Moore, Besser, Munro, and Kirby 1983). Others found that small doses of dexamethasone (0.5 mg at night) for three months increased SHBG (Cunningham, Loughlin, Culliton, and McKenna 1983) and some evidence for a direct agonistic effect of dexamethasone on SHBG synthesis comes from *in vitro* studies in a hepatoma-derived cell line (Mercier-Bodard and Baulieu 1986).

Decreased SHBG capacities in men with chronic asthmatic bronchitis taking large doses of dexamathasone were found by Vermeulen *et al.* (1969).

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More recently, Wu (1982) found that the mean percentage of testosterone bound to SHBG in hirsute women treated with dexamethasone (2 mg/day) was significantly decreased from pretreatment levels. Further support for the lowering of SHBG by dexamethasone comes from studies in cycling rhesus macaques (Stanczyck, Petra, Senner, and Novy 1985). In children and adolescents, long term (3 day) ACTH stimulation which resulted in massive increases in blood cortisol levels, was accompanied by significant reductions in SHBG binding capacities (Forest *et al.* 1986). The reasons for the conflicting literature are not clear but dosage and length of treatment are probably important.

8 ANTI-OESTROGENS

i Tamoxifen

Tamoxifen has been widely used in the treatment of breast cancer. The mode of action of the drug is thought to be that of an anti-oestrogen blocking oestradiol at the oestrogen receptor sites in the tumour. However the biological activity of the compound varies with the dose from species to species and even from organ to organ and it can act as an oestrogen (Nicholson, Walker, and Davies 1986). As an anti-oestrogen, it might have been expected to inhibit SHBG synthesis, but this is not the case. Tamoxifen caused significant increases, not only in SHBG but also in other oestrogen-sensitive proteins such as CBG and pregnancy-zone protein in patients under treatment for breast cancer (Sakai, Cheix, Clavell, Colon, Mayer, Pommatau, and Saez 1978; Fex, Adielsson, and Mattson 1981; Caleffi, Fentiman, Clark, Wang, Needham, Clark, La Ville, and Lewis 1988). In human hepatoma cells in culture tamoxifen significantly induced SHBG synthesis (Mercier-Bodard and Baulieu 1986).

ii Clomiphene

Clomiphene citrate is structurally related to tamoxifen and has a similar oestrogenic effect on SHBG synthesis. Increases in this protein have been observed in women undergoing treatment for infertility (Lobo *et al.* 1982) and in men being treated for oligozoospermia (Adamopoulos, Vassilopoulos, Kapolla, and Kontogeorgos 1981). As pointed out by the latter group, this is an unwanted side effect of the drug in the treatment of this type of male infertility where the need is usually to increase testicular androgen production. Although gonadotrophins increase and testicular androgen production improves, testicular oestrogen production also increases and the rise in SHBG upsets the endocrine balance by diminishing the bioavailability of testosterone to a greater extent than that of oestradiol.

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9 ANTI-CONVULSANT DRUGS

SHBG binding capacity was shown to be increased in male and female epileptic patients being treated with phenytoin (Victor, Lundberg, and Johansson 1977; Dana-Haeir, Oxley, and Richens 1982; Beastall, Cowan, Gray, and Fogelman 1985). In the study carried out by Beastall *et al.* (1985), binding capacities for CBG, as well as SHBG, were increased during treatment and there was a strong positive association between the capacities of these proteins and serum levels of phenytoin. Levels of TBG or the binding capacity of vitamin D binding protein were not affected, but there were reductions in levels of cortisol and thyroid hormones which suggests that anticonvulsant therapy causes widespread disturbances in hormonal homeostatsis.

In new-born infants, far from inducing synthesis of SHBG, a single intramuscular injection of phenobarbital was associated with a marked delay in the post-natal rise in SHBG, which suggested that the drug may retard synthesis in the neonate (Forest, Lecoq, Salle, and Bertrand 1981).

In summary, administration of oestrogenic compounds increase whereas androgenic medications and some progestogens decrease the concentration of SHBG. The combined oestrogen/progestogen contraceptives have a variety of effects depending on the type and dose of progestogen used. The situation regarding the effect of glucocorticoids is still controversial but the weight of evidence suggests that large doses inhibit synthesis. Anticonvulsants appear to induce SHBG synthesis except in neonates.

VII SHBG and disease

1 HYPERANDROGENISM AND THYROID DISEASES

One of the few areas where SHBG measurements are widely used clinically is in the diagnosis and management of diseases associated with hyperandrogenization in women such as hirsutism, polycystic ovary syndrome and acne vulgaris. In these conditions blood levels of testosterone, other 17βOH-steroids and adrenal androgens may not be consistently elevated but SHBG is usually significantly depressed. This results in increased levels of biologically available testosterone (Anderson 1974; Lawrence, Katz, Robinson, Newman, McGarrigle, Shaw, and Lachelin 1981; Odlind *et al.* 1982; Carter, Holland, Alaghband-Zadeh, Rayman, Dorrington-Ward and Wise 1983; Cunningham *et al.* 1983; Darley *et al.* 1983).

Hyperthyroidism and the administration of large doses of thyroid hormones is associated with increased circulating SHBG activity, and hypothyroidism with decreased SHBG levels (see Yosha, Fay, Longcope, and Braverman 1984 for references). Although SHBG measurements are of minor importance in the diagnosis of common thyroid diseases (Lindstedt, Lundberg, Hammond, and Vihko 1985), they are reported as being valuable

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in the diagnosis of thyroid hormone resistance where SHBG levels are normal, despite markedly elevated thyroid hormone levels (Cooper, Ladenson, Nisula, Dunn, Chapman, and Ridgway 1982; de Nayer, Lambot, Desmons, Rennotte, Malvaux, and Beckers 1986).

2 BENIGN PROSTATIC HYPERPLASIA AND PROSTATIC CANCER

Growth and function of the prostate gland are regulated predominantly by testosterone and its metabolites, particularly 5α-dihydrotestosterone, although other hormones such as oestradiol and prolactin are also involved (Pasqualini 1982; Peeling and Griffiths 1986; Griffiths, Davies, Eaton, Harper, Peeling, Turkes, Wilson, and Pierrepoint 1987).

SHBG inhibits the uptake, metabolism and action of testosterone in rat prostate glands in organ culture (Lasnitzki and Franklin 1975) and in excised human hypertrophic tissue (Pachman 1984). The possibility that peripheral SHBG capacities might be different in men with benign prostatic hyperplasia compared with normal has been examined by several authors. No differences were observed by some (Dennis, Horst, Kreig, and Voight, 1977; Bartsch, Becker, Pinkenburg, and Krieg 1979). Pachman (1984*a*), however, showed that the mean SHBG binding capacity was significantly higher and testosterone levels lower in patients and suggested that the increase in SHBG might represent a defence mechanism against further androgenic stimulation of the gland. Curiously, excision of the hypertrophied tissue resulted in a significant fall in SHBG without any alteration in testosterone concentration.

Prostatic tissue contains oestradiol receptors (Chaisiri and Pierrepoint 1980). Although the importance of endogenous oestrogens in prostatic cancer is uncertain there is some evidence that they are protective. Thus, autopsy studies of patients with cirrhosis of the liver, where testosterone levels are low (Gordon, Altman, Southren, Rubin, and Lieber 1976) and oestradiol levels are high (Siiteri and MacDonald 1973), indicate a lower incidence of prostatic cancer compared with controls of the same age (Glantz 1964). Blood oestradiol concentrations and SHBG binding capacity measurements in 116 patients with prostatic cancer supports this view. Significantly higher SHBG capacities and lower oestradiol levels were associated with poorly differentiated tumours while the mean amounts of free oestradiol were 30 per cent higher in those with the most differentiated tumours (Haapiainen, Rannikko, Adlercreutz, and Alfthan 1986).

3 BREAST CANCER

Early work on mammary cancer in murine species (Noble 1964), epidemiological studies in the human (Kelsey 1979; Pike and Ross 1984) and clinical experience with endocrine treatment of breast cancer (Hayward and Rubens

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1987) has led to the conclusion that ovarian hormones, especially the oestrogens, are intimately involved in the induction of breast cancer in the human. Despite this, however, there is very little convincing evidence that there are consistent abnormalities in blood levels of ovarian, adrenal, pituitary or hypothalamic hormones in patients either with breast cancer or at high risk of developing breast cancer (see Moore, Thomas, and Wang 1986, for review).

Recently, attention has focused on the biologically available fraction of oestradiol in the blood of patients with breast cancer and several laboratories have found significantly increased percentages of non-protein-bound oestradiol in patients with breast cancer compared with matched controls (Siiteri *et al.* 1981; Moore *et al.* 1982; Reed, Cheng, Noel, Dudley, and James 1983; Langley, Hammond, Bardsley, Sellwood, and Anderson 1985; Ota, Jones, Jackson, Jackson, Kemp, and Bauman 1986). Significantly increased percentages of the albumin-bound fraction of oestradiol were also found in two of these studies (Langley *et al.* 1985; Ota *et al.* 1986). In all of these investigations, the available fractions of oestradiol (free and albumin-bound) were correlated with the SHBG binding capacity. In two studies (Moore *et al.* 1982 and Ota *et al.* 1986) but not in the others (Siiteri *et al.* 1981; Reed *et al.* 1983; Langley *et al.* 1985) where no differences were observed, the increase in the available oestradiol fraction was partially dependent on diminished SHBG binding. Adami, Johansson, Vegelius, and Victor (1979) also found marginally but significantly lower capacities in 122 patients with newly diagnosed breast cancer compared to the same number of age matched controls. On the other hand Sulkes, Fuks, Gordon, and Gross (1984) and Meyer, Brown, Morrison, and MacMahon (1986) found that women with breast cancer had higher SHBG binding capacities than normal women of the same nutritional status.

The reason for the increased percentage of free oestradiol in the presence of normal or only slightly diminished SHBG capacities is not clear and has been ascribed to interference of oestradiol binding by free fatty acids (see Moore *et al.* 1982). Credence to this hypothesis is given by Bruning and Bonfrer (1986) who, though unable to confirm that the percentage of free oestradiol is elevated in patients with breast cancer (Bruning, Bonfrer, and Hart 1985) have shown *in vitro* that the polyunsaturated free fatty acids (arachidonic, linoleic and linolenic) tend to displace oestradiol from both SHBG and albumin. They also found, in a study involving 56 women with breast and other forms of cancer, that an increase in free fatty acids during an overnight fast was accompanied by an increase in the percentage free oestradiol. A similar increase was observed after lipase activation by intravenous injection of heparin (500 i.u.). Further evidence that fatty acids can displace steroids from blood binding proteins is presented by Umstot and Andersen (1986) and by Reed and his colleagues (Reed, Baranek, Cheng, and James 1986; Reed, Cheng, Beranek, Few, Franks, Gilchik, and James 1986).

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A prospective study involving 5000 women living on the island of Guernsey (see below) has shown that those who developed breast cancer usually had a higher percentage of free oestradiol than matched controls and that, again, this was at least in part explained by diminished SHBG levels (Moore *et al.* 1986).

Siiteri *et al.* (1981) were the first to demonstrate the increase in percent free oestradiol in patients with breast cancer but they have subsequently been unable to confirm their original findings. They suggest that this was due to the age of the serum samples used in the first study (Siiteri, Simberg, and Murai, 1984). Certainly, Langley *et al.* (1985), whose study was confirmatory of the free oestradiol hypothesis, showed a change in affinity of SHBG for 5α-dihydrotestosterone during prolonged storage at −20°C and matched their samples from cases and controls appropriately.

Studies in Japan and the USA have led to the claim that there are positive correlations between tumour oestradiol receptor levels and SHBG binding capacities indicating that SHBG may be a strong indicator of response to hormone therapy (Murayama *et al.* 1978; Murayama, Utsonomiya, Takahashi, Kitamura, Tominga 1979; Plymate, Stutz, and Farris 1984). High SHBG binding capacities were also associated with a long disease free interval (Muryama, Utsonomiya, Asano, and Bulbrook 1979). At least two studies have failed to confirm the relationship between SHBG and receptor status (Mason, Miller, Hawkins, and Forrest 1981; Sulkes *et al.* 1984) and Harris, Smith, Dowsett, Jeffcoate, Coombes, Powles, and Neville (1981) were unable to confirm the relationship between SHBG capacity and recurrence of breast cancer in British women. The differences may be ascribed to differing study populations or to the methods used (Harris *et al.* 1981).

The evidence that raised levels of plasma oestradiol are important in the aetiology and clinical course of breast cancer is weak and results on bioavailable oestrogens are still not definitive. Whether risk of breast cancer, free fatty acid concentrations and bioavailable oestradiol are interrelated remains to be determined.

4 ENDOMETRIAL AND CERVICAL CANCER

Ratajczak, Twaddle and Hahnel (1980) found significantly higher mean SHBG binding capacity in postmenopausal patients with oestrogen-receptor positive endometrial or cervical cancer than in those with oestrogen-receptor negative tumours.

Gambone, Pardridge, Lagasse, and Judd (1982) used the blood/brain assay (which measures the transport of labelled steroid through the brain capillary wall and, hence, the proportion of steroid bound to SHBG) to compare the availability of oestradiol in patients with endometrial cancer and weight-matched controls. They found no significant differences in the two groups but brain uptake was related to body size. Thus obese women show a

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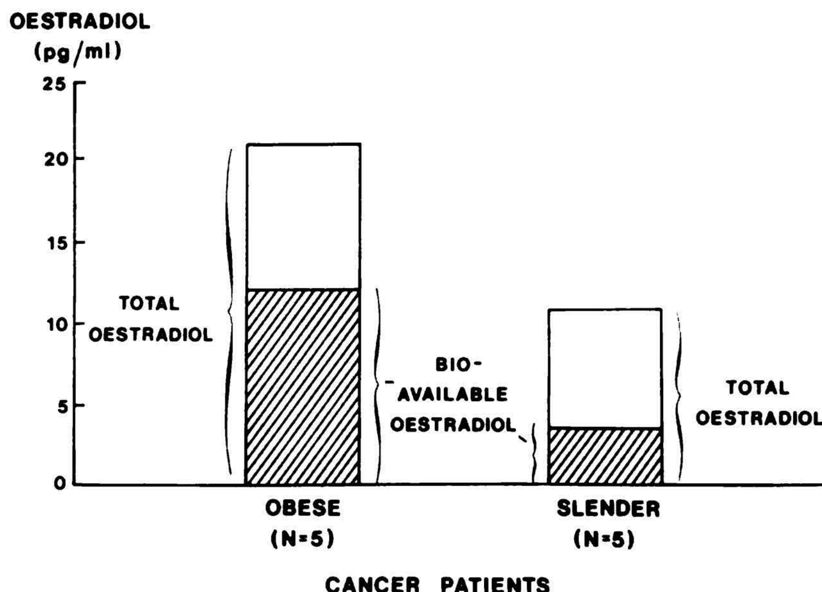

Fig. 5.6. Available oestradiol in patients with endometrial cancer. (Reprinted with permission from Gambone, Pardridge, Lagasse, and Judd (1982).

dual effect: their total plasma oestradiol is twice as high as that of slender controls and, because of diminished binding, their available oestradiol levels are four times higher (see Figure 5.6).

Weight is a known risk factor for endometrial cancer and the use of weight-matched controls is questionable. In a subsequent publication, Laufer and his colleagues showed that patients with endometrial cancer had higher levels of oestradiol and lower levels of SHBG than women with osteoporotic hip fractures who were used as, somewhat unsatisfactory, controls (Laufer, Davidson, Ross, Lagasse, Siiteri, and Judd 1983).

5 OTHER DISEASES

i Osteoporosis

It is widely accepted that endocrine function is of primary importance in osteoporosis, especially the oestrogens, but attempts to implicate particular hormonal abnormalities with the disease have not been strikingly successful (see, for example Davidson, Riggs, Coulam, and Toft 1980). This situation is similar to that found for breast cancer.

Osteoporosis may be a disease in which SHBG is important. Davidson, Ross, Paganini-Hill, Hammond, Siiteri, and Judd (1982) compared elderly women who had a hip fracture with controls and, while they found no differences in the total amounts of plasma oestrogens, SHBG levels were higher and unbound oestradiol was lower in the fracture group.

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There has been a proposal that the anti-oestrogen Tamoxifen, might be used for the prevention of breast cancer and that SHBG assays could be used for identifying women at high risk since there is some evidence that low levels of this protein are related to enhanced risk (Cuzick *et al.* 1985). One of the drawbacks to this proposal is that women might become osteoporotic and it would be a matter of importance to monitor bone densities if preventitive experiments were carried out.

ii Multiple myeloma

De Moor, Louwagie, Faict, and Vanham (1986) showed that male patients with multiple myeloma (Kahler's disease) have higher serum SHBG and lower CBG binding activity than normal, age-matched blood donor controls. Serum CBG in patients whose myeloma was secreting immunoglobulin light chains of the lambda variety were significantly lower whereas those patients secreting kappa light chains had a significantly higher mean SHBG activity. De Moor *et al.* (1986) suggest that low CBG levels are more likely to arise from the lambda repertoire of clones and the high SHBG from the kappa variety, and suggest that immunoglobulin light chain *V* genes may be genetic determinants for low CBG and high SHBG binding capacity in serum.

iii Cardiovascular disease

SHBG has been measured in several studies aimed at clarifying the relationships between the endocrine environment and risk factors for cardiovascular disease. Lapidus *et al.* (1986) have shown an intriguing relationship between SHBG concentration and risk of myocardial infarction. The plot of the 12-year incidence against SHBG was in fact U-shaped, indicating that high or low values are associated with higher risk.

Although Heller, Wheeler, Micallef, Miller, and Lewis (1983) found a negative association between SHBG and HDL-C, positive relationships have usually been observed (Semmens, Rouse, Beilin, and Maserei 1983; Hämäläinen, Adlercreutz, Enholm, and Puska 1986). In our population study (Moore *et al.* 1987) we observed that premenopausal women who smoked had, on average, serum levels of SHBG (corrected for weight and other covariates) which were 14 per cent higher than non-smokers. Postmenopausally, the trend was the same although not statistically significant. Lapidus *et al.* (1986) made similar observations in postmenopausal women, although in men SHBG was not related to smoking habits (Lindholm *et al.* 1982).

VIII Concluding comments

In this review, we have examined some of the recent advances in knowledge

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of SHBG over a fairly broad front. At the fundamental level, the elucidation of the structure of human SHBG represents a highly significant landmark and the culmination of years of intensive effort. These advances will result in the introduction of many new and exciting techniques such as those of immunocytochemistry and molecular biology which could, in a short time, clarify questions concerning synthesis and localization of SHBG within tissues.

New methods of analysis where the actual concentration of SHBG can be measured with high precision, accuracy, sensitivity and speed, has already enabled the assay of SHBG in large populations which have helped to identify some of the factors which affect the considerable variation in SHBG. Our own study in normal women in Guernsey has shown that in addition to weight, which has a strong effect on SHBG, other factors which influence peripheral levels of the protein include age at menarche and parity in pre-menopausal women, age in postmenopausal women and possibly smoking history. The mechanisms by which these factors affect SHBG are totally unknown.

The unconfirmed report (Uriel *et al.* 1982) that SHBG variants exist which show defective binding during pregnancy raises the question about the relationship between assays which measure the binding capacity and those which measure concentration. Hammond *et al.* (1985) in a limited study could not demonstrate any discrepancies between SHBG concentration measured by their immunoradiometric assay and the 5α-dihydrotestosterone binding capacity. It would be helpful to know, in a large population, if such variants exist and if so, their frequency.

With respect to the effects of drugs on SHBG, although stimulation or suppression of synthesis by the liver is widely assumed, it remains a possibility that the changes in peripheral SHBG activity brought about by drug administration could be the result of alterations in degradation of the protein. The use of the hepatoma-derived cell line (Hep G2) has already confirmed that oestradiol and thyroid hormones stimulate SHBG synthesis (Mercier-Bodard and Baulieu 1986; Lee, Dawson, Wetherall, and Hahnel 1987). The surprising finding that androgens also stimulate SHBG synthesis in these cells (Lee *et al.* 1987) is contrary to the traditional concept of hormonal control and obviously needs to be investigated further.

The prevalent concepts about transport of steroidal hormones into cells has been thoroughly covered by Pardridge in this volume and it would be superfluous to add significantly to his discussion. However, the finding of very high intra-cellular concentrations of several steroid hormones (200-fold for oestradiol in extreme cases), and the lack of correlation between these levels and the amount of oestrogen receptor protein, leads us to the supposition that some form of intra-cellular control mechanism exists. The cellular levels of steroids are well within the range at which they would have a pronounced effect on several key enzymes (for example glucose-6-phosphate

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dehydrogenase). It would be illogical to agonize over 2- or 3-fold differences in availability of steroids in the blood brought about by changes in SHBG concentration, while ignoring the fact that tissue levels of oestradiol (in the breast for example) average 20 times the blood levels. If the key events in the final biological action of steroids is mainly affected by intra-cellular availability, it would not be surprising that so many of the results that we have reviewed are indecisive. This is exactly what would be expected if blood levels of a binding protein are only poorly correlated to intra-cellular events.

Present indications point to the fact that concentrations of the protein may be related to the aetiology of diseases which account for a substantial proportion of the early deaths in man (hormone-related cancers, cardiovascular disease). Indeed Lapidus and his colleagues (1986) have found an inverse correlation between SHBG levels and the 12 year mortality rates in women. The importance of elucidating the precise role of SHBG cannot be underestimated.

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