6 Selective delivery of sex steroid hormones to tissues by albumin and by sex hormone-binding globulin

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I Introduction

Sex steroids such as testosterone or oestradiol circulate in human serum in three physical states: free, albumin-bound, and sex hormone-binding globulin (SHBG)-bound (Westphal 1971). About 2 per cent of the total sex steroid is free and the remaining 98 per cent is distributed to either albumin or to SHBG in varying proportions, depending on the relative concentrations of the two plasma proteins. It has generally been regarded over the last thirty years that only the free fraction is biologically available for uptake by tissues *in vivo*, and this view has been called the free hormone hypothesis (Tait and Burstein 1964; Westphal 1971; Siiteri 1981). The free hormone hypothesis has both basic and clinical implications. With regard to basic endocrinology, the free hormone hypothesis predicts that the concentration of free cytoplasmic or nuclear hormone, that is, the hormone pool that drives nuclear receptor occupancy and hormone biological action, may be reliably determined by measurements of free hormone concentrations in human serum *in vitro* (Pardridge 1987). With regard to clinical endocrinology, the free hormone hypothesis predicts that clinical status in patients can also be assessed by measurements of *in vitro* concentrations of free hormone in human serum (Pardridge 1981). In addition, the free hormone hypothesis predicts that, in the absence of cellular metabolism, all tissues are exposed to an equal concentration of circulating free hormone and the function of hormone-binding plasma proteins, such as albumin or SHBG, is to sequester passively steroid hormone within the circulating plasma. As is true in other areas of science, theories that posit a passive role to a biologic process have little, if any, heuristic value and generally lead to little progress, often for long periods of time.

This chapter will review and develop further the protein-bound hormone hypothesis (Pardridge 1981; Pardridge 1987), which assumes that plasma proteins such as albumin or SHBG selectively and dynamically deliver hormones to tissues via mechanisms that vary from tissue to tissue, from species to species, during development and among differing clinical conditions. Since it has been known for nearly thirty years that plasma proteins *per se* do not significantly leave the plasma compartment during a single circulatory pas-

sage through the organ (Dewey 1959), then it is clear that the finding that albumin-bound or SHBG-bound sex steroid is available to tissues *in vivo* indicates that the hormone is stripped away from the plasma protein as it courses through the organ microcirculation. In this way, the plasma protein-bound hormone is operationally available for transport into tissue without significant exodus of the plasma protein *per se*. Therefore, the important parameter is not the concentration of free hormone in serum measured *in vitro*, for example, by equilibrium dialysis but the concentration of exchangeable hormone measured *in vivo* in a particular organ (Pardridge 1981; Pardridge 1987). There are several implications of the protein-bound hormone hypothesis with respect to both basic and clinical endocrinology. The protein-bound hormone hypothesis predicts that the concentrations of free cytosolic and nuclear hormone, which have never been directly measured *in vivo*, may be log orders higher than the concentration of free hormone, but approximate the concentration of plasma exchangeable hormone measured with *in vivo* techniques. For example, if it is found that albumin-bound steroid hormone is readily available for transport into a particular organ, then the concentration of free cytosolic hormone in that organ, in the absence of significant cellular metabolism of hormone, will be equal to the free plus albumin-bound concentration as measured *in vitro* (Pardridge and Landaw 1985). The albumin-bound concentration is often ten-fold higher than the free concentration and is approximately equal to the dissociation constant (K_D) of testosterone or oestradiol nuclear receptors (Fig. 6.1). In order to generate 50 per cent nuclear receptor occupancy, the concentration of free cellular hormone must equal the K_D of the nuclear receptor. As shown in Fig. 6.1, the concentration of albumin-bound testosterone or oestradiol does, in fact, approximate the K_D of the respective receptor, whereas the concentration of free testosterone or oestradiol only leads to very low occupancy of the nuclear receptor and does not allow for saturation of hormone biological response (Siiteri, Murai, Hammond, Nisker, Raymoure, and Kuhn 1982). With regard to clinical endocrinology, the protein-bound hormone hypothesis predicts that either the concentration of albumin-bound hormone or the total concentration of hormone in plasma will reliably predict the hormone biological response in a particular organ for a given clinical condition. Whether the albumin-bound or the total concentration of hormone is used to predict clinical status depends on whether both albumin-bound and SHBG-bound hormone are available for uptake by a particular organ. The protein-bound hormone hypothesis has heuristic value since it leads to the discovery of organ selective transport mechanisms involving albumin or SHBG. In addition, this hypothesis should also lead to the discovery of the biochemical mechanisms mediating the catalysis of hormone dissociation from the binding proteins within the organ microcirculation (Pardridge 1987).

The need for a unified transport theory is illustrated by the diversity of opinion regarding the mechanisms of steroid hormone delivery to tissues *in*

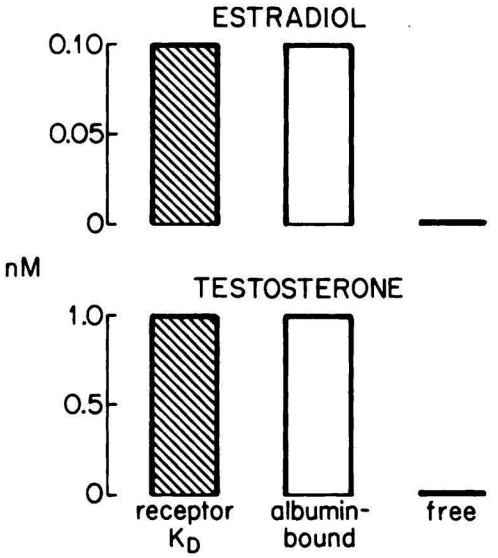

Fig. 6.1 Comparison of nuclear receptor dissociation constant (K_D), albumin-bound fraction, and free fraction for testosterone and oestradiol. These comparisons show that the concentration of free sex steroid is very small compared to the K_D, that is, the concentration of free nuclear steroid required to cause 50 per cent occupancy of the nuclear receptor. In contrast, the concentration of albumin-bound sex steroid approximates the K_D. Therefore, the availability of circulating plasma protein-bound sex steroid to tissues allows for significant occupancy of the nuclear sex steroid receptor and for saturation of hormone biological response. Data from Siiteri *et al.* (1982); Griffin and Wilson (1985); Pardridge (1986*b*).

vivo. Tait and Burstein (1964) recognized twenty years ago that albumin-bound steroid hormones are available for uptake by liver, since this organ extracts 10–50 per cent of circulating steroid hormone on a single passage. They proposed that globulin-bound hormone is not available for uptake by tissues, including liver. Implicit in their hypothesis is the idea that only free hormone is available for uptake by tissues that only minimally extract steroid hormone from the circulation. This view was explicitly stated by Riad-Fahmy and co-workers (1982) who proposed that free hormone concentration is important in tissues where metabolic clearance rate (MCR) is low, but concentrations of bound hormone are important in tissues where MCR is high. This view is actually a restatement of the free hormone hypothesis, since it predicts that the concentration of free cellular hormone cannot exceed the concentration of free hormone measured *in vitro*, and significant hormone nuclear receptor occupancy would not be attainable (Fig. 6.1). In contrast, the protein-bound hormone hypothesis assumes that the delivery of albumin-bound or SHBG-bound hormone to tissues is independent of the rate of hormone metabolism by the organ and is a function of conformational changes about the plasma protein hormone binding site caused by interactions between the protein and the surface of the microcirculation (see Biochemical Model below).

II Quantifiable physiological model

A quantifiable physiological model of sex steroid transport into tissues is shown in Fig. 6.2. The concentration of free cellular hormone (L_M) is believed to be the single-most important parameter determining either nuclear receptor occupancy and hormone biological response or hormone metabolism (Tait and Burstein 1964). For example, hormone biological response is a function of the degree of nuclear receptor occupancy, and this occupancy is proportional to the total concentration of nuclear receptor and to the concentration of free nuclear hormone, which is assumed to be in equilibrium with free cytoplasmic hormone (L_M). Similarly, the rate of steroid hormone metabolism, which determines the MCR, is proportional to the total concentration of cytosolic enzymes and to the concentration of free cytoplasmic hormone (Tait and Burstein 1964).

Despite the importance of the free cytoplasmic hormone concentration (L_M), this parameter has never been measured directly *in vivo* since this would require the development of a testosterone or oestradiol sensitive electrode that can be placed into the cellular cytoplasm. The concentration of free cytoplasmic hormone, however, can be measured indirectly by determination of the concentration of plasma exchangeable hormone (L_F, Fig. 6.2). Given the simplified case of a single plasma protein-binding system where hormone dissociation is rapid compared to capillary transit time, the concentrations of free cytoplasmic hormone (L_M) and capillary exchangeable hormone (L_F) are given by (Pardridge and Landaw 1985):

$$L_M = \frac{k_3}{k_4 + k_9}(V_P/V_T)\cdot L_F \tag{1}$$

$$L_F = \left[\frac{K_A^a}{A_F + K_A^a(1 + R)}\right] L_T^\circ \tag{2}$$

$$R = \frac{k_3 \cdot k_9}{(k_4 + k_9)k_{10}} \tag{3}$$

where k_3, k_4, k_9, and k_{10} are defined in Fig. 6.2 and Table 6.1, and V_P = the organ capillary plasma volume, V_T = the organ extravascular water volume, K_A^a = the apparent *in vivo* molar dissociation constant of ligand binding to the plasma protein within the microcirculation, and A_F = the concentration of unoccupied plasma protein binding sites (Table 6.1). The above equations are applicable to the rat since there is little SHBG circulating in this species (Corvol and Bardin 1973). However, in other species, such as humans, which possess SHBG, the above equations are simplifications of more complex relationships (Pardridge and Landaw 1985).

The above equations are simple illustrations of important principles. First, when organ metabolism is nil, for example, R is ≈ 0, plasma exchangeable hormone (L_F) = free cytosolic hormone (L_M). Although L_M is not presently

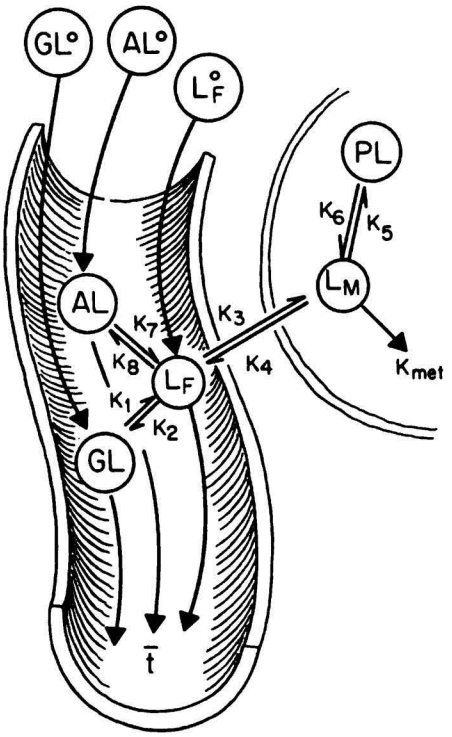

Fig. 6.2 Steady state model of testosterone transport through the brain capillary wall and into brain cells. Rate constants are defined in Table 6.1, where rate constant of hormone metabolism (k_{met}) is defined as k_9. Pools of globulin-bound, albumin-bound, and free ligand in the systemic circulation are denoted as GL°. AL°, and L_F°, and pools of globulin-bound, albumin-bound and exchangeable ligand in the brain capillary are GL, AL, and L_F, respectively. Pools of free and cytoplasmic-bound steroid in cells are given by L_M and PL, respectively; $\bar{t}$ is mean capillary transit time. (From Pardridge and Landaw 1985 with permission).

amenable to direct empiric measurement, L_F can be determined for a particular organ using the tissue sampling single injection technique (Pardridge 1981). Second, the above equations illustrate that the concentration of cytoplasmic free hormone is fully independent of cytoplasmic binding proteins and is simply a function of the concentration of capillary exchangeable hormone, the rates of membrane transport (k_3, k_4), and cytoplasmic metabolism (k_9). Third, as emphasized previously by Tait and Burstein (1964), hormone volume of distribution (V_D) and MCR are not linked if, as depicted in Fig. 6.2, cellular enzymes act only on free cytoplasmic hormone. Hormone volume of distribution is a function of hormone binding to cytoplasmic proteins. However, MCR is independent of hormone binding to cytoplasmic proteins, for example:

$$MCR = E^{SS} \cdot F \tag{4}$$

$$E^{SS} = \frac{f(R)}{1 + fR} \tag{5}$$

Table 6.1

Testosterone basal parameters

Parameter No.	Parameter symbol U	Parameter name	Parameter value
1	k_1 (s^{-1})	Capillary globulin-ligand dissociation	0.03
2	k_2 ($M^{-1}\cdot s^{-1}$)	Capillary globulin-ligand association	1.5×10^7
3	k_3 (s^{-1})	Plasma to tissue influx	1.9
4	k_4 (s^{-1})	Tissue to plasma efflux	0.0271
5	k_5 ($M^{-1}\cdot s^{-1}$)	Cytosolic protein-ligand association	1.0×10^7
6	k_6 (s^{-1})	Cytosolic protein-ligand dissociation	0.03
7	k_7 (s^{-1})	Capillary albumin-ligand dissociation	2.5×10^3
8	k_8 ($M^{-1}\cdot s^{-1}$)	Capillary albumin-ligand association	1.0×10^6
9	k_9 (s^{-1})	Ligand tissue metabolism	0 or 0.1
10	k_{10} (s^{-1})	Plasma transit	1.0
11	K_G (M)	Systemic globulin dissociation constant	2.0×10^{-9}
12	K_A (M)	Systemic albumin dissociation constant	5.3×10^{-5}
13	G_T° (M)	Total globulin plasma concentration	2.8×10^{-8}
14	A_F (M)	Free (total) albumin plasma concentration	6.4×10^{-4}
15	P_T (M)	Total cytosolic protein concentration	2.5×10^{-9}
16	L_T°	Total ligand plasma concentration	1.0×10^{-8}
17	V_P (1/kg)	Brain capillary plasma volume	0.01
18	V_T (1/kg)	Brain extravascular water volume	0.70

The assignments of k_7 and k_8 are somewhat arbitrary, because only the ratio of k_7/k_8 is known (Pardridge and Landaw, 1984). However, simulation studies showed that increasing k_7 and k_8 to 2.5×10^5 s^{-1} and 1.0×10^8 $M^{-1}\cdot s^{-1}$, respectively, had no effect on the results in Table 6.3. The range in varying k_8 was restricted to 10^6 to 10^8 ($M^{-1}\cdot s^{-1}$) because this is the known range of hormone-plasma protein association rate constants (Westphal, 1978). However, decreasing k_7 and k_8 to 53 s^{-1} and 21,200 ($M^{-1}\cdot s^{-1}$), respectively, also had no significant effect on the results in Table 6.3. From (Pardridge and Landaw 1985 with permission).

where E^{SS} = the net steady state hormone extraction by the organ, F = organ blood flow, and $f = K_A^*/(A_F + K_A^*)$, that is, the fraction of capillary exchangeable hormone. Thus, MCR, like the free cytoplasmic hormone (and nuclear receptor occupancy), is strictly a function of the concentration of capillary exchangeable hormone, membrane transport, and organ metabolism, and is independent of tissue-binding proteins.

While it is possible to derive the relatively simple equations listed above that predict the concentrations of capillary exchangeable and cytoplasmic free hormone for a single, rapidly dissociating plasma protein system, the equations describing the two protein (albumin and SHBG) models, as shown in Fig. 6.2, are more complex and are best analyzed using a small computer. A program in Basic for performing such an analysis has recently been published (Pardridge and Landaw 1987), and this program was used in previous studies to estimate the concentration of free cytoplasmic testosterone in brain under a variety of conditions using the model parameters listed in Table 6,1 (Pardridge and Landaw 1985). The reader is encouraged to copy this program in Basic for use on any microcomputer since it provides a useful interactive approach to learning transport theory. The model is composed of eighteen parameters (Table 6.1) and it is important to understand each of these parameters since they all describe discrete physiological events.

1 STEROID DISSOCIATION RATES IN VIVO

The parameters k_1, k_2, k_7, and k_8 refer to the capillary-globulin or albumin-ligand dissociation or association rates *in vivo* (Table 6.1). Since previous studies have shown that SHBG-bound testosterone is not available for uptake by brain (Pardridge 1981), k_1 is set at 0.03 sec^{-1}, which is the measured rate of testosterone dissociation from SHBG *in vitro* at 37°C (Heyns and De Moor 1971). Since the SHBG dissociation constant (K_G) is 2 nM (Mickelson and Petra 1978) and since $k_2 = k_1/K_G$, then $k_2 = 1.5 \times 10^7$ $M^{-1}\,sec^{-1}$. Previous studies have shown that, owing to enhanced rates of dissociation of testosterone from albumin within the brain microcirculation, the capillary albumin dissociation constant for testosterone (K_A^*) = 2500 μM (Pardridge and Landaw 1984). Assigning $k_8 = 1 \times 10^6 M^{-1} sec^{-1}$, then $k_7 = K_A^* \times k_8 = 2500\,sec^{-1}$. The assignments of k_7 and k_8 are somewhat arbitrary because only the ratio of k_7/k_8 is known. However, simulation studies have shown that increasing k_7 and k_8 by up to two log orders of magnitude has no effect on the results (Pardridge and Landaw 1985). The range in varying k_8 was restricted to $10^6 - 10^8\ M^{-1} sec^{-1}$ because this is the known range of hormone-plasma protein association rate constants (Westphal 1978). The assignment of k_7 in Table 6.1 only pertains to brain since other studies have shown that the rate of testosterone dissociation from albumin varies from organ to organ (Table 6.2). As discussed below, the degree to which testosterone dissociation is enhanced in the organ microcirculation is

Table 6.2

In vivo dissociation constant (K_D^a) *of bovine albumin binding of testosterone or oestradiol in three organs*

Organ	K_D^a (μM)	
	Testosterone (T)	Oestradiol (E_2)
Brain	2,500 ± 700	710 ± 100
Salivary Gland	602 ± 40	N.D.
Lymph Node	300 ± 90	1,500 ± 500

The *in vitro* K_D is 53 ± 1 μM and 23 ± 1 μM, respectively, for T and E_2. N.D. = not determined. (From Pardridge and Landaw 1984; Cefalu, Pardridge, Chaudhuri, and Judd 1986; Cefalu and Pardridge 1987).

believed to be a function of the conformational change about the testosterone binding site that, in turn, is caused by interactions between the plasma protein and the microcirculatory surface (Pardridge and Landaw 1984). Since this interaction may differ among organs, the conformational change and the ultimate rates of testosterone dissociation may also vary from organ to organ (see **III Biochemical model**). It is assumed that interactions between the binding protein and the surface of the large systemic arteries is minimal and that the rates of hormone dissociation from binding proteins in the arterial circulation is approximately equal to the values found *in vitro*. Therefore, the systemic globulin or albumin dissociation constants (K_G or K_A) are fixed at the respective *in vitro* values of 2 nM (Mickelson and Petra 1978) and 53 μM (Pardridge 1981), respectively (Table 6.1).

2 ALBUMIN AND SHBG CONCENTRATIONS

The concentration of albumin (A_F) or SHBG (G_T°) is fixed at the normal male values of 640 μM or 28 nM, respectively (Pardridge and Landaw 1985). These values may vary among clinical conditions. For example, SHBG is elevated and albumin is decreased in cirrhosis (see **VII Clinical endocrinology**).

3 MEMBRANE TRANSPORT

The plasma to tissue influx and the tissue to plasma efflux of hormone is given by k_3 and k_4, respectively (Table 6.1). Previous studies have shown that the k_3 value for testosterone transport through the brain capillary endothelial wall, that is, the blood-brain barrier (BBB), is 1.9 sec^{-1} (Pardridge and Landaw 1984). It is assumed that cell membrane permeability to hormone is symmetric (that is, there is no active transport) such that the ratio of transport rate constants $k_3/k_4 = V_P/V_T$, where $V_P = 0.01$ L/kg and $V_T = 0.70$ L/kg, respectively (Pardridge and Landaw 1985). Therefore,

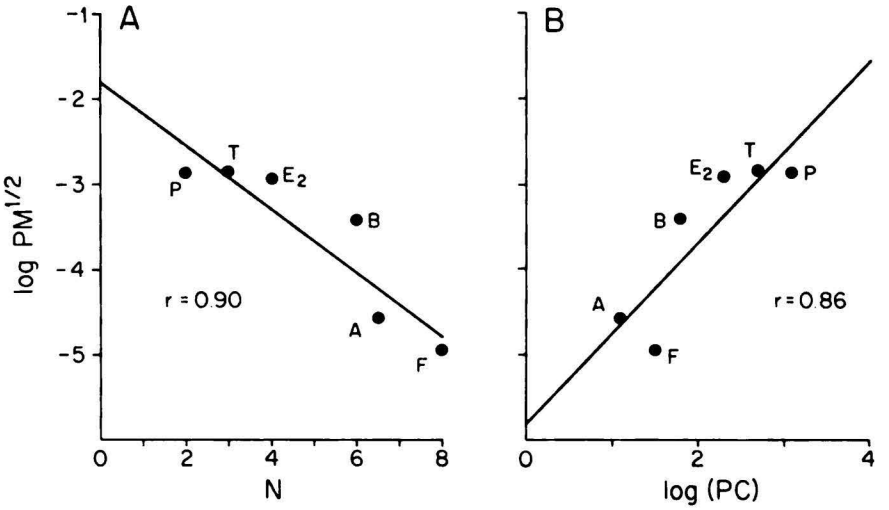

Fig. 6.3 (A) The log P (mol wt)1/2 for each hormone is plotted versus the number (N) of hydrogen bonds the steroid makes in aqueous solution, where P is the blood-brain barrier permeability constant. The N value was assigned according to the rules of Stein (1967): 2 for hydroxyl groups, 1 for carbonyl, aldehyde, or ketone groups, and 0 for ether moieties. (B) The log P (mol wt)1/2 per ionic is plotted versus the log of the 1-octanol/Ringer's partition coefficient. Steroids have been abbreviated as follows: P—progesterone, T—testosterone, E_2—oestradiol, B—corticosterone, A—aldosterone, F—cortisol. (From Pardridge and Mietus 1979*b* with permission).

$k_4 = k_3\ (V_P/V_T) = 0.0271\ sec^{-1}$. Previous studies have shown that BBB transport of testosterone or oestradiol is nonsaturable up to 25 μM concentrations of steroid hormone (Pardridge 1981) and this transport process is presumed to be free diffusion. Although some studies have suggested that steroid hormones traverse cell membranes via carrier-mediated transport (Milgrom, Atger, and Baulieu 1973; Rao 1981), this has not been confirmed (Muller and Wotiz 1979; Kilvik, Furu, Haug, and Gautvik 1985), and would seem unlikely for steroid hormones that are highly lipid soluble. The major factor regulating cell membrane transport of steroid hormones appears to be the polarity of the molecule as determined by the hydrogen bond number (Pardridge 1981). As illustrated by the linear regression plot in Fig. 6.3, membrane transport of steroid hormones can be predicted by determining the hydrogen bond number, that is, the number of hydrogen bonds formed between the water solvent and the polar functional groups on the steroid hormone nucleus. The hydrogen bond number is computed on the basis of the steroid hormone structure (Fig. 6.4), given the rules of hydrogen bonding: hydroxyl group forms two hydrogen bonds, carbonyl, aldehyde, or ketone groups form one hydrogen bond, and ether moieties form no hydrogen bonds (Stein 1967).

Active transport of steroid hormones across the cell membrane would allow for an enrichment of the pool size of the cytoplasmic hormone relative to concentration of plasma exchangeable hormone, and would be repre-

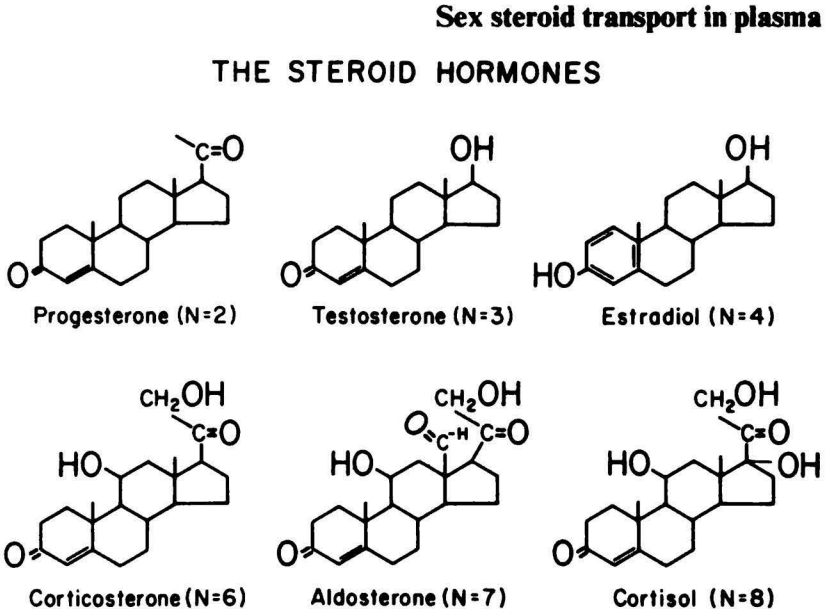

Fig. 6.4 Molecular structures for steroid hormones with emphasis placed on hydrogen bond forming functional groups. The addition of one hydroxyl to corticosterone results in the formation of cortisol and a log-order decrease in BBB permeability (see Fig. 6.3). N = number of hydrogen bonds. (From Pardridge 1981 with permission).

sented by a k_4 value that is disproportionately low given the known k_3, V_P, and V_T values. Thus far, there have been no studies published which support active transport of steroid hormones across cell membranes. Transport studies performed in isolated cells *in vitro* show saturable uptake of steroid hormones by the cells against the concentration gradient (Milgrom *et al.* 1973; Rao 1981). However, it is likely that these studies are actually measuring cytoplasmic binding and not membrane transport, since the latter process probably reaches equilibrium within a second in an isolated cell system where the extracellular water volume is log orders greater than the intacellular water volume. For example, initial studies suggesting active transport of thyroxine into isolated liver cells have been recently re-evaluated to show that thyroxine crosses the hepatocyte cell membrane by free diffusion (Rao and Rao 1983).

4 CAPILLARY TRANSIT TIME

The capillary transit time (t) = $1/k_{10}$, where k_{10} is the rate constant of plasma transit through the organ, equals 1.0 sec for brain (Pardridge and Landaw 1985). Organ blood flow = $V_P \times k_{10}$. Therefore, organ blood flow may increase owing to either increased plasma velocity (decreased transit time) or to increased plasma volume. There may be either a direct or inverse relationship between organ blood flow and capillary exchangeable hormone, depending on whether blood flow is increased owing to increased plasma

volume or to decreased plasma transit time. An increased plasma volume would have no effect on the *fraction* of capillary exchangeable hormone, but would decrease the concentration of total hormone in plasma (L_T°) and, thus, would decrease the capillary exchangeable hormone in proportion. However, if organ blood flow is increased owing to decreased plasma transit time (that is, increased k_{10}), then the R fraction would decrease (equation #3) and the concentration of capillary exchangeable hormone would increase (see equation #2).

5 CYTOPLASMIC HORMONE BINDING

The values for the dissociation constant of testosterone binding to brain cytoplasmic binding systems (K_P) has not been measured directly *in vivo*. However, the P_T/K_P ratio, where P_T = the concentration of cytoplasmic binding systems, has been measured in rat brain *in vivo* and is 0.67 for testosterone (Pardridge, Moeller, Mietus, and Oldendorf 1980). Moreover, the rate constant of testosterone dissociation from the brain cytosolic protein has also been measured directly, that is, $k_6 = 0.03\ sec^{-1}$ (Pardridge *et al.* 1980*b*). Because $K_P = k_6/k_5$ and a typical $k_5 = 10^7\ M^{-1}\ sec^{-1}$ (Westphal 1978), then $K_P = 3$ nM and $P_T = 2$ nM (Table 6.1). If k_5 were arbitrarily increased or decreased, then both K_P and P_T would change in proportion to each other. The individual k_5 and k_6 rate constants are not used in the mathematical model, as only the k_6/k_5 ratio is important (Pardridge and Landaw 1985).

The cytoplasmic binding systems should not be confused with nuclear hormone receptors, since the sequestration of hormone by the nuclear receptor is probably trivial compared to cytoplasmic binding. It is known that both testosterone and oestradiol are avidly bound by cytoplasmic fatty acid binding proteins such as ligandin or Z-protein (Ketterer, Tipping, and Hackney 1976; Ketterer, Carne, and Tipping 1978).

Owing to the cytoplasmic binding, the volume of distribution of steroid hormone may be greatly in excess of the cellular water volume, and this explains why the tissue concentration of steroid hormones is oftentimes many-fold greater than the total plasma concentration of hormone. The volume of distribution (V_D) is given by

$$V_D = \frac{(L_M + PL)V_T}{L_T^\circ} \tag{6}$$

where PL = the pool of cytoplasmic-bound steroid hormone (Fig. 6.2). Therefore, hormone volume of distribution is a function of both plasma and cytoplasmic binding systems, membrane transport (k_3, k_4), and organ metabolism (k_9). While a primary increase in MCR (for example, owing to an increase in k_9) may result in a decreased hormone volume of distribution, the reverse is not true. A primary increase in hormone volume of distribution

(for example, owing to either increased P_T or decreased K_P) is predicted to have no effect on the concentration of cytoplasmic free hormone or MCR (Pardridge and Landaw 1985).

6 ORGAN METABOLISM

The rate constant of tissue metabolism of steroid hormone is given by k_9 (Table 6.1). If metabolic clearance of steroid hormone by a given organ is essentially nil, then $k_9 = 0$ and $R = 0$ (equation #3). When metabolic clearance by an organ is high, then k_9 is much greater than k_4 and R reduces to $k_3/k_{10} = k_3t$. Under these conditions, steroid hormone transport into the organ from plasma is rate limiting for overall hormone metabolic clearance and the steady state MCR is equal to the unidirectional clearance determined by the tissue sampling single injection technique. Although the use of the single injection technique and measurements of hormone undirectional clearance are the best estimate of *in vivo* plasma protein binding effects, measurements of unidirectional clearance may also predict hormone MCR if hormone metablism is rate limited by hormone transport. That this is, in fact, the case, is suggested by the good correlation between androgen MCR in humans and androgen unidirectional clearance measurements in rats. For example, the ratio of testosterone to dihydrotestosterone MCR in humans is 1.2–1.9 (Vermeulen and Ando 1979), and the ratio of unidirectional testosterone clearance to unidirectional dihydrotestosterone clearance by rat brain is 0.8–2.0 (Pardridge, Mietus, Frumar, Davidson, and Judd 1980). Other studies have shown that oestradiol MCR in the rat is rate limited by the transport of oestrogen out of the plasma compartment (Larner and Hochberg 1985).

7 STEADY STATE MODEL PREDICTIONS

Table 6.3 shows the predicted concentrations of testosterone in the various tissue pools (Fig. 6.2) for both the free hormone model (simulations #2 and #11) and the protein-bound model (simulations #1 and #3–10). In simulations #1–9, the rate constant of testosterone metabolism (k_9) is set at 0 and k_9 is set at 0.1 for simulations #10 and #11. Simulation #1 is the basal state and is described by the parameters listed in Table 6.1. These results show that the concentration of cytoplasmic free hormone is equal to the concentration of capillary exchangeable hormone but is nine-fold greater than the free hormone concentration (Fig. 6.5). Simulation #2 is the situation wherein the albumin dissociation constant (k_7/k_8) in the capillary is identical to the albumin dissociation constant *in vitro*, and this results in the concentration of cytoplasmic free hormone equalling the concentration of free hormone *in vitro*. Simulation #3 represents the case in which blood flow is increased five-fold owing to a five-fold increase in the velocity of capillary

Table 6.3
Steady state model predictions

Simulation No.	Parameter* Change	Arterial† G_L^0 nM	Arterial† A_L^0 nM	Arterial† L_F^0 nM	Capillary GL nM	Capillary AL nM	Capillary L_F nM	Cytosolic L_M nM	Cytosolic PL nM	V_D 1/kg	E^{ss}
1	basal	4.7	4.9	0.40	5.7	0.88	3.4	3.4	1.3	0.33	0
2‡	$k_7 = 53\ s^{-1}$	4.7	4.9	0.40	4.7	4.9	0.40	0.40	0.30	0.049	0
3	$k_{10} = 5\ s^{-1}$	4.7	4.9	0.40	5.0	1.0	4.0	4.0	1.4	0.38	0
4	$k_1 = 3\ s^{-1}$	4.7	4.9	0.40	1.8	1.7	6.5	6.5	1.7	0.58	0
5	$G_T^0 = 50$ nM	6.3	3.5	0.29	7.4	0.54	2.1	2.1	1.0	0.22	0
6	$P_T = 25$ nM	4.7	4.9	0.40	5.7	0.88	3.4	3.4	13.3	1.17	0
7	$G_T^0 = 50$ nM $A_F = 200\mu M$	8.1	1.5	0.39	8.7	0.10	1.2	1.2	0.73	0.14	0
8	$k_1 = 3\ s^{-1}$ $G_T^0 = 50$ nM $A_F = 200\ \mu M$	8.1	1.5	0.39	3.1	0.51	6.3	6.3	1.7	0.56	0
9	$G_T^0 = 0$	0	9.2	0.76	0	2.0	8.0	8.0	1.8	0.68	0
10	$k_9 = 0.1\ s^{-1}$	4.7	4.9	0.40	5.2	0.45	1.8	0.38	0.28	0.046	0.26
11‡	$k_7 = 53\ s^{-1}$ $k_9 = 0.1\ s^{-1}$	4.7	4.9	0.40	4.7	4.4	0.36	0.078	0.063	0.0098	0.054

*Basal parameters are listed in Table 6.1. Simulations #2–11 include basal parameters plus the respective change in individual parameters for each simulation. †Because the total ligand concentration is 10 nM (Table 6.1), the percent albumin-bound, globulin-bound, or free hormone in the arterial plasma may be computed by multiplying GL^0, AL^0, and L_F^0, respectively, by 10. ‡These simulations assume hormone-binding protein dissociation constant in the microcirculation is identical to the *in vitro* constant. (From Pardridge and Landaw 1985 with permission).

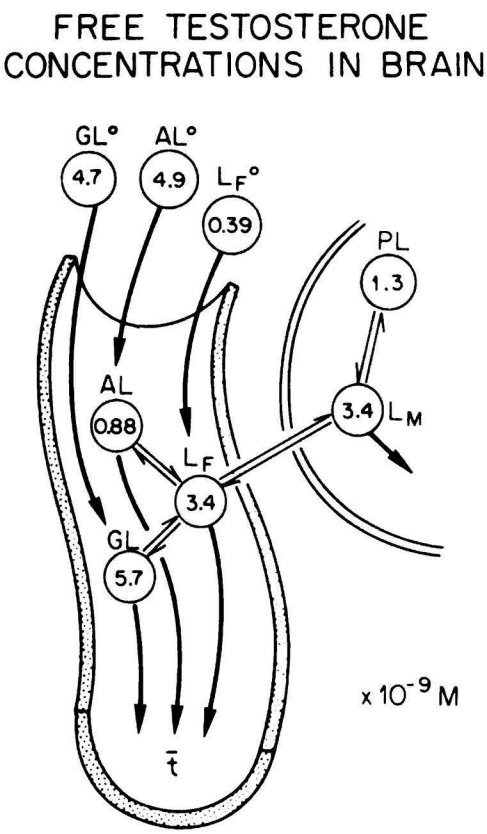

Fig. 6.5 Predicted steady state concentrations of testosterone in the various pools of the brain capillary and in brain cells that are depicted in Fig. 6.2. The pool sizes shown in this figure represent the basal state, which is simulation #1 in Table 6.3. The concentration of free cytosolic testosterone in brain cells is predicted to approximate the concentration of albumin-bound hormone in the circulation and to be more than ten-fold greater than the concentration of free hormone (L_F°). (From Pardridge and Landaw 1985 with permission).

plasma flow (that is, five-fold decrease in capillary transit time). In the case of zero metabolism, intracellular hormone concentrations are increased 18 per cent over the basal state, which indicates both the capillary exchangeable and cytosolic free hormone are a function of the capillary transit time.

Simulation #4 is the case of enhanced dissociation of testosterone from the SHBG binding site within the brain microcirculation. In this situation, testosterone is operationally available for transport out of the brain capillary from the circulating SHBG-bound pool without significant exodus of the globulin *per se*. In this case, the concentration of cytoplasmic free testosterone is increased approximately two-fold above the concentration found in simulation #1. Simulation #5 represents the case in which the concentration of SHBG is nearly doubled and, in this case, the systemic free hormone (L_F°), systemic albumin-bound hormone (A_L°), capillary exchangeable hormone (L_F), and cytoplasmic free hormone (L_M) are all decreased propor-

tionately relative to the basal state. Simulation #6 shows that a ten-fold increase in cellular hormone-binding protein results in a ten-fold increase in the concentration of cytoplasmic-bound hormone, but there is no change in the concentration of cytoplasmic free hormone. The concentration of bound hormone is directly related to the concentration of cytosolic binding sites and is inversely related to the dissociation constant (K_P).

Simulation #7 represents a case such as cirrhosis wherein the concentration of SHBG is increased and albumin concentrations are decreased (Sakiyama, Pardridge, and Judd 1982). In this case, the cytoplasmic free hormone is decreased to 35 per cent of control levels, although there is no change in the concentration of free hormone. On the other hand, if the SHBG concentration is increased, albumin concentration is decreased, and SHBG-bound testosterone is available for uptake by the tissue (for example, owing to an increase in k_1 over the basal state), then the concentration of cytoplasmic free hormone is actually increased. Simulation #9 represents the case wherein SHBG concentrations are zero. The concentration of free cytoplasmic testosterone is predicted to be ten-fold greater than the free hormone concentration and to approximate the concentration of albumin-bound testosterone.

Simulation #10 gives the hormone pool sizes when the metabolism of testosterone by the tissue is moderate, for example, $k_9 = 0.1$. Under these conditions, the steady state net extraction (E^{SS}) is 26 per cent. A marked concentration gradient exists across the cell membrane since the concentration of free cytoplasmic hormone is only 21 per cent of the concentration of plasma capillary exchangeable hormone. Moreover, increased hormone metabolism results in an 86 per cent decrease in hormone volume of distribution (Table 6.3). Simulation #11 represents the free hormone model (that is, no enhanced dissociation of testosterone from albumin-binding sites *in vivo*) and gives the pool sizes when $k_9 = 0.1$. In this situation, E^{SS} and V_D are approximately five-fold less than the values predicted for the protein-bound hormone model (simulation #10).

Simulations #2 and #11 expose two fundamental flaws of the free hormone hypothesis. First, if only free hormone is available for uptake by tissues, then the concentration of free cytosolic hormone does not exceed the concentration of free hormone measured *in vitro*, and this concentration is at least ten-fold lower than that needed to cause 50 per cent occupancy of nuclear steroid hormone receptors (Fig. 6.1). Second, in an organ of net metabolic clearance of testosterone (simulation #11), the net steady state extraction (E^{SS}) barely exceeds the dialyzable fraction and, thus, the high extraction values for testosterone in organs such as liver could not be generated (Tait and Burstein 1964).

The comparison of simulations #1 and #10 show that the free cytosolic concentration of testosterone in an organ such as brain, where hormone metabolism is minimal (simulation #1), is nearly tenfold freater than the free

Table 6.4
Species diversity of serum SHBG or TeBG

SHBG	TeBG	None
humans	rabbit	rat
baboon	dog	pig
chimpanzee	goat	horse
	bull	

SHBG = sex hormone-binding globulin (binds both testosterone and oestradiol with high affinity); TeBG = testosterone-binding globulin (binds only testosterone with high affinity). (From Corvol and Bardin 1973; Renoir, Mercier-Bodard, and Baulieu 1980).

cytosolic testosterone concentration in organs such as liver (simulation #10) that actively metabolizes testosterone. However, this discrepancy in free cytosolic hormone concentration could be rectified if, in the organ of high steroid metabolism, there is transport of globulin-bound steroid hormone into the tissue (for example, simulation #4). As will be discussed in Section **IV (Organ specificity)**, SHBG-bound oestradiol is available for transport into metabolic organs such as liver or salivary gland, and SHBG-bound testosterone is available for transport into tissues such as the testis or prostate. Thus, the selective delivery of steroid hormones to tissues by albumin and by SHBG allows for maintenance of high cytosolic concentrations of free hormone in the face of active organ metabolism of the hormone.

III Biochemical model

1 SHBG

Human SHBG is an 88,000 Dalton heterodimer that is composed of a 47,000 Dalton heavy chain and a 41,000 Dalton light chain (Cheng, Musto, Gunsalus, and Bardin 1983; Joseph, Hall, and French 1987). The protein electrophoreses in the β-globulin fraction, as opposed to the α-globulin fraction wherein CBG or α-fetoprotein migrate (Swartz and Soloff 1974). SHBG in humans and primates binds both testosterone and oestradiol with high affinity (Table 6.4). In contrast, the globulin is primarily a testosterone-binding globulin (TeBG) in species such as the bull, goat, dog, or rabbit. The serum concentration of SHBG or TeBG is essentially negligible in species such as the rat, pig, horse, donkey, cat, or bird (Table 6.4). However, in the rat, and presumably other species, the testes secretes androgen-binding protein (ABP) and small amounts of this protein are secreted into the bloodsteam so that the circulating rat ABP concentration is about 1 nM (Gunsalus, Musto, and Bardin 1978). Recent studies have shown a 68 per cent homology between the amino acid sequences of human SHBG and rat ABP (Joseph *et al.* 1987).

Both the heavy and light chains of rat ABP are encoded by a single copy gene and a 1600 base pair mRNA transcript (Joseph *et al.* 1987). The heavy and light chains are formed by posttranslational modification of a 45,000 Dalton precursor. It is believed that a 4000 Dalton signal peptide is cleaved from the precursor to generate the 41,000 Dalton light chain and that the heavy chain is formed by the addition of carbohydrate moieties on asparagine 274 and asparagine 397 residues (Joseph *et al.* 1987). The estimates of the carbohydrate moiety range from 10 to 30 per cent of the total protein molecular weight (Westphal 1978). Heterogeneity in the carbohydrate added to the SHBG polypeptide accounts for several SHBG isoforms seen on isoelectric focusing gels (Petra, Stanczyk, Senear, Namkung, Novy, Ross, Turner, and Brown 1983). There appear to be species differences in the posttranslational modification of the protein, since the rabbit TeBG isoforms are more acidic than the human SHBG isoforms (Petra *et al.* 1983). These structural species differences may explain the functional species differences when comparing the activity of human SHBG or rabbit TeBG in delivering steroid hormones to tissues (see Section V. Species Differences).

2 ALBUMIN

Albumin is a 66,000 Dalton single polypeptide that is normally not glycosylated (Brown 1977). The 3-dimensional structure of albumin has not yet been determined since crystals appropriate for X-ray diffraction cannot be generated with the albumin molecule. However, a model of the 3-dimensional structure of albumin has been deduced from the primary sequence by Brown (1977). Albumin is composed of three major domains that form six hemicylinders which are believed to constitute the six primary binding sites on albumin (Fig. 6.6). These binding sites have specific affinities for different classes of substrate. For example, it is known that four of the binding sites selectively bind free fatty acids, bilirubin, warfarin, or indole compounds/diazepam (Muller.and Wollert 1979). The interior of the hemicylinder acts as the binding site. Albumin is a highly flexible molecule that literally "breathes" as it fluctuates back and forth between conformations (Kragh-Hansen 1981). The binding of ligands to albumin is mediated by an induced conformational fit (Muller and Wollert 1979). That is, the binding of the ligand to albumin causes a conformational change about the binding site that allows the ligand to stay within the binding hemicylinder. The concept that albumin exists in different conformations, depending on the ligand bound to it, has recently been confirmed when it was shown that the isoelectric point of bovine albumin carrying ^{125}I-D-T_3 is more acidic than the isoelectric point of bovine albumin carrying ^{125}I-L-T_3 (Terasaki and Pardridge 1987*a*). Moreover, stereospecificity of plasma protein-mediated transport of T_3 into liver was detected using the *in vivo* single injection technique, since D-T_3

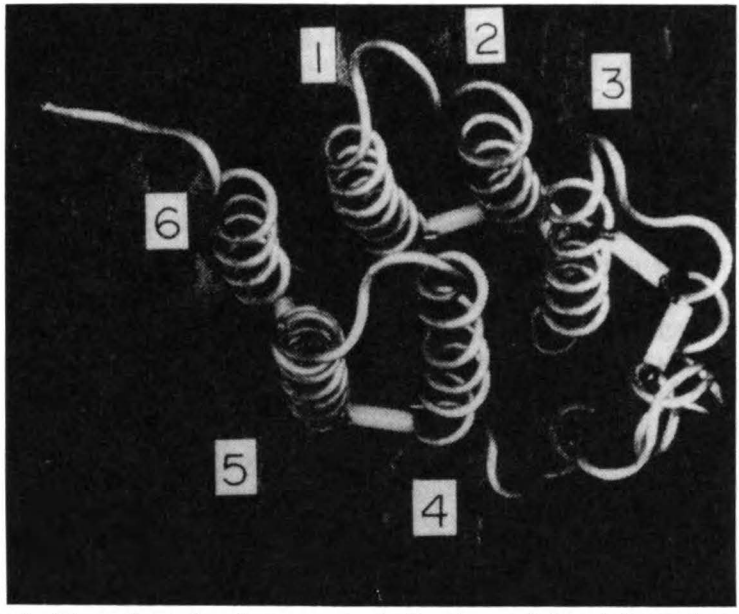

Fig. 6.6 Three-dimensional structure of albumin as deduced from the primary sequence by Brown (1977). Albumin is composed of three domains and six hemicylinders. The ligand binding sites are interiors of the six hemicylinders of the albumin molecule. This model illustrates the high flexibility of albumin and a marked increase in ligand dissociation is expected with a slight uncoiling caused by conformational changes about the binding site. Conformational changes are likely to be ligand- and tissue-specific. For example, steroid or thyroid hormone dissociation from albumin is enhanced in brain capillaries, whereas propranolol or lidocaine dissociation is not. Conversely, the dissociation of these two drugs from albumin is markedly enhanced in the liver microcirculation. (See Pardridge 1987).

bound to albumin was only partially available for uptake by liver. In contrast, albumin-bound L-T_3 was freely available for transport into liver (Terasaki and Pardridge 1987*a*).

Thermodynamic studies of ligand binding to albumin have shown that the free energy (ΔG), that is, the binding dissociation constant, changes little as the temperature is increased from 8°C to 37°C, and that enthalpy (ΔH) plays a minimal contribution in the overall free energy of the albumin-ligand interaction (Burton and Westphal 1972). The dominant term is a positive entropy (ΔS) that arises from a disordering of the water lattice surrounding amino acid residues comprising the interior of the binding site. This disordering of the water structure and the positive entropy is generated by the conformational change associated with ligand binding within the interior of the albumin binding site (Fig. 6.6). Moreover, relatively minor conformational changes about the ligand binding site can have rather drastic effects on the free energy and the dissociation constant of albumin binding of a particular

Table 6.5

Comparison of bovine albumin and human α_1-acid glycoprotein (AAG) dissociation constant in vivo in brain capillary (K_D^a) and in vitro

Plasma Protein	Ligand	K_D (μM)	K_D^a (μM)
Bovine albumin	testosterone	53 ± 1	2520 ± 710
	tryptophan	130 ± 30	1670 ± 110
	corticosterone	260 ± 10	1330 ± 90
	dihydrotestosterone	53 ± 6	830 ± 140
	oestradiol	23 ± 1	710 ± 100
	propranolol	290 ± 30	220 ± 40
	bupivacaine	141 ± 10	211 ± 107
	T_3	4.7 ± 0.1	46 ± 4
Human AAG	propranolol	3.3 ± 0.1	19 ± 4
	bupivacaine	6.5 ± 0.5	17 ± 4

T_3 = triiodothyronine. (From Pardridge and Landaw 1984; Pardridge 1986*b*; Terasaki, Pardridge, and Denson 1986).

steroid hormone or any other ligand. Moreover, this principle is true for other proteins such as SHBG or even enzymes. For example, recent X-ray diffraction studies showed that the loss of a single hydrogen bond between the ligand and an enzyme binding site results in 2–3 log order differences in binding dissociation constant (Bartlett and Marlowe 1987).

A conformational change about the albumin binding site (caused by interactions with the organ microcirculation, see below) would be expected to cause a partial uncoiling about the binding site that would result in a markedly increased rate of ligand dissociation (Pardridge 1987). The enhanced dissociation can be quantified *in vivo* for a particular organ by using tracer kinetic models to estimate the apparent dissociation constant of ligand-albumin interaction (Table 6.5). These experiments have shown that the conformational changes about the albumin binding site may be restricted to particular regions of the albumin molecule (Fig. 6.6), since some albumin-bound ligands do not undergo enhanced dissociation in some organs. For example, the *in vivo* dissociation constant (K_D^a) of lipophilic amine drug binding to bovine albumin is not significantly different from the K_D measured *in vitro* (Table 6.5). Secondly, conformational changes about a particular binding site may differ among organs (Table 6.2).

3 PLASMA PROTEIN-MEDIATED TRANSPORT MECHANISMS

There are at least three possible mechanisms by which plasma protein-bound hormone may be transported into tissues *in vivo*. First, the plasma protein-ligand complex, *per se*, may undergo net transport through the cellular barrier lining the microcirculation, for example, the capillary endothelium in

most tissues or the hepatocyte plasma membrane in liver. Siiteri *et al.* (1982) have hypothesized this mechanism with little supportive experimental data. However, Rosner and co-workers (1985) have recently provided evidence for an SHBG receptor on human prostatic cell membranes, and Bordin and Petra (1980) have shown that SHBG is found intracellularly in several tissues of the monkey, for example, prostate, testis, adrenal, and liver, but not other organs. Therefore, if receptor-mediated uptake of SHBG does occur, this process may be restricted to a few organs. There are several observations that mitigate against receptor-mediated uptake of the binding protein *per se*, as the principle mechanism underlying the transport of plasma protein-bound hormones into tissues *in vivo*. First, it is known that the rate of egress of proteins from the plasma compartment is log orders slower than the rate of exodus of steroid hormones and other ligands from the circulating plasma protein-bound pool (Dewey 1959). Although a substantial amount of plasma protein is found extravascularly, this plasma protein resides in the lymphatic compartment and not in the tissue interstitial space (Reeve and Chen 1970). (The low concentration of plasma protein in the interstitium arises from the rapid uptake of plasma protein into the lymphatic compartment, compared to the relatively slow exodus of plama protein from the plasma compartment into the interstitium.) Second, previous studies have shown that the uptake of circulating binding globulins, such as CBG, by organs such as rabbit brain or rabbit uterus is immeasurably low compared to the transport of corticosterone from the circulating rabbit CBG-bound pool (Pardridge, Eisenberg, Fierer, and Kuhn 1986; Chaudhuri, Steingold, Pardridge, and Judd 1987). Similarly, the uptake of labelled albumin by tissues is trivial compared to the uptake of ligands from the circulating albumin-bound pool (Pardridge, Eisenberg, and Cefalu 1985). Therefore, while the receptor-mediated uptake of plasma proteins by tissues undoubtedly occurs, this process operates at rates that are log orders lower than the movement of circulating sex steroids into tissues from the plasma protein-bound pools.

There are two possible mechanisms by which plasma protein-bound ligands may be transported out of the plasma compartment without significant exodus of the plasma protein *per se*. First, the permeability of the ligand, when presented to the membrane in the bound form, may be much greater than the permeability of the membrane to the free ligand. Second, tissue-mediated conformational changes at the binding site may allow for markedly enhanced rates of ligand dissociation from the plasma protein within the organ microcirculation as compared to dissociation rates that exist in the unperturbed or *in vitro* state. Previously reported tracer kinetic modelling studies have shown that the enhanced membrane permeability mechanism cannot explain the experimental data, whereas the enhanced dissociation model does allow for a fit of the experimental data to the tracer kinetic model (Pardridge and Landaw 1984). Thus, the important parameter is the dissociation constant of the plasma protein-ligand binding interaction *in vivo* in a particular organ (Tables

6.2 and 6.5). The enhanced dissociation model also fits with the known thermodynamics and biophysics of albumin-ligand binding interactions and the importance of conformational changes about the ligand binding site in determining the rates of ligand dissociation from the plasma protein (see above).

The biochemical mechanism by which plasma proteins such as albumin or SHBG rapidly and transiently interact with the surface of the organ microcirculation to cause conformational changes about the binding site is at present unknown. Other workers have postulated the operation of receptors for plasma proteins such as albumin lining the organ microcirculation (Weisiger, Golland, and Ockner 1981; Forker and Luxon 1983). These putative receptors would transiently immobilize the circulating plasma protein, cause conformational changes, and increase rates of ligand dissociation from the plasma protein. However, the initial report of an albumin receptor has not been confirmed (Stollman, Gartner, Theilmann, Ohmi, and Wolkoff 1983; Stremmel, Potter, and Berk 1983). On the other hand, it is known that albumin normally transiently interacts with the surface of the organ microcirculation, possibly through electrostatic mechanisms involving positively charged residues (for example, arginine) on the plasma protein surface with negative charges on the surface of the organ microcirculation (Michel, Phillips, and Turner 1985). Indeed, the adsorption of plasma proteins such as albumin to the surface of the microcirculation as plasma traverses the organ is an important mechanism by which normal vascular permeability is maintained (Turner, Clough, and Michel 1983). Finally, recent biochemical studies using spin-labelling techniques have provided direct evidence for conformational changes in plasma proteins such as albumin that are mediated by interactions between the binding protein and the cells comprising the surface of the organ microcirculation (Mizuma, Horie, Hayashi, and Awazu 1986). The biochemical nature of the constituents lining the microcirculatory surface that interact with albumin and other plasma proteins is not known at present but may represent membrane carbohydrate or glycolipid moieties. Since it is known that the composition of carbohydrate moieties that line the endothelial glycocalyx differs from organ to organ (Ponder and Wilkinson 1983), it would be predicted that organ differences might exist in terms of plasma protein-mediated transport of steroid hormones and other ligands into tissues *in vivo*.

IV Organ specificity of sex steroid transport

1 BRAIN

The *in vivo* dissociation constant (K_D^a) of bovine albumin binding of testosterone in the rat brain capillary was determined by fitting the unidirectional

extraction data shown in Fig. 6.7 to the classical Kety-Renkin-Crone equation of capillary physiology (Pardridge and Landaw 1984),

$$E = 1 - e^{-f \cdot k_3 t} \tag{7}$$

$$f = \frac{K_D^a}{A_F + K_D^a} \tag{8}$$

where E = the first pass unidirectional extraction of ligand by the tissue, k_3 = the membrane permeability constant (Table 6.1), t = the capillary transit time, and A_F = the albumin concentration. The use of the Kety-Renkin-Crone equation to estimate the K_D^a parameter assumes maintenance of near equilibrium of the plasma protein-ligand binding reaction as the plasma protein traverses the organ microcirculation (Pardridge and Landaw 1984). This proviso only requires that the rates of ligand dissociation and/or association with the plasma protein are much faster than the rates of ligand movement through the endothelial membrane. Since testosterone dissociation from albumin (k_7, Table 6.1) ranges from 53–5300 sec^{-1} and since the $k_8 (A_F)$ product (Table 6.1) ranges from 100–10 000 sec^{-1}, then the assumption of maintenance of near equilibrium *in vivo* is valid because the rate constant of testosterone transport through the brain capillary endothelium is only 1.9 sec^{-1} (Table 6.1).

The data in Figure 6.7 indicate that albumin-bound testosterone is readily available for transport through the rat brain capillary wall (Pardridge 1981). The transport of testosterone or oestradiol into brain from the circulating human SHBG-bound pool was assayed using serum obtained from nearly 80 patients representing seven different clinical conditions (Pardridge, Mietus, Frumar, Davidson, and Judd 1980). These seven conditions differed in the serum concentration of SHBG (Table 6.6). As the SHBG concentration decreased, the unidirectional clearance of either oestradiol or testosterone increased (Fig. 6.8). In addition, the oestradiol/testosterone unidirectional clearance ratio decreased as the concentration of SHBG decreased (Table 6.6). Thus, SHBG acts as an oestradiol amplifier relative to testosterone delivery to tissues, and the *in vivo* data in Figure 6.7 support the original concept put forward by Anderson (1974) regarding the role of SHBG as an oestradiol amplifier. The close relationship between oestradiol or testosterone clearance and the SHBG concentration is shown in Figure 6.9, which describes the linear relationship between the unidirectional clearance and the SHBG concentration for either of the two sex steroids. The relationship between steroid clearance and SHBG concentration is five-fold greater for testosterone as compared to oestradiol (see slopes of the plots, Fig. 6.8).

The data in Figures 6.7–6.9 indicate that only albumin-bound oestradiol or testosterone is available for transport into brain, whereas SHBG-bound sex steroid is not available. Since these studies were some of the first results published with the tissue sampling single injection technique, there is a tend-

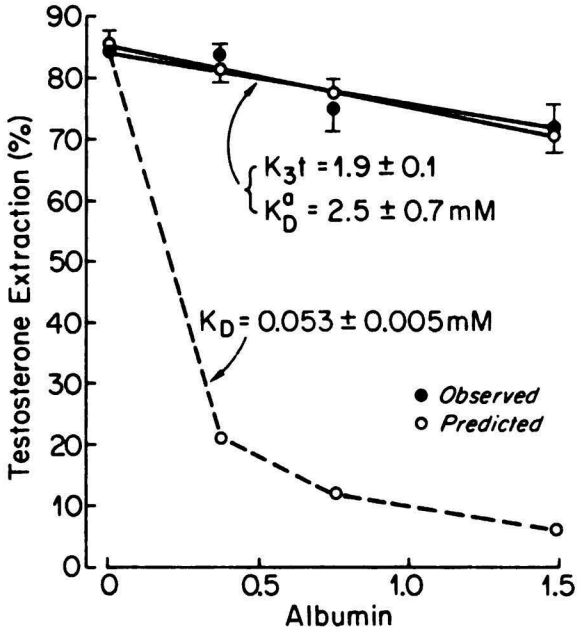

Fig. 6.7 The unidirectional extraction of ^{3}H-testosterone by rat brain is plotted versus the concentration of arterial bovine albumin. The experimentally observed values are given by the closed circles (mean ± S.E., n = 3–6 animals per point). The extraction values predicted on the basis of fitting the experimental data to equation #7 are shown by the open circles and the curve fitting gives the two parameters k_3t and K_D^a (see equation #7). The parameters k_3 and t are defined in Fig. 6.2 and Table 6.1, where $t = 1/k_{10}$. The dashed line represents the extraction values predicted by substituting into equation #7 the albumin concentration, the k_3t product, and the *in vitro* albumin-testosterone dissociation constant, $K_D = 53 \pm 1$ μM. Therefore, the dashed curve gives the expected inhibition of testosterone transport caused by hormone binding to albumin *if* testosterone was not available for transport into brain from the circulating albumin-bound pool. However, since albumin-bound testosterone is available via an enhanced dissociation mechanism, the upper curve is observed and the K_D^a *in vivo* (2500 ± 700 μM) is much greater than the K_D *in vitro*. (From Pardridge 1986*b* with permission).

ency to extrapolate these data to other organs and to make the general conclusion that while albumin-bound sex steroids are available for uptake by tissues, SHBG-bound sex steroids are not available for transport into tissues. However, as other organs have been studied directly in subsequent experiments, it has become clear that such generalizations are false and there are marked organ specificities in terms of the transport of albumin or SHBG-bound sex steroid into tissues *in vivo*.

2 LIVER

Albumin-bound testosterone or oestradiol is freely available for transport

Table 6.6
Oestradiol amplifier function of SHBG[a]

Patient category	SHBG (nM)	E_2/T unidirectional clearance ration
Pregnancy (9)	323 ± 28	3.9 ± 0.5
Oral contraceptives (8)	126 ± 16	2.6 ± 0.3
Thin postmenopausal (9)	74 ± 9	2.1 ± 0.2
Normal female (9)	65 ± 9	2.1 ± 0.2
Obese postmenopausal (9)	43 ± 4	1.7 ± 0.2
Normal male (8)	28 ± 3	1.3 ± 0.1
Hirsute (5)	17 ± 2	1.3 ± 0.1

[a]From Pardridge (1981) with permission. Data are mean ± S.E.M. E_2, oestradiol, T, testosterone. Numbers in parentheses are number of patients.

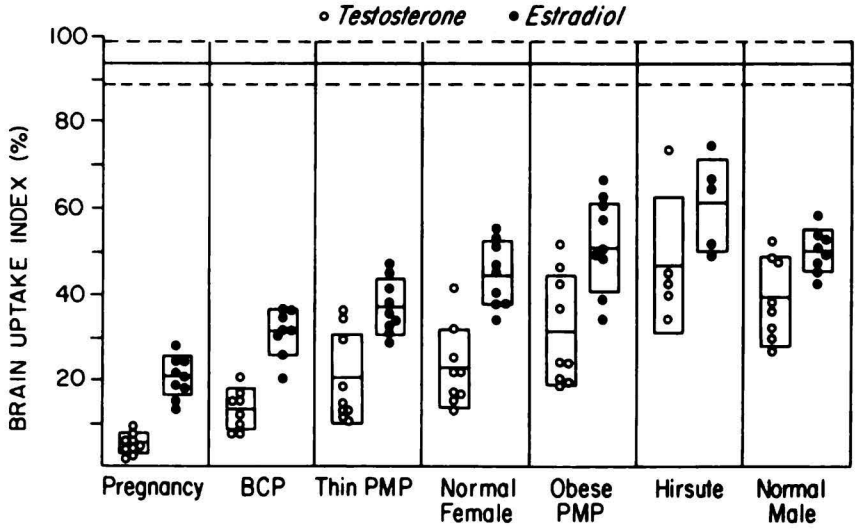

Fig. 6.8 Brain uptake index (BUI) for ^{3}H-testosterone and ^{3}H-oestradiol relative to ^{14}C-butanol is shown for 5–9 patients in seven clinical conditions. Vertical rectangles are mean ± S.D.; horizontal line is mean of testosterone or oestradiol BUI in absence of plasma proteins. BCP, birth control pill-treated women; PMP, postmenopausal women. (From Pardridge *et al.* 1980*a* with permission).

into liver (Baird, Longcope, and Tait 1969; Pardridge and Mietus 1979*a*). However, studies using human serum indicated that SHBG-bound oestradiol is also readily available for uptake by liver (Pardridge and Mietus 1979*a*). As shown in Figure 6.10, the concentration of bioavailable testosterone in human male serum in brain or liver is not significantly different from the frac-

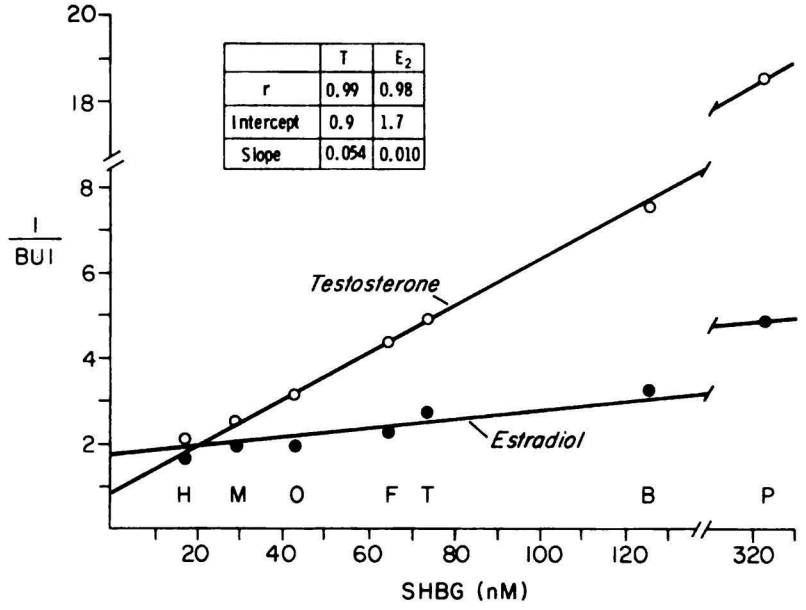

Fig. 6.9 Reciprocal of brain uptake index (BUI) for ^{3}H-testosterone (T) and ^{3}H-oestradiol (E_2) relative to ^{14}C-butanol is plotted versus level of sex hormone-binding globulin (SHBG) in human serum. BUI data are shown in Fig. 6.8. P—pregnancy, B—birth control pills, T—thin postmenopausal female, F—normal follicular phase female, O—Obese postmenopausal female, M—normal male, H—hirsute female. Data obtained by linear regression are shown in inset for both plots. (From Pardridge *et al.* 1980*a* with permission).

tion of free plus albumin-bound hormone measured *in vitro*. Similarly, the concentration of brain bioavailable oestradiol or corticosterone is no different from the free plus albumin-bound steroid fraction *in vitro*. However, the hepatic bioavailable fraction of either oestradiol or corticosterone is markedly increased over the free plus albumin-bound fraction, indicating SHBG-bound oestradiol or CBG-bound corticosteroid is readily available for transport into rat liver *in vivo*. Thus, it is believed that transient interactions between SHBG and the hepatocyte surface cause conformational changes about the oestradiol binding site that result in markedly increased rates of oestradiol dissociation from SHBG within liver capillaries. Similarly, thyroid hormone-binding globulin (TBG)-bound T_3 is readily available for transport into rat liver, whereas human TBG-bound T_4 is not available (Pardridge 1981). The selective transport of SHBG-bound oestradiol or TBG-bound T_3 into liver would appear to be at odds with the conventional view that testosterone or oestradiol bind to a single competitive binding site on SHBG, or that T_3 or T_4 bind to a single competitive binding site on TBG. One would have to postulate an unusual conformational change that would allow for selective enhancement of oestradiol dissociation from the binding site without enhanced dissociation of testosterone. The heterogeneity of SHBG or TBG function in terms of hormone delivery to liver, however, also has an

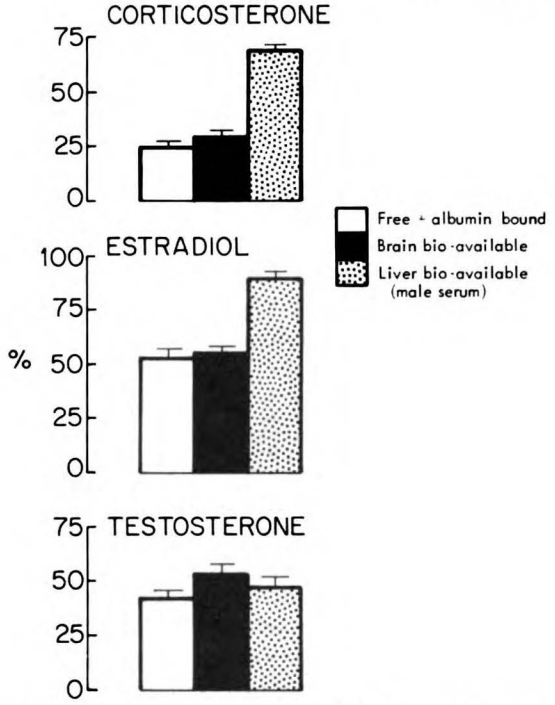

Fig. 6.10 The *in vitro* free plus albumin-bound, *in vivo* brain bioavailable, and liver bioavailable fractions for human male serum are shown for three steroid hormones. (From Pardridge 1986*b* with permission).

analogue in terms of structural heterogeneity of either the SHBG or TBG molecule. Recent studies have shown that the multiple isoforms of TBG in human serum do not bind T_3 and T_4 equally (Terasaki and Pardridge 1988). The most acidic isoforms of TBG selectively bind T_4 as compared to T_3. Whether a similar heterogeneity exists amongst the different SHBG isoforms in human serum in terms of selective binding of testosterone and oestradiol is not known at present but is being investigated in the author's laboratory.

The finding that SHBG-bound oestradiol is selectively available for transport into liver as compared to SHBG-bound testosterone provides a second order amplification of oestradiol delivery to tissues caused by the presence of SHBG in plasma. The first order of amplification is shown by the brain studies (Table 6.6) and arises from the combination of increased binding of oestradiol to albumin as compared to testosterone, and decreased binding of oestradiol to SHBG as compared to testosterone (Anderson 1974). Therefore, the albumin-bound fraction of testosterone decreases faster as SHBG increases. This allows for amplification of oestradiol delivery to tissues, even if SHBG-bound oestradiol is not available *per se*. However, in liver further amplification of oestrogen delivery occurs because the binding globulin

selectively delivers this sex steroid to liver as compared to the androgen (Pardridge 1981).

3 SALIVARY GLAND

Since recent trends in endocrine practice involve the use of measurements of steroid hormone concentrations in human saliva as an index of the biologically active steroid hormone in the circulation (Riad-Fahmy *et al.* 1982), studies were initiated to measure steroid hormone delivery mechanisms in rat salivary gland. This was felt important because other studies had shown that the concentration of testosterone in human saliva closely parallels the concentration of free testosterone *in vitro* (Riad-Fahmy *et al.* 1982). Such a correlation is compatible with one of two explanations. First, only free testosterone may be available for transport across salivary gland capillaries (Riad-Fahmy *et al.* 1982). A second possible explanation, however, is that free plus albumin-bound testosterone is available for transport into salivary gland epithelium from the circulation, but the pool size of cytosolic free androgen in salivary gland is markedly restricted compared to the pool size of capillary exchangeable hormone owing to rapid androgen metabolism in this organ (for example, see simulation # 10, Table 6.3). As shown in Table 6.2, albumin-bound testosterone is partially available for transport into salivary gland as the rate of androgen dissociation from albumin is increased approximately twelve-fold in this tissue (Cefalu, Pardridge, Chaudhuri, and Judd 1986). Moreover, the salivary gland bioavailable testosterone using human serum approximates the free plus albumin-bound fraction measured *in vitro* (Figure 6.11). Thus, the marked discrepancy between the concentration of free cytosolic testosterone in salivary gland (which is presumed to be in equilibrium with the salivary fluid testosterone), and the capillary exchangeable testosterone concentration suggested that rapid metabolism of testosterone in salivary tissue occurs. This prediction was confirmed by studies in which ^{3}H-testosterone was injected into the common carotid artery of rats followed by removal of the organ 60 seconds later and rapid processing of the tissue homogenate for thin-layer chromatography (Cefalu *et al.* 1986). As shown in Figure 6.12, testosterone is rapidly metabolized by salivary gland tissue as compared to two other organs, lymph node or brain, in which no measurable metabolism of androgen within 60 seconds of pulse administration can be detected. Two-dimensional thin-layer chromatography studies showed that the major metabolite formed is androstenedione at 60 seconds and is 5α-androstane-3α,17β-diol (3α-diol) at 5 minutes after a single carotid injection of ^{3}H-testosterone (Cefalu *et al.* 1986). These studies suggest that enzymes such as 17β-hydroxysteroid dehydrogenase (which interconverts testosterone and androstenedione), 5α-reductase (which converts testosterone to dihydrotestosterone or androstenedione to androstanedione), and 3α-hydroxysteroid dehydrogenase (which converts androstanedione to

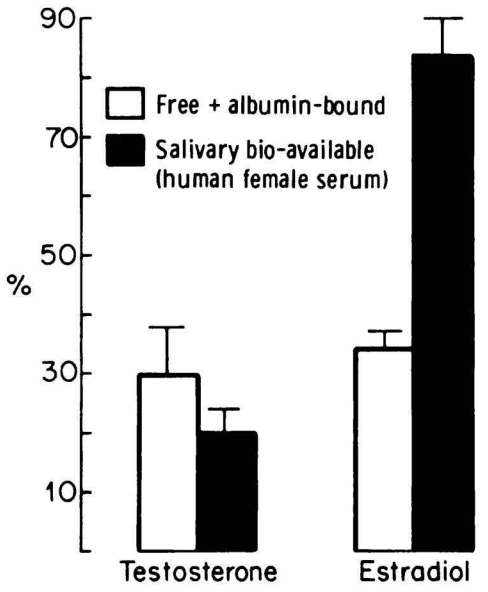

Fig. 6.11 The fractions of free plus albumin-bound testosterone and oestradiol *in vitro*, and testosterone and oestradiol bioavailable in rat salivary gland *in vivo* are compared and show that SHBG-bound oestradiol, but not testosterone, is readily available for transport into rat salivary gland. (From Pardridge 1986*b* with permission).

androsterone or dihydrotestosterone to 3α-diol) are present in salivary gland tissue.

The results in Figure 6.12 showing the organ diversity of androgen metabolic rates underscores the limited utility of MCR measurements on the whole organism. The whole body MCR is the average of interorgan differences in androgen metabolism and these differences can be profound. Tissues such as liver, prostate gland, or salivary gland rapidly metabolize androgen, and this situation may create marked concentration differences between the capillary exchangeable hormone and the cytosolic free hormone. On the other hand, organs such as brain, lymph node, and others metabolize testosterone at markedly reduced rates compared to testosterone transport into and out of the organ. Consequently, in these tissues, the concentration of free cytosolic hormone is nearly equal to the concentration of capillary exchangeable hormone. Moreover, one can envisage profound differences in individual organ MCRs that would not be detected in whole body MCR measurements. In addition to interorgan differences in sex steroid transport, similar interorgan differences most likely occur for the rates of hormone metabolism. Both of these processes, hormone transport from the plasma compartment and intracellular hormone metabolism, are the dominant forces controlling the cytosolic free steroid hormone concentration.

The finding that salivary gland, like liver or prostate, is an organ of active androgen metabolism contradicts the proposal of Riad-Fahmy *et al.* (1982),

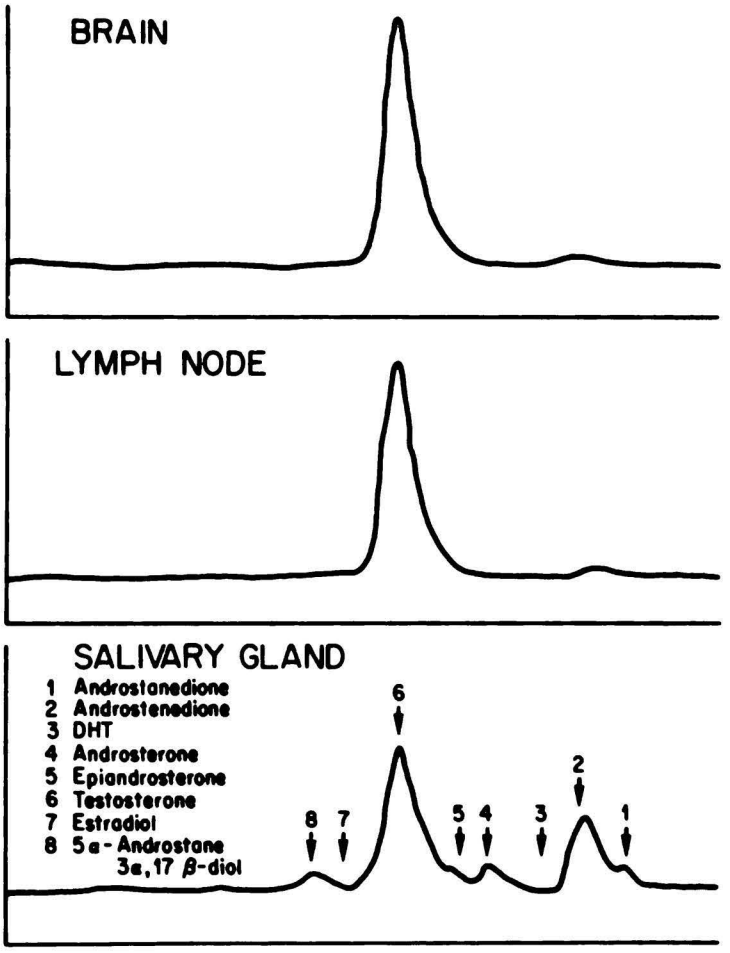

Fig. 6.12 One-dimensional thin-layer chromatographic separation of brain, cervical lymph node, and salivary gland homogenates of tissue obtained 60 seconds after a single carotid injection of ^{3}H-testosterone (50 μCi/ml) in Ringer-Hepes buffer (0.1 g/dl bovine albumin) in the rat. Migration of testosterone or several other metabolites in the one-dimensional system is shown in the figure. The minor peak in the brain and lymph node studies that co-migrated with androstenedione (peak 2) represented an impurity in the isotope, as this was also found in the ^{3}H-testosterone obtained from the manufacturer. (From Cefalu *et al.* 1986 with permission).

who proposed that only free testosterone is available for transport into salivary gland because androgen metabolism in this organ is low. These workers proposed that salivary gland is an organ of low androgen metabolism on the finding of low testosterone concentrations in saliva and the assumption that only free testosterone is available for transport into the tissue. As discussed previously, the low concentration of salivary fluid testosterone arises from active androgen metabolism in this organ.

Finally, the data in Figure 6.11 indicate that, like liver, SHBG-bound oestradiol is selectively available for transport into salivary gland, whereas

SHBG-bound testosterone is not (Cefalu *et al.* 1986). The finding of a high bioavailable oestradiol concentration in salivary gland indicates that if free salivary oestradiol concentrations are found to be low relative to the total concentration of serum hormone, it is likely that active organ metabolism of oestradiol occurs in this tissue.

4 LYMPH NODE

Albumin-bound testosterone is partially available for transport into lymph node (Cefalu and Pardridge 1987). However, the *in vivo* dissociation constants listed in Table 6.2 indicate the dissociation of testosterone from bovine albumin is only enhanced six-fold in lymph node capillaries as compared to a twelve-fold or fifty-fold enhancement of testosterone dissociation in salivary gland or cerebral capillaries. Conversely, oestradiol dissociation from albumin in lymph node capillaries is increased sixty-fold, but only about thirty-fold in brain capillaries. These findings indicate that albumin-bound oestradiol is freely available for transport into lymph node capillaries, but albumin-bound testosterone is only partially available for transport. The plasma protein *per se* does not undergo significant exodus from the capillary compartment as shown by the autoradiography data in Figure 6.13, whereas oestradiol rapidly escapes the organ microcirculation and distributes into the total organ water during a single circulatory passage (Cefalu and Pardridge 1987). Therefore, the transport of albumin-bound steroid hormone into the tissue represents a process of enhanced hormone dissociation from the plasma protein within the microcirculation.

With regard to the transport of testosterone or oestradiol into lymph node from the circulating SHBG-bound pool, recent studies have shown that the lymph node is similar to salivary gland or liver. SHBG-bound oestradiol, but not SHBG-bound testosterone, is readily available for transport into lymph node tissue (Cefalu and Pardridge 1987).

While this review is emphasizing organ diversity in regard to sex steroid transport and metabolism, it is also possible that there is a similar diversity in a particular organ over a variety of pathologic or physiologic conditions. For example, recent studies of testosterone transport and metabolism in lymphoid neoplasia have shown that both androgen transport and metabolism are enhanced in the neoplastic condition (Cefalu and Pardridge 1987). Although SHBG-bound testosterone is not available for transport into lymph nodes under normal conditions, SHBG-bound testosterone is partially available for transport into the neoplastic lymph node. Similarly, normal lymph node does not rapidly metabolize testosterone (Fig. 6.12). However, neoplastic lymph node rapidly metabolizes ^{3}H-testosterone and the major metabolites formed 60 seconds after pulse administration of ^{3}H-testosterone are epiandrosterone and androsterone, as well as dihydrotestosterone and 5α-androstane-3α,17β-diol (Cefalu and Pardridge 1987). These

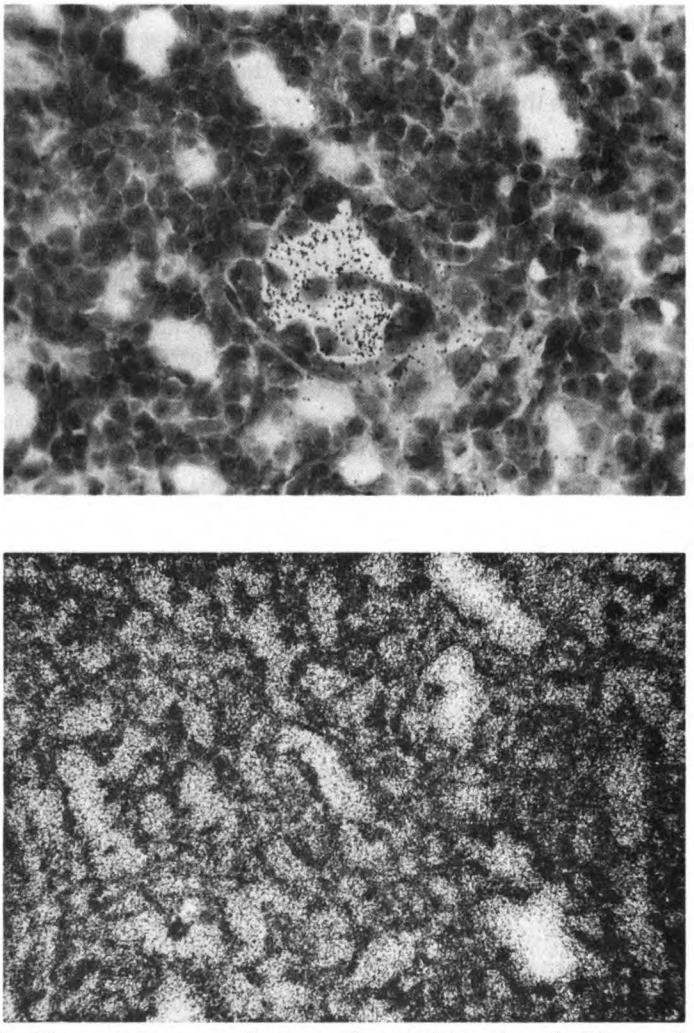

Fig. 6.13 Thaw-mount autoradiogram of control lymph node 15 seconds after a single arterial injection of either ^{3}H-albumin (upper) or ^{3}H-oestradiol (lower) in Ringer-Hepes buffer containing 0.1 g/dl bovine albumin. The albumin radioactivity is confined to the microvasculature compartment, showing that the plasma protein does not measurably cross the capillary wall on a single pass. Conversely, the ^{3}H-oestradiol radioactivity is found throughout the lymph node indicating complete extravascular distribution of the steroid hormone and absence of sequestation to the capillary endothelium. The tissue was counterstained after autoradiography with methyl green-pyronin. Magnification × 100. (From Cefalu and Pardridge 1987 with permission).

studies suggest that pivotable androgen metabolizing enzymes such as 17β-hydroxysteroid dehydrogenase, 5α-reductase, and 3α-hydroxysteroid dehydrogenase are activated in the lymphoid neoplastic state. If the products of testosterone metabolism, for example, dihydrotestosterone or 5α-androstane-3α-17β-diol, mediate in part the mitogenesis of the neoplastic state, then inhibitors of these enzymes, for example, 5α-reductase, may prove in the future to be of beneficial effect in controlling lymphoid neoplasia.

5 TESTIS/PROSTATE GLAND

Recent studies have investigated the bioavailability of testosterone and oestradiol in rat testis (Sakiyama, Pardridge, and Musto 1988). These studies afforded the opportunity to investigate the transport properties of the blood-testis barrier (BTB), which is comprised of the Sertoli cell epithelium surrounding the tubules in the testis (Setchell and Waites 1975). The results indicate both albumin-bound testosterone and oestradiol are readily available for transport into the testis or prostate gland (Sakiyama, Pardridge, and Musto 1988). Similarly, SHBG-bound oestradiol is freely available for uptake by the two organs. The surprising finding, however, was that SHBG-bound testosterone is also readily available for transport into the testis or prostate. The observation that both SHBG-bound testosterone and SHBG-bound oestradiol were transported through the BTB suggested that the SHBG-bound steroid complex may be transported from the testicular microcirculation into the Sertoli cell on a single pass through the organ. This was confirmed by showing the first pass extraction of ^{3}H-TeBG was approximately 75 per cent, as opposed to the extraction of ^{3}H-albumin or ^{113m}In-transferrin (that is, plasma space markers), which was approximately 35 per cent (Sakiyama, Pardridge, and Musto 1988). Thus, in some organs, the rapid uptake of circulating steroid hormones from the circulating SHBG-bound pool may occur via rapid movement of the plasma protein-hormone complex.

6 KIDNEY: SELECTIVE STEROID HORMONE CONJUGATE TRAFFICKING

Steroid hormones are inactivated in part by conjugation to either sulfate or glucuronate moieties (Pan, Woolever, and Bhavnani 1985). Recent studies have shown that oestrone sulfate or oestradiol glucuronate undergo selective trafficking between the plasma, liver, and kidney compartments (Chaudhuri, Verheugen, Pardridge, and Judd 1987). Albumin-bound oestrone sulfate or oestradiol glucuronate are both readily available for transport into liver. Similarly, oestradiol glucuronate is readily available for uptake by kidney from the circulation. However, albumin-bound oestrone sulfate is poorly, if at all, available for uptake by kidney (Fig. 6.14). Thus, the placement of a

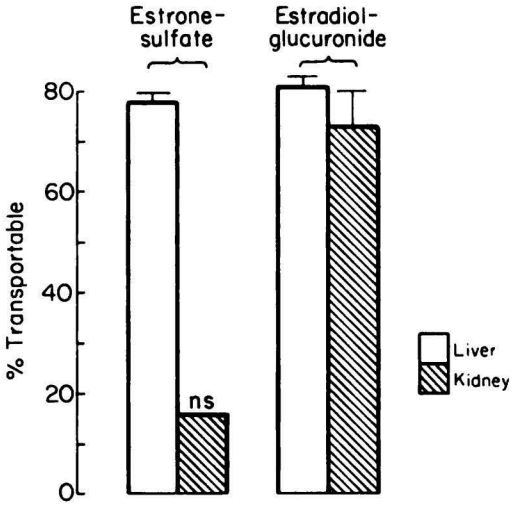

Fig. 6.14 Comparison of kidney and hepatic bioavailable fractions of oestrone sulfate or oestradiol glucuronide after aortic or portal vein injection of the labelled steroid mixed in 4 g/dl bovine albumin. The study shows that albumin-bound sulfate or glucuronate conjugate is readily available for transport into liver but only albumin-bound glucuronate is available for transport in the kidney. Therefore, sulfate conjugation causes selective trafficking of the steroid hormone to the liver. ns = not different from zero. (From Chaudhuri *et al.* 1987).

sulfate moiety on the oestrogen nucleus by peripheral tissues selectively trafficks this oestrogen to the liver, whereas the placement of a glucuronate moiety on the steroid nucleus allows for uptake by either kidney or liver and ultimately allows for either biliary or urinary excretion.

In tissues such as brain or uterus, the placement of a negatively charged sulfate or glucuronate moiety on the sex steroid molecule results in a markedly diminished microvascular permeability to the steroid hormone (Verheugen, Pardridge, Judd, and Chaudhuri 1984; Pardridge, Eisenberg, Fierer, and Musto 1987). Thus, oestrone sulfate, which is in high concentrations in postmenopausal serum (Noel, Ree, Jacobs, and James 1981), is poorly available for uptake by tissues other than liver (Verheugen *et al.* 1984). The high concentration of oestradiol or oestrone in breast cancer tissue (Santner, Feil, and Santen 1984) probably does not arise from the tissue uptake of oestrone sulfate. The low circulating concentrations of unconjugated oestrone or oestradiol may be taken up by breast cancer tissue and then sequestered within that tissue by the formation of oestrone or oestradiol sulfate. This would allow for the development of a large tissue pool of biologically inactive but sequestered oestrogen that could be slowly activated by conversion to free oestrogen due to the action of tissue sulfatases. Similar processes probably also occur in brain for dehydroepiandrosterone sulfate (DHEAS). Studies have shown that brain DHEAS is very high, particularly in olfactory lobe, compared to plasma concentrations (Corpechot, Robel,

Table 6.7

SHBG delivery of sex steroids to tissues: organ diversity in the rat

Organ	Oestradiol	Testosterone
Brain and uterus	−	−
Liver, salivary gland and lymph node	+	−
Testis	+	+

(−) = little, if any, transport of hormone into tissue under normal conditions; (+) = hormone is partially or freely transported into tissue under normal conditions. (From Pardridge and Mietus 1979*a*; Pardridge 1981; Verheugen, Pardridge, Judd, and Chaudhuri 1984; Cefalu, Pardridge, Chaudhuri, and Judd 1986; Cefalu and Pardridge 1987; Sakiyama and Pardridge 1988).

Axelson, Sjovall, and Baulieu 1981). However, DHEAS is poorly transported through the brain capillary endothelium (Pardridge *et al.* 1988). The unconjugated DHEA, however, is rapidly transported and may then be sequestered in brain tissue by formation of the DHEAS conjugate.

7 SUMMARY OF INTER-ORGAN DIVERSITY

Table 6.7 summarizes the inter-organ differences in terms of bioavailability of circulating SHBG-bound testosterone or oestradiol. Neither SHBG-bound testosterone nor oestradiol is available for transport into rat brain or uterus. However, SHBG-bound oestradiol is selectively available for uptake by tissues such as liver, salivary gland, or lymph node, and both SHBG-bound testosterone and SHBG-bound oestradiol are available for uptake by tissues such as testis or prostate. Thus, the presence of SHBG in human serum allows for selective amplification of either oestradiol or testosterone delivery across the entire spectrum of organs in the body. This allows for enhanced delivery of sex steroids to organs that are more sex steroid dependent than others and also allows for maintenance of cytosolic pool sizes of free hormone in the face of active organ metabolism of testosterone or oestradiol.

V Species differences

The rabbit, unlike the rat, has high serum concentrations of circulating TeBG which selectively bind testosterone and not oestradiol, as opposed to human SHBG, which avidly binds both sex steroids (Table 6.4). Although the rat is a species in which high concentrations of SHBG or TeBG do not exist in the circulation, this species has proved to be a good model for assessing the biological functions of human sex steroid-binding proteins. This is probably true because, as reviewed in **Section III (Biochemical Model)**, spe-

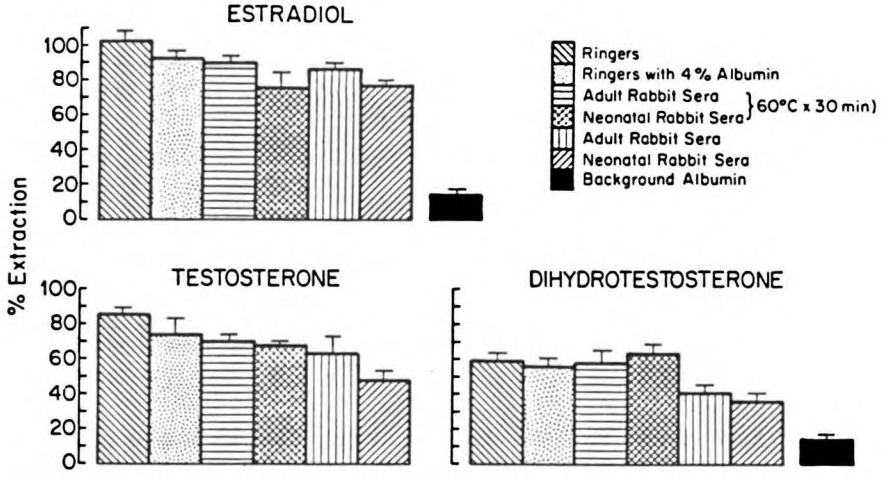

Fig. 6.15 The mean uterine extractions ± S.E.M. of ^{3}H-oestradiol (E_2), testosterone (T), dihydrotestosterone (DHT), and ^{3}H-bovine albumin by rabbit uterus are shown for different injection vehicles. Adult rabbit serum, or neonatal rabbit serum in some experiments, was heat treated at 60°C for 30 minutes prior to aortic injection of the serum. These studies show that the presence of rat serum causes no inhibition of the unidirectional extraction of E_2, T, or DHT by rabbit uterus as compared to the control injections using Ringer's solution. Therefore, the binding of androgens to TeBG causes no inhibition of the clearance of the hormone, and all three hormones are cleared by the rabbit uterus at approximately the same rate. (From Chaudhuri *et al.* 1988 with permission).

cific albumin or SHBG receptors do not appear to mediate the transport of hormones into tissues from the circulating plasma protein-bound pools in most tissues. Moreover, the rat does have high concentrations of CBG and the same general patterns have been observed for gonadal steroid and corticosteroid transport processes. Nevertheless, recent studies of steroid hormone transport in rabbits have been completed in order to investigate the role of rabbit TeBG or CBG.

The unidirectional clearance of oestradiol, testosterone, or dihydrotestosterone by rabbit uterus is high and is not significantly inhibited by either bovine albumin or by rabbit serum (Fig. 6.15). These studies predict that the MCR of the three sex steroid hormones in rabbits might be approximately the same. Indeed, this is what is observed, as two different studies have shown the MCR of oestradiol, testosterone, or dihydrotestosterone are not significantly different in the rabbit (Bourget, Flood, and Longcope 1984; Mahoudeau, Corvol, and Bricaire 1973). The studies shown in Fig. 6.15, and other results (Chaudhuri, Steingold, Pardridge, and Judd 1988) have shown that rabbit TeBG-bound testosterone or dihydrotestosterone is readily available for transport into rabbit tissues, since the bioavailable fraction is much greater than the free plus albumin-bound fraction. Similarly, the bioavailable

corticosterone in rabbit brain is much greater than the free plus albumin-bound fraction of corticosteroid in rabbit serum (Pardridge, Eisenberg, Fierer, and Kuhn 1986). These results indicate that CBG-bound corticosteroid is readily available for uptake by rabbit brain. The findings on unidirectional clearance are in agreement with other studies which show that the concentration of CBG exerts no inhibitory influence on the MCR of corticosterone in rabbits (Daniel, Leboulenger, Vaudry, Floch, and Assenmacher 1982). Moreover, the unidirectional clearance of corticosterone by rabbit uterus is six-fold greater than the unidirectional clearance of cortisol by rabbit uterus in the presence of rabbit serum (Chaudhuri *et al.* 1988). These measurements of unidirectional clearance are also in agreement with MCR estimates, as other workers have shown that the MCR of corticosterone in rabbits is six-fold greater than the MCR of cortisol (Daniel *et al.* 1982). The much lower unidirectional clearance of cortisol in rabbit uterus is due to the combined effects of reduced membrane permeability (owing to increased polar functional groups, see Fig. 6.3 and 6.4) and to a greater inhibitory effect of rabbit CBG on cortisol transport as compared to corticosterone (Chaudhuri *et al.* 1988).

The possible role of CBG receptors on brain capillaries in the transport of CBG-bound corticosterone into rabbit brain was investigated recently (Pardridge, Eisenberg, Fierer, and Kuhn 1986). A ^{3}H-CBG preparation that retains normal ^{14}C-cortisol binding properties was prepared by reductive tritiation of the protein. Saturable CBG receptors in isolated brain capillaries were not detectable. These types of experiments further support the proposal that specific globulin receptors do not mediate the enhanced dissociation of steroid hormones from the circulating globulin-bound pools.

The propensity for rabbit TeBG to deliver testosterone to tissues, whereas human SHBG retards testosterone uptake by most, but not all, tissues may be related to the differential kinetics of androgen binding to the two plasma proteins. For example, the K_D of androgen binding to the two proteins is approximately the same at 4°C: K_D = 0.4 nM for human SHBG and 0.5 nM for rabbit TeBG (Tabei, Mickelson, Neuhaus, and Petra 1978). However, the rate of androgen dissociation from rabbit TeBG at 4°C is 27-fold faster than the rate of androgen dissociation from human SHBG. Similarly, the rate of androgen association with rabbit TeBG is 21-fold faster than the rate of androgen association with human SHBG (Tabei *et al.* 1978). Therefore, while the two plasma proteins have approximately the same equilibrium dissociation constant, the kinetics are dramatically different between the two binding proteins. There is a 68 per cent homology between the primary amino acid sequence of rat ABP and human SHBG (Joseph *et al.* 1987), and the binding kinetics of rat ABP and rabbit TeBG are similar (Kotite and Musto 1982). These considerations suggest that minor amino acid substitutions in the binding proteins about the androgen binding site results in markedly different conformational states and steroid binding kinetics.

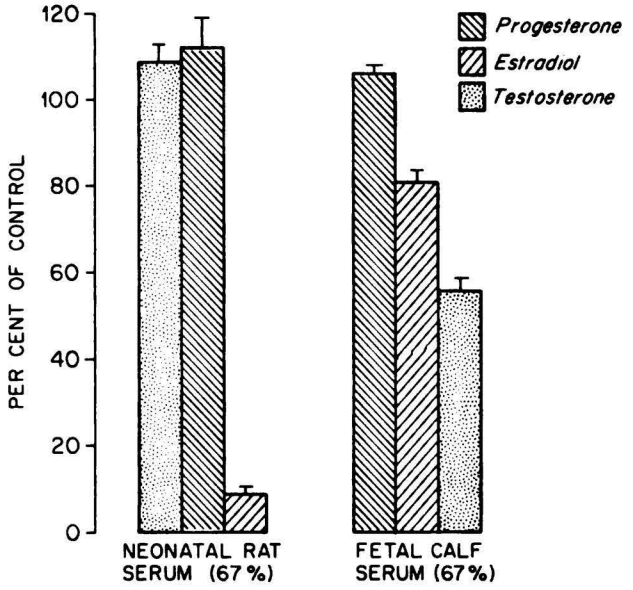

Fig. 6.16 The bioavailable fraction (shown on the y-axis as percent of control) of ^{3}H-progesterone, ^{3}H-oestradiol, or ^{3}H-testosterone is shown for adult rat brain. The bioavailable fraction was determined from the ratio of the first pass steroid extraction after arterial injection of hormone mixed in either 67% neonatal rat serum or 67% fetal calf serum divided by the first pass extraction of hormone after arterial injection in Ringer's solution. Data are mean ± S.E.M. (n = 4–6 rats). Confidence limits for the neontal rat serum: progesterone and testosterone (NS), oestradiol ($p < 0.0005$) and for fetal calf serum: progesterone (NS), oestradiol ($p < 0.05$), testosterone ($p < 0.0005$). (From Pardridge and Mietus 1979*b* with permission).

VI Developmental modulations

1 PLASMA PROTEIN BINDING

The development of sexually dimorphic behaviour in rodents and, possibly, in humans is believed to be the result of uptake and action in brain of circulating testosterone (Jacobson, Scernus, Shryne, and Gorski 1981; Holloway 1982). Testosterone action in developing rat brain may be mediated via aromatase conversion of testosterone to oestradiol (MacLusky and Naftolin 1981). The masculinization of the female brain by circulating oestradiol is believed not to occur owing to the avid oestradiol binding properties of α-fetoprotein (MacLusky and Naftolin 1981). The α-fetoprotein of developing rats, but not of developing humans, avidly binds oestradiol and oestrone but not testosterone (Raynaud, Mercier-Bodard, and Baulieu 1971; Crandall 1978). Moreover, the α-fetoprotein in the serum of developing rats markedly impairs the transport of oestradiol but not testosterone into adult rat brain (Fig. 6.16). However, human cord serum results in a slight depression of testosterone transport into adult rat brain as compared to oestradiol transport owing to the relatively low concentration of SHBG in human serum. Human

α-fetoprotein, which is in high concentrations in human cord serum, does not retard oestradiol transport into brain (Pardridge 1982).

2 CYTOPLASMIC BINDING

As reviewed in **Section II (Quantifiable physiological model)**, an important determinant of hormone volume of distribution is binding to nonreceptor cytoplasmic proteins. The kinetics of steroid hormone binding to these cytoplasmic proteins can be conveniently quantified *in vivo* using the tissue sampling single injection technique (Pardridge *et al.* 1980*b*). This method was originally used to quantify the avid cytosolic binding of testosterone and oestradiol in adult rat brain, and preliminary studies indicated there was no measurable cytosolic sequestration of sex steroids by newborn rabbit brain (Pardridge *et al.* 1980*b*). These studies have recently been extended to developing rabbits of varying postnatal ages. The brain sequestration index (BSI) is a semi-quantitative measure of the activity of the cytoplasmic binding of testosterone by rabbit brain. As shown in Fig. 6.17, there is no measurable sequestration by newborn rabbit brain but the binding of testosterone to cytoplasmic proteins is induced between 10–15 days postnatally and reaches adult levels by three weeks of development (Pardridge, Eisenberg, Fierer, and Musto 1988). The increased sequestration of testosterone by cytoplasmic binding systems in rabbit brain parallels the time course of increased serum binding of testosterone in rabbits (Fig. 6.17), owing to a developmental increase in circulating TeBG (Hansson, Ritzen, Weddington, McLean, Tindall, Nayfeh, and French 1974). The postnatal induction of cytoplasmic and plasma binding systems of testosterone also parallel the developmental induction of the rat brain nuclear androgen receptor. This receptor is low in newborn rats but is greatly increased between 10–15 days postnatally (Lieberburg, MacLusky, and McEwen 1980). Thus, the androgen binding systems in plasma, brain cytoplasm, and brain nucleus appear to be orchestrated in parallel at a critical stage of development in rats and rabbits.

3 CAPILLARY TRANSPORT OF ANDROGEN-BINDING PROTEINS

The possibility that androgen-binding proteins such as albumin or TeBG are actually transported through the brain capillary wall in developing rabbits was investigated with isolated brain capillaries and a preparation of ^{3}H-TeBG that retains normal testosterone binding properties. Capillaries were isolated from 28-day old rabbit brain (Fig. 6.18) and uptake of peptides and plasma proteins by the isolated brain capillary preparation have been shown in other studies to predict reliably peptide or plasma protein transport through the BBB *in vivo* (Pardridge 1986*a*). Since iodination of hormone-binding plasma proteins invariably leads to denaturation of the protein, puri-

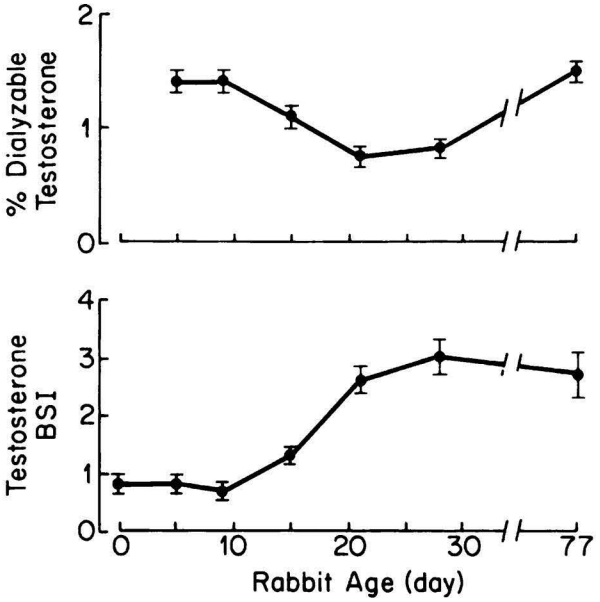

Fig. 6.17 *Bottom*: The brain sequestration index (BSI) of ^{3}H-testosterone is shown in rabbits of varying postnatal ages. A BSI of 1 suggests there is no sequestration (that is, binding) of testosterone by brain, whereas a rising BSI indicates the development of a testosterone sequestration system in brain. *Top*: The percent dialyzable ^{3}H-testosterone in rabbit serum of various ages is shown. The dialyzable percentage was determined by equilibrium dialysis at 37°C. (From Pardridge *et al.* 1988 with permission).

fied rabbit TeBG was radiolabelled by reductive methylation using ^{3}H-sodium borohydride (Pardridge *et al.* 1988). As shown in Figure 6.19, this preparation of ^{3}H-TeBG retained testosterone binding properties. This non-denatured preparation of TeBG was then used in *in vitro* uptake studies using the isolated brain capillaries. The results indicated that ^{3}H-TeBG and, to a lesser extent, ^{3}H-albumin were rapidly taken up by 28-day old rabbit brain capillaries via a process that was time- and temperature-dependent and was saturable by serum. Moreover, the uptake of the plasma proteins was much faster than the uptake of fluid phase markers such as ^{14}C-sucrose (Pardridge *et al.* 1988). The uptake of the plasma proteins appeared to be a function of development, since the uptake of ^{3}H-TeBG was several-fold greater by capillaries isolated from 28-day old rabbit brain as compared to capillaries isolated from adult rabbit brain. Why the developing rabbit brain takes up TeBG from the circulation is at present unknown. Owing to the rapid rates of androgen uptake by brain from the circulation via the enhanced dissociation mechanism, it would appear unlikely that the function of brain uptake of circulating TeBG is androgen delivery to brain cells. It may be that the apoprotein, *per se*, has a specific neuromodulator role in brain development. For example, transferrin, which mediates the brain uptake of circulating iron, is believed to have a neuromodulator role in brain that is separate from its iron

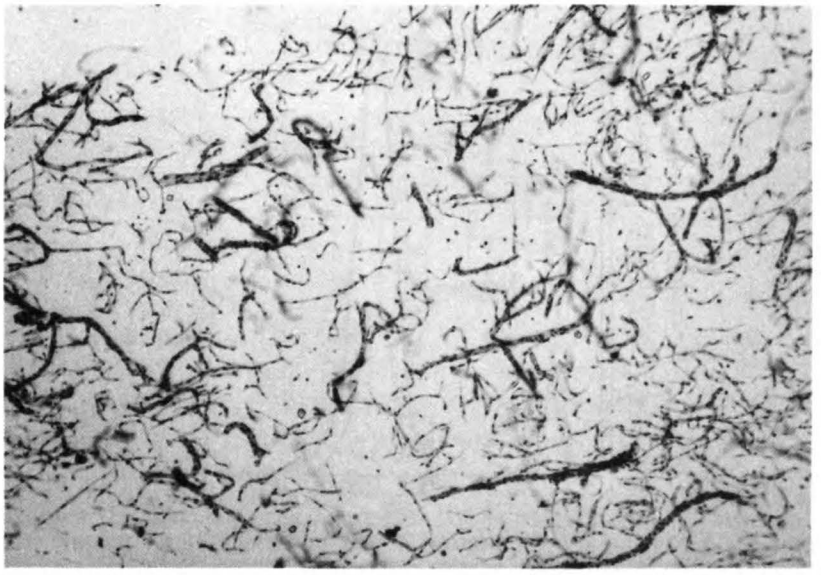

Fig. 6.18 Photomicrograph of isolated microvessels obtained from 28-day old rabbit brain. These capillaries were used in radioreceptor assays with ^{3}H-TeBG (see Fig. 6.19) to detect developmental modulations in uptake systems for TeBG in rabbit brain capillaries. Magnification × 25. (From Pardridge *et al.* 1988 with permission).

delivery function. Transferrin receptors in brain show little topographic correlation with iron distribution in brain cells (Hill, Ruff, Weber, and Pert 1985).

VII Clinical endocrinology

1 CIRRHOSIS

The gynaecomastia and feminization seen in cirrhotic men is a general observation, but the aetiology of this process is poorly understood (van Thiel 1979). Generally, either the total or free oestradiol level is normal in cirrhotic men, although the total testosterone level is decreased owing to hypogonadism (van Thiel 1979). A part of the feminization process includes a marked increase in the serum concentration of SHBG (van Thiel, Gavaler, Lester, Loriaux, and Braunstein 1975). The increased SHBG results in a decrease in the free plus albumin-bound fraction of testosterone in cirrhotic serum, and this correlates with a 35 per cent decrease in the unidirectional clearance of testosterone by rat brain using cirrhotic serum (Pardridge *et al.* 1980*a*), and a 35 per cent decrease in the MCR of testosterone in cirrhotic men (Gordon, Olivo, Rafii, and Southren 1975). The combination of increased SHBG and decreased albumin also results in an increase in the free plus albumin-bound fraction of oestradiol (Pardridge *et al.* 1980*a*). However,

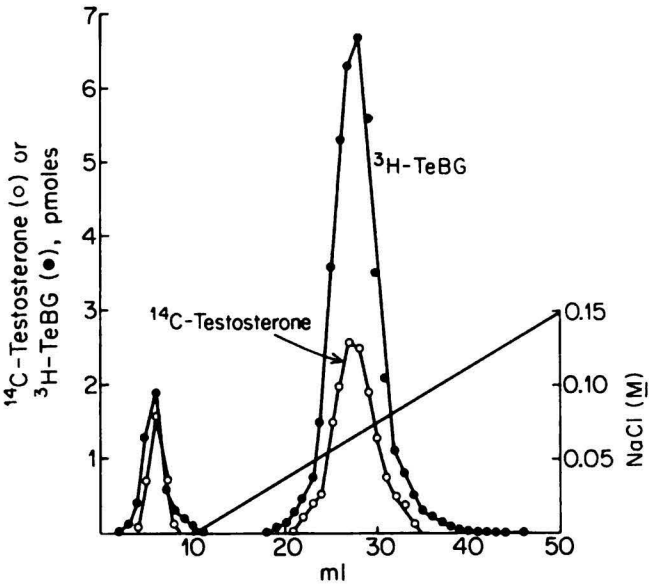

Fig. 6.19 Elution of ^{14}C-testosterone bound to ^{3}H-rabbit testosterone-binding globulin (TeBG) from a DEAE agarose column with a linear NaCl gradient. Testosterone co-eluted with labelled TeBG at 4°C indicating that the ^{3}H-TeBG molecule retained testosterone binding properties after the tritiation procedure. Less than 10 per cent of either the ^{3}H-TeBG or the ^{14}C-testosterone eluted in the low salt void volume of the column. (From Pardridge *et al.* 1988 with permission).

there is no decrease in the unidirectional clearance of oestradiol by rat brain using cirrhotic serum (Fig. 6.20). These measurements of unidirectional clearance confirm previous studies showing the MCR of oestradiol is not decreased in cirrhosis despite the elevation in SHBG (Olivo, Gordon, Rafii, and Southren 1975). The results in Figure 6.20 suggest the SHBG molecule in cirrhosis is structurally modified by the disease process and that this modification results in a change in the SHBG function with regard to oestradiol delivery to tissues. The hypothesis of structural differences in the SHBG molecule in cirrhosis has apparently not been tested to date. However, differences in glycosylation of TBG are known to occur in cirrhosis and this results in a change in the distribution of TBG isoforms in cirrhotic serum (Gartner, Henze, Horne, Pickardt, and Scriba 1981). Whether a similar modulation of the SHBG isoforms occurs in cirrhosis is not known but is presently being investigated in the author's laboratory. However, the data in Figure 6.20 and the study showing no decrease in the MCR of oestradiol in cirrhosis (Olivo *et al.* 1975) suggest that the increased SHBG in cirrhosis abnormally delivers oestradiol to tissues, and this may account for the feminization in this disease.

2 HYPERTHYROIDISM

Hyperthyroid men also show evidence of feminization and gynaecomastia

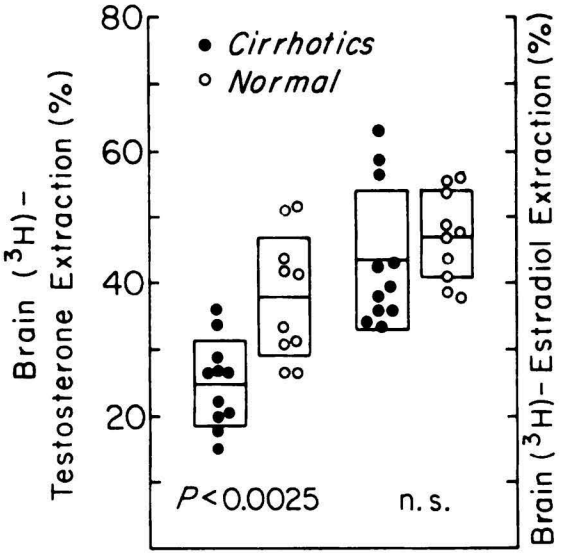

Fig. 6.20 The percentage of brain extraction of ^{3}H-testosterone or ^{3}H-oestradiol is shown after arterial injection of the labelled hormone mixed in either male cirrhotic or control human male serum. Rectangles represent the mean (horizontal line) ± S.D. The data show the unidirectional testosterone clearance by rat brain is decreased 33 per cent using cirrhotic serum, and this parallels a 2.6-fold increase in SHBG and a 40 per cent decrease in serum albumin in cirrhosis. However, the unidirectional clearance of oestradiol by rat brain was not decreased using cirrhotic serum, despite the marked increase in SHBG, decrease in albumin, and 41 per cent decrease in the non-SHBG-bound fraction of oestradiol. The brain bioavailable oestradiol using cirrhotic serum, 54 ± 4 per cent, is substantially greater than the non-SHBG-bound oestradiol, 37 ± 6 per cent, in cirrhotic serum, indicating SHBG-bound oestradiol is available for transport into brain from cirrhotic serum but not from control male serum. (From Sakiyama *et al.* 1982 with permission).

Table 6.8
Clinical alterations in serum SHBG

Increase	Decrease
Pregnancy	dexamethasone androgens
Estrogens	obesity
Hyperthyroidism	hirsutism
Cirrhosis	hyperprolactinemia
Hypogonadism	acromegaly

From DeMoor and Joossens (1970); Anderson (1974); Vermeulen (1977); Lobo and Kletzky (1983).

and an increase in the concentration of SHBG (Tulchinsky and Chopra 1973). Other conditions that result in an increase in serum SHBG in humans include hypogonadism, pregnancy, and oestrogen administration (Table 6.8). Serum SHBG concentrations are decreased in hyperprolactinemia,

acromegaly, hirsutism, obesity, dexamethasone administration, and danazol treatment (Table 6.8). There are conflicting reports as to whether the MCR of oestradiol is decreased in hyperthyroidism (Ruder, Corvol, Mahoudeau, Ross, and Lipsett 1971; Olivo *et al.* 1975). Therefore, hyperthyroidism may resemble cirrhosis, and the feminization of these conditions may arise from enhanced oestradiol delivery to tissues owing to posttranslational modifications (for example, in the carbohydrate moiety) of the SHBG molecule.

3 CRITICAL ILLNESS

Acute illness is known to be associated with a rapid decrease in the serum testosterone concentration, similar to the low T_4 syndrome seen in nonthyroidal illness (Goussis, Pardridge, and Judd 1983). However, unlike nonthyroidal illness, which is associated with an increase in the dialyzable fraction of circulating thyroid hormones (Pardridge 1983), the low testosterone syndrome of illness is not associated with an inhibition of plasma protein binding. In fact, serum SHBG concentrations are slightly elevated (Goussis *et al.* 1983). Since serum luteinizing hormone concentrations are increased in the late phase of anesthesia and surgery (Nakashima, Koshiyama, Uozumi, Monden, Hamanaka, Kurachi, Aono, Mizutani, and Matsumoto 1975), the low testosterone observed with acute illness may reflect a primary testicular effect of stress (Moberg 1987).

4 SPIRONOLACTONE ADMINISTRATION

A side effect of spironolactone administration for the treatment of hypertension is gynaecomastia. This feminization appears to arise from a drug inhibition of the androgen receptor. Spironolactone and its active metabolite, canrenone, also inhibit androgen binding to serum SHBG (Manni, Pardridge, Cefalu, Nisula, Bardin, Santner, and Santen 1985). The brain bioavailable fraction of testosterone is increased proportionately with increasing concentrations of either spironolactone or canrenone (Fig. 6.21). Thus, these drugs inhibit androgen binding both to SHBG and to the androgen receptor. The effect on the androgen receptor is more profound since the inhibition of androgen binding to SHBG would be expected to increase cytoplasmic free testosterone and, thus, increase androgen receptor occupancy, whereas the drug actually inhibits the receptor.

5 DANAZOL ADMINISTRATION

Danazol is used in the treatment of endometriosis since the drug causes a "medical oophorectomy", and results in a marked diminution in serum oestradiol levels (Meldrum, Pardridge, Karow, Rivier, Vale, and Judd 1983). The androgen-like effects of this drug also result in a marked decrease in

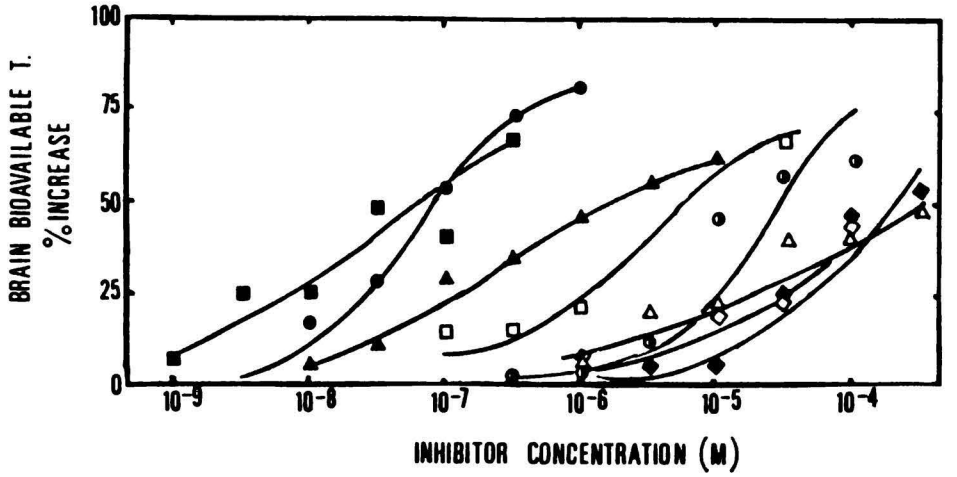

Fig. 6.21 The percent increase in brain bioavailable testosterone is plotted versus the concentration of inhibitors added to male serum ●, T(testosterone) ■, dihydrotestosterone; ▲, oestradiol; □, canrenone; △, SC24813; ◇, SC26519; ○, SC26962; ◆, spironolactone. Increasing concentration of these compounds result in gradual displacement of ^{3}H-testosterone from SHBG to human albumin and result in a progressive increase in the brain bioavailable steroid fraction. (From Manni *et al.* 1985 with permission).

SHBG production by the liver (Fig. 6.22). The diminished SHBG concentrations would be expected to result in a marked increase in testosterone MCR and the maintenance of normal plasma testosterone levels in danazol therapy (Fig. 6.22) indicate androgen production is increased in proportion to the increase in MCR. Recent studies indicate the "medical oophorectomy" may also be induced by chronic administration of gonadotropin-releasing hormone agonists, and that this therapy markedly lowers serum oestradiol concentrations without affecting the serum concentration of SHBG (Fig. 6.22).

6 DIETHYLSTILBESTROL ADMINISTRATION

Diethylstilbestrol (DES) was widely used in the past as a synthetic oestrogen (Steingold, Cefalu, Pardridge, Judd, and Chaudhuri, 1986). This oestrogenic drug does not bind to SHBG or α-fetoprotein, but does bind to albumin (Sheehan and Young 1979). In fact, the albumin binding of DES is about four-fold greater than albumin binding of oestradiol (Sheehan and Young 1979). However, the importance of measuring ligand binding to albumin with *in vivo* techniques is illustrated in Fig. 6.23, which shows the extraction of DES, oestradiol, oestrone, oestrone sulfate, and ethinyl oestradiol by rat uterus *in vivo* after aortic injection of labelled steroid hormone in either Ringer's solution, 4 g/dl bovine albumin, human pregnancy serum, or postmenopausal serum (Steingold *et al.* 1986). The results show that bovine albumin inhibits oestradiol extraction by more than 50 per cent but has no

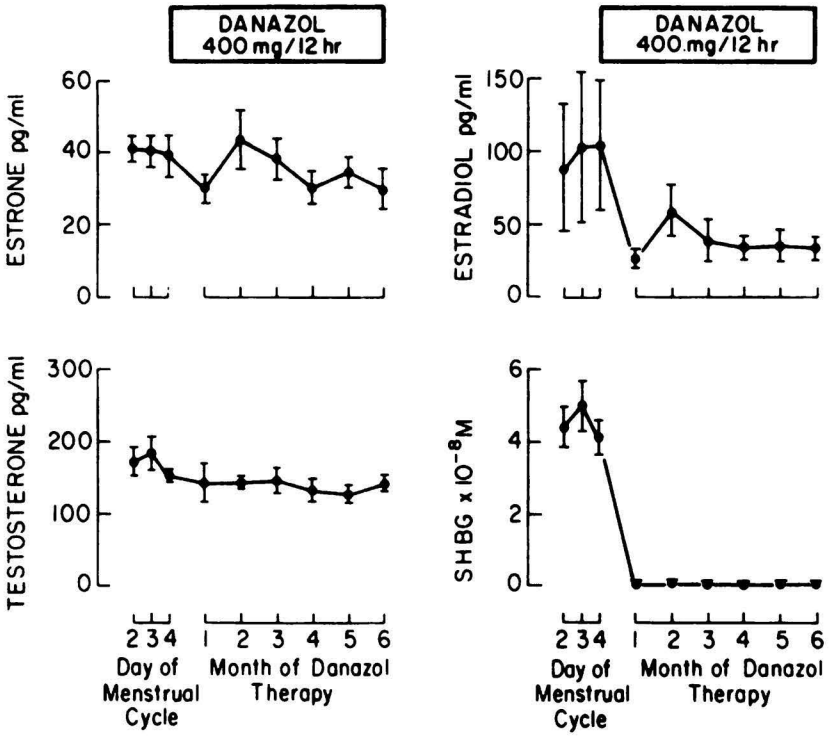

Fig. 6.22 Mean ± S.E. 8:00 a.m. levels of oestrone, oestradiol, testosterone, and sex hormone-binding globulin before and after treatment at the end of each month of danazol therapy. (From Meldrum *et al.* 1983 with permission).

measurable effect on uterine uptake of DES. Conversely, the brain extraction of DES was inhibited about 60 per cent by 4 g/dl bovine albumin, whereas the brain extraction of oestradiol was inhibited only about 25 per cent by this concentration of albumin. Therefore, albumin tends to retard DES delivery to an organ such as brain but mediates the avid uptake of DES by an organ such as the uterus.

VIII Other sex steroid-binding proteins

1 PROGESTERONE-BINDING GLOBULIN

Progesterone-binding globulin (PBG) is a protein that is found in the serum of the pregnant guinea pig, but is absent in the non-pregnant guinea pig. This protein, like SHBG, is a hormone-binding globulin secreted by the liver that has up to a 70 per cent carbohydrate content (Westphal 1980). Because PBG has a very high affinity for steroids such as progesterone or androgens, and is found in nearly micromolar concentrations in pregnant guinea pig serum, the dialyzable fraction of these sex steroids in pregnant guinea pig serum is extremely low (Westphal 1980). Moreover, the unidirectional clearance of progesterone and androgens by rat brain is greatly retarded by pregnant guinea

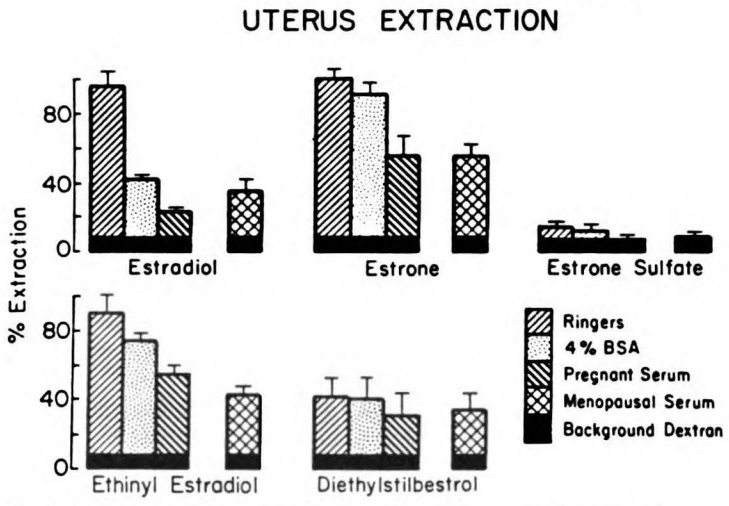

Fig. 6.23. Mean unidirectional extraction of various oestrogens by the rat uterus during a single pass through the organ microvasculature using the different aortic injection vehicles shown. The data show that albumin-bound diethylstilbesterol is readily available for transport into the uterus (i.e. the extraction with Ringers or 4 per cent BSA is comparable), whereas albumin-bound oestradiol is only partially available *in vivo* (i.e. the extraction with 4 per cent BSA is about 40 per cent of the Ringer's extraction). *In vitro*, however, albumin binds diethylstilbesterol several-fold greater than the plasma protein binds oestradiol. (From Steingold *et al.* (1986) with permission).

pig serum (Fig. 6.24). Although PBG greatly restricts the brain uptake of progesterone, dihydrotestosterone, testosterone, and 17-hydroxyprogesterone, but not oestradiol (Fig. 6.24), a substantial fraction of plasma progesterone (10 per cent) or testosterone (25 per cent) is still available for transport into the brain (Pardridge and Mietus 1980). These results indicate that PBG-bound testosterone or progesterone is partially available for transport from the circulating globulin-bound pool. Since the rate of steroid hormone dissociation from PBG *in vitro* is particularly fast, $t_{\frac{1}{2}} \approx 1$ second (Westphal 1980), it is not surprising that the rates of steroid hormone dissociation from PBG *in vivo* are also sufficiently fast so that a portion of the PBG-bound hormone is operationally available for uptake by tissues (Pardridge and Mietus 1980).

2 α_1-ACID GLYCOPROTEIN (OROSOMUCOID)

α_1-acid glycoprotein (AAG), like PBG, binds both progesterone and testosterone (Burton and Westphal 1972), and exists normally in human serum in relatively high concentrations, for example, about 10 μM in normal human serum (Pardridge, Sakiyama, and Fierer 1983). However, the affinity of SHBG for testosterone is much higher than the affinity of AAG and the

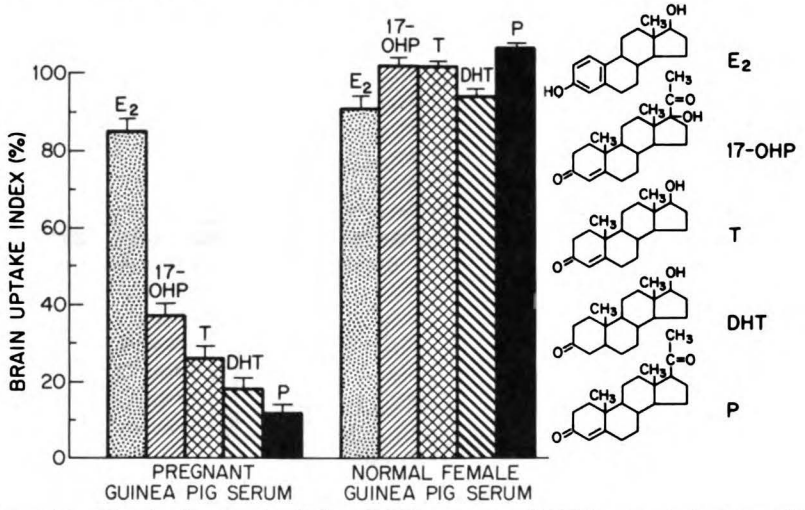

Fig. 6.24 The brain uptake index (BUI), mean ± S.E.M., n = 3–4 rats, of five ^{3}H-labelled steroid hormones is shown after carotid injection of the hormone mixed in either 67 per cent pregnant guinea pig serum or 67 per cent normal female guinea pig serum. Abbreviations used: oestradiol, E2; 17-hydroxyprogesterone, 17OHP; testosterone, T; dihydrotestosterone, DHT; progesterone, P. (From Pardridge and Mietus 1980 with permission).

capacity of albumin binding of testosterone is much higher than that of AAG. Consequently, very little testosterone normally circulates in the AAG-bound pool (Westphal 1971). However, the recent introduction of the progesterone antagonist, RU-486 (Nieman, Choate, Chrousos, Healy, Morin, Renquist, Merrian, Spitz, Bardin, Baulieu, and Loriaux 1987), as a contraceptive agent signals the need for physiologic studies regarding the role of AAG in tissue uptake of this progesterone antagonist. RU-486 is a lipophilic amine and, like lidocaine, propranolol, or bupivacaine (Table 6.5), probably actively binds AAG (Moguilewski and Thilibert 1985). While AAG binding of drugs such as propranolol or lidocaine results in little inhibition of drug transport to brain *in vivo* (Pardridge *et al.* 1983), AAG binding of other lipophilic amine drugs such as bupivacaine does result in a marked decrease in brain uptake of the drug (Terasaki, Pardridge, and Denson 1985). That is, enhanced dissociation from AAG binding sites does not occur for all lipophilic amines in all organs. Conceivably, AAG binding of RU-486 may facilitate hepatic uptake of this drug (and account for reduced bioavailability of orally administered drug) but markedly restrict uptake of RU-486 by other tissues such as brain, uterus, or ovary.

IX Conclusions

(1) The concentration of free cytosolic steroid hormone is believed to be the

driving force of both steroid hormone nuclear receptor occupancy and steroid hormone MCR. The free cytosolic testosterone or oestradiol has never been directly measured *in vivo* since this would require the development of a testosterone or oestradiol sensitive electrode that could be placed into the living cytoplasm. Consequently, there is a great need in endocrine research for a quantifiable physiological model of steroid hormone transport so that the factors regulating the concentration of free cellular hormone may be understood. The free hormone hypothesis is inadequate as a transport theory since the pool of free hormone is too small to explain saturation of nuclear receptor occupancy and is too small to allow net extractions of 10–50 per cent of steroid hormones by organs such as liver. The free cellular hormone is a predictable function of membrane transport, hormone metabolism, and the concentration of plasma exchangeable hormone. The plasma exchangeable hormone is a predictable function of *in vivo* hormone dissociation rates from albumin or SHBG, capillary transit time, and membrane transport kinetics (Pardridge and Landaw 1985; Pardridge and Landaw 1987).

(2) The plasma capillary exchangeable hormone is many-fold greater than the free hormone measured *in vitro*, owing to enhanced rates of steroid hormone dissociation from plasma proteins *in vivo* within the organ microcirculation. The enhanced rates of dissociation are believed to arise from conformational changes about the ligand binding site, which are initiated by transient interactions between the binding proteins and the surface of the organ microcirculation. Enhanced dissociation of steroid hormones from albumin binding sites occurs in all organs studied thus far, but to a quantitatively different extent among the organs. The molecular basis of the interaction between the binding protein and the microcirculatory surface is unknown at present but may be electrostatic in nature and, possibly, related to carbohydrate moieties on either the surface of the microcirculation or the binding globulins.

(3) There is a marked organ specificity underlying the transportability of testosterone or oestradiol into tissues from the circulating SHBG-bound pool. SHBG-bound testosterone and SHBG-bound oestradiol are not available for transport into tissues such as brain or uterus in the rat. However, SHBG-bound oestradiol but not SHBG-bound testosterone is selectively available for transport into liver, salivary gland, and lymph node in the rat. In tissues such as testes, both SHBG-bound oestradiol and SHBG-bound testosterone are readily available for transport. Thus, the presence of SHBG in serum allows for varying degrees of amplification of hormone delivery to tissues. In this way, sex steroid delivery is amplified to sex steroid dependent tissues and cytosolic pools of free hormone may be maintained in the face of active steroid hormone metabolism by particular organs such as liver, prostate gland, or salivary gland. Oestrogen sulfate or glucuronate conjugates are readily available for uptake by liver, but only oestrogen-glucuronate conjugates, and not sulfate conjugates, are freely transported into kidney. There-

fore, the conjugation of steroid hormones with a sulfate moiety selectively traffics this compound for biliary excretion as opposed to urinary excretion. This selective trafficking of conjugates occurs because oestrogen sulfates undergo enhanced dissociation from the sulfate binding site on albumin in the hepatic microcirculation but not in the renal microcirculation.

(4) In addition to organ specificities, there are also species and developmental differences in the transport of plasma protein-bound hormones into tissues. Rabbit TeBG and CBG deliver testosterone and corticosterone to rabbit tissues *in vivo*, and this explains why oestradiol, testosterone, and dihydrotestosterone MCR in the rabbit are all the same, despite marked differences in *in vitro* binding of these steroid hormones to TeBG. The functional differences between human SHBG and rabbit TeBG have kinetic and structural counterparts. For example, the multiple isoforms of rabbit TeBG are more acidic than the human SHBG isoforms. Although the equilibrium dissociation constant of androgen binding to human SHBG and rabbit TeBG are approximately the same, the rates of androgen association and dissociation with rabbit TeBG are more than twenty-fold greater than the reaction rates with human SHBG.

(5) Albumin or SHBG may be modified in pathologic states in humans. For example, cirrhotic men are feminized and the MCR of oestradiol is not diminished in cirrhosis despite the marked elevation in serum SHBG. Transport studies indicate the SHBG molecule in cirrhosis is modified in such a way that the binding globulin delivers oestradiol to organs such as brain, where normally SHBG retards oestradiol delivery to this organ in the rat. Therefore, pathologic delivery of oestradiol to tissues may lead to feminization.

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