Sex differences in hepatic oestrogen-binding proteins

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Gel-filtration (Sephadex G-75) analysis of hepatic cytosol reveals both qualitative and quantitative sex differences in oestrogen-binding proteins. The elution profile of [³H]oestradiol-labelled cytosol shows four species of oestrogen-binding proteins (peaks I, II, IV and V) common to both sexes. The amount of [³H]oestradiol binding in peak I is equivalent in both males and females and corresponds quantitatively to the specific oestrogen receptor. The amount of binding in the remaining three peaks is greater in males than females. In addition, an oestrogen-binding protein (peak III) is present that is unique to male cytosol. Proteinase-inhibition studies demonstrate that the observed multiplicity of oestrogen-binding proteins is not an artefact of proteolytic breakdown. Sex differences in oestrogen-binding proteins are absent in immature male and female animals; the oestrogen-binding protein profile in immature rats resembles that of an adult female. Gonadectomy of adult animals does not affect the oestrogen-binding-protein profile. In contrast, neonatal (day 1) castration results in partial feminization of the characteristic oestrogen-binding protein profile seen in the adult male; the appearance of Peak III is suppressed and marked decreases in the amount of oestradiol binding occurs in the remaining peaks. Hypophysectomy of adult animals results in near abolishment of the observed sex differences; the male oestrogen-binding protein profile is partially feminized and the female profile is partially masculinized, as characterized by the appearance of [³H]oestradiol binding in the region of peak III and increased amounts of binding in peaks IV and V. The present studies demonstrate a multiplicity of oestrogen-binding proteins in liver cytosol and raise the possibility that the presence of some of these proteins may be imprinted at birth through the hypothalamic–pituitary axis, by a mechanism requiring neonatal androgen exposure.

During the past several years it has become evident that mammalian liver function changes after oestrogen administration (Song *et al.*, 1969). More recently, abnormal liver function after oral-contraceptive use has suggested that oestrogens might act directly on the liver (Eisenfeld *et al.*, 1978). Increases in hepatic synthesis of plasma renin substrate in rat (Eisenfeld *et al.*, 1977) as well as increases in mRNA sequences for lipoprotein and vitellogenin in avian liver (Deeley & Goldberger, 1979; Chan *et al.*, 1979) have been directly correlated with the presence of oestrogens. The demonstration that hormone binds to a specific cytoplasmic receptor in rat liver (Eisenfeld, 1974; Powell-Jones *et al.*, 1976; Chamness *et al.*, 1975; Viladiu *et al.*, 1975) followed by nuclear translocation of the hormone–receptor complex (Aten *et*

al., 1978; Powell-Jones *et al.*, 1978) suggests that the liver is a target organ for oestrogens.

In contrast with the situation in males, certain hepatic enzyme systems in females are relatively unresponsive to oestrogen treatment (Berg & Gustafsson, 1973; Einarsson *et al.*, 1974; Dieringer & Lucier, 1978; Gustafsson *et al.*, 1977) although the concentrations of specific oestrogen receptors in males and females are similar (Dickson *et al.*, 1978; Powell-Jones *et al.*, 1980). However, a second class of liver oestrogen-binding sites have been shown to exist in greater numbers in males than in females (Dickson *et al.*, 1978; Eagon *et al.*, 1978; Powell-Jones *et al.*, 1980). These binding sites are characteristically different from the classical receptor, and are not a result of plasma contamination. Studies have indicated that the presence of this second class

of oestrogen-binding sites may effect nuclear translocation of hormone and hence may be involved in the sex differences seen in oestrogen responsiveness (Powell-Jones *et al.*, 1980). Moreover, the superior role played by the pituitary in the maintenance of sex differences in hepatic oestrogen-binding proteins has raised the possibility that their presence may be imprinted at birth (Thompson *et al.*, 1979; Powell-Jones *et al.*, 1980).

The present studies were undertaken to: (1) characterize further the second class of oestrogen-binding sites and investigate the possible multiplicity of oestrogen-binding proteins in rat liver; (2) investigate the contribution of specific oestrogen-binding proteins to the observed sex differences in total oestrogen binding in the liver; and (3) determine if the possible sex differentiation of individual hepatic oestrogen-binding proteins is under pituitary-hypothalamic control.

Materials and methods

Buffers and chemicals

The following buffers were used: 10 mM-Tris (Ultrapure; Bethesda Research Laboratories, Rockville, MD, U.S.A.), 1 mM-EDTA, 0.5 mM dithiothreitol adjusted to pH 7.4 at 4°C (Buffer A); 0.5% (w/v) activated untreated charcoal powder and 0.05% (w/v) dextran (Clinical grade) in Buffer A (DCC solution).

[2,4,6,7-³H]Oestradiol-17 β (94–113 Ci/mmol) was purchased from New England Nuclear Corp., Boston, MA, U.S.A. Unlabelled steroids were supplied by Steraloids, Wilton, PA, U.S.A. Purity of radioactive and unlabelled steroid was checked routinely by t.l.c. with benzene/ether (1:1, v/v) as solvent. Biofluor was purchased from New England Nuclear Corp. Sephadex G-75, ribonuclease A, chymotrypsinogen, ovalbumin, and Blue Dextran were obtained from Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A. Phenylmethanesulphonyl fluoride was supplied by Pierce, Rockford, IL, U.S.A. Proteinase (*Streptomyces griseus*) was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN, U.S.A. All other chemicals were purchased from Sigma, St. Louis, MO, U.S.A., unless otherwise indicated.

Animals

Intact adult (63–65 days old) and immature (28–29 days old) CD rats (Sprague-Dawley-derived) were used. For some studies, animals were gonadectomized or hypophysectomized (under diethyl ether anaesthesia) 5 weeks before being killed. All surgically manipulated adult animals other than neonatal castrated ones were obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.). Castrations of neonatal animals were per-

formed on hypothermic 1-day-old males. Controls were sham-operated.

Preparation of cytosol

Animals were killed by decapitation. Livers were quickly excised and placed on ice. All subsequent procedures were performed at 4°C. Livers were minced finely by using scissors and were weighed. Tissue was then homogenized, in Buffer A, (10%, w/v) by using four strokes on a Potter-Elvehjem homogenizer equipped with Teflon pestle. The liver preparation was centrifuged at 100 000 *g* for 60 min. The clear supernatant (cytosolic fraction) was removed, the fat layer being avoided. Protein determinations were by the method of Lowry *et al.* (1951).

Detection of total oestrogen binding

An incubation mixture (final volume 1.0 ml), consisting of equal volumes of cytosol and [³H]-oestradiol to give a final cytosolic protein concentration of 5–6 mg/ml and an [³H]oestradiol concentration of 30 nM (optimal conditions for resolving oestrogen-binding proteins by column chromatography), was incubated for 2 h at 4°C (the assay was done in triplicate). Independent studies have shown that the maximum extent of [³H]-oestradiol binding is obtained within a 1 h incubation of either male or female cytosol with [³H]oestradiol and remains essentially stable up to 18 h. After the incubation, the cytosol samples were added to a DCC pellet previously prepared from 500 μ l of DCC solution (see under 'Buffers and chemicals') that was centrifuged at 800 *g* for 10 min and the pellet retained. The amounts of total [³H]oestradiol binding seen in both males and females remains constant when charcoal-absorption times of 10 to 30 min are used. In these experiments the charcoal/cytosol mixtures were routinely vortex-mixed once and left on ice for a 30 min incubation time. Charcoal was then pelleted (800 *g* for 10 min). Portions (100 μ l) of supernatant were added to Biofluor liquid scintillant (10.0 ml) and assessed for the amount of total [³H]oestradiol binding by counting for radioactivity in a Beckman LS 7000 liquid-scintillation counter.

Gel-filtration analysis of oestrogen-binding protein

Sephadex G-75 was packed in a glass column (1.5 cm $\times$ 90 cm; bed volume 125 ml) and equilibrated in Buffer A. Blue Dextran (mol.wt. 2 000 000) was used to ascertain the void volume and the elution positions of ribonuclease A (mol.wt. 13 700), chymotrypsinogen (mol.wt. 25 000), ovalbumin (mol.wt. 45 000) and bovine serum albumin (mol.wt. 65 000) were used to calibrate the column. Samples of cytosol prepared from a minimum of two livers per group under investigation were incubated (the

assay was done in triplicate) for 2 h at 4°C with [^3H]oestradiol (60 nM). Unbound [^3H]oestradiol was removed from bound by DCC treatment as previously described. Samples of labelled cytosol were then pooled and a portion (1.0 ml) applied to the Sephadex G-75 column. By using Buffer A as the elution buffer, fractions (3.0 ml) were collected at a flow rate of 15–18 ml/h. A portion (1.0 ml) from each fraction was assessed for radioactivity. Protein content of each fraction was monitored by measuring u.v. absorption at 280 nm.

Phenylmethanesulphonyl fluoride and proteinase studies

The preparation of cytosol from adult male liver and its subsequent incubation (2 h at 4°C) with [^3H]oestradiol (60 nM) was carried out in the presence or absence of the proteinase inhibitor phenylmethanesulphonyl fluoride (100 μM) at optimal conditions for phenylmethanesulphonyl fluoride activity (James, 1978). Unbound [^3H]oestradiol was separated from bound by DCC as described above. A portion (1.0 ml) was then analysed for [^3H]oestradiol binding by gel filtration on Sephadex G-75 in which phenylmethanesulphonyl fluoride (100 μM) was either present or absent from the elution buffer.

Samples of prepared cytosol from adult male liver were incubated with [^3H]oestradiol (30 nM) in the presence or absence of *Streptomyces griseus* proteinase (0.25 mg/ml) for 1 h at 25°C. Samples were placed on ice and unbound [^3H]oestradiol separated from bound by DCC treatment as described above. A portion (1.0 ml) was then analysed by gel-filtration chromatography.

Specificity studies

Cytosol preparations (500 μl , 10–12 mg of protein/ml) were incubated (2 h at 4°C) with an equal volume of [^3H]oestradiol (60 nM) in the presence or absence of a 100-fold excess of various unlabelled ligands. Unbound [^3H]oestradiol was separated from bound by DCC treatment as described above. A portion (1.0 ml) was then analysed for [^3H]oestradiol binding by gel filtration on Sephadex G-75.

Sedimentation analysis of oestrogen-binding protein

Samples (400 μl) of cytosol labelled with [^3H]oestradiol (described under 'Detection of oestradiol binding' above) were layered on 5–20% sucrose gradients prepared in Buffer A. Gradients were centrifuged in a SW-56 swinging-bucket rotor at 100 000 g for 18 h. Bovine serum albumin (4.6 S) was used as a sedimentation standard. After centrifugation, gradients were fractionated and each fraction (approx. 100 μl) assessed for bound radioactivity.

Results

Sedimentation analysis (Fig. 1) of [^3H]oestradiol-labelled cytosol from male and female rats demonstrates that the amount of [^3H]oestradiol retention in the 4S region is approx. 10 times greater in males than females, whereas the amount of binding in the 8S region is equivalent. Analysis of [^3H]oestradiol binding to hepatic cytosol by gel filtration (Fig. 2) reveals multiplicity of cytosolic oestrogen-binding proteins not evident by sedimentation analysis. Results given in Fig. 2 show both qualitative and quantitative sex differences in cytosolic oestrogen-binding-protein profiles. Whereas peaks I, II, IV and V are present in both sexes, a fifth binding species, peak III, is apparent only in male cytosol. The characteristic elution profile of oestrogen-binding proteins in male cytosol, shown in Fig. 2, remains essentially unchanged after the addition of phenylmethanesulphonyl fluoride (100 μM) to both the homogenization and elution buffers (results not shown). These results would suggest that the oestrogen-binding proteins do not arise from proteolytic breakdown.

The amount of [^3H]oestradiol associated with peak I is equivalent in both males and females and corresponds quantitatively to the amount of binding demonstrated for the specific 8S hepatic oestrogen

Fig. 1. Sedimentation analysis of the binding of [^3H]oestradiol by cytosolic components of male and female liver

Samples of cytosol prepared from male (●) or females (○) livers were incubated (2 h at 4°C) with [^3H]oestradiol (30 nM). After treatment with DCC, portions (400 μl) of cytosol were analysed on 5–20% sucrose gradients. The cytosol protein concentration was 5–6 mg/ml.

Fig. 2. Gel-filtration analysis of the binding of [^3H]oestradiol by cytosolic components of male and female liver

Samples of cytosol prepared from male (●) or female (○) livers were incubated (2 h at 4°C) with [^3H]oestradiol (30 nM). After treatment with DCC, samples from each group were pooled and a portion (1.0 ml) analysed on a Sephadex G-75 column. The cytosol protein concentration was 5–6 mg/ml. Marker-protein abbreviations: BSA, bovine serum albumin; Oval, ovalbumin; Chym., chymotrypsin; RNAase, ribonuclease.

receptor (Fig. 1). In contrast, the amount of [^3H]oestradiol binding to peaks II, IV and V is greater in males than females. The contribution of radioactivity from these peaks, with the addition of peak III (in males) is equivalent to both the amounts of [^3H]oestradiol retention seen in the 4S region of sucrose gradients (Fig. 1) and the amount of total [^3H]oestradiol binding determined before chromatographic separation.

Results suggest that each of the radioactive regions represents [^3H]oestradiol-bound protein. Studies have indicated (results not shown) that free [^3H]oestradiol applied to the column elutes after peak V at a volume of approx. 165 ml. U.v. spectroscopy demonstrates that protein is associated with peaks I–V (Fig. 3). In addition, a marked decrease (80–100%) in the amount of [^3H]oestradiol retained in each of the peak regions results from proteinase treatment, as illustrated in Fig. 4.

Fig. 3. Gel-filtration analysis of the protein profile of cytosol from adult male liver

Samples of cytosol prepared from adult male livers were incubated (2 h at 4°C) with [^3H]oestradiol (30 nM). After treatment with DCC, samples were pooled and a portion (1.0 ml) analysed on a Sephadex G-75 column. Each fraction was monitored for both [^3H]oestradiol (●) and protein (○) (u.v. absorbance at 280 nm). The cytosol protein concentration was 6–7 mg/ml.

Fig. 4. Gel-filtration analysis of the effect of *Streptomyces griseus* proteinase on the binding of [^3H]oestradiol by cytosolic components

Samples of cytosol prepared from adult male livers were incubated (1 h at 25°C) with [^3H]oestradiol in the presence of proteinase (0.25 mg/ml). After treatment with DCC, samples from each group were pooled and a portion (1.0 ml) analysed on a Sephadex G-75 column. The cytosol protein concentration was 5–6 mg/ml.

Ligand specificity of oestrogen-binding proteins

The ligand specificity exhibited by each of the radioactive peaks obtained after gel filtration of male

Table 1. *Competitive binding of steroids for individual oestrogen-binding proteins*

Male liver cytosol was incubated for 2 h at 4°C with 60 nM [3 H]oestradiol in the presence or absence of 100-fold excess of non-radioactive competitor. Unbound ligand was removed by treatment with DCC solution. A portion (1.0 ml) of labelled cytosol was analysed by gel filtration. Values represent single determinations.

Competitor	[3 H]Oestradiol-17 β bound (%)				
	Peak I	Peak II	Peak III	Peak IV	Peak V
Control	100	100	100	100	100
Diethylstilboestrol	15	100	100	100	100
Oestradiol-17 β	17	34	12	30	23
Dihydrotestosterone	166	58	48	48	53
Cortisol	142	101	100	100	100

Fig. 5. Gel-filtration analysis of the binding of [3 H]-oestradiol by cytosolic components of pre-pubescent male and female liver

Samples of cytosol prepared from immature (day-28) (●) or female (○) livers were incubated (2 h at 4°C) with [3 H]oestradiol (30 nM). After treatment with DCC, samples from each group were pooled and a portion (1.0 ml) analysed on a Sephadex G-75 column. The cytosol protein concentration was 4–5 mg/ml.

cytosol is presented in Table 1. Both oestradiol and diethylstilboestrol compete effectively for [3 H]-oestradiol-binding sites in peak I, whereas cortisol and dihydrotestosterone do not compete. In contrast with peak I, the remaining four peaks (II, III, IV and V) do not demonstrate oestrogen specificity, since dihydrotestosterone competes well for binding sites in those regions. The increased amount of [3 H]oestradiol binding seen in peak I after the addition of the competitors dihydrotestosterone and cortisol may reflect lack of saturation in this region. More [3 H]oestradiol becomes available in the presence of these competitors and subsequently binds to peak I.

Oestrogen-binding proteins in pre-pubescent rats

Immature male and female rats were killed at 28 days of age and oestrogen-binding proteins analysed by gel filtration. The sex differences seen in the cytosolic oestrogen-binding proteins of the adult (Fig. 2) are not apparent in the immature animal (Fig. 5). The elution profiles of oestrogen-binding

proteins (peaks II–V) from immature male and female cytosol are quantitatively as well as qualitatively similar and resemble that of the adult female. It has been noted that the amount of [3 H]oestradiol binding in the region of peak V is greater in the immature female than male; however, the significance of this is not apparent at the present time. Interestingly, the male-specific peak III, seen in adult cytosol, is absent in the immature male. The amount of binding in peak I in both immature males and females is approx. 50% of that seen in the adult.

Effect of gonadectomy on oestrogen-binding proteins

The characteristic elution profile of cytosolic oestrogen-binding proteins of adult animals is not markedly altered after gonadectomy, as seen in representative profiles given in Figs. 6(a) and 6(b). Results from several experiments indicate that neither castration nor ovariectomy greatly affects the amount of [3 H]oestradiol binding seen in peak I or in the male-specific peak III. However, some minor changes are evident in other peaks, although sex differences are still clearly apparent. For example, a slight elevation of amounts of [3 H]-oestradiol binding is seen in peaks IV and V of both sexes, whereas binding to peak II is decreased in males.

In contrast with the lack of effects on the oestrogen-binding protein profile after adult gonadectomy, profiles of adult males castrated on day 1 are decidedly different from the characteristic elution profile described for the intact male. The elution profile of oestrogen-binding proteins from an adult that was castrated on day 1 is feminized (Fig. 7); the appearance of peak III is suppressed and the amounts of binding in the regions of peaks II, IV and V are similar to those in females. Binding in Peak I in the neonatally castrated group is similar to control values (sham-operated).

Effect of hypophysectomy on male and female oestrogen-binding proteins

Sex differences observed between males and females are nearly abolished after hypophysectomy.

Fig. 6. Gel-filtration analysis of the effect of gonadectomy on the binding of [^3H]oestradiol by cytosolic components of male and female liver

Samples of cytosol prepared from the livers of (a) adult male castrated animals (○) or sham-operated adult males (●) or (b) adult female ovariectomized (○) or sham-operated adult females (●) were incubated (2 h at 4°C) with [^3H]oestradiol (30 nM). After treatment with DCC, samples from each group were pooled and a portion (1.0 ml) analysed on a Sephadex G-75 column. The cytosol protein concentration was 5–6 mg/ml.

Partial feminization of the male oestrogen-binding protein profile results from adult hypophysectomy (Fig. 8a). This is characterized by the absence of [^3H]oestradiol retention in the region of peak III, as well as a decrease in the amounts of [^3H]oestradiol binding to the remaining peaks, II, IV and V. In contrast, the elution profile of female cytosol after

Fig. 7. Gel-filtration analysis of the effect of neonatal castration on the binding of [^3H]oestradiol by cytosolic components of the adult male liver

Samples of cytosol prepared from the livers of adult males castrated on day 1 (○) or adult males sham-operated on day 1 (●) were incubated (2 h at 4°C) with [^3H]oestradiol (30 nM). After treatment with DCC, samples from each group were pooled and a portion (1.0 ml) analysed on a Sephadex G-75 column. The cytosol protein concentration was 5–6 mg/ml.

hypophysectomy (Fig. 8b) exhibits more masculine-like characteristics. This is evidenced by the appearance of binding in the region of the male-specific peak III, with concomitant increased amounts of binding in the regions of peaks IV and V that approach male values. In addition, there is a slight elevation in [^3H]oestradiol binding to peak II as compared with the sham-operated female control. After hypophysectomy, the amount of binding in peak I is decreased in both sexes by approx. 50%.

Discussion

Recent studies have investigated binding of oestradiol to hepatic cytosol by sedimentation analysis and a quantitative sex difference in amounts of oestrogen binding to cytosolic components was demonstrated (Powell-Jones *et al.*, 1980). In the current study, sex differences in [^3H]oestra-

Fig. 8. Gel-filtration analysis of the effect of hypophysectomy on the binding of [^3H]oestradiol by cytosolic components of adult male and female liver

Samples of cytosol prepared from the livers of (a) adult male hypophysectomized (O) or sham-hypophysectomized (●) or (b) adult females hypophysectomized (O) or sham-hypophysectomized (●) 3 weeks before being killed were incubated (2 h at 4°C) with [^3H]oestradiol (30 nM). After treatment with DCC, samples from each group were pooled and a portion (1.0 ml) analysed on a Sephadex G-75 column. The cytosol protein concentration was 5–6 mg/ml.

diol binding to the 4S region of sucrose gradients was also observed (Fig. 1). However, this technique is not applicable to a more thorough examination of the multiplicity of oestrogen-binding proteins. Our studies have demonstrated: (1) multiple forms of oestrogen-binding proteins in the liver of male and female rats; (2) quantitative sex differences in the amount of [^3H]oestradiol binding to the individual proteins; and (3) the presence of oestrogen-binding protein (peak III) unique to male cytosol. The observed oestrogen-binding protein multiplicity does not appear to be an artefact of proteolytic breakdown, since the addition of the proteinase inhibitor phenylmethanesulphonyl fluoride has no appreciable effect on the elution profile of the oestrogen-binding proteins. Furthermore, each of the radioactive regions contains protein as determined by both u.v. spectroscopy (Fig. 3) and proteinase treatment (Fig. 4). Although it is apparent that multiple forms of oestrogen-binding proteins exist in rat liver and that these multiple forms (peaks II–V) bind dihydrotestosterone as well as oestradiol, the nature and function of these proteins have not been demonstrated *in situ*. It is unlikely that any of the oestrogen-binding proteins represent the sex-hormone-binding globulin, since this latter protein is not present in the rat. The similarity of both the amount of oestrogen binding and the ligand specificity of the individual oestrogen-binding proteins to previous reports describing a second class of oestrogen-binding sites (Dickson *et al.*, 1978; Powell-Jones *et al.*, 1980) suggests that the oestrogen-binding proteins (peaks II–V) are analogous to both the unusual sex-steroid-binding protein and the male-specific steroid-binding protein.

The first protein to elute from the column (peak I) is similar in males and females (Fig. 2). Several lines of evidence indicate that this oestrogen-binding protein represents the oestrogen receptor characterized in rat liver: (1) the amount of [^3H]oestradiol retained in the region of peak I (Fig. 2) is equivalent to that reported for the specific oestrogen receptor sedimenting in the 8S region of sucrose gradients (Powell-Jones *et al.*, 1980); (2) the absence of any sex difference in the amount of [^3H]oestradiol binding to peak I is in agreement with previous studies (Dickson *et al.*, 1978; Powell-Jones *et al.*, 1980); (3) the ligand specificity of peak I (Table 1) is analogous to that described for the specific receptor (Chamness *et al.*, 1975; Beers & Rosner, 1977; Aten *et al.*, 1978; Powell-Jones *et al.*, 1980); (4) the decrease in the amount of [^3H]oestradiol binding to peak I resulting from hypophysectomy (Fig. 8) is in accord with previous reports that hypophysectomy markedly decreases concentrations of hepatic oestrogen receptors (Chamness *et al.*, 1975; Beers & Rosner, 1977; Powell-Jones *et al.*, 1980).

Several enzyme systems exhibit large post-

pubertal sex differences (Gustafsson *et al.*, 1977; Denef & DeMoor, 1972; Lamartiniere *et al.*, 1979). This sexual differentiation appears to result from irreversible imprinting ('programming') of the hypothalamic-pituitary axis by neonatal exposure to testicular androgens (McEwen, 1976; Gustafsson *et al.*, 1977). The present results suggest that the sex differences observed in the oestrogen-binding proteins of the adult (Fig. 2) may have developed through a similar mechanism. For example, the binding of [³H]oestradiol in the region of peak III is detected in the adult male, whereas in the adult female and the immature animal of either sex, no such binding is seen. Further evidence for the imprinting theory of individual hepatic oestrogen-binding proteins is supplied by our findings that (a) neonatal castration of male rats prevents the appearance of peak III in the subsequent post-pubertal animal, and (b) that hypophysectomy of adult animals nearly abolishes the sex differences in oestrogen-binding protein profiles. This suggests that the pituitary secretes a factor that stimulates peak-III synthesis. Other studies on the regulation of hepatic metabolism suggests that this pituitary factor may be controlled by a hypothalamic releasing factor. One noteworthy result was that hypophysectomy of adult females resulted in the appearance of [³H]oestradiol binding in the region of peak III, whereas in the hypophysectomized male rat, [³H]oestradiol binding in this region was not detectable. Peak V appears to be under a somewhat different form of control than the oestrogen-binding protein associated with peak III, since hypophysectomy increases the amount of this oestrogen-binding protein to male values. Gustafsson *et al.* (1977) have postulated the secretion of a pituitary feminizing factor that plays an important role in directly regulating sex differences in hepatic protein synthesis. In our system, the secretion of such a factor could maintain the 'feminized' concentration of peak V by acting on the liver to repress its synthesis.

In summary, our studies have demonstrated that in rat hepatic cytosol: (1) multiple forms of oestrogen-binding proteins exist in both males and females; (2) sex differences are apparent in the oestrogen-binding protein profiles as determined by Sephadex G-75 column chromatography; (3) sex differences of certain oestrogen-binding proteins might be programmed at birth by neonatal exposure to testicular androgens; and (4) different regulatory models may be constructed for different oestrogen-binding proteins. Further investigations employing both whole animals and isolated hepatocytes will more specifically characterize the role that individual oestrogen-binding proteins may play in the nuclear translocation process of hepatic cytosolic receptors and correlate changes seen in oestrogen binding

with responsiveness by employing cellular indicators of oestrogen response.

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