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RAT THYMIC ESTROGEN RECEPTOR—I. PREPARATION, LOCATION AND PHYSIOCHEMICAL PROPERTIES

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SUMMARY

Specific, high affinity estrogen receptor has been shown to be present in the thymus tissue from both rat and bovine sources. The receptor is located in the reticuloepithelial portion of the organ in the rat and is not present in the T-lymphocyte fraction. The equilibrium association constant and concentration of specific estrogen receptor was determined in thymic cytosol by Scatchard plot analysis. The equilibrium association constant (K_A) for bovine was $1.72 \pm 0.12 \times 10^9\ M^{-1}$ and for rat was $7.14 \pm 0.52 \times 10^9\ M^{-1}$. The receptor concentration for bovine thymus was 0.298 ± 0.03 pmol/g tissue and rat was 0.362 ± 0.017 pmol/g tissue or for bovine 6.57 ± 0.13 fmol/mg protein and for rat was 7.18 ± 0.61 fmol/mg protein. From competition assays, the receptor possessed specificity for estradiol and the estrogen-like compound diethylstilbestrol, but not for progesterone, testosterone, triamcinolone or cortisol, Dihydrotestosterone was shown to bind slightly (0.04% with respect to estradiol) and only at high concentrations (4×10^{-6} M). By sucrose gradient centrifugation studies the thymic estrogen receptor was shown to have a sedimentation value of 7–8s in low salt or 3–4s in high salt buffer. A possible mechanism for estradiol suppression of the cell mediated immune system is discussed.

INTRODUCTION

Estrogens are known to suppress the immune response. For example, tuberculin skin sensitivity is depressed when guinea pigs are treated with estradiol (Lurie *et al.*, 1949; Kappes *et al.*, 1963). Estrogen treatment can also suppress, or delay, corneal allograft rejection in rabbits (Waltman *et al.*, 1971) and skin allograft rejection in mice (Graff, Hildemann & Snell, 1966). Reduction in circulating steroids following gonadectomy substantially enhances tissue rejection in male and female mice (Graff, Hildemann & Snell, 1966; Graff, Lappe & Snell, 1969). It has also been reported that treatment of male and female mice with estradiol results in a decrease in thymic weight and an acute lymphopenia (Franks, Perkins & Bishop, 1975), while castration produces a significant increase in thymic weight (Westphal, 1971; Chiodi, 1940 and 1976). Treatment of castrate animals with estradiol reverses the increased thymic weight (Westphal, 1971; Chiodi, 1940).

The findings listed above could be interpreted to mean that the thymus possess an estrogen binding mechanism which might mediate weight and functional effects (Grossman, 1977, Grossman & Nathan 1977a, b). Such a binding mechanism has been shown to exist in a variety of other steroid sensitive tissues such as rat uterus (Baulieu *et al.*, 1971; Jensen *et al.*, 1971), hamster uterus (Steggles & King, 1972), calf uterus (Best Belpomme *et al.*, 1970), Stumpf *et al.*, 1970), and oviduct (Gorski *et al.*, 1968; Stumpf *et al.*, 1970). We therefore felt it worthwhile to determine if a specific estrogen receptor was present in thymic tissue.

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MATERIALS AND METHODS

Animal model and steroid treatment. Thymus tissue was obtained either fresh in the slaughter house from young cattle or it was acquired in the laboratory from sexually mature 1–2 month old male or female rats (body weight 150–200 g). The rats were maintained on a standard laboratory diet and a 13:11 photoperiod (lights on 0500 h to 1800 h). Castrations or ovariectomies in the rats were carried out under phenobarbital anesthesia (90 mg/kg body wt) at least one week before the animals were to be tested. For certain experiments, castrated animals were treated daily with 15 μg estradiol-17β (15 μg dissolved in 0.3 ml of corn oil) injected subcutaneously for 3 days and the rats were sacrificed on the 4th day by cervical dislocation. The thymus or, in certain experiments, the uterus was removed and freed from extraneous tissue. When beef thymus was used, it was prepared in the same manner as rat thymus. The fresh tissue was blotted, weighed and then placed in ice-cold saline. To prepare cytosol, the thymus was minced and homogenized in twice the tissue volume (w/v) of TE buffer (0.01 M Tris, pH 7.5, 1 mM EDTA) at 2°C

with a Vertis 45 microhomogenizer. The tissue was exposed to three bursts of homogenization for 5 s each at maximum setting and 60 seconds allowed between bursts for cooling. The homogenate was centrifuged in polyallomer tubes at 120,000 *g* for 30 min at 0°C in a Beckman Model L2-65B. The supernatant fraction (termed cytosol) was decanted into a separate container and the precipitate was frozen and saved to be used later in the diphenylamine procedure for measurement of deoxyribonucleic acid concentration.

Measurement of estrogen receptor. To measure the amount of estrogen receptor present in the tissue, Scatchard plot analysis was employed (Scatchard, 1949; Rosenthal, 1967; Chamness & McGuire, 1975). The thymic cytosol was diluted 1:6 using TE buffer and 0.3 ml cytosol fractions were then incubated with five concentrations of [^{3}H]-estradiol from 8.8×10^{-10} M to 5.4×10^{-11} M with or without the addition of unlabelled estradiol (4×10^{-6} M) for 4 h as described originally by Toft & O'Malley (1972). After incubation, each sample was treated with 0.5 ml of dextran-charcoal solution to remove free estradiol. Dextran-charcoal solution (DCC) was prepared by dissolving 0.05% dextran T-70 w/v (Pharmacia) in 400 ml of 0.01 M Tris, pH 7.5, 1 mM EDTA and then adding 0.5% Norite A activated charcoal. Each sample was stirred on a vortex mixer and allowed to stand for 10 min at 0°C and then centrifuged for 3 min at 800 *g*. From those tubes containing labelled estradiol the amount of total binding could be determined, and from the tubes containing unlabelled estradiol the amount of nonspecific binding could be measured. Specific binding of [^{3}H]-estradiol was calculated by subtracting nonspecific binding from total binding. The relationship between the bound to free ratio and the concentration of specifically bound [^{3}H]-estradiol (nanomolar) was then subjected to linear regression analysis. The results provided a correlation coefficient, measurement of binding affinity and the concentration of binding sites (pmol/ml cytosol) where one binding site per receptor molecule was assumed.

Measurement of specificity of estrogen receptor-hormone binding. To determine the specificity of thymic estrogen receptor to bind various steroid hormones, competition assays were performed. Aliquots of 0.3 ml of cytosol (1:4, w/v) were added to assay tubes containing 0.1 ml of [^{3}H]-estradiol at a final concentration of 1.4×10^{-9} M (22,000 c.p.m.) and 0.1 ml of unlabelled competitor steroids. Six concentrations of competitor steroids were used (varying from 4×10^{-6} M to 4×10^{-11} M). All assays were prepared 5 times and after 4 h of incubation at 0°C the free [^{3}H]-estradiol was separated from the bound by dextran-charcoal treatment.

Measurement of the sedimentation value of estrogen receptor by centrifugation. To determine the sedimentation value of thymic estrogen receptor, 0.3 ml cytosol (1:4, w/v) was incubated for 2 hr at 0°C with 8×10^{-6} M [^{3}H]-estradiol. In certain experiments, thymic cytosol was also incubated with other unlabelled steroids at a final concentration of 4×10^{-6} M prior to the addition of the labelled compound. Sucrose gradients (5–20%) were prepared in 5 ml polyallomer tubes with a Beckman gradient former. The labelled cytosol (0.2 ml/gradient) was layered at the top of each gradient and centrifuged at 2°C in a Spinco SW 50 L rotor at 202,000 × g for 18 h using a Beckman Model L2-65B preparative ultracentrifuge. Gradients were divided using a Buchler tube piercer into 22 fraction/gradient. Each fraction was collected in 0.5 ml of TE buffer, and 0.5 ml of dextran-coated charcoal was added. The 1.0 ml suspension was vigorously stirred and after 10 min centrifuged. Bovine serum albumin (BSA) or rhodamine bovine albumin (®BA) was employed as a sedimentation standard (4.8s). The supernatant fractions were counted in 7 ml toluene Triton solution in a Beckman Model LS-31557 scintillation counter. Toluene Triton solution was prepared by adding 15 g PPO (2,5-Diphenyloxazole) and 0.15 g POPOP (1.4-bis{2-(5-Phenyloxazolyl)}-Benzene) (Eastman) to 2 l. of toluene, scintillation grade (MCB) and 1 l. of Triton X-100. The concentration of DNA in samples was measured by the diphenylamine method of Burton (1956). Protein concentration was determined by the Lowry method (Lowry *et al.*, 1951).

Statistics. Statistical analyses utilized the Student's *t*-test.

Materials. Rats were purchased from Harlen Industries of Indiana. All reagents were purchased from Sigma Chemical Co. unless otherwise specified. Radioactive estradiol-6,7-^{3}H(N) (specific activity 40–60 ci/mmol) was obtained from New England Nuclear Corp.

Preliminary experiments. Thymic estrogen receptor was initially prepared from bovine thymus. During these preliminary experiments, it was discovered that the best fit of Scatchard points to a linear plot was obtained if the DCC remained in contact with the cytosol for 10 min. It was also shown that the use of soft, colored plastic pipette tips resulted in variability in results. White or clear polyethylene tips were judged more satisfactory for the test system.

RESULTS

Comparison of estrogen receptor from bovine and rat thymus

Cytosol fractions were prepared from bovine and male rat thymus at dilutions of 1:6 w/v in TE buffer. From Scatchard plot analysis (Fig. 1), it can be seen that the amount of estrogen receptor for both rat and beef thymus was approximately 0.07 pmol/ml but the association constant (KA), as determined by slope, was greater for rat (11.96×10^9 M^{-1}) than for beef (1.9×10^9 M^{-1}) thymic estrogen receptor. These results for estrogen receptors are reported in Table 1 as fmol/mg protein and pmol/g tissue wet wt and again there is no significant difference between rat

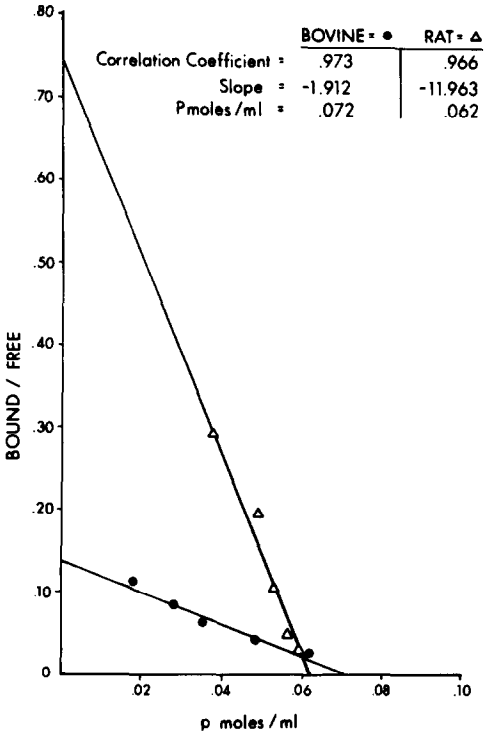

Fig. 1. Sample Scatchard plot of [^{3}H]-estrogen binding in rat and bovine thymic cytosol fractions.

and beef preparations. Once these initial experiments had been completed, rat thymus was used exclusively since it was felt that this preparation would lend itself most easily to the study of variations in physiological parameters.

A comparison of binding and saturation parameters and sedimentation properties of estrogen receptors in the cytosol of the thymi and uteri from castrate rats

Female rats, 6 weeks of age, were ovariectomized and 1 week after the surgery cytosol fractions were prepared from both uterine and thymic tissues. For comparison, cytosol was also prepared from the male rat thymus. The results of Scatchard plot analysis of these cytosol fractions are reported in Table 2. The K_A measured for uterine estrogen receptor is $18.4 \times 10^9\,M^{-1}$ and for both castrate females and intact males the K_A is approximately $9 \times 10^9\,M^{-1}$. These values are well within the range reported in the literature for these tissues (King & Mainwaring, 1974; Baulieu *et al.*, 1971; Jenson *et al.*, 1971). The results (Table 2) also indicate that there is approximately 100 fold more uterine than thymic estrogen receptor as measured in pmol/g tissue, fmol/mg protein, or pmol/mg DNA. The data used for thymic Scatchard plot analysis has also been presented in terms of saturation binding curves (Fig. 2). By definition nonspecific binding is unsaturable and rises at a constant slope as the [^{3}H]-estradiol concentration increases. Total binding being a combination of nonspecific and specific binding first rises rapidly at low [^{3}H]-estradiol concentrations and then at the point when all specific binding sites become saturated, the total binding curve is then a reflection of the slope of the non-specific binding curve. A saturation binding curve for uterine estrogen receptor taken from the uterine Scatchard plot analysis runs is included for comparison (Fig. 3). Note that the thymic and uterine saturation curves are similar in characteristics, but there is a greater level of specific binding in uterus than thymus. Sucrose gradient centrifugation studies (Fig. 4) using cytosol from both castrate female uteri (1:14, w/v) and male thymus (1:6, w/v) showed that sedimentation values for estrogen receptor from both uterus and thymus were in the 7–8s range (fractions 14–16) using rhodamine bovine albumin as a 4.8s standard. These sedimentation values are in agreement with those reported for uterine estrogen receptor (King & Mainwaring, 1974).

Table 1. Estrogen Receptor in rat and bovine thymus as measured in total cytosol by Scatchard plot analysis

	K_A ($X10^9\,M^{-1}$)	pmol/g Tissue	fmol/mg Protein
Rat	7.14 ± 0.519* $n = 29$†	0.326 ± 0.017	7.18 ± 0.61
Bovine	1.72 ± 0.12	0.298 ± 0.031	6.57 ± 0.48

* $\bar{X}$ ± SEM.
† n = separate animal preparations.

Table 2. A comparison of association constants and concentration of estrogen receptor in cytosol prepared from male rat thymus and ovariectomized remale rat thymus and uterus

Type	$K_A(10^9\,M^{-1})$	pmol/g Tissue	fmol/mg Protein	pmol/mg DNA
Male thymus	9.92 ± 3.34* $n = 3$†	0.246 ± 0.015	6.06 ± 0.365	0.032 ± 0.002
Female thymus	9.14 ± 1.40 $n = 4$	0.219 ± 0.012	5.60 ± 0.308	0.022 ± 0.001
Female uterus	18.48 ± 1.36	17.55 ± 0.26	531.25 ± 7.706	4.892 ± 0.072

* $\bar{X}$ ± SEM.
† n = separate animal preparations.

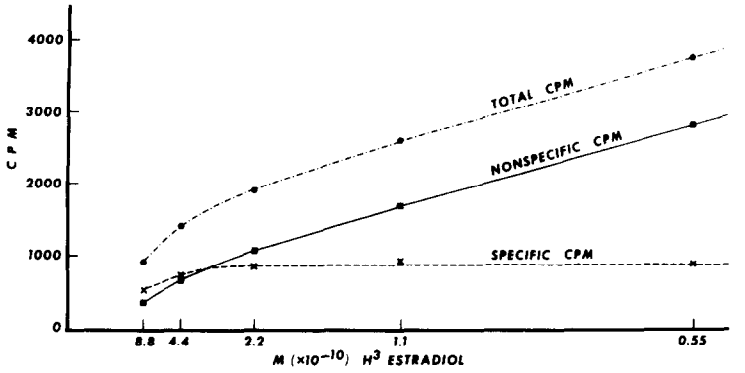

Fig. 2. Saturation binding curve for male rat thymic estrogen receptor.

Specificity of binding of thymic estrogen receptor as measured by competition studies

Competition studies were used to determine if steroids other than estradiol could bind to thymic estrogen receptor. When rat thymic cytosol was prepared at a dilution of 1:4 (w/v) and run in a competition assay, the curves of Fig. 5 were generated. Interpretation of these curves is simplified by drawing the horizontal line at the point on the estradiol binding curve at which 50% of the total specific radioactive estradiol binding is blocked. The point of intersection of any other curve with this line is taken to indicate significant competition by the competitor steroid. Curves that do not intersect and remain above this line indicate that these steroids do not compete sig-

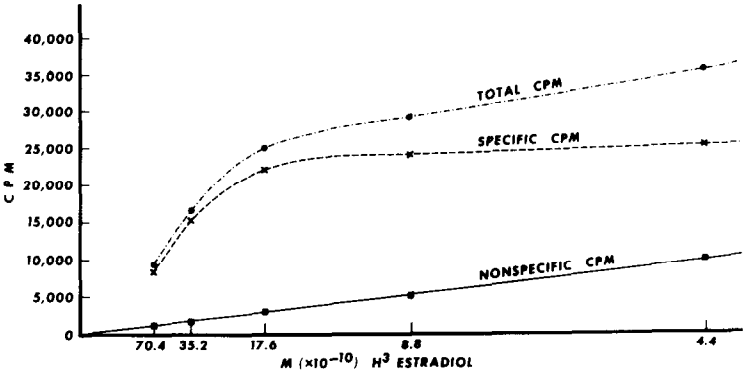

Fig. 3. Saturation binding curve for uterine estrogen receptor prepared from castrate female rats.

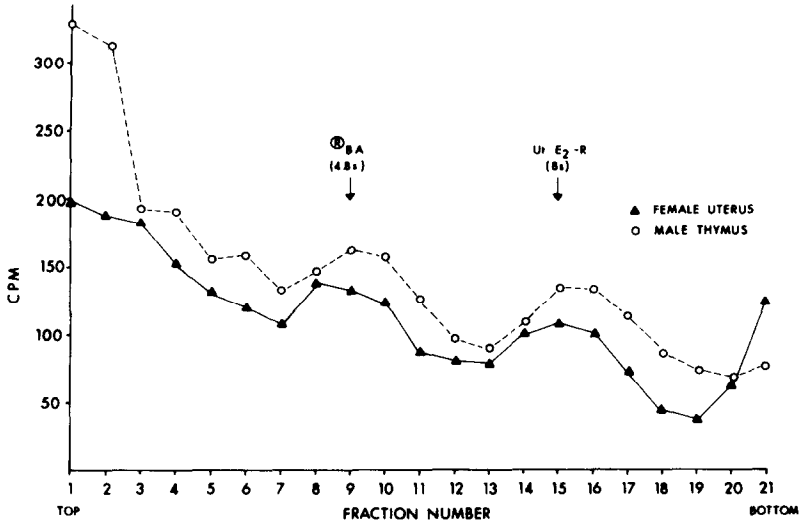

Fig. 4. Sucrose gradient centrifugation study of estrogen, receptor from female rat uterus and male rat thymus. CPM = Specific counts/min bound. ®BA = Rhodamine bovine albumin. UT E_2 – R = Uterine estrogen receptor.

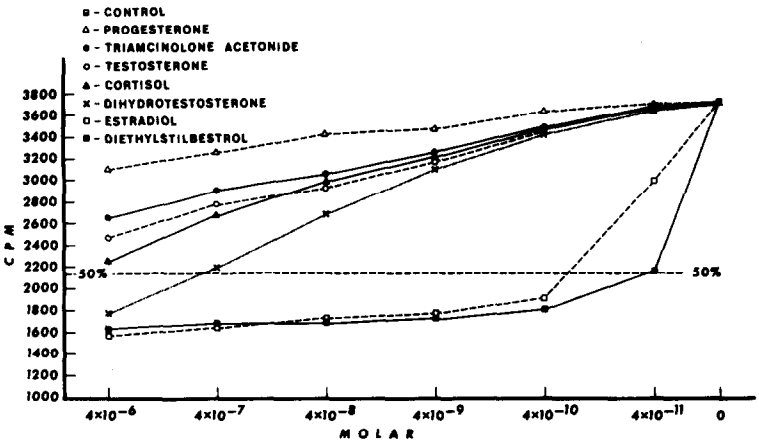

Fig. 5. Competition study of [^{3}H]-estradiol binding on rat thymic cytosol in the presence of varying concentrations of unlabelled steroids. CPM = Specific counts/min bound. Each point represents the mean of five values.

nificantly. The results shown in Fig. 5 are reported in Table 3. Steroid concentrations for competition at the 50% level were obtained from the curves. Relative binding affinities were calculated by setting the molar concentration of estradiol to achieve 50% competition equal to 100% and calculating the other steroids relative to estradiol. For rat thymus, diethylstilbestrol binds best followed by estradiol. The fact that diethylstilbestrol binds four fold better than estradiol is not unexpected since diethylstilbestrol is a potent non-steroidal estrogen. Dihydrotestosterone binds only 0.04% as well as estradiol while progesterone, testosterone, triamainolone acetonicle and cortisol do not bind significantly.

Distribution of estrogen receptor within the thymus

Thymic tissue was removed from 6 week old male rats, placed in cold saline and minced with scissors. The mince was strained through cheese cloth and the suspension of free thymic lymphocytes was weighed, counted, and also observed by microscopic analysis. The remaining matrix tissue probably consisting mostly of reticuloepitelial cells was weighed and saved. Cytosol was prepared from both the thymic lymphocyte (T-lymphocyte) fraction and the reticulo-epithelial cell fraction at 1:6 (w/v). Measurement of the amount of receptor in the separate fractions of cytosol prepared from 1 g of matrix tissue or 1 g of T-lymphocytes (Table 4) indicates that, while there is a similar concentration of estrogen receptor in matrix cytosol (0.32 pmol/g tissue) as in cytosol from intact thymus preparations, there is no detectable receptor present in the T-lymphocyte cytosol fraction. The separated lymphocytes represent only 0.01% of the total tissue weight. However, a sufficient weight of T-lymphocytes was used so that the test was not

Table 3. Competition studies of [H^3]-estradiol binding on rat thymic cytosol receptor in the presence of varying concentrations of unlabelled steroids

Steroids tested	50% Level concentration	RBA
Estradiol (E_2)	2.3×10^{-10} Molar	100%
Diethylstilbestrol (DES)	4.5×10^{-11} Molar	488.8%
Dihydrotestosterone (DHT)	5.6×10^{-7} Molar	0.04%
Testosterone (T)	None	
Progesterone (P)	None	
Cortisol (F)	None	
Triamcinolone acetonide (TA)	None	

Table 4. Rat estrogen receptor in thymic matrix and thymic lymphocyte cystosol as measured by Scatchard plot analysis

Reticulo-epithelial matrix			
K_A (10^9 M^{-1})	pmol/g tissue	fmol/mg protein	Thymic lymphocyte
6.05 ± 0.73* n = 10†	0.315 ± 0.027	6.69 ± 0.58	None

* $\bar{X}$ ± SEM.
† n = separate animal preparations.

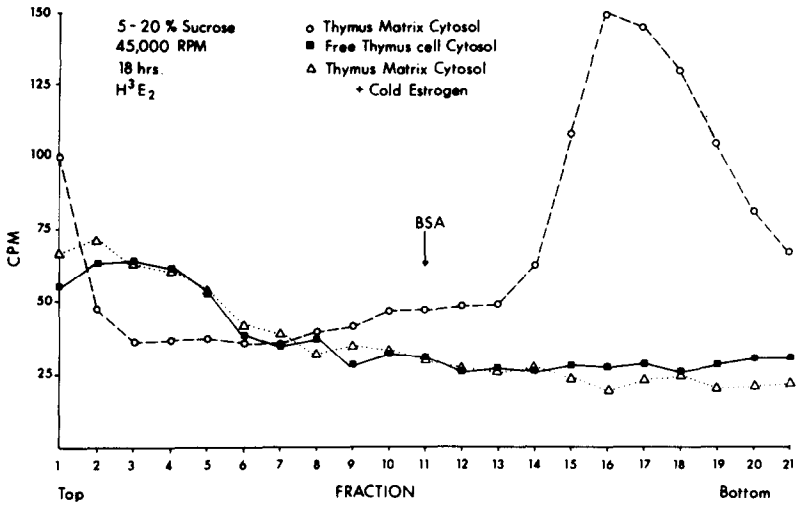

Fig. 6. Sucrose gradient sedimentation pattern of rat thymic cytosol fractions. CPM = Specific counts/min bound. BSA = bovine serum albumin.

limited by the available cell mass. It is unlikely that preparation of thymic lymphocytes as described here could have resulted in selective recovery of a subpopulation of cells which lack estrogen receptor. This is supported by the fact that upon microscopic examination two T-lymphocyte populations consisting of large and small cells were observed. This technique, therefore, does not appear to be enriching one population. Furthermore, we have been able to show that peripheral lymphocytes do not contain estrogen receptor (data not presented).

When thymic matrix and T-lymphocyte cytosol were analyzed on 5–20% sucrose gradients, the patterns shown in Fig. 6 was obtained. The estrogen receptor of matrix cytosol sedimented in the 7–8s range as has already been reported for this receptor from intact thymus (Fig. 4). If the matrix cytosol was preincubated with 1.5×10^{-5} M unlabelled estradiol and then run on the gradient, the 7–8s peak is not present. This indicates that the [^{3}H]-estradiol was unable to bind to the receptor since the excess unlabelled estradiol was competing for the sites. If T-lymphocyte cytosol is evaluated on the gradient, no 7–8s peak is present. These results are in agreement with those of the Scatchard plot analyses and indicate that at the limits of sensitivity of these methods T-lymphocytes do not contain estrogen receptor while thymic reticuloepithelial cells have the estrogen receptor.

Changes in the gradient sedimentation patterns of thymic estrogen receptors in the presence of solutions containing high salt concentrations

To ascertain if the rat thymic estrogen receptor could be dissociated into smaller subunits as reported for uterine estrogen receptor (Jensen *et al.*, 1971; King & Mainwaring, 1974), thymic tissue was homogenized on 5–20% sucrose gradients (Fig. 7) and the sedimentation pattern indicates the presence of a 4s fraction

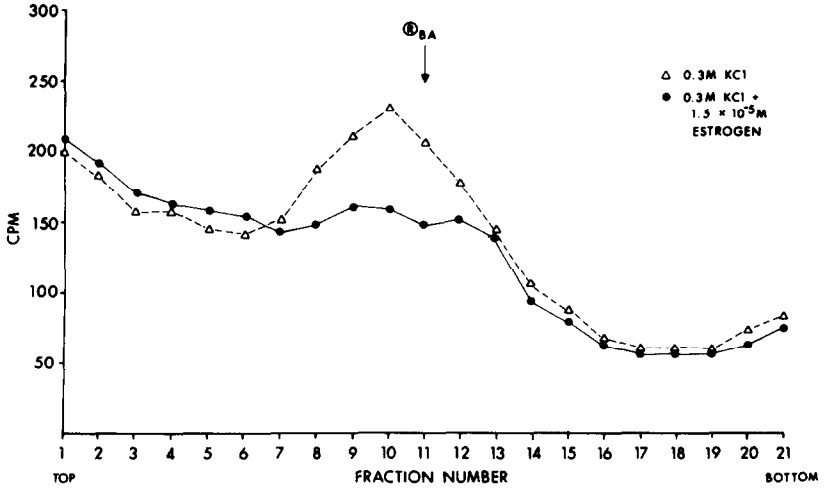

Fig. 7. Sucrose gradient centrifugation study of estrogen receptor of rat thymic cytosol, prepared in high salt solution (0.3 M KCL) with and without unlabelled estrogen. CPM = specific counts/min bound. ®BA = Rhodamine bovine albumin.

and the absence of the 7–8s peak. This 4s peak vanishes when the cytosol is incubated with excess amounts of unlabelled estradiol. These results are interpreted to mean that thymic estrogen receptor dissociates into smaller subunits in the presence of high salt concentrations and the smaller subunits retain their estrogen binding sites.

DISCUSSION

Castration has been shown to affect the thymus and increase its weight by removing a major source of gonadal steroids. Estradiol administration decreases the weight of thymus tissue in either intact or castrate animals (Westphal, 1971). As reported earlier, the cell mediated immune system can be suppressed by estradiol administration (Lurie *et al.*, 1974; Graff, Hildemann & Snell, 1966; Graff, Lappe & Snell, 1969; Franks, Perkins & Bishop, 1975). It is not surprising, therefore, that we are able to demonstrate the presence of a high affinity, specific estrogen receptor in rat and beef thymus.

High affinity ($K_A \geq 1 \times 10^9\,M^{-1}$) estrogen receptors have been shown to be present in uterus (Baulieu *et al.*, 1971; Jensen *et al.*, 1971; Steggle & King, 1972; Best-Belpomme *et al.*, 1970), vagina (Baulieu *et al.*, 1971), pituitary gland (King *et al.*, 1965; Stumpf *et al.*, 1970), and oviduct (Gorski *et al.*, 1968, Stumpf *et al.*, 1970) to list only a few tissues. The receptor is present in the cytoplasm and upon binding to the steroid translocates into the nucleus (Jensen *et al.*, 1968; Gorski *et al.*, 1968) where it is thought to stimulate RNA synthesis which, in turn, activates protein synthesis (Clark, Andersen & Peck, 1973).

This receptor was shown by Scatchard plot analysis to be present in both rat and beef thymic cytosol in a concentration of approximately 0.3 pmol/g tissue or 7 fmol/mg protein. Comparison of this tissue with uterus from castrate animals indicates that there is approximately 100 times more estrogen receptor in uterine tissue than in thymic tissue.

With regard to the affinity of thymic estrogen receptor (as measured by the slope of the Scatchard plots), the equilibrium association constant (K_A) which is a measure of affinity was $6.6 \times 10^9\,M^{-1}$ for rat thymic estrogen receptor and $1.7 \times 10^9\,M^{-1}$ for beef thymic estrogen receptor. From *in vitro* competition assays, the rat thymic estrogen receptor specifically bound diethylstilbestrol and estradiol-17β but not progesterone, testosterone, triamicinolone or cortisol. Dihydrotestosterone at high concentrations of 4×10^{-6} M was shown to bind slightly with respect to estradiol. Using 5–20% sucrose gradient centrifugation, rat thymic estrogen receptor had a sedimentation value of 7–8s in low salt and 3–4s in high salt buffer.

The properties of uterine estrogen receptor are well documented (Jensen *et al.*, 1968; Gorski *et al.*, 1968; Jensen *et al.*, 1971; King & Mainwaring, 1971; Jensen & DeSombre, 1972; Baulieu *et al.*, 1975). Thymic estrogen receptor appears to be similar in all physiochemical respects to uterine estrogen receptor.

Thymic incorporation of radioactive estradiol was studied by Mauer & Chalkley (1967). These workers were able to show that [^{3}H]-estradiol enters calf thymus cells but only very low quantities bind in this tissue. They did not show specificities, affinity or characteristics of the estradiol uptake and were of the opinion that it might be nonspecific. Their finding of low [^{3}H]-estradiol binding in thymus is in agreement with our results showing specific estrogen receptor is present but in fmol quantities.

Recently Reichman & Villee (1978) reported that female rat thymus contained a high affinity, specific estrogen receptor. The receptor was shown by competition analysis to be highly specific for estradiol, to bind dihydrotestosterone slightly and to sediment on sucrose gradients in the 7–8s region. The results are similar to those reported here and also in our earlier studies on male rat thymus (Grossman & Nathan, 1977a, b), however, Reichman & Villee (1978) have not localized the receptor to a specific cell population.

An interesting observation of the present study was the demonstration that thymic derived lymphocytes possess no measurable estrogen receptor. The specific estrogen receptor is located in the portion of the thymus which is composed predominantly of reticuloepithelial cells. If cytosol fractions from both reticuloepithelial matrix and T-lymphocytes are prepared from equivalent amounts of tissue at equal dilutions, estrogen receptor is present only in matrix. Thus, the fact that there is only about 0.01 mg T-lymphocytes/100 mg thymus can be ruled out as the cause of the undetectable levels of estrogen receptor in T-cells and, therefore, wet weight of the original tissue is not a factor which determines the sensitivity of Scatchard plot analyses.

Although T-lymphocyte function and number are depressed by estrogens (Lurie *et al.*, 1949; Kappes *et al.*, 1963; Waltman *et al.*, 1971; Graff, Hildemann & Snell, 1966; Graff, Lappe & Snell, 1969; Franks, Perkins & Bishop, 1975), these cells contain no cytoplasmic estrogen receptor. This estrogen receptor is present in the supporting matrix of the thymus. A similar situation is evident in the testes where sperm maturation in the seminiferous tubules is under the control of testosterone. It has been reported (Williams, 1974) that testosterone directly affects the seminiferous tubule cells and acts only indirectly on the sperm cells. This link between the seminiferous tubule cells and sperm cells is unknown; however, it could be mediated by a hormone or through direct cellular contact. Thus, for thymus-T cell and seminiferous tubule-sperm cell, hormone receptors, are located in the supportive matrix portion of the glands and interactions with the "free cell" portion is through as yet unknown means. Since estrogen has been reported to produce lymphocytopenia (Franks, Perkins & Bishop, 1975), one question which remains

to be answered is related to the possible direct effect of estrogen on the specific presence of receptors in peripheral T-lymphocytes. Preliminary results indicate that these cells (like thymic derived lymphocytes) also have no estrogen receptor (unpublished work). Thus, it is possible that lymphocytopenia in response to estrogen treatment might be mediated by a thymic hormone. Metcalf (1956) reported that the plasma from patients with certain diseases (i.e., lymphatic leukemia, lymphosarcoma) was capable of inducing lymphocytosis when inoculated into baby mice.

The substance, LSS (Lymphocyte stimulating substance) would also be prepared from an aqueous emulsion of normal thymus, but not from other organs. The production of thymic LSS was depressed greatly over controls in thymic homogenates prepared from mice pretreated with estradiol (Metcalf, 1956). Since estrogen receptor is present in the reticuloepithelial matrix of the thymus, it follows that the target of estrogen action is probably the reticuloepithelial cells. If these cells are shown to produce thymic hormone, then estradiol action could depress thymic hormone production and result in a decrease in the production or activity of T-lymphocytes. We are presently investigating this hypothesis.

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