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Rapid Analysis of Estrogen and Progesterone Receptors Using Gel-Exclusion High-Performance Liquid Chromatography†

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ABSTRACT: Estrogen and progesterone receptors prepared from mouse, rat, and human uteri, as well as from human breast cancers, have been characterized by gel-exclusion high-performance liquid chromatography. The qualitative relationships previously established by sedimentation analysis between the cytoplasmic [aggregated (~8S), deaggregated (~4S), and trypsinized (~3.6S)] and nuclear (~5S) forms of the rat uterine estrogen receptor were maintained by this technique. Differences in the partition of estrogen and progesterone receptors from the same species as well as interspecies differences in these receptors were reproducibly observed. Multiple forms of human estrogen and progesterone receptors could clearly be resolved in a single analysis and were distinct from serum steroid binding tissue contaminants. Separation analyses, performed at flow rates up to 2 mL min^{-1}, were capable of resolving all receptor forms in 10-12 min with the column returning to base line in 25 min. With this exclusion gel column (TSK-G3000SW) as a background upon which to reference different receptor forms, eight distinct partitions or elution positions have been enumerated. This approach has considerable promise for the rapid characterization of different forms of steroid-receptor proteins. Moreover, it should provide a critical advantage in minimizing the opportunities for receptor modification during separation analysis and in maximizing the opportunity to study short-lived interactions between receptors and physiologic or pharmacologic ligands.

Estrogenic and progestational steroid hormones exert their effects upon target tissues by passing through the cell membrane, interacting with a cytoplasmic-binding protein, and, as an activated binding complex, translocating to the nucleus where interaction with chromatin presumably initiates certain events responsible for alterations in the rates of transcription, translation, and even replication (Jensen & DeSombre, 1972, 1973; Katzenellenbogen & Gorski, 1975; O'Malley & Means, 1974; Yamamoto & Alberts, 1976). These intracellular steroid-binding proteins are referred to as steroid "receptors", and their presence or expression provides a method for identifying the potential for endocrinologic response in various tissues. Since endocrine therapy can be of considerable benefit in the management of sex hormone responsive cancers, there is an obvious value to performing receptor determinations in order to distinguish those patients who, because of their receptor-positive status, are likely to respond to endocrine therapy (Block et al., 1975; Horwitz et al., 1975; Osborne et al., 1980). Steroid-receptor determinations can be based upon principally quantitative analysis by employing assays involving dextran-coated charcoal, hydroxylapatite, or protamine sulfate (Garola & McGuire, 1977a,b, 1978; Rosner et al., 1980; Zava et al., 1976). Moreover, receptor assays involving sucrose- or glycerol-gradient analysis, gel filtration, column chromatography,

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and electrophoresis can also provide a qualitative characterization (Sherman et al., 1980; Miller et al., 1975, 1981; Tilzer et al., 1981; Carlson et al., 1977; Katzenellenbogen et al., 1980). Time and convenience are generally limiting factors in the application of qualitative receptor analysis. Sucrose-gradient sedimentation analysis appears to be the most popular qualitative approach but has a limited resolution and often requires overnight centrifugation. The value of qualitative receptor analysis is that (1) the data are displayed in a more convincing multipoint format that seems less vulnerable to the variability that accompanies quantitative estimates of receptor and (2) the potential for documenting physicochemical heterogeneity in receptors may significantly contribute to more precise assignments of patient therapy by defining those receptor forms that are associated with therapeutic failures or successes.

In this report, we describe a very rapid method for the qualitative analysis of estrogen- and progesterone-receptor proteins, which utilizes the gel-exclusion principal and a high-performance liquid chromatograph (HPLC). In addition to rapid analysis time, this approach presents a method of improved resolution and high reproducibility.

Experimental Procedures

Materials. Radiolabeled steroids [17β-[2,4,6,7-$^3H_4$]estradiol (111 Ci/mmol) and 17α-[*methyl*-$^3H_3$]R5020 (87 Ci/mmol)] were obtained from New England Nuclear. The following biochemicals were used: estradiol, diethylstilbestrol, progesterone, trypsin (type XI), soybean trypsin inhibitor (type 11S) (Sigma Chemical Co.), and hydroxylapatite (Bio-Gel HTP, Bio-Rad Laboratories). Immature female Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) and immature female CD-1 mice (Charles River, Wilmington, MA) were obtained at days 19–21 of age. Animals used to make estrogen-receptor preparations were sacrificed between 20 and 23 days. Animals used to make progesterone-receptor preparations were primed with subcutaneous injections of estradiol (1 μg (0.2 mL of saline)$^{-1}$ animal^{-1}; 1 injection per day for 2 days; sacrifice on the fourth day).

Preparation of Cytosol and Nuclear Extraction. Animal uteri were dissected free of fat and mesentery; they were then homogenized (2 uterine equiv/mL) in 10 mM Tris[1]–1.5 mM EDTA, pH 7.4 (25 °C) (TE buffer), containing 10 mM sodium molybdate. A high-speed supernatant was prepared by centrifugation at 105000g for 45 min. All manipulations were performed at 0–4 °C. Human tumor tissue was received on ice from Surgical Pathology, stored in liquid nitrogen, manually minced, and homogenized with a Polytron (Brinkmann Instruments) in 10 mM Tris, 1.5 mM EDTA, 10 mM monothioglycerol, and 10% glycerol, pH 7.5 (25 °C), at a concentration of 100 mg of tissue/mL. Human cytosols prepared in this way generally contained 5–10 mg of protein/mL as determined spectrophotometrically (Hartree, 1972). Human cytosol, which was stored at −70 °C with very little loss of binding activity, was used within 30 days. Cytoplasmic receptor was charged with either [^{3}H]estradiol (40 mM) or [^{3}H]R5020 (40 nM) overnight at 0–4 °C. For assessment of nonspecific binding, parallel incubations contained nonradioactive DES or progesterone as competitor (8 μM). The differences between total binding measured in the presence of [^{3}H]steroid alone and unsaturable, nonspecific binding measured in the presence of [^{3}H]steroid and excess competitor were used to determine specific binding (Williams & Gorski, 1973).

Cytoplasmic receptors were translocated to the nucleus by incubating whole rat and mouse uteri in Dulbecco's modified Eagle's medium containing 10 nM [^{3}H]steroid with or without excess unlabeled competitor (2 μM) for 60 min at 37 °C. After homogenization in TE buffer, the crude nuclear-myofibrillar pellet was washed centrifugally 3 times with TE buffer (800g, 10 min), and nuclear receptor was extracted with TE buffer containing 400 mM KCl (1 h, 0–2 °C). A high-speed supernatant was prepared as described above.

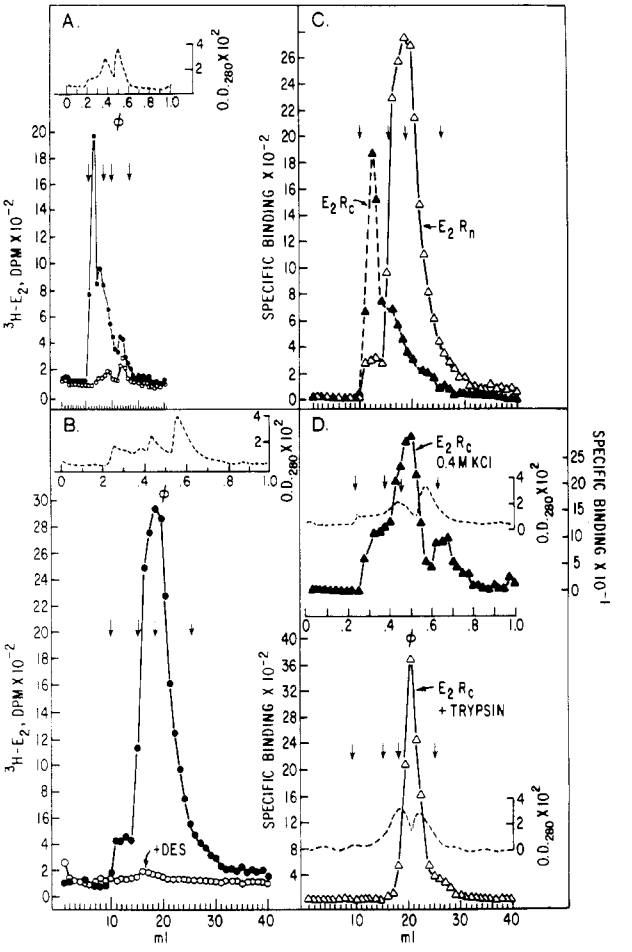

FIGURE 1: Analysis of estrogen-receptor protein forms from rat uterus. High-speed supernatant that has been exposed to [^{3}H]estradiol (10 nM) with (○) or without (●) nonradioactive DES (2 μM) was applied to the gel-permeation column (250 μL) after removing unbound ligand by treatment with dextran-coated charcoal. Cytoplasmic estrogen-receptor elution proceeded in phosphate buffer (200 mM), pH 7.4 (1 mL min^{-1}; V_e = 12 mL) (A). Nuclear estrogen receptors were extracted with 400 mM KCl (1 h, 0–2 °C) and after treatment with dextran-coated charcoal were applied to the column (250 μL). Elution was in PK$_{400}$ buffer, pH 7.4 (0.5 mL min^{-1}; V_e = 18 mL) (B). Specific binding by cytoplasmic (▲) and nuclear (△) estrogen receptors is overlayed (C). Specific binding by cytoplasmic estrogen receptors (▲) deaggregated by bringing the preparation to 400 mM KCl (4 h, 0–2 °C) (0.5 mL min^{-1}; V_e = 19 mL) and (△) treated with trypsin (40 μg/mg of cytosol protein, 1 h, 0–2 °C) [0.5 mL min^{-1}; V_e = 20 mL (△)] (D). Reference absorbance determinations at 280 nm are shown by the dashed line. Arrows designate the V_e for high molecular weight calf thymus DNA (V_0), bovine and human γ-globulins, ovalbumin, and free steroid (V_T) (from left to right). The column partition (ϕ) is here defined to begin at the first fraction, to include the void volume, and to terminate at the total column volume (V_T). Receptor recovered from the column ranged from 60 to 94% of receptor activity estimated by the hydroxylapatite adsorption assay.

[1] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Me_2SO, dimethyl sulfoxide; DES, diethylstilbestrol; TE buffer, 10 mM Tris and 1.5 mM EDTA, pH 7.4.

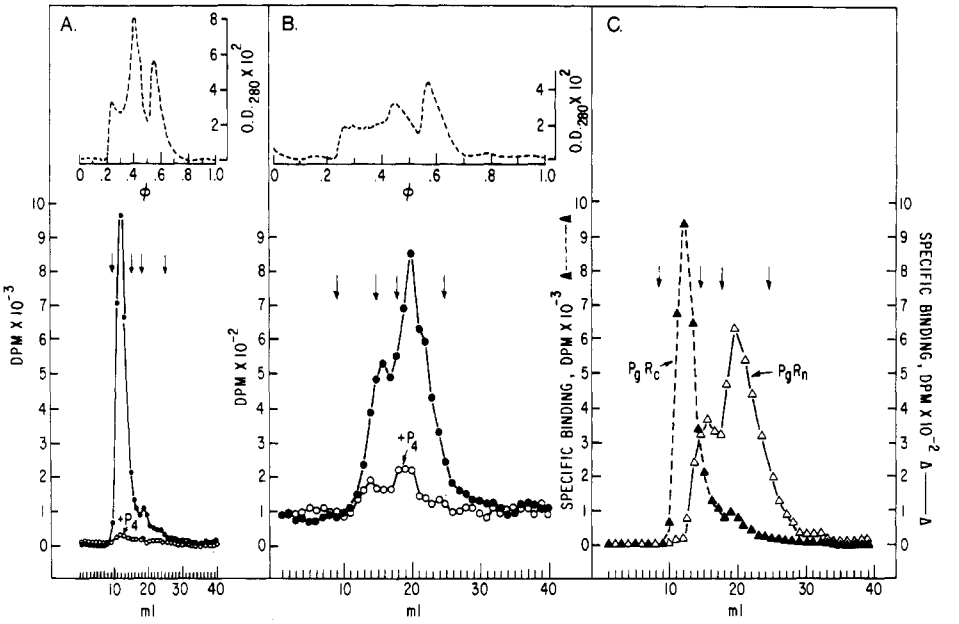

FIGURE 2: Analysis of progesterone-receptor protein forms from rat uterus. High-speed supernatant prepared from estrogen-primed Sprague-Dawley rats was exposed to [^{3}H]R5020 (10 nM) with (○) or without (●) nonradioactive progesterone and was applied to the column (250 μL) after treatment with dextran-coated charcoal. Cytoplasmic progesterone receptor was eluted in phosphate buffer (200 mM), pH 7.4 (1 mL^{-1}; V_e = 12 mL) (A). Nuclear progesterone receptors were extracted with 0.4 M KCl and subjected to dextran-coated charcoal treatment before being injected to the column (250 μL). Elution was in PK_{400} buffer, pH 7.4 (0.5 mL min^{-1}; V_e = 20 mL) (B). Specific binding by cytoplasmic (▲) and nuclear (△) progesterone receptors is overlayed (C). All else is as described in Figure 1. Greater than 90% of the hydroxylapatite-estimated receptor was recovered from the column.

Ligand Binding Determinations. Batch binding determinations were made on soluble steroid receptors in cell preparations by two methods. First, dextran-coated charcoal (5 g of charcoal and 0.5 g of dextran C in 100 mL of buffer; slurry–cytosol, 1:9 v/v) was used to strip unbound ligand as previously described (Katzenellenbogen et al., 1973; Pavlik & Katzenellenbogen, 1980). Activity that remained soluble was regarded as "bound" radioactivity. Second, adsorption to hydroxylapatite was used to immobilize steroid receptors, and radioactivity that was associated with hydroxylapatite after washing was regarded as bound radioactivity (Pavlik & Coulson, 1976). Liquid scintillation counting was performed in a Triton X-100–xylene fluor with automatic correction for quench made on each sample (Pavlik & Rutledge, 1980).

Sucrose Gradient Analysis. Conditions for the separation of steroid receptors on 5–20% sucrose gradients (sucrose in TE buffer) have been described elsewhere (Katzenellenbogen et al., 1981; Pavlik & Rutledge, 1980). Cytoplasmic receptors were resolved by sedimentation at 225000*g* for 13 h.

Rapid Separation Analysis Using High-Performance Liquid Chromatography on an Exclusion Gel. Isocratic elution on a Spherogel-TSK-G3000SW exclusion column (7.5 × 600 mm; Beckman Instruments) fitted with a guard column (Spherogel-TSK precolumn 3000SW; 7.5 × 100 mm) was obtained through buffer delivery with a solvent metering pump (Model 100A; Beckman instruments). Each sample was applied with a syringe-loaded injector (Model 210; Beckman Instruments) fitted with an interchangeable 20- or 250-μL sample loop. Sample elution was monitored with a variable wavelength detector (Varian Instruments; Model UV50) connected to a strip chart recorder, and samples were collected in a Gilson microfractionator. The system was housed and operated at 2–5 °C in a refrigerated chromatography cabinet (Kelvinator). Receptor–column interactions were minimized in phosphate buffer [K_2HPO_4 (200 mM), pH 7.4 (25 °C)]. When indicated, phosphate buffer was made to 400 mM KCl (PK_{400} buffer). All buffer was subject to membrane filtration to remove particulate contaminants. Column exposure to elevated KCl (400 mM) was kept minimal by purging the column immediately after use with distilled water. The column was routinely purged twice weekly to maintain performance. For removal of buildup of organic material, the column was flushed with distilled water, followed by dimethyl sulfoxide (20%) in methanol (both HPLC grade; 2–3 L).

Results

Analysis of Estrogen and Progesterone from Rat and Mouse Uteri. All elutions were performed in phosphate-based buffer, and the samples contained sodium molybdate (10 mM). Cytoplasmic rat estrogen receptors eluted with 200 mM phosphate buffer did not appear in the void volume but in a later fraction on the exclusion gel column (Figure 1A). Extracted nuclear estrogen receptors were eluted in phosphate buffer containing KCl (400 mM) as substantially smaller forms (Figure 1B). For both cytoplasmic and nuclear estrogen receptors, total binding was markedly reduced by excess nonradioactive DES so that specific receptor binding for both cytoplasmic and nuclear estrogen receptors could be expressed (Figure 1C). Hence, the well-defined cytoplasmic estrogen receptor, which sediments at ~8 S on sucrose gradients, and the nuclear estrogen receptor, which sediments at ~5 S, could both be readily resolved on this exclusion gel. In addition, the cytoplasmic receptor that after deaggregation with 400 mM KCl sediments at ~4 S and the small form of the cytoplasmic receptor (~3.6 S) generated by exposure to trypsin (40 μg/uterine equiv, 1 h, 0–2 °C; reaction terminated with soybean trypsin inhibitor, 2.5 μg/μg of trypsin) have been resolved on this column (Figure 1D). [For reference to sedimentation analysis, see Jensen & DeSombre (1972), Carlson et al. (1977), and Katzenellenbogen et al. (1980).] Moreover, the relationship between these forms previously established by sucrose-gradient analysis persists on this column: cytoplasmic estrogen receptor > extracted nuclear estrogen receptor > KCl-deaggregated cytoplasmic estrogen receptor > trypsin-treated cytoplasmic estrogen receptor. A similar analysis applied to cytoplasmic and nuclear progesterone receptors

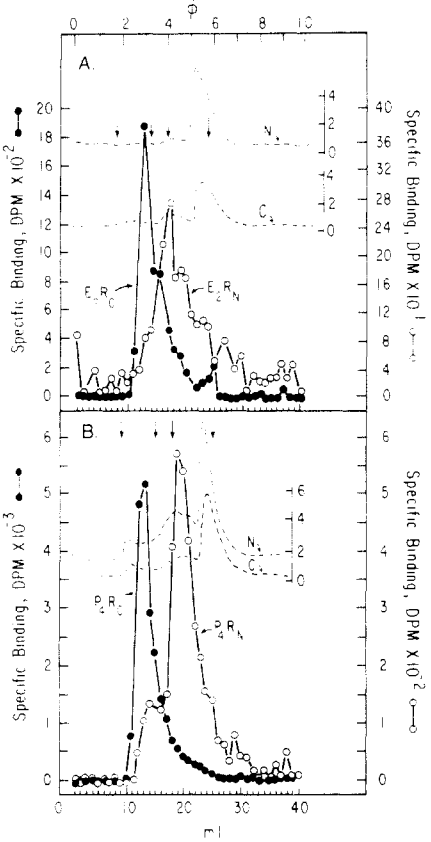

FIGURE 3: Analysis of estrogen- and progesterone-receptor protein forms from CD-1 uterus. Specific binding for cytoplasmic estrogen receptors (●), eluted in phosphate buffer, pH 7.4 (1 mL min^{-1}; V_e = 13 mL), and nuclear estrogen receptors (○), eluted in PK_{400} buffer, pH 7.4 (0.5 mL min^{-1}; V_e = 17 mL), was determined after injecting 20-μL volumes (A). Estrogen-primed CD-1 mice were used to prepare cytoplasmic progesterone receptor. Cytoplasmic receptors (●) were eluted in phosphate buffer (20 μL injected, 0.5 mL min^{-1}; V_e = 12 mL), and nuclear progesterone receptors (○) were eluted in PK_{400} buffer, pH 7.4 (20 μL injected, 0.5 mL min^{-1}; V_e = 19 mL) (B). Absorbances by cytoplasmic (C) and nuclear (N) preparations are indicated by the dashed line in OD_{280} units × 10^2. Receptor recoveries from the column ranged from 70 to 100% of hydroxylapatite-estimated receptor binding.

prepared from rat uteri clearly shows that both cytoplasmic and nuclear forms emerge at different positions in the eluant (Figure 2).

In Figure 3, different forms of estrogen and progesterone receptors prepared from mouse uteri are analyzed. Cytoplasmic and nuclear forms of estrogen (Figure 3A) and progesterone (Figure 3B) receptors are clearly and individually resolved. In addition, it becomes apparent that mouse estrogen receptors partition differently from mouse progesterone receptors on this column.

Analysis of Estrogen and Progesterone Receptors from Human Tumors. Sucrose-gradient analysis was performed on all fresh preparations. HPLC gel-exclusion analysis was made on samples preserved at −70 °C that were rapidly thawed and made to 10 mM sodium molybdate. This procedural process was dictated by the logistics of a formal institutional tumor collection and receptor-analysis protocol, from which the human experimental material has been obtained. Cytoplasmic estrogen and progesterone receptors prepared from a carcinoma of the human uterus were analyzed (Figure 4). Elevated nonspecific binding in the presence of competitor (DES) was obvious in estrogen-receptor preparations (Figure 4A); nevertheless, specific binding was determinable (Figure 4B), and two large forms and one smaller form of receptor

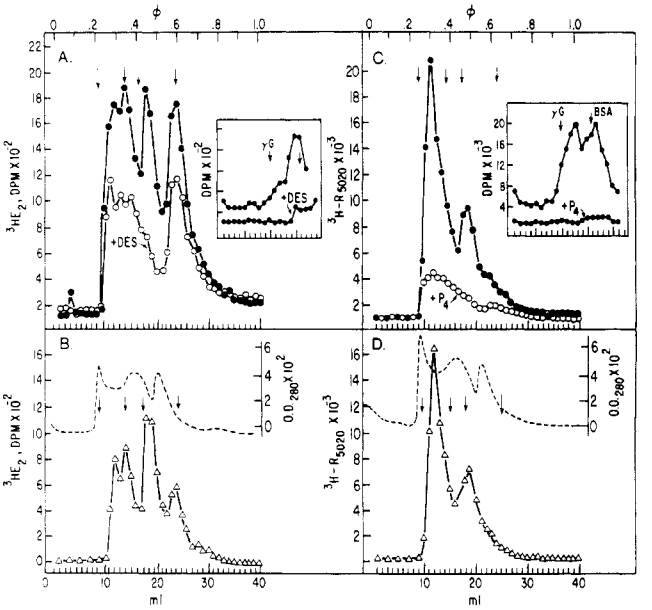

FIGURE 4: Analysis of estrogen- and progesterone-receptor protein forms from a human endometrial adenocarcinoma. High-speed supernatant that had been exposed to [^{3}H]estradiol (40 nM) with (○) or without (●) nonradioactive DES (8 μM) was injected (250 μL) on the column after treating with dextran-coated charcoal. Elution was in phosphate buffer, pH 7.4 (1 mL min^{-1}; V_e = 12, 14, and 18 mL). Coordinate sucrose-gradient analysis is included in the inset (A). Specific estrogen-receptor binding and absorbance profile are shown (B). Preparation that had been exposed to [^{3}H]R5020 (40 nM) with (○) or without (●) nonradioactive progesterone was injected (250 μL) on the column after treating with dextran-coated charcoal. Elution was as above (V_e = 12 mL and 19 mL) (C). Specific binding and absorbance of the preparation are shown (D).

were resolved in the same analysis. Nonspecific binding was more moderate in progesterone-receptor preparations (Figure 4C), allowing for the clear determination of specific binding and the resolution of large and small progesterone receptors in the same analysis (Figure 4D). Quantitative agreement with coordinate sucrose-gradient analysis for both estrogen and progesterone receptors was good (unpublished results). However, HPLC gel-exclusion analysis was clearly superior in identifying large forms of the receptors for two probable reasons: (1) endogenous proteases may convert the receptors to smaller forms during the protracted (13 h) sedimentation analysis (Miller et al., 1981; Sherman et al., 1980) while rapid separation by HPLC minimizes the opportunity for these conversions, and (2) preparations analyzed by HPLC gel exclusion received some stabilization from sodium molybdate included in the buffers.

In similar analyses performed on cytosol prepared from human breast carcinoma, the estrogen-receptor preparations were again characterized by elevated nonspecific binding (Figure 5A) and by the presence of two larger forms and one smaller form of estrogen receptors (Figure 5B). Nonspecific binding in the progesterone-receptor preparation was again moderate, and large and small receptor forms were resolved in the same analysis (Figure 5C,D). Because serum-binding components appeared slightly larger than the small steroid-receptor forms and were clearly distinct from the large forms of steroid receptors (Figure 6) and because serum binding was not reduced by competition (data not shown), it is likely that the smaller receptor forms identified in these analyses were genuine receptor forms and not the result of serum-binding components that contaminated the preparation.

Performance Analysis of HPLC Gel-Exclusion Separations of Steroid Receptors. The use of phosphate buffer (200 mM),

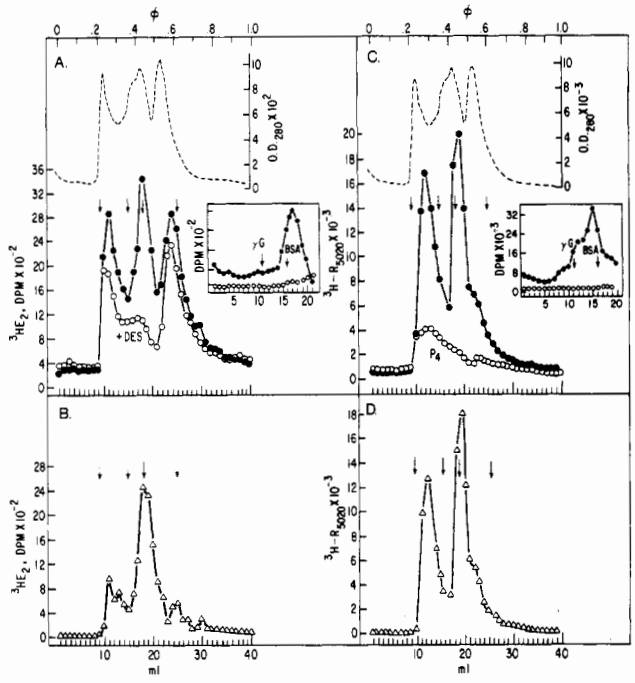

FIGURE 5: Analysis of estrogen- and progesterone-receptor protein forms from an adenocarcinoma of the human breast. The preparation was prepared and analyzed as described in Figure 4. Estrogen receptor: binding analysis (A) and specific binding (B) (V_e = 11, 13, and 18 mL). Progesterone receptor: binding analysis (C) and specific binding (D) (V_e = 12 and 19 mL).

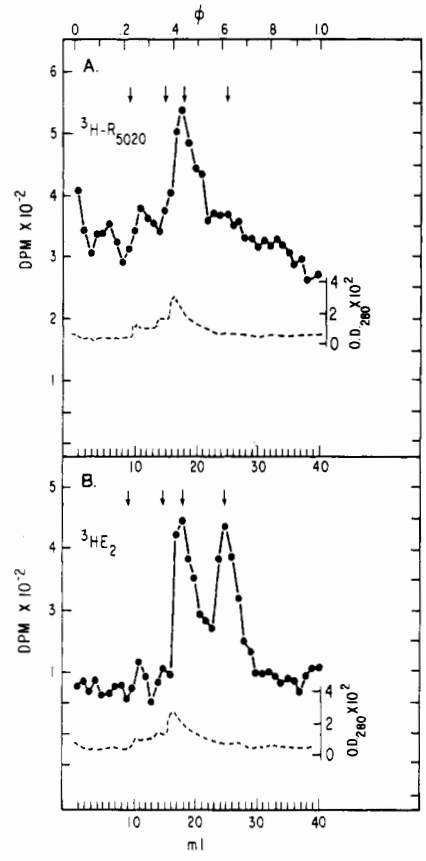

FIGURE 6: Analysis of binding components in human serum. Human serum, diluted in TE buffer (1:10), was exposed overnight to [^{3}H]-R5020 (40 nM) or [^{3}H]estradiol (40 nM) and treated with dextran-coated charcoal. The preparations were injected (20 μL) and eluted at 1 mL min^{-1}. Human serum binding by [^{3}H]R5020 (A) and by [^{3}H]estradiol (B).

pH 7.4, was observed to be very important for minimizing receptor–column interactions and for achieving discrete receptor profiles. No differences in resolution were observed in analyses performed with 20- or 250-μL sample injection loops; however, the increased receptor activity delivered to the column with the 250-μL loop clearly presented an advantage in terms of sufficiency. Samples up to 20 mg of protein/mL were run with no effect upon receptor resolution or partition. It was generally advantageous to limit sample concentration to 1–5 mg of protein/mL because the column required cleaning with 20% Me_2SO in methanol less frequently. Rises in column back-pressure in excess of 30% of purged column base pressure were interpreted as indications that columns needed to be cleaned. Estimates of receptor recovery were always greater than 50% and many times greater than 90%. Surprisingly, receptor recovery did not vary as a function of injected protein load, within the ranges of protein concentration used; however, poorer receptor recoveries appeared to be associated with analyses performed on freshly cleaned and purged columns, as well as on columns clearly in need of cleaning as judged by doubling of column back-pressure. Column condition had no discernible influence on receptor partition analysis or resolution of different receptor forms. The speed at which receptor separation analysis could be performed was analyzed (Figure 7). Preparations run at up to 2 mL min^{-1} showed receptor resolution and partition that appeared identical with those of slower column elutions. At this rate of elution, all forms of human estrogen and progesterone receptors will have passed the column in ~10 min, and a return to base line column activity can be achieved in ~25 min. Lastly, preparations containing both bound and free steroid can be injected; however, an interaction between the column and unbound [^{3}H]estradiol and [^{3}H]R5020 causes the trailing edge of the free steroid peak to return to base line more slowly than ex-

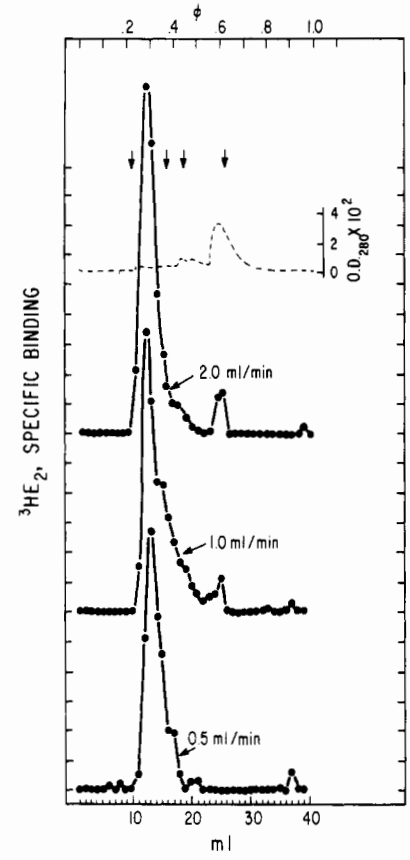

FIGURE 7: Flow rate and resolution of estrogen receptors. Cytosol was prepared from CD-1 mice and treated with dextran-coated charcoal before injection (20 μL). Each division on the ordinate is 200 dpm.

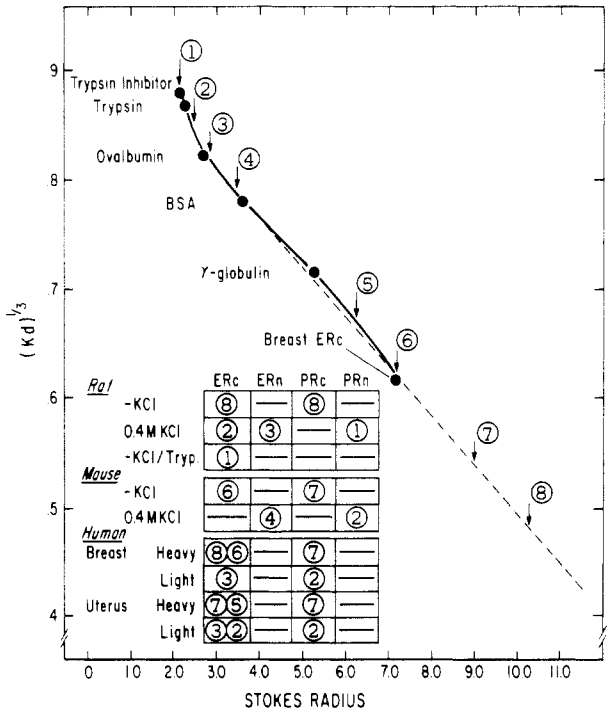

FIGURE 8: Referenced elution behavior of estrogen- and progesterone-receptor protein forms. The partition coefficients (K_d) were determined as defined by Ackers (1975); $K_d = (V_e - V_0)/V_T - V_0)$ on at least three replicate determinations. Estimates of Stokes' radii were referenced for soybean trypsin inhibitor, ovalbumin, bovine serum albumin (BSA) (Carlson et al., 1977), γ-globulin (Glick, 1970), human breast estrogen receptor, molybdate stabilized (Miller et al., 1981), and trypsin, calculated from f/f_0 (Walsh, 1970) and molecular weight (Glick, 1970) according to the relationship $f/f_0 = a/[3\bar{v}M)/(4\pi N)]^{1/3}$; $\bar{v} = 0.725$ cm^2 g^{-1} (Siegel & Monty, 1966). The dashed line is the best fit estimate determined by linear-regression analysis from points including ovalbumin, BSA, γ-globulin, and human breast estrogen receptor. Units for Stokes' radii are nanometers. Each V_e was determined on the basis of peak-height elution. The inset table matches the different receptor forms with the corresponding position on the line relating partition to Stokes' radius.

pected. As a consequence, when significant free steroid is injected, more buffer must be pumped through the column in order to restore the low cpm base line.

Resolution of Different Receptor Forms by HPLC Gel-Exclusion Separation Analysis. Through peak-height analysis involving replicate rodent receptor preparations and ten different human receptor preparations, we have observed the reproducibility of this separation analysis (data not shown). Receptor partition on the column remained unaltered even when preparations were diluted. Multiple analyses of the same sample resulted in identical peak-height partitions. Thus, the separation analysis performed by HPLC gel exclusion can be precisely referenced to very stable elution profiles for a variety of receptor proteins. This reference characterization for the exclusion gel column (TSK-3000SW) used in these studies is shown in Figure 8. With this column as a reference background, it is apparent that certain forms of estrogen and progesterone receptors from the same species demonstrate distinctly different partition behavior on this column and that certain species-related differences appear when "homologous" receptors are examined. Finally, because these differences can be referenced in Figure 8 to a hydrodynamic molecular property (Stokes' radius), the potential of this methodology for documenting physicochemical differences in receptors is very apparent.

Discussion

In these studies we show that qualitative steroid-receptor analysis can be performed by a method that is much more rapid than sucrose-gradient sedimentation analysis. Moreover, the qualitative relationships defined by sucrose-gradient analysis between different forms of steroid receptors persist on this rapid gel-exclusion system. In addition, the reproducibility of the separations performed by the gel-exclusion column indicates that this methodology has a powerful potential as a reference background upon which steroid-receptor proteins can be characterized.

This report is significant because of its contribution to rapid steroid-receptor analysis: separations that require 13 h of centrifugation can be obtained in 10 min. Because cytosols can contain endogenous proteases that modify receptors (Schneider & Dao, 1977; Notides, 1978; Tilzer et al., 1981; Miller et al., 1981), rapid separations can more accurately define native-receptor forms. Since protease-treated receptors and "processed" receptors appear to bind ligand less well, rapid separation times can minimize the ligand dissociation that can occur during prolonged sedimentation analysis (Weichman & Notides, 1977; Pavlik & Rutledge, 1980). Moreover, rapid separation analysis can facilitate the characterization of steroid-receptor interactions with low-affinity ligands that appear to elicit physiological or pharmacological responses. Thus, interactions, which are too short-lived to be detected by sedimentation analysis, may be defined by HPLC gel-exclusion analysis. Finally, because of the rapid separation times, receptor peak broadening due to ligand dissociation is minimized, and receptors become more narrowly resolved.

Estrogen- and progesterone-receptor assays are used clinically to identify those breast and endometrial cancer patients who might benefit from endocrine therapy (Osborne et al., 1980; Mortel et al., 1981). While some investigators have suggested that a category of estrogen receptors exists that can only be identified by sucrose-gradient sedimentation analysis (Savlov et al., 1977), the majority of determinations are conducted with the dextran-coated charcoal assay, so that sucrose gradients are used in only ~26% of clinical determinations (Segaloff, 1980). The protracted sedimentation time (13 h) and limited number of rotor tubes (six) generally cannot be tolerated by cancer centers with large receptor-assay requirements. When assay requirements are modest (i.e., 1–2 assays/day), the application of sucrose-gradient technology is much slower than the dextran-coated charcoal assay (Smith, 1980). HPLC gel-exclusion separation analysis provides a very rapid alternative to sedimentation analysis and should allow for up to 16 samples to be analyzed in a work day. Moreover, isocratic systems are economical and could be dependably used in a laboratory where the number of assays is limited. Finally, the capacity of this gel-exclusion column for resolving different receptor forms may be of significant use for defining differences in receptors from patients who do not respond to endocrine therapy.

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Effects of Thyroxine Binding on the Stability, Conformation, and Fluorescence Properties of Thyroxine-Binding Globulin†

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ABSTRACT: The effects of thyroxine (T_4) on several molecular properties of human thyroxine-binding globulin (TBG) have been evaluated. Changes in the sedimentation constant and relaxation time indicate that TBG becomes more symmetric and compact when T_4 is bound. This modification in structure is associated with an increase in the stability of TBG to denaturation by either acid or guanidinium chloride. T_4 binding also produces changes in the emission and excitation spectra of TBG, reflecting different environments of the four tryptophanyl residues. T_4 preferentially quenches residues in a less polar environment. In addition, it alters the effect of the collisional quencher, acrylamide, so as to indicate a shift in the environment of some of the exposed tryptophanyl residues.

Thyroxine-binding globulin (TBG)[1] is the plasma protein found in higher vertebrates which has the greatest affinity for thyroid hormones and transports the majority of T_4 and T_3 in the circulation (Robbins et al., 1978). Human TBG is a single polypeptide chain (M_r 54 000) (Gershengorn et al., 1977a) with about 20% of its weight in carbohydrates which are organized in four oligosaccharide chains (Zinn et al., 1978a,b). One mole of hormone is bound per mole of protein. Recent studies have shown that TBG loses its ability to bind hormones in dilute acid (pH <5) and in dilute guanidinium chloride (<2 M) solutions (Gershengorn et al., 1977b). In the present work, we have shown that T_4 enhances the stability

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[1] Abbreviations used: TBG, thyroxine-binding globulin; TBG·T_4, thyroxine-binding globulin complexed with T_4; GdmCl, guanidinium chloride; Dns, dansyl (5-dimethylamino-1-naphthalenesulfonyl); T_4, L-thyroxine; T_3, L-3,5,3′-triiodothyronine.