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Seasonal Changes in the Molecular Species and Nuclear Binding of the Chick Oviduct Progesterone Receptor†

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ABSTRACT: A seasonal variation in the capacity of the progesterone–receptor complex to bind to isolated chromatin and partially purified acceptor protein bound to deoxyribonucleic acid (DNA) from the developed oviducts of estrogen-treated immature chicks has been described. Receptors isolated during the late winter fail to bind to these sites, whereas receptors isolated during the rest of the year show a marked binding. The level of binding to DNA remains unchanged throughout the year. A similar seasonal variation in the nuclear translocation in vivo of [^{3}H]progesterone is demonstrated. Further, the effect of progesterone on the endogenous ribonucleic acid polymerase II (B) activity in vivo also displays a seasonal variation. The variations in the in vivo binding and transcriptional effects show a similar periodicity and timing as the in vitro binding. The level of one of the two molecular species of the progesterone–receptor complex is markedly reduced during the winter, thus explaining the overall decrease in receptor amounts during this same period. The receptor preparations isolated during the late winter–early spring show greatly reduced amounts of the "A" species as compared to those isolated during the other periods of the year. The ability of a particular receptor preparation to bind to the chromatin in vivo and in vitro or to the nuclear protein acceptor sites in vitro correlates with the level of the "A" receptor species. These data support the role of certain nonhistone protein–DNA complexes as acceptor sites for the progesterone receptor in the chick oviduct and that either the "A" receptor species or a combination of the "A" and "B" receptor species is required for nuclear binding of the progesterone–receptor complex.

This laboratory has been investigating the interaction of steroid–receptor complexes with chromatin and its components. The binding of steroid–receptor complexes to target cell nuclei is required for the steroid-induced alteration of gene expression (Jensen & DeSombre, 1972). Various methods have been studied for analyzing nuclear binding of progesterone in the chick oviduct in order to chemically identify the nuclear acceptor site which binds the progesterone receptor. Details of these studies have been published in a recent review (Thrall et al., 1978). During these investigations, seasonal variations in oviduct weights, cytosol receptor levels, and the capacity of the receptor to bind to nuclear acceptor sites were observed (Spelsberg et al., 1979a,b; T. C. Spelsberg and F. Halberg, unpublished experiments). A circannual rhythm in the binding of [^{3}H]P–R[1] to nuclear acceptor sites was observed by using receptor preparations isolated throughout the year. The binding of [^{3}H]P–R to pure DNA was essentially constant throughout the year (Spelsberg et al., 1979a,b; T. C. Spelsberg and F. Halberg, unpublished experiments). The period of the isolation of the receptor, and not the nuclear preparation, was found to be the cause of this rhythm. Thus, the variability is a function of the receptor. Maximal [^{3}H]P–R binding to the nuclear acceptor sites was observed with receptor preparations isolated in the summer and fall, while minimal binding of [^{3}H]P–R was observed with receptor preparations isolated in the winter and early spring. The seasonal variation in the receptor binding to the NAP, but not to the pure DNA, was verified as a circannual rhythm by using computer-determined cosine-fitted curves (T. C. Spelsberg and F. Halberg, unpublished experiments). Although circannual rhythms were confirmed, the cycles did not repeat on an exact year basis (Spelsberg et al., 1979a,b; T. C. Spelsberg and F. Halberg, unpublished experiments).

Interestingly, as the [^{3}H]P–R lost its capacity to bind the acceptor sites, the level of receptor in the cytosol decreased ~50% (Spelsberg et al., 1979a,b; T. C. Spelsberg and F. Halberg, unpublished experiments). This report further describes the biological relevance of these rhythms and the cause of the overall decrease in receptor levels. First, evidence is presented that the seasonal variations in nuclear binding of the progesterone–receptor complex occur in vivo. Second, this loss of nuclear translocation of [^{3}H]P in vivo during the winter is accompanied by a marked decrease in the effect of the hormone treatment on RNA polymerase II activity. Finally, a correlation is shown between the capacity of [^{3}H]P–R to bind nuclear acceptor sites and its effect on transcription and the

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[1] Abbreviations used: [^{3}H]P, [^{3}H]progesterone; [^{3}H]P–R, [^{3}H]-progesterone–receptor; IEF, isoelectric focusing; Tris, tris(hydroxymethyl)aminomethane; EDTA, disodium ethylenediaminetetraacetate; NAP, nucleoacidic protein.

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level of one of the two molecular species of the progesterone receptor quantitated by isoelectric focusing (Boyd & Spelsberg, 1979).

Materials and Methods

Animals. Immature chicks (Shaver pullets) were obtained from the Silver Lake Hatcheries (Silver Lake, MN), where they were maintained in incubators for a few days at 27 °C after hatching. Open-air boxes containing 100 (2-day-old) chicks were shipped to the Mayo Clinic via the U.S. Postal Service (~3-h trip). Upon arrival, the animals were placed immediately in cages (50 chicks/cage) for 5 weeks in a room containing no other animals. The temperature of the room was maintained at 22 ± 1 °C with a relative humidity of 61 ± 1%. The room received 16 h of light and 8 h of darkness throughout the year. At 7–8 days of age, the chicks were started on a 4-week regimen of daily injections (sc) with 5 mg of diethylstilbestrol (DES) in sesame oil 5 times/week (Monday–Friday). The chicks were sacrificed on a Friday of the fourth week of estrogen treatment, and the fully developed oviducts were removed for isolation of the progesterone receptor as described previously (Schrader & O'Malley, 1972; Pikler et al., 1976).

Isolation of Nuclei and Chromatin. Shortly after the chicks or hens were sacrificed, the oviducts were excised, cleaned, and frozen in dry ice. When needed, the tissue was homogenized while still frozen in a Waring blender in buffered sucrose solutions and the nuclei and chromatin were purified as described previously (Pikler et al., 1976; Spelsberg et al., 1972, 1977). All steps were performed at 0–4 °C. Briefly, the nuclei were sedimented in a buffered 0.5 M sucrose solution (0.05 M Tris, 0.025 M KCl, 0.002 M $MgCl_2$, pH 7.5) at 40000*g* for 10 min, resuspended in a buffered 2.0 M sucrose solution (0.05 M Tris, 0.025 M KCl, 0.002 M $MgCl_2$, pH 7.5) by using a Teflon pestle-glass homogenizer, and resedimented at 60000*g* for 1 h. The nuclei were resuspended in 0.5 M sucrose solution containing 0.2% Triton X-100, passed through 100-mesh organza cloth, and resedimented at 12000 rpm for 20 min. These nuclei were used to isolate chromatin via suspension in solutions containing (1) 0.08 M NaCl and 0.02 M EDTA, pH 6.3, (2) 0.3 M NaCl, and (3) 2 mM Tris-HCl and 0.1 mM EDTA, pH 7.5, as described elsewhere (Spelsberg & Hnilica, 1971; Spelsberg et al., 1971).

Preparation of the Cellulose Resins. The nuclear binding studies were conducted by using the cellulose method which is described in detail elsewhere (Spelsberg et al., 1975, 1978; Webster et al., 1976). Chromatin was linked to cellulose in ethanol with high-intensity ultraviolet light (Litman, 1968; Spelsberg et al., 1975, 1978; Webster et al., 1976). The chromosomal proteins were then dissociated selectively with a gradient of guanidine hydrochloride according to their affinity for the DNA. Extraction of the chromatin–cellulose by 4 M Gdn·HCl yields residual protein–DNA, called nucleoacidic protein (NAP), bound to the cellulose (i.e., NAP–cellulose). DNA–cellulose was prepared from the chromatin–cellulose by extracting the latter twice for 30 min at room temperature either with a 0.2% $NaDodSO_4$ solution or with a 7 M Gdn·HCl (pH 6.0) solution to remove most of the chromosomal proteins and RNA. After each extraction the suspension was centrifuged at 1000 rpm for 5 min and the supernatant decanted. The pellet was washed 5 times with dilute buffer (0.01 M NaCl + 0.01 M Tris, pH 7.5) and the DNA–cellulose resin lyophilized to dryness. Some cross-linking of protein to DNA probably occurs but it does not appear to be significant, nor does it interfere with the binding of the [^{3}H]P–R. Greater than 70% of the total chromatin protein is recovered from the chromatin–cellulose resin even after dialysis and lyophilization (Spelsberg et al., 1978). Further, only a few percent of the protein can be detected by using the Lowry analysis (Lowry et al., 1951) of alkaline hydrolysates of the "DNA"–cellulose. More importantly, the binding of the [^{3}H]P–R to the "DNA"–cellulose is almost identical with the binding to purified DNA [containing less than 1% (w/w) protein] by using the streptomycin assay with respect to capacity and with a lack of any seasonal variation in receptor binding (Spelsberg et al., 1979a,b). All cellulose preparations were stored dry at room temperature (22 °C). When needed, the resins were hydrated in dilute Tris buffer at 4 °C for 4–6 h with gentle mixing.

In Vitro Binding Assay. The binding of the [^{3}H]P–R to the chromatin–, NAP–, or DNA–cellulose in vitro has been described (Spelsberg et al., 1975, 1976b; Webster et al., 1976). Briefly, the binding reactions were initiated by the addition of 400 μL of the [^{3}H]P–R preparations to the chromatin–cellulose, NAP–cellulose or DNA–cellulose using 25 μg of DNA/assay mixture. The assays contained 0.18 M KCl buffered at pH 7.5 in the 1.0-mL final volume. In all experiments, blanks containing pure cellulose were included to serve as background radioactivities and were subtracted from the assays containing the chromatin–, NAP–, or DNA–cellulose. The binding assays were terminated after 90 min of incubation at 4 °C, which was previously shown to saturate the nuclear acceptor sites. The resins were then collected on membrane filters (0.45-μM pore size, 24-mm diameter; Millipore Corp., Bedford, MA) under vacuum and washed several more times with cold dilute Tris buffer. The filters were dried, and the radioactivity was measured by scintillation spectrometry using 5.0 mL of a toluene-based scintillation solution per vial at 40% counting efficiency. The fluor contained 6.0 g of PPO (2,5-diphenyloxazole) and 0.075 of POPOP [1,4-bis[2-(5-phenyloxazolyl)benzene]] per L. After counting, the filters were dried and the DNA per filter was quantitated as described previously (Spelsberg et al., 1971). Briefly, the DNA was hydrolyzed by incubating the filters at 90 °C for 30 min in 0.3 M $HClO_4$. The DNA in the hydrolysate was assessed by the diphenylamine reaction (Burton, 1956). Standards for the diphenylamine assay consisted of spot drying 20 μg of DNA on several filters followed by hydrolysis. The radioactivity per filter was correlated with the amount of DNA on the same filter to calculate the counts per minute per milligram of DNA.

In Vivo Binding of [^{3}H]Progesterone to Oviduct Nuclear Chromatin. DES-treated chicks were injected with 200 μCi of [^{3}H]progesterone ([^{3}H]P) in 50 μL of ethanol–H_2O (1:1) in the wing vein. Evans Blue dye was included as a marker for the accuracy of the injection. One-half hour after injection, the birds were sacrificed and the oviducts quickly excised. The nuclei were then immediately isolated as described elsewhere (Spelsberg, 1976). The DNA was then quantitated and the radioactivity in the nuclei measured by using a liquid scintillation spectrometer. The counts per minute per milligram of DNA was then calculated for each group. The nuclear bindings show a mean of 3 groups (3 birds/group).

Progesterone Effects on RNA Polymerase Activity in Vivo. The preparation of the progesterone for injections into chicks is described elsewhere (Spelsberg, 1976). Progesterone was administered subcutaneously in 200 μL of sesame oil. Groups of 6–10 chicks were injected subcutaneously with the hormone. Certain groups were injected with vehicle only and used for determining the controls at each time interval. The oviducts from each group were excised and combined, the nuclei were

isolated, and the activity was assayed as described elsewhere (Glasser et al., 1972; Knowler et al., 1973). This polymerase assay was previously shown to represent DNA-dependent RNA synthesis, which requires all four nucleotides and divalent cations and synthesizes DNA-like RNA with a linear incorporation of [^{3}H]UMP during the assay which is 90% inhibited with α-amanitin (Glasser et al., 1972; Spelsberg, 1976; Spelsberg & Cox, 1976). The counts per minute per milligram of DNA and the percent of control (vehicle only) were then calculated. The data are plotted as the mean and standard deviation of four replicate analyses for each time period.

Isoelectric Focusing of [^{3}H]P–R. The isoelectric focusing method using flat beds of superfine Sephadex G-75 is described in detail in the preceding paper (Boyd & Spelsberg, 1979). Briefly, [^{3}H]P–R samples were focused for 8 h at 8 W/plate at 0–4 °C in flat beds of superfine prewashed Sephadex G-75 containing LKB (pH 3.5–10) ampholine solution and 10% glycerol. Following focusing, the pH gradient of the resin was measured, the gel was fractionated into 30 sections, and each section was eluted with 9 mL of TEGSH buffer (0.10 M Tris-HCl, 0.01 M EDTA, 10% v/v glycerol, 0.12 M thioglycerol, pH 7.4) by using small columns. An aliquot of each fraction was assayed for specifically bound [^{3}H]P by using a modified dextran-coated charcoal assay. The two species of receptor were then quantitated.

Results

This laboratory has previously described the binding of progesterone–receptor complexes to nuclear acceptor sites of the chick oviduct in vitro and in vivo (Spelsberg et al., 1976b, 1977; Webster et al., 1976). A large percentage (~80%) of these acceptor sites were found to be masked in intact chromatin isolated from the chick oviduct (Spelsberg et al., 1971, 1976a,b; Webster et al., 1976). The removal of the histones and some nonhistone proteins from chromatin yields a residual protein–DNA complex called nucleoacidic protein (NAP) which is capable of binding 5 times the amount of [^{3}H]P–R as whole chromatin (Spelsberg et al., 1976b,c, 1977). These formerly masked sites display a requirement for intact activated receptor for binding the steroid as do the unmasked sites in whole chromatin (Webster et al., 1976; Spelsberg et al., 1976b, 1977; T. C. Spelsberg and F. Halberg, unpublished).

During studies of the [^{3}H]P–R binding to the NAP, a seasonal variation in the capacity of the steroid–receptor complexes to bind to the NAP was observed. Figure 1 illustrates the binding of two preparations of [^{3}H]P–R isolated at different times of the year to NAP–cellulose. The values for DNA binding were subtracted from the values obtained for the NAP–cellulose to analyze only the binding to acceptor sites involving the nucleoprotein. The patterns of [^{3}H]P–R binding to DNA have been published previously (Webster et al., 1976; Spelsberg et al., 1976a–c). The receptor preparation isolated in the summer demonstrated a high level of binding to the NAP which was saturated at 400 μL of [^{3}H]P–R. However, little binding to the NAP was observed by the preparation of [^{3}H]P–R isolated in the winter.

To assess the biological relevance of these variations, we performed an analysis of the nuclear transport of [^{3}H]P in vivo. Figure 2A shows the level of nuclear binding of [^{3}H]P in the oviduct within 0.5 h after an injection (iv) of 200 μCi of the hormone. A marked seasonal variation in the level of binding was observed. The nuclear levels of the steroid reached maximal values during the summer and fall, whereas very low levels were observed in the late winter.

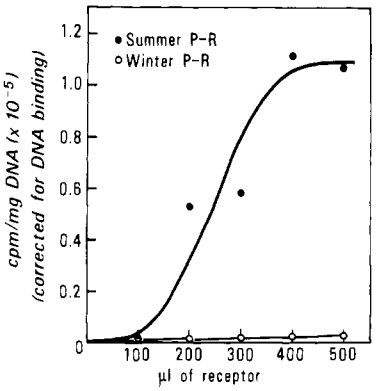

FIGURE 1: Binding of two preparations of [^{3}H]progesterone–receptor complexes to NAP–cellulose. NAP–cellulose was prepared as described under Materials and Methods. Various amounts of partially purified [^{3}H]P–R, isolated in either the summer (August, ●) or the winter (March, ○), were assayed for nuclear acceptor binding by using the cellulose method as described under Materials and Methods. The nuclear material was collected on Millipore filters and dried. The filters were counted, the DNA per filter was quantitated, and the counts per minute per milligram of DNA was calculated. The values obtained for DNA binding were subtracted from those for NAP binding to analyze the degree of binding of [^{3}H]P–R to nucleoprotein. The mean of three replicates for each level of receptor is shown.

Using saturating levels of [^{3}H]P–R (400 μL) isolated at various periods throughout the year, we analyzed the levels of binding of [^{3}H]P–R to the nuclear sites on chromatin, NAP, and DNA in vitro for comparative purposes. Parts B and C of Figure 2 show the seasonal variations in the capacities of [^{3}H]P–R preparations to bind to the chromatin and NAP, respectively. The data are plotted with respect to the dates on which the [^{3}H]P–R samples were isolated. The binding of [^{3}H]P–R reached maximal levels in the summer and fall periods and decreased markedly during the winter–spring months (February–April) for both the chromatin and NAP. As shown in Figure 2D, the binding of [^{3}H]P–R to DNA displayed no seasonal pattern of binding.

Therefore, the seasonal variations in the nuclear binding of [^{3}H]P are observed both in vivo and in vitro. The in vitro patterns are based on the timing of the isolation of the [^{3}H]P–R and not the isolation of the nuclear material. These results strongly support the validity of the in vitro binding assays and indicate that the DNA–protein complexes (i.e., the NAP), and not DNA alone, represent the native nuclear acceptor sites for this steroid receptor. It should be stated that the levels and seasonal variations in binding to NAP using the cellulose binding method are similar to those observed when using the streptomycin method (Webster et al., 1976; Spelsberg et al., 1979a,b). Further, the levels of binding and a lack of any seasonal variation in binding to DNA were comparable by using both of these methods. Therefore, these patterns of binding are probably not due to the method of preparing the cellulose resins or to the method of binding.

To verify the loss of nuclear translocation in the late winter as a true biological event, the effect of progesterone on DNA-dependent RNA synthesis was examined. Figure 3 shows the effect of an injection of 1 mg of progesterone into DES-treated chicks on the RNA polymerase II (or B) activity in the developed oviducts. This enzyme is the nucleoplasmic enzyme which synthesizes the DNA-like RNA. In the fall, the steroid greatly reduced the activity of this RNA polymerase as described previously (Spelsberg & Cox, 1976). However, in the late winter–early spring, the effect of the hormone was greatly diminished. Thus, when the in vivo nuclear translocation of [^{3}H]P decreased in the late winter–

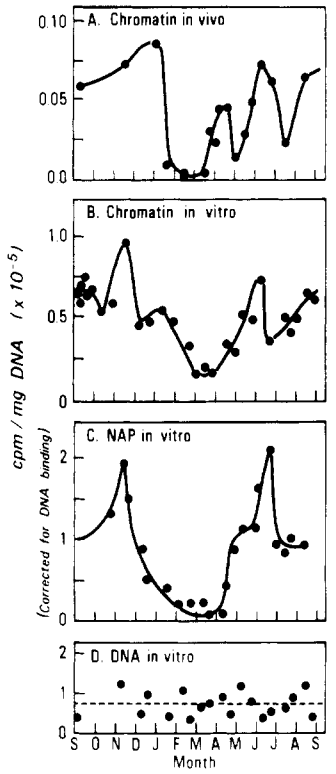

FIGURE 2: Seasonal variations in the capacity of [^{3}H]progesterone-receptor complex to bind to nuclear acceptor sites in vivo and in vitro. Part A represents the in vivo binding of [^{3}H]P to nuclear chromatin in fully developed oviducts of immature chicks conducted at various periods of the year. DES-treated chicks were injected with 200 μCi of [^{3}H]progesterone ([^{3}H]P) in 50 μL of ethanol–H_2O (1:1) in the wing vein. Evans Blue dye was included as a marker for the accuracy of the injection. One-half hour after injection, the birds were sacrificed and the oviducts quickly excised. The nuclear chromatin was immediately isolated and quantitated for DNA and assayed as described uner Materials and Methods. Part B represents the [^{3}H]P–R binding to chromatin–cellulose. [^{3}H]Progesterone–receptor complexes isolated at various periods of the year were assayed within 1 week in June by the cellulose method. Chromatin binding assays in vitro were conducted as described elsewhere (Spelsberg et al., 1976a–c; Pikler et al., 1976). The receptor preparations were isolated at specific times during the year, stored at −80 °C as ammonium sulfate precipitates, and resuspended in TEGSH buffer on the day of the binding assay. Saturating levels of [^{3}H]P–R (400 μL/assay) were used. Each point represents the mean of four replicates of the binding of [^{3}H]P–R to the chromatin. Part C represents the in vitro binding to NAP–cellulose by the [^{3}H]progesterone–receptor complex isolated at various periods of the year. The receptor preparations and the nuclear binding assays were conducted by using the cellulose method as described above. The NAP–cellulose was prepared as described under Materials and Methods. Saturating levels of [^{3}H]P–R (400 μL/assay) were used in the binding assays. Each point represents the mean of four replicates of the binding of [^{3}H]P–R to NAP–cellulose corrected for DNA binding as described under Materials and Methods and the legend to Figure 1. Part D represents the binding to DNA–cellulose by the [^{3}H]P–R complex (400 μL/assay) isolated at various periods of the year. The receptor preparations and the nuclear binding assays were conducted by using the cellulose method as described above. The DNA–cellulose was prepared as described under Materials and Methods. The mean of four replicates of the binding is plotted at each date. The data are plotted as [^{3}H]P–R bound to the nuclear material vs. the date on which each particular [^{3}H]P–R was isolated.

early spring, the hormone's effect on RNA polymerase also decreased and these results correlate with the patterns of the in vitro nuclear bindings.

Previous reports from our laboratory have demonstrated that the amount of progesterone receptor in the cytosol also displays a circannual rhythm (Spelsberg et al., 1979a,b; T. C. Spelsberg and F. Halberg, unpublished experiments). Further, it appeared that the loss in nuclear binding was due to the receptor preparations since it appeared to be based on the time of isolation of the receptor. Since the receptor was varying in amount and function (i.e., binding to the nuclear acceptor), we initiated studies to identify any changes which might occur in the receptor during this period. The sedimentation rates of the [^{3}H]P–R in sucrose gradients, its elution from molecular sieve columns, and the binding affinity between the steroid and the receptor from both the summer and winter periods were found to be indistinguishable. However, when various receptor preparations were analyzed by isoelectric focusing to quantitate the "A" and "B" receptor species, marked differences were detected between the winter and summer periods.

Figure 4 shows the IEF pattern of summer and winter preparations of [^{3}H]P–R. The preparation isolated during the summer contained equivalent amounts of the two peaks of

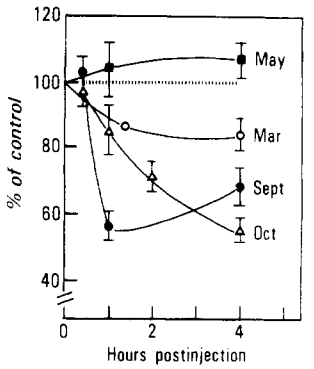

FIGURE 3: Response of the RNA polymerase II (or B) activity in vivo to injected progesterone at various periods of the year. Immature chicks were injected with DES for 4 weeks to obtain fully developed oviducts. Unlabeled progesterone (1 mg) was injected into these chicks. Control animals received vehicle (sesame oil) only. At selected times after injection, the birds were sacrificed, the oviducts removed, and the nuclei immediately isolated and assayed for RNA polymerase II activity as described under Materials and Methods. The values for the polymerase activities are calculated as the percent of control values (vehicle only). The mean and standard deviation of four replicate polymerase analyses are presented. These experiments were performed in May (■), March (○), September (●), and October (△).

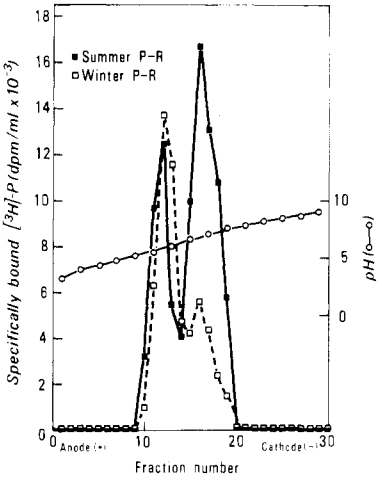

FIGURE 4: Isoelectric focusing of a summer and a winter preparation of the [^{3}H]progesterone receptor. Partially purified receptor preparations isolated in either the summer (June) or the winter (March) were focused for 8 h at 8 W/plate in a superfine Sephadex G-75 support medium as described in the preceding paper (Boyd & Spelsberg, 1979). Specifically bound [^{3}H]P from the gel was determined by the charcoal assay after focusing the summer (■) or the winter (□) receptor preparations. The open circles represent the pH gradient of the fractions.

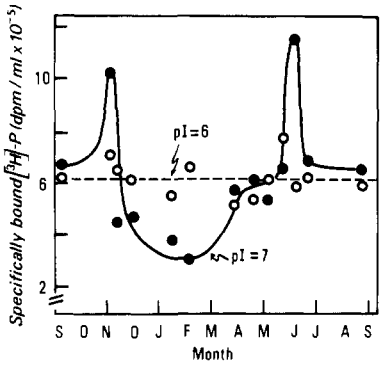

FIGURE 5: Seasonal changes in the molecular species of the [^{3}H]-progesterone–receptor complex. Partially purified receptor preparations were focused as described in the legend to Figure 4 and in the preceding paper (Boyd & Spelsberg, 1979). The specifically bound radioactivity was corrected for loss in recovery during IEF, and the distribution of radioactivity between the two peaks was determined. Generally, the two peaks represented fractions 10–14 and 15–20 of the focusing plate. The amounts of each of the receptor species were then plotted against the date of the isolation of the [^{3}H]P–R. The open circles represent the amount of specific binding which focused as the p*I* 6 receptor species, and the closed circles represent the specific binding of the p*I* 7 receptor species.

specifically bound [^{3}H]P focusing with p*I* values of 6 (the "B" receptor species) and 7 (the "A" receptor species). However, the [^{3}H]P–R preparation isolated during the winter showed markedly reduced amounts of the species focusing with a p*I* value of 7 ("A" species). The levels of the receptor species focusing at a p*I* of 6 (or "B" species) were very similar between the winter and summer preparations. To analyze further this apparent change in the amount of the "A" receptor, we focused [^{3}H]P–R samples isolated throughout the year and quantitated the two species. Figure 5 illustrates the seasonal change observed in the molecular species of [^{3}H]P–R. The amounts of each of the two species are plotted with respect to the date of receptor isolation. The amount of the p*I* 7 ("A" receptor) species fluctuated significantly with a pattern very similar to that observed for [^{3}H]P–R binding in vivo and in vitro to the nuclear acceptor sites on chromatin and in vitro to NAP (see parts A, B, and C of Figure 2). Maximal amounts of the p*I* 7 or "A" receptor were measured during the fall and summer months, whereas during the winter the level of this species was greatly diminished. Therefore, the binding of [^{3}H]P–R to the chromatin or NAP and the effect of progesterone on RNA polymerase activity correlate with the amount of the "A" receptor present in the preparation. The binding of [^{3}H]P–R to DNA shows no correlation with these events.

Discussion

Using IEF, it was demonstrated that the progesterone–receptor complex is not constant throughout the year. The seasonal variation observed for the levels of the "A" receptor species (p*I* 7) shows a significant correlation with the effect of progesterone on the RNA polymerase II activity and the capacity of [^{3}H]P–R to bind to the acceptor sites on chromatin both in vivo and in vitro. Similarly, the binding of [^{3}H]P–R in vitro to NAP, but not to DNA, correlated with the levels of the "A" species. The seasonal loss of one of the species of receptor explains the decrease observed in the overall [^{3}H]P–R levels measured in the cytosol during the winter period (Spelsberg et al., 1979a,b; T. C. Spelsberg and F. Halberg, unpublished experiments). These seasonal variations in receptor levels and receptor binding to nuclear protein acceptor sites have been shown to be statistically significant circannual rhythms (T. C. Spelsberg and F. Halberg, unpublished experiments). No such rhythm in binding to pure DNA was detected by the computer-fit cosine curves. These results represent the first evidence of a molecular regulation of endocrine function at the level of the molecular species of a steroid receptor. The data also support a role of proteins in chromatin and NAP in the nuclear acceptor sites for the progesterone–receptor complex. The seasonal variation in the capacity of the [^{3}H]P–R to bind to the nuclear sites observed with chromatin in vivo and in vitro, and with the NAP but not the DNA in vitro, indicates that the DNA alone does not serve as the acceptor site. The DNA does play a role in the acceptor sites, for it is a constituent of chromatin and NAP.

It has been suggested that the intact dimer (A–B) is the functional progesterone receptor unit in vivo in the chick oviduct (Schrader et al., 1972, 1977; Buller et al., 1976). The results reported here support their hypothesis or at least indicate that the "A" receptor species (p*I* 7) is required for nuclear translocation and/or binding of the [^{3}H]P–R to the nucleoprotein and chromatin acceptor sites.

The changes in the receptor species reported here may represent another level of control of endocrine functions. The loss of one receptor subunit may inactivate the subsequent steps in the steroid receptor mechanism. If this method of regulation occurs in other steroid systems, it would explain the failure of target tissues to respond to their respective steroid despite the presence of measurable steroid in the serum and receptor levels in the cytosol. This theory has been proposed for the failure of certain breast tumors to respond to endocrine therapy (Wittliff et al., 1977; Kute et al., 1978).

Further, this level of regulation might explain the molting phenomenon in birds. Once a year for a month or more, adult hens will molt (the shedding and renewal of feathers) with a loss of egg production (Bahr & Nalbandov, 1977; Imai et al., 1972). This molt occurs even under controlled environmental conditions, thus supporting an endogeneous clock. During molting the oviducts decrease in weight from ~40 to ~2 g. However, the serum levels of estrogen in molting hens are equal to or greater than those in nonmolting (laying) hens (Peterson & Common, 1972). Since estrogen is responsible for maintaining oviduct weight and function, the oviduct appears to be refractory to the action of the estrogen in the serum during the molting period. Hawkins et al. (1969) injected [^{3}H]estrogen into molting and nonmolting hens and found that the half-life of the steroid in the sera was equivalent for the two groups. However, the oviducts of the molting hens retained much less of the steroid compared to the nonmolting hens even when differences in oviduct weights were considered. These results support the lack of a normal functioning estrogen receptor during the once a year molting stage in hens. This may also be the case for the progesterone receptor. Thus, during molt, one of the molecular species (e.g., the p*I* 7 or "A" species) may disappear, resulting in (1) reduced steroid receptors and a lower tissue uptake and retention and (2) an inability of the receptor to translocate to the nucleus and alter transcription (i.e., RNA polymerase activity) and thus a marked refractoriness of the target cell to respond to the steroid. Studies are currently in progress to analyze the receptor species and function during molting in laying hens.

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