Glucocorticoid binding in the hen oviduct

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[³H]Triamcinolone acetonide (15 nM) was incubated with cytosol (150 000 g fraction) prepared from oviducts of egg-laying hens. The extent of steroid binding, as determined by charcoal assays, was greatest between 2–4 h at 4°C. A similar time curve was obtained when cytosol preparations were first fractionated with (NH₄)₂SO₄ before labelling. The addition of 10 mM-Na₂MoO₄ or 10 mM-ATP during the incubation of hen oviduct cytosol with [³H]triamcinolone acetonide lowered the extent of steroid binding. The presence of glycerol (20%), however, increased the extent of [³H]triamcinolone acetonide binding in cytosol fractions from chick (330%) and hen (160%) oviducts. The [³H]triamcinolone acetonide–receptor complex was stable for over 4 h at 4°C, but dissociated rapidly at 37°C, exhibiting a half-life of about 10 min. The presence of 10 mM-Na₂MoO₄ and 10 mM-ATP or both had a small protective effect on the dissociation of [³H]triamcinolone acetonide–receptor complex. The receptor from hen oviduct showed significant affinity for unlabelled triamcinolone acetonide, cortisol, compound R5020 and dihydrotestosterone and, to a lesser extent, for oestradiol, oestrone and progesterone. Diethylstilboestrol treatment of immature chicks appeared to induce a more specific binder, which showed affinity for unlabelled triamcinolone acetonide, cortisol and compound R5020 only. Scatchard analysis of [³H]triamcinolone acetonide binding in hen oviduct cytosol revealed a *K_d* value of 6.4 nM. The steroid–receptor complex sedimented as a 7–8S and a 4S entity on low-salt (0.01 M-KCl)- and high-salt (0.3 M-KCl)-containing sucrose gradients respectively. The cytosol [³H]triamcinolone acetonide–receptor complex showed no affinity for ATP–Sephadex or DNA–cellulose, but acquired this ability on heat activation (23°C, 40 min). The data indicate the avian oviduct possesses a high-affinity binding molecule that fulfils the criteria of a glucocorticoid receptor.

Since their initial detection in the thymus (Munck & Brinck-Johnsen, 1968), glucocorticoid receptors have been reported in numerous tissues of laboratory animals in various stages of development (Ballard *et al.*, 1974; Giannopoulos *et al.*, 1974; Feldman, 1974). Most tissues in the body appear to possess receptors for glucocorticoids (Calk & Litwack, 1975). A detailed account of the data from studies on glucocorticoid receptors in various tissues has been presented (Baulieu *et al.*, 1975). Affinities of receptors for natural and synthetic glucocorticoids have been measured and compared with the biological activities in hormone-responsive cell cultured *in vitro*. In general, glucocorticoid receptors tend to be more difficult to detect than receptors for other steroid hormones because of the higher ratio of non-specific to specific glucocorticoid binding in target cells, the rapid dissociation of glucocorti-

coid–receptor complexes and the highly unstable nature of the receptors themselves (King & Mainwaring, 1974).

Oviducts, from diethylstilboestrol-primed chicks or egg-laying White Leghorn hens, have been the target of numerous investigations in the area of steroid-hormone receptors. The chick oviduct provides an excellent system to study the synthesis of several major egg-white proteins and their regulation by administration and withdrawal of steroid hormones (Schimke *et al.*, 1975). Specific proteins for both progesterone and oestrogen have been reported in avian oviduct (O'Malley *et al.*, 1970; Sherman *et al.*, 1970; Harrison & Toft, 1975; Best *et al.*, 1975). Diethylstilboestrol administration in the immature chicks causes a manyfold increase in the content of progesterone receptors in the oviducts (Toft & O'Malley, 1972). Although the effects of

oestrogen and progesterone in influencing the growth of this tissue have been studied quite extensively (O'Malley *et al.*, 1969), the involvement of other steroids had not been demonstrated. Recently, Hager *et al.* (1980) have shown that glucocorticoids could induce both ovalbumin (mRNA_{ov}) and conalbumin (mRNA_{con}) mRNA species when added to oviduct explants derived from oestrogen-withdrawn chicks. We report here our data on the binding of glucocorticoid in the oviducts of diethylstilboestrol-primed chicks and egg-laying hens. We have used the synthetic glucocorticoid, triamcinolone acetonide {9-fluoro-11 β ,21-dihydroxy-16 α ,17-[1-methylethylidenebis(oxy)]pregna-1,4-diene-3,20-dione} because of its higher affinity for cellular receptors and its slower rate of metabolism compared with that of natural glucocorticoids. In addition, the incidence of binding to serum proteins (transcortin) was also decreased by the use of triamcinolone acetonide. The information on the new receptor systems should assist the discovery of new regulatory pathways.

Materials and methods

All reagents were of analytical grade. Activated charcoal, CNBr, monothioglycerol, glycerol, unlabelled steroids, ATP and calf thymus DNA were purchased from Sigma; [6,7(n)-³H]triamcinolone acetonide (30–50 Ci/mmol) was from New England Nuclear Corp. and was re-purified by t.l.c., methylene chloride/methanol (24:1, v/v) being used as solvent. Sodium molybdate was purchased from Merck. Sepharose 4B and Dextran T-70 were from Pharmacia.

Preparation of buffers

All buffers were pH 8.0 at 23°C. Buffer A comprised 10 mM-Tris/HCl, 12 mM-monothioglycerol, 2.5 mM-EDTA, 0.01 M-KCl and 20% (v/v) glycerol, pH 8.0. Buffer B was the same as buffer A, but contained 1 M-KCl. Buffer C comprised 10 mM-Tris/HCl and 12 mM-monothioglycerol.

Preparation of glucocorticoid receptors

Oviducts were obtained from laying hens or from 3–4-week-old White Leghorn chicks, which received daily injection of 5 mg of diethylstilboestrol in sesame oil for a period of 2 weeks. The freshly excised tissue was rinsed with cold 0.9% NaCl, transferred to 2 vol. (w/v) of buffer (40 mM-Tris/HCl/12 mM-monothioglycerol/10% glycerol, pH 8.0) and homogenized with a Tisumizer (Tekmar, model SDT). The homogenate was first centrifuged at 12 000 g for 10 min and then at 150 000 g for 1 h. The resultant clear supernatant was used immediately for various assays. For some experiments the fresh cytosol was incubated with [³H]triamcinolone aceto-

nide for 4 h at 4°C and fractionated with 50%-satd. (NH₄)₂SO₄. The precipitate containing the glucocorticoid receptor complex was used immediately for various assays or stored at –85°C for no more than a week without any loss of steroid from the complex. The use of EDTA was omitted in experiments dealing with the activation of cytosolic receptor complexes, since its presence is known to interfere with the process of receptor activation (Jensen *et al.*, 1973).

Preparation of ATP–Sepharose and DNA–cellulose

ATP was covalently linked to Sepharose 4B as described previously (Moudgil & Toft, 1975, 1977) by the method of Lamed *et al.* (1973). In this procedure, adipic acid dihydrazide was attached to CNBr-activated Sepharose. ATP was oxidized by NaIO₄ and then linked to Sepharose hydrazide. Our preparations contained 4–6 μ mol of ATP/ml of packed resin, as determined by phosphate analysis (King, 1932; Moudgil & Toft, 1975).

DNA–cellulose was prepared as described by Alberts & Herrick (1971). Calf thymus DNA (Sigma, type II) was linked to Cellex-410 (Bio-Rad), and our preparations contained 1–2 mg of DNA/g of cellulose. The affinity resin was stored as a slurry and hydrated in buffer C for 2 days before use.

Affinity chromatography

ATP–Sepharose and DNA–cellulose affinity chromatography was performed essentially as described previously (Moudgil & Toft, 1975, 1977; Moudgil & John, 1980a,b). Small columns containing 1–2 ml of ATP–Sepharose and DNA–cellulose were equilibrated with buffer A. Portions containing steroid–receptor complexes (0.5 ml–1 ml) were applied and the columns were washed with 20 vol. of buffer A and the complexes were eluted from the affinity columns by using buffer B. The specific details are given in the Figure legends.

Sucrose-density-gradient analysis

Linear 5–20% sucrose gradients (4.4 ml) were prepared in buffer C by using a Beckman gradient former and contained either 0.01 M-KCl (low-salt) or 0.3 M-KCl (high-salt). Samples (0.2 ml) containing labelled cytosol were layered and the gradients were centrifuged at 150 000 g for 16 h at 4°C. [¹⁴C]-Ovalbumin was used as marker protein (Rice & Means, 1971) and layered on a separate gradient as a standard for the determination of sedimentation coefficients (Martin & Ames, 1961). The fractions (0.2 ml) were collected by piercing the bottom of each tube.

Determination of steroid binding

Charcoal-adsorption assays were performed to determine the extent of steroid binding in different

preparations. Duplicate or triplicate samples of receptor preparations were incubated with 15 nM [3 H]triamcinolone acetone. To determine the non-specific binding, a 100–500-fold excess of unlabelled steroid (cortisol or triamcinolone acetone) was added to other duplicate samples before the addition of labelled steroid. In cases where complexes were already formed, non-specific binding was determined by heating the samples at 60°C for 30 min in presence of excess unlabelled steroid to destroy specific binding (Buller *et al.*, 1976). An equal volume of Dextran-coated charcoal (1% Norit A and 0.05% Dextran T-70, made in buffer C) was added to each tube and the contents were mixed gently. After a 5 min incubation at 4°C, tubes were centrifuged at 1000 *g* for 5–10 min and the supernatants were used to measure radioactivity. The specific binding represented the total radioactivity minus the values from heated or unlabelled hormone-treated samples.

Radioactivity in samples was determined by combining aqueous samples with 5 ml of scintillation fluid consisting of 4.2% (v/v) Spectrafluor (Amersham/Searle) in toluene.

Results

Fig. 1(a) illustrates results of a time-course study of glucocorticoid binding in cytosol and $(\text{NH}_4)_2\text{SO}_4$ -fractionated preparations from laying-hen oviducts. The binding of [3 H]triamcinolone acetone increased in a time-dependent manner and reached a maximum value when incubated with receptor preparations for 2–4 h at 4°C. There was no apparent loss of steroid binding in the cytosol preparations 4 h after it reached the maximum value. The [3 H]triamcinolone acetone–receptor complex from $(\text{NH}_4)_2\text{SO}_4$ -fractionated preparations seems somewhat unstable after reaching a plateau. In this experiment, the receptor was labelled after the salt precipitation. Although the steroid-binding capacity did not seem to have altered, the kinetic behaviour of receptor might have undergone a change. Previous studies (Nielsen *et al.*, 1977a,b; Moudgil & John, 1980a) have reported that the glucocorticoid receptor is highly unstable in the absence of steroid. It is also possible that some dissociation of the complex would occur at 4°C.

In order to establish optimal assays for studying glucocorticoid receptors in the avian oviduct, efforts

Fig. 1. [3 H]Triamcinolone acetone (${}^3\text{H-TA}$) binding in hen oviduct preparations: effects on dissociation and stability of glucocorticoid–receptor complex

(a) Portions of freshly prepared hen oviduct cytosol (O) or cytosol fractionated by $(\text{NH}_4)_2\text{SO}_4$ (●) were incubated with 15 nM [3 H]triamcinolone acetone for different time periods at 4°C in a total volume of 0.5 ml. The mixture contained 20% glycerol. An equal volume of dextran-coated charcoal was added at the end of time periods shown and the contents were mixed briefly. The mixtures were then centrifuged at 1000 *g* for 10 min and supernatants were used to measure radioactivity. Non-specific binding was determined by including a 100-fold excess of unlabelled cortisol or triamcinolone acetone before the addition of labelled steroid, and was subtracted from the total radioactivity. (b) The hen oviduct cytosol was allowed to form a complex with 15 nM [3 H]triamcinolone acetone as described above. Triplicate samples were incubated at 4°C (O) or 37°C (●) for the time periods shown. Non-specific binding was determined for each point by including excess unlabelled steroid. The extent of steroid binding was then determined by charcoal assays. 100% specific binding represents 6500 c.p.m. (c) The complexes were incubated in triplicate in a series of tubes, separately, with buffer C (O), 10 mM-ATP (●), 10 mM-sodium molybdate (Δ) or 10 mM-ATP and 10 mM-sodium molybdate (▲) for 30 min at 4°C. After this the tubes were incubated at 37°C for the time periods shown. Charcoal assays were performed at the end of each time period. Specific binding represented the total macromolecule-bound radioactivity in the charcoal-treated supernatant minus the radioactivity in samples preincubated with excess unlabelled steroid. 100% specific binding represents 4550 c.p.m. in this Figure. Abbreviation used: ${}^3\text{H-TA}$, [3 H]triamcinolone acetone.

were made to define its stability under various conditions. Fig. 1(b) shows that the [^3H]triamcinolone acetonide–receptor complex was stable at 4°C up to more than 4 h after its formation. A rapid decrease in the number of steroid–receptor complexes was observed when the mixtures were incubated at 37°C. By 1 h at 37°C, the amount of glucocorticoid–receptor complex remaining was decreased to background values. Whether the loss of quantifiable steroid–receptor complex was due to a dissociation of the steroid from the complex or due to a conformation change in the receptor protein is not apparent from these studies. Also, whether this is a reversible process is not known.

In recent years, several chemical agents have been identified that stabilize the steroid receptors both in their occupied and unoccupied states (Nielsen *et al.*, 1977a,b; Pratt *et al.*, 1979; Nishigori & Toft, 1980; Moudgil & John, 1980a). The specific glucocorticoid-binding capacity of cytosol preparations from rat thymus, liver and mouse fibroblasts is rapidly lost on incubation at 25°C in the absence of ligand (Pratt *et al.*, 1979). This inactivation can be prevented by inhibitors of phosphatase action such as molybdate, fluoride and glucose 1-phosphate. The receptor can also be partially re-activated or stabilized by addition of exogenous ATP in the

cytosol (Pratt *et al.*, 1979; Moudgil & John, 1980a). The results of our attempts to determine the effects of these compounds on the stability of glucocorticoid–receptor complex from the chick oviduct are shown in Fig. 1(c). The complex showed a rapid dissociation at 37°C by a 30 min incubation. Presence of 10 mM-sodium molybdate or 10 mM-ATP or both had some protective effect in that they appeared to prolong the life of the glucocorticoid–receptor complex. Although the various additions do not seem to affect the initial rate of loss of steroid binding, they did prevent the amount of binding falling to the basal plateau value. These observations do not seem to represent simple stabilization effects of molybdate and ATP, as suggested by Pratt *et al.* (1979). However, interesting results were obtained when molybdate or ATP were included in the incubation mixture during the formation of glucocorticoid–receptor complex. Fig. 2 demonstrates the effects of glycerol, molybdate and ATP on the steroid-binding capacity of glucocorticoid receptor from hen and chick oviducts. Both ATP and molybdate appeared to have no effect on the ability of [^3H]triamcinolone acetonide to bind to chick cytosol receptor; instead, they appeared to interfere with the process of steroid binding when included in the cytosol preparation from hen

Fig. 2. Effects of glycerol, ATP and molybdate on the [^3H]triamcinolone acetonide (^3H -TA) binding capacity of chick and hen oviduct

Triplicate samples of freshly prepared cytosol from chick (a) or hen oviduct (b) were mixed with 15 nM- ^3H -triamcinolone acetonide in a total volume of 0.5 ml, and contained different concentrations of glycerol (●, 0–20%), ATP (△, 0–10 mM) or sodium molybdate (○, 0–10 mM). The mixtures were incubated at 4°C for 4 h. The extent of steroid binding was determined by charcoal-adsorption assays.

oviducts. A 60% decrease in the formation of glucocorticoid-receptor complex could be observed in the hen oviduct cytosol in the presence of 20mM-sodium molybdate. Steroid-binding capacity of glucocorticoid receptor increased dramatically in the presence of glycerol, more dramatic effects being observed with chick cytosol (Fig. 2). A 3-fold increase in [^3H]triamcinolone acetonide binding in the chick cytosol occurred in the presence of 20% glycerol. It appears likely that the glucocorticoid receptors in hen oviduct might have remained undetected, owing to an unstable nature of the molecules. The mechanism by which glycerol influences steroid binding is not clear. It could be inactivating a cytosol inhibitor of glucocorticoid binding or stabilizing a portion of receptor protein most optimal for this interaction.

The specificity of glucocorticoid binding of the cytosolic oviduct receptor from laying hens was characterized by examining the ability of different concentrations of unlabelled steroids to compete for specifically bound [^3H]triamcinolone acetonide. As

shown in Fig. 3, both triamcinolone acetonide and compound R5020 (a synthetic progestational agent) were found to exhibit significant competition. Cortisol was also effective, but at higher concentrations. However, compound R5020 appeared to exhibit a biphasic inhibition. Whether this is due to its interaction with more than one type of binding sites on the receptor is not clear. It is also possible that compound R5020 interacts with other macromolecules in the incubation mixture at higher concentration and thus shows an altered affinity for [^3H]triamcinolone acetonide receptor. To further investigate the comparative potencies of different unlabelled steroids at equimolar concentrations to compete for [^3H]triamcinolone acetonide, more steroids were tested, as shown in Fig. 4. Cortisol,

Fig. 3. Steroid-binding-specificity analysis of glucocorticoid binding in hen oviduct

The $(\text{NH}_4)_2\text{SO}_4$ -fractionated hen oviduct cytosol was redissolved in buffer C and incubated with 12nM-[^3H]triamcinolone acetonide (^3H -TA) in the presence of different concentrations of unlabelled triamcinolone (Δ), cortisol ($\bullet$) or compound R5020 ($\circ$) for 4 h at 0–4°C. The extent of steroid binding was then determined by charcoal-adsorption assays. 100% binding equals 9538 c.p.m.

Fig. 4. Steroid-binding specificity of [^3H]triamcinolone acetonide receptor from hen and diethylstilboestrol-primed chick oviducts

The cytosol samples from hen (a) or chick (b) oviduct were taken in triplicate in a series of tubes and labelled with 16nM-[^3H]triamcinolone acetonide. Other triplicate sets were each added with a different unlabelled steroid (2mM) before the addition of ^3H -labelled steroid. The incubation mixture (0.5 ml), which also contained 20% glycerol, was then set at 4°C for 4 h with brief mixing every 30 min. The extent of steroid binding was then determined by charcoal assays. 100% Binding in (a) equals 11050 c.p.m. and in (b) 37000 c.p.m. Abbreviations used: TA, triamcinolone acetonide; F, cortisol; T, dihydrotestosterone; E₃, oestriol; E₁, oestrone; E₂, oestradiol; P, progesterone; C, control; R, compound R5020.

Fig. 5. *Sedimentation rate and Scatchard (1949) analysis of [^3H]triamcinolone acetonide binding in hen oviduct cytosol* (a) Portions (0.1 ml) of cytosol were incubated with [^3H]triamcinolone acetonide (1–100 nM) for 4 h at 0–4°C. The non-specific binding was determined by including excess unlabelled steroid in the incubation mixtures. (b) Samples (0.2 ml) containing the [^3H]triamcinolone acetonide (^3H -TA) receptor complex were layered on linear 5–20% sucrose gradients (4.4 ml) containing 0.01 M-KCl (●) or 0.3 M-KCl (○). [^{14}C]Ovalbumin was layered on a separate gradient and served as a sedimentation standard. The gradients were centrifuged at 150000g for 16 h (0–4°C). Fractions (0.2 ml) were collected by piercing the bottom of each gradient tube.

Fig. 6. *ATP-Sepharose and DNA-cellulose affinity chromatography of [^3H]triamcinolone acetonide (^3H -TA) receptor complex*

(a) Hen oviduct cytosol was allowed to form a complex with 12 nM- ^3H -triamcinolone acetonide for 4 h at 0–4°C in the presence of 20% glycerol. This preparation containing cytosol receptor complex was divided into two equal portions of 0.5 ml each. One of the samples was heat-activated (23°C, 40 min) (●), while others remained on ice (○). The activated and non-activated cytosol complexes were then subjected to chromatography in 2 ml ATP-Sepharose columns pre-equilibrated with buffer A. The columns were washed with 20 vol. of buffer A and the adsorbed complexes were eluted with buffer B and are shown in the Figure. In this experiment, 46% of the original steroid-receptor complexes could be bound to the column (●) and subsequently recovered with buffer B. (b) Freshly prepared cytosol was allowed to form a complex with 10 nM- ^3H -triamcinolone acetonide (^3H -TA) for 4 h in the presence of 20% glycerol. The preparation (3 ml) containing the resulting glucocorticoid-receptor complexes was divided into three groups. Group 1 (○) received buffer C and was kept at 0–4°C for 40 min. Groups 2 (●—●) and 3 (●—●—●) were incubated at 23°C for 40 min to heat-activate the glucocorticoid-receptor complexes. Group 3 received 10 mM-sodium molybdate during the 23°C incubation period. Contents of all three groups were separately mixed with 2 ml of hydrated packed DNA-cellulose equilibrated with buffer A. The mixtures were stirred every 10 min for 1 h. After this, the affinity resin was poured into columns and the packed resin washed with 20 vol. of buffer A to remove unbound complexes and free steroid. The DNA-cellulose-bound complexes were recovered with buffer B.

triamcinolone acetonide, dihydrotestosterone and compound R5020 appeared to bind the [^3H]triamcinolone acetonide-binding site with an equal effectiveness, whereas oestradiol, oestriol, oestrone and progesterone were weak competitors. However, when a similar binding-specificity analysis was performed with the cytosol from diethylstilboestrol-primed chicks, a remarkable specificity was exhibited by the [^3H]triamcinolone acetonide-binding component. Triamcinolone acetonide showed maximum competition, followed by cortisol and compound R5020, whereas dihydrotestosterone, oestradiol, oestriol, oestrone and progesterone failed to bind to the [^3H]triamcinolone acetonide site. Therefore it appears that diethylstilboestrol administration either induced a new form of receptor or altered the proportions in the receptor population in favour of one with greater specificity.

The binding of [^3H]triamcinolone acetonide in the hen oviduct cytosol appeared to be of high affinity. Scatchard (1949) analysis of the [^3H]triamcinolone acetonide binding is shown in Fig. 5(a). There was a linear correlation between the steroid binding and steroid concentration, with an apparent K_d of 6.4 nM. The dissociation constant (K_d) for steroid binding in chick oviduct was observed to be 4.8 nM. Although diethylstilboestrol appears to alter the specificity of steroid binding in chick and hen, it does not cause any significant change in the K_d values in the two systems. These values are indicative of a strong ligand binding by the cytosolic receptor.

The sedimentation characteristics of [^3H]triamcinolone acetonide binding in hen oviduct cytosol are illustrated in Fig. 5(b). The steroid-receptor complex sedimented as a 4S entity in the salt-containing gradients, but appeared to aggregate somewhat in the salt-free medium (~8S). These findings are consistent with the known sedimentation characteristics of steroid receptors in general and glucocorticoid receptors in particular, and, therefore, validate these observations as representing a typical steroid-receptor system.

An interaction between ATP and progesterone receptor has been demonstrated (Moudgil & Toft, 1975, 1977). This interaction, which was demonstrated by the uses of ATP-Sepharose affinity chromatography, appears to be a general phenomenon and has been reported for oestradiol (Moudgil & Weekes, 1978; Miller & Toft, 1978; Moudgil & Eessalu, 1980), glucocorticoid (Moudgil & John, 1980b) and progesterone receptors in rat brain (Moudgil *et al.*, 1979) and hamster uterus (Leavitt *et al.*, 1979). It was observed in previous studies that the freshly prepared cytosol contained a receptor which, when complexed with the labelled steroid, had very little affinity for ATP-Sepharose (Miller & Toft, 1978; Moudgil & John, 1980b). The complex acquired this ability to bind to ATP-Sepharose on

heating at 23°C for various time periods. This increased ATP binding on activation of steroid-receptor complex has been shown to parallel the nuclear uptake of progesterone-receptor complex (Miller & Toft, 1978). Therefore ATP binding is a property of an activated receptor, and the binding to ATP-Sepharose represents the extent of receptor activation. Results in Fig. 6(a) illustrate the ATP-Sepharose binding of [^3H]triamcinolone acetonide receptor complex from hen oviduct cytosol with and without heat activation. As for other receptor systems, the hen oviduct cytosol receptor showed no affinity for ATP-Sepharose, but acquired this ability on heat activation. In this respect the glucocorticoid-receptor complex behaves in a manner similar to other steroid receptors.

Activated receptors have been shown to acquire DNA-binding properties (Yamamoto & Alberts, 1972; Milgrom *et al.*, 1973). Rat liver glucocorticoid-receptor complexes activated by heat can be retained on DNA-cellulose columns (Moudgil & John, 1980a). This DNA-binding capacity can be blocked by the presence of molybdate during the time of receptor activation. The data in Fig. 6(b) demonstrate DNA-cellulose chromatography of hen oviduct cytosol [^3H]triamcinolone acetonide-receptor complex. Heat activation of cytosol receptor caused a 10-fold increase in DNA-cellulose binding, which was blocked by 10 mM-sodium molybdate. Here again, the hen oviduct glucocorticoid receptor exhibited characteristics demonstrated by other steroid receptors.

Discussion

Glucocorticoids influence almost all tissues in the body, and most tissues are known to possess glucocorticoid receptors. On their initial binding to the steroid hormones, the cytoplasmic receptor proteins are known to translocate at certain sites in the nucleus (O'Malley & Means, 1974). the interaction between the steroid-receptor complex and nuclear binding sites is known to trigger alterations in gene expression. Chick oviduct has been known to be a target tissue for many classes of steroid hormones. These steroids have been known to influence the gene expression and general development in this tissue. Oestrogens affect tubular gland cells by inducing cell division and differentiation, which results in increased synthesis of the egg-white proteins ovalbumin and conalbumin. An oestrogen-primed oviduct becomes responsive to the action of progesterone. Only recently have glucocorticoids been shown to induce both mRNA_{ov} and mRNA_{con} (see the introduction) when added to oviduct explants derived from oestrogen-withdrawn chicks (Hager *et al.*, 1980). The action of oestrogen and progesterone on chick oviduct are known to be

receptor-mediated. The question of whether glucocorticoids have any effect (receptor-mediated) on chick oviduct has remained unanswered. The purpose of the present study was to determine the presence of glucocorticoid receptors in avian oviduct and to compare the data with the characteristics of glucocorticoid receptors established in other conventional target tissues.

At low temperature (0–4°C) the glucocorticoids interact with the target-cell receptors to form non-activated complexes that do not bind to isolated nuclei (Munck *et al.*, 1972). The cytosol receptor complexes can be activated by warming at 23°C in the absence of nuclei. Such activated receptor complexes have been known to develop increased affinity for isolated nuclei or DNA (Baxter *et al.*, 1972; Beato *et al.*, 1973; Milgrom *et al.*, 1973, 1976; Atger & Milgrom, 1976; Goidl *et al.*, 1977; Cidlowski & Munck, 1978) and ATP–Sephacrose (Miller & Toft, 1978; Moudgil & John, 1980a). Results of Fig. 6 are consistent with these general observations in that the hen oviduct glucocorticoid receptor exists in a non-activated form in the freshly prepared cytosol and can be activated by heat treatment in presence of the ligand.

The [³H]triamcinolone acetonide binding can be stabilized in the presence of molybdate and ATP. Our data in Fig. 1 support that generality. The dissociation of glucocorticoid–receptor complex is prevented by the addition of molybdate and ATP or both agents. However, the steroid-binding capacity of hen oviduct glucocorticoid receptor appears to be decreased in the presence of both ATP and molybdate (Fig. 2). This finding is not in agreement with previous observations (Sando *et al.*, 1979; Moudgil & John, 1980a), which suggested that ATP and molybdate enhance the steroid-binding capacity of glucocorticoid receptors from rat thymocytes and liver. Whether molybdate effects are indirect (through an inhibition of phosphatases), or direct (via a receptor system) is unclear. Also, whether molybdate and ATP actions involve the same mechanism in the avian oviduct glucocorticoid receptor system as with progesterone receptor, remains to be determined. Finally, the demonstration of [³H]triamcinolone acetonide binding in chick and hen oviduct should open up new avenues for understanding the steroid-hormone-dependent regulation of gene expression in these tissues.

We have presented data that demonstrate the existence of glucocorticoid-binding sites in oviducts of laying hens and diethylstilboestrol-primed chicks. The binding sites in the diethylstilboestrol-primed chick oviducts appear to be more specific when compared with those from laying hens. Whether diethylstilboestrol induces a new population of receptors with greater ligand specificity or it changes the proportion of specific to non-specific sites is not

clear at this point. The data on binding sites by Scatchard (1949) analysis, sedimentation analysis and steroid-binding specificity are consistent with the glucocorticoid receptors demonstration in conventional target tissues. However, the observation that compound R5020 is a better competitor than cortisol and triamcinolone acetonide is perplexing and difficult to explain. The possibility that [³H]-triamcinolone acetonide might be binding to a progesterone receptor was also considered, but appeared unlikely, as [³H]R5020 binding in hen or chick oviduct was not decreased by pretreatment of cytosol with triamcinolone acetonide or cortisol. It is also possible that the steroid binder in the present studies possesses sites for both glucocorticoids and compound R5020. The compound R5020 binding in unlabelled cytosol preparation may involve a conformational change in the receptor that decreases the affinity for subsequent binding by a glucocorticoid. At this time the relationship between compound R5020 and triamcinolone acetonide binding in hen and chick oviduct is far from clear and requires further investigation.

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