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A COMPARATIVE STUDY OF RECEPTORS FOR NATURAL AND SYNTHETIC GLUCOCORTICOIDS IN FETAL RABBIT LUNG*

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SUMMARY

The cytosol fraction of fetal rabbit lung contains three glucocorticoid-binding proteins. One of these binds only natural glucocorticoids and shares some of the properties of serum transcortin. The other two proteins, sedimenting at 4 S and 7 S, bind both cortisol and dexamethasone but they are stabilized to a different extent by each steroid. The 7 S component is detectable by sucrose density gradient centrifugation, charcoal adsorption or gel filtration when complexed with dexamethasone. However, 7 S cortisol-protein complexes could not be detected by any of these methods. The presence of the latter complexes is indicated by the ability of cytosol fractions sedimenting at 7 S to enhance specific nuclear uptake of cortisol in cell-free systems. In contrast to the 7 S receptor, the 4 S protein is more stable when complexed with cortisol and does not enhance significantly specific transfer of glucocorticoid into the nucleus. Binding of dexamethasone to the 4 S component can be demonstrated by its ability to displace pre-bound cortisol.

The nuclear cortisol- and dexamethasone-receptor complexes formed after incubation of fetal lungs at 37°C are similar, both sedimenting at 4 S. The cortisol-receptor complexes are very labile and are partially stabilized in the presence of excess hormone.

INTRODUCTION

Previous studies[1, 2] demonstrated that the fetal rabbit lung contains cytoplasmic and nuclear proteins with high binding affinity and specificity for glucocorticoids. Competition experiments indicate that both natural and synthetic steroids interact with the same binding sites. However, the physical properties of cytoplasmic cortisol- and dexamethasone-protein complexes are distinctly different[1]. In solutions of low ionic strength, the dexamethasone–protein complexes sediment at 7 S and are excluded from Sephadex G-200. Under the same experimental conditions, the cortisol–protein complexes sediment at 4 S and are retained by Sephadex G-200. Nuclear dexamethasone-receptor complexes sediment at 4 S in sucrose gradients but similar complexes with cortisol are not detectable by this technique.

A number of explanations could account for the apparent differences in the physical properties of cortisol- and dexamethasone-protein complexes. These include ligand-specific changes in the conformational or aggregation properties of a single protein with a binding affinity for both steriods, failure to detect certain steroid-protein complexes by the assay used due to rapid dissociation of the hormone, or the presence of a 4 S protein with a binding affinity for cortisol but not dexamethasone. In the present studies we have attempted to distinguish among these possibilities.

EXPERIMENTAL

Steriods. [1,2-^{3}H]-Cortisol (44 Ci/mmol) was purchased from New England Nuclear Corporation and [1,2-^{3}H]-dexamethasone (27 Ci/mmol) from Amersham–Searle. Dexamethasone was a gift from the Upjohn Company. Cortisol was purchased from Ikapharm.

Animals. Pregnant New Zealand White rabbits at 28–31 days of gestation were obtained from the Canadian Breeding Farm and Laboratories Ltd.

Preparation of cytosol and nuclei. Fetuses were delivered by laparotomy and immediately decapitated[1]. Excised fetal lungs were homogenized with a Teflon homogenizer in batches of 1 g in 2–5 ml of 0.01 M Tris–HCl buffer, pH 7.6, or in the same buffer containing 0.0015 M Na_2EDTA. The homogenate was centrifuged for 30 min at 224,000 *g* at 2°C to obtain the cytoplasmic supernatant fraction (cytosol). For the preparation of nuclei, lungs were homogenized in 0.01 M Tris–HCl buffer, pH 7.6, containing 0.25 M sucrose and 0.003 M $MgCl_2$. The homogenate was filtered through four layers of cheesecloth and centrifuged at 800 *g* for 10 min. The crude nuclei were washed three times and purified by centrifugation through 2.2 M sucrose as previously described[2].

Tissue incubations. Fetal lungs were incubated for 2 h at 37°C in Eagle's HeLa tissue culture medium containing [^{3}H]-dexamethasone or [^{3}H]-cortisol and purified nuclei were prepared as described above. Nuclear steroid-protein complexes were extracted with 0.01 M Tris–HCl buffer, pH 7.6, containing 0.0015 M Na_2EDTA and 0.4 M KCl[2]. Since gluco-

* Trivial name used: dexamethasone, 11β,17,21-trihydroxy-9α-fluoro-16α-methyl-1,4-pregnadiene-3,20-dione.

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corticoid-protein complexes are stabilized in the presence of excess hormone[1], in some cases the extraction buffer contained an appropriate concentration of labeled steroid.

Measurement of specific binding. Cytoplasmic steroid-protein complexes were formed by incubating lung cytosol for 2 h at 0°C with [^{3}H]-dexamethasone or [^{3}H]-cortisol alone or together with excess nonlabeled steroid[1]. Specifically bound steroid was estimated by the charcoal assay[1] or by gel filtration on small columns of Sephadex G-50[3].

Precipitation of steroid-protein complexes. Saturated ammonium sulfate solution, adjusted to pH 7.6, was added slowly under stirring to cytosol preveously incubated with the appropriate hormone(s) until a 33 or 66% saturation was obtained. After 30 min, the mixture was centrifuged at 70,000 *g* for 15 min and the precipitate was assayed for radioactivity. In some cases the precipitate was dissolved in 0.01 M Tris–HCl buffer, pH 7.6, containing 0.0015 M Na_2EDTA and centrifuged at 70,000 *g* for 15 min. The clear supernatant containing steroid–protein complexes was desalted by passage through Sephadex G-50 prior to further analysis.

Sucrose density gradient analysis. Aliquots of samples (0.2–0.3 ml) were applied on 3.6 ml linear gradients of 5–20% sucrose. The sucrose solutions were prepared in 0.01 M Tris–HCl buffer, pH 7.6, containing 0.0015 M Na_2EDTA or 0.001 M $MgCl_2$ and in some cases they were supplemented with 10% glycerol or appropriate concentrations of non-labeled and/or labeled steroid(s). Centrifugation was carried out at 290,000 *g* for 7 or 16 h. The gradients were fractionated and the fractions were either assayed directly for radioactivity[1] or they were assayed for bound steroid by gel filtration[3]. Sedimentation coefficients of steroid–protein complexes were estimated by comparison with that of BSA (4.6 S).

Uptake of glucocorticoids by isolated lung nuclei. Lung cytosol preincubated with [^{3}H]-cortisol or [^{3}H]-dexamethasone in the presence or absence of the appropriate non-labeled steroid was centrifuged in sucrose gradients for 7 h. The sucrose solutions were prepared in 0.01 M Tris–HCl buffer, pH 7.6, containing 0.01 M $MgCl_2$ and the same steroid(s) used to incubate the cytosol. After centrifugation, the gradients were fractionated to 15 fractions and each fraction was mixed with purified lung nuclei (200–300 μg DNA) and incubated under agitation for 30 min at 25°C. The nuclear suspensions were then centrifuged at 800 *g* for 10 min and the pellets were assayed for radioactivity and DNA after repeated washing[4].

Chemical assays. DNA and protein were estimated by the diphenylamine method[5] and by the method of Lowry *et al.*[6], respectively.

RESULTS

Separation of proteins binding both cortisol and dexamethasone from "transcortin-like" proteins of fetal lung cytosol. Transcortin present in fetal rabbit serum, and possibly as a serum contaminant in fetal lung cytosol, has a high affinity for natural but not synthetic glucocorticoids and it retains its capacity to bind the steroid at 37°C[1]. On the other hand, intracellular glucocorticoid receptors would be expected to bind both natural and synthetic steroids. In addition, most of the steroid receptors described to date are heat labile and lose their binding capacity after brief heating in solution at 37°C[7]. These two properties, namely heat stability and steroid binding specificity, can be exploited to distinguish between two classes of glucocorticoid binding proteins in lung cytosol preparations. The first class of proteins, which are thought to represent the cellular glucocorticoid receptors, bind both natural and synthetic glucocorticoids and are labile at 37°C. The second class of proteins bind only natural glucocorticoids and are stable at 37°C. The latter proteins, however, may not necessarily be identical to serum transcortin and the possibility that they may represent cellular proteins is not excluded; they will therefore be referred to in this report as "transcortin-like" proteins.

Table 1 (column 1) compares the binding capacity of cytosol prepared from fresh lungs for [^{3}H]-cortisol and [^{3}H]-dexamethasone. The specificity of binding for each labeled steroid was established by determining the effect of a 100-fold amount of the analogous nonlabeled steroid. As shown in Table 1, this cytosol preparation has about two times higher specific binding capacity for [^{3}H]-cortisol than [^{3}H]-dexamethasone (18.8 and 9.5 pmol/g tissue, respectively). The binding of [^{3}H]-dexamethasone is virtually abolished by an excess amount of either dexamethasone or cortisol (Table 1) or by prior heating of the cytosol at 37°C[1]. Thus these binding sites represent glucocorticoid receptors. However, binding of [^{3}H]-cortisol is decreased by only 14% in the presence of a 100-fold amount of nonlabeled dexamethasone (Table 1) or by prior heating of the cytosol at 37 C[1]. It is therefore concluded that only 14% (2.9 pmol/g tissue) of the bound [^{3}H]-cortisol is associated with glucocorticoid receptor sites. The remaining fraction (86%) of [^{3}H]-cortisol binding is not affected by prior exposure of the cytosol to 37°C and is mostly specific for cortisol but not dexamethasone. Thus this fraction of bound [^{3}H]-cortisol (15.9 pmol/g tissue) (Table 1, column 1, C-B) represents binding to "transcortin-like" proteins.

The data shown on Table 1, column 1, demonstrate that the concentration of detectable binding sites with an affinity for both steroid (glucocorticoid receptors) differs significantly depending on whether [^{3}H]-cortisol or [^{3}H]-dexamethasone is used as the ligand (2.9 and 9.5 pmol/g tissue weight, respectively). This suggests that detection of cortisol-receptor complexes by the charcoal assay is much less quantitative than that of dexamethasone-receptor complexes, probably due to a more rapid dissociation of cortisol from the binding sites. Similar results were obtained when hormone

Table 1. Separation of "glucocorticoid receptors" from "transcortin-like" binding proteins by preincubation of fetal lungs in the absence of hormone or by precipitation with ammonium sulfate

	pmol [^{3}H]-steroid/g fresh weight tissue			
	Fresh lung			Preincubated lung[b]
Steroids(s)[a]	Cytosol[c]	0–33% AS ppt[d]	33–66* AS ppt[d]	Cytosol[c]
A. [^{3}H]-Cortisol	21.2	2.1	12.1	7.2
B. [^{3}H]-Cortisol + Cortisol	2.4	0.2	0.8	0.5
A–B[e]	18.8	1.9	11.3	6.7
C. [^{3}H]-Cortisol + Dexamethasone	18.3	0.2	11.6	4.4
A–C[f]	2.9	1.9	0.5	2.8
C–B[g]	15.9	0.0	10.8	3.9
D. [^{3}H]-Dexamethasone	10.0	5.1	2.4	7.5
E. [^{3}H]-Dexamethasone + Dexamethasone	0.5	0.2	0.3	0.4
F. [^{3}H]-Dexamethasone + Cortisol	0.8	0.4	0.5	0.5
D–E[h]	9.5	4.9	2.1	7.1
D–F[j]	9.2	4.7	1.9	7.0

[a] Lung cytosol was incubated for 2 h at 0°C with a saturating concentration of labeled steroid (2×10^{-8} M [^{3}H]-dexamethasone or 1×10^{-7} M [^{3}H]-cortisol) alone or together with a 100-fold amount of nonlabeled steroid.

[b] Lungs were preincubated for 2 h at 37°C in the absence of hormone prior to homogenization.

[c] Bound labeled steroid in the cytosol was estimated by the charcoal assay.

[d] Labeled steroid precipitated with ammonium sulfate at the 0–33% or 33–66% saturation range.

[e] Total amount of specifically bound [^{3}H]-cortisol.

[f] [^{3}H]-Cortisol binding inhibited by dexamethasone. This value represents the amount of binding sites with an affinity for both hormones ("glucocorticoid receptors") detectable with [^{3}H]-cortisol as the ligand.

[g] [^{3}H]-Cortisol binding inhibited by cortisol but not dexamethasone. This value represents the amount of "transcortin-like" binding sites.

[h,j] [^{3}H]-Dexamethasone binding inhibited by dexamethasone and cortisol, respectively. These values represent the amounts of "glucocorticoid receptors" detectable with [^{3}H]-dexamethasone as ligand using either dexamethasone or cortisol as competitor.

binding was assayed by gel filtration on Sephadex G-50 (not shown).

To investigate the properties of proteins binding both steroids, an attempt was made to separate them from "transcortin-like" proteins which are present in cytosol preparations in relatively high concentrations. A partial separation was achieved by incubating fetal lungs for 2 h at 37°C in a large volume of Eagle's HeLa medium in the absence of hormone (Table 1). Cytosol prepared from preincubated lungs retains most (75%) of the initial [^{3}H]-dexamethasone binding capacity but has only about 35% of the initial [^{3}H]-cortisol binding capacity. The large decrease in the binding of [^{3}H]-cortisol is at least in part due to the release of "transcortin-like" proteins into the incubation medium where they can be easily detected (not shown). Binding of [^{3}H]-cortisol in cytosol from preincubated lungs is inhibited by nonlabeled dexamethasone to a much greater extent (39%) than in cytosol from fresh lungs (14%). The main reason for this increase in the proportion of proteins binding both steroids relative to the total [^{3}H]-cortisol specific binding seems to be due to release of "transcortin-like" proteins into the incubation medium but it may also partly reflect dissociation of endogenous steroids from receptor sites during tissue preincubation.

Fractionation of cytosol by ammonium sulfate precipitation is a satisfactory method for virtually complete separation of "transcortin-like" proteins from proteins binding both steroids (Table 1). Ammonium sulfate in the 0–33% saturation range precipitates proteins binding both steroids but little or no "transcortin-like" proteins. The latter proteins are precipitated in the 33–66% saturation range of salt.

Sedimentation behavior of cortisol- and dexamethasone-protein complexes in sucrose gradients. Cytosol from lungs preincubated at 37°C in the absence of hormone contains [^{3}H]-dexamethasone-binding proteins sedimenting at 7 S but binding of [^{3}H]-cortisol to such proteins is not detected in sucrose gradients (Fig. 1a). However, such a cytosol preparation contains proteins which form complexes with [^{3}H]-cortisol sedimenting at 4 S. An excess of non-labeled dexamethasone or brief heating of the cytosol at 37°C inhibits the formation of the 4 S [^{3}H]-cortisol-protein complexes by 30–40%. These results indicate that a fraction of the 4 S sedimenting components are different from transcortin and they may represent a form of an intracellular glucocorticoid receptor.

[^{3}H]-Cortisol-protein complexes precipitated with ammonium sulfate in the 0–33% saturation range are very labile and dissociate during centrifugation in suc-

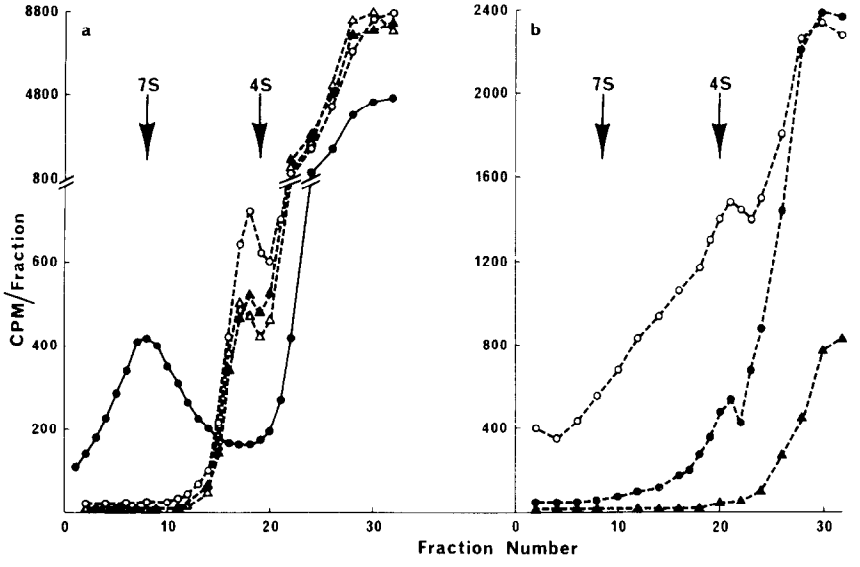

Fig. 1. Sucrose density gradient centrifugation of cytosol from lungs preincubated at 37°C in the absence of hormone and of cytosol fraction precipitated with ammonium sulfate at 33% saturation. (a) Cytosol was prepared from lungs preincubated for 2 h at 37°C in the absence of hormone. Aliquots of the cytosol were incubated for 2 h at 0°C with 1×10^{-8} M [^{3}H]-dexamethasone (●——●), 1×10^{-8} M [^{3}H]-cortisol (○---○) (similar results were obtained with a saturating concentration of labeled cortisol), or with 1×10^{-8} M [^{3}H]-cortisol plus 1×10^{-6} M dexamethasone (▲----▲). Another aliquot of cytosol was first heated for 30 min at 37°C and then incubated as above with 1×10^{-8} M [^{3}H]-cortisol (△----△). The samples (0.2 ml) were layered on 5–20% sucrose gradients in 0.01 M Tris–HCl buffer, pH 7.6, containing 0.0015 M Na_2EDTA (Tris–EDTA) and centrifuged at 290,000 *g* for 16 h at 2°C. (b) Aliquots of cytosol were incubated for 2 h at 0°C with 1×10^{-8} M [^{3}H]-cortisol alone (●----●, ○----○) or together with 1×10^{-6} M dexamethasone (▲----▲) and precipitated with ammonium sulfate at 33% saturation. The precipitate was dissolved in Tris–EDTA buffer, centrifuged at 70,000 *g* for 15 min, and the supernatant was desalted by passage through Sephadex G-50 and centrifuged in 5–20% sucrose gradients prepared in Tris–EDTA buffer in the presence (○---○) or absence (●---●, ▲---▲) of 10% glycerol. Centrifugation was carried out as in (a).

rose gradients. Only small amounts of [^{3}H]-cortisol remain associated with proteins sedimenting at about 4 S while the major fraction of hormone does not appear to be bound to macromolecules (Fig. 1b). Again, 7 S sedimenting [^{3}H]-cortisol-protein complexes were not detected. The presence of 10% glycerol in the gradients results in partial stabilization of the complexes but they spread over the entire gradient with no resolved peaks (Fig. 1b).

Evidence for the presence of 4 S and 7 S proteins in lung cytosol with a binding affinity for both cortisol and dexamethasone. Binding of [^{3}H]-dexamethasone to 4 S proteins was not detected following centrifugation of [^{3}H]-dexamethasone–cytosol mixture in sucrose gradients, although non-labeled dexamethasone inhibited partially the formation of [^{3}H]-cortisol-protein complexes (Fig. 1a). These observations suggested that lung cytosol contains 4 S proteins having a binding affinity for both steroids but forming more stable complexes with cortisol. This suggested that it could be possible to demonstrate the formation of 4 S [^{3}H]-dexamethasone-protein complexes by first isolating 4 S cortisol-protein complexes and then exchanging bound cortisol with [^{3}H]-dexamethasone. Such as experiment is shown in Fig. 2. Cytosol was incubated for 2 h at 0°C with a saturating concentration (1×10^{-7} M) of non-labeled cortisol and centrifuged in sucrose gradients prepared in buffer containing the same concentration of the non-labeled hormone. The gradients were fractionated, each fraction was treated briefly with charcoal to remove unbound cortisol, incubated for 2 h at 0°C with [^{3}H]-dexamethasone in the presence or absence of non-labeled dexamethasone and specifically bound [^{3}H]-dexamethasone was estimated by gel filtration. Under these conditions [^{3}H]-dexamethasone forms complexes sedimenting predominantly at 4 S while only minute amounts of complexes are observed in the 7 S region of the gradient.

To explain the failure to detect significant amounts of 7 S [^{3}H]-dexamethasone-protein complexes under the experimental conditions described in Fig. 2 the following possibilities were considered. (a) If cortisol does not form 7 S complexes with cytosol proteins as the observations in Fig. 1a seem to indicate, the 7 S proteins may lose their capacity to bind [^{3}H]-dexamethasone after prolonged centrifugation in sucrose gradients since unoccupied receptors are much less stable than receptors complexed with hormone[1]. (b) Despite the absence of a 7 S [^{3}H]-cortisol-protein peak in Fig. 1a, such complexes may be formed and subsequently dissociated during centrifugation. How-

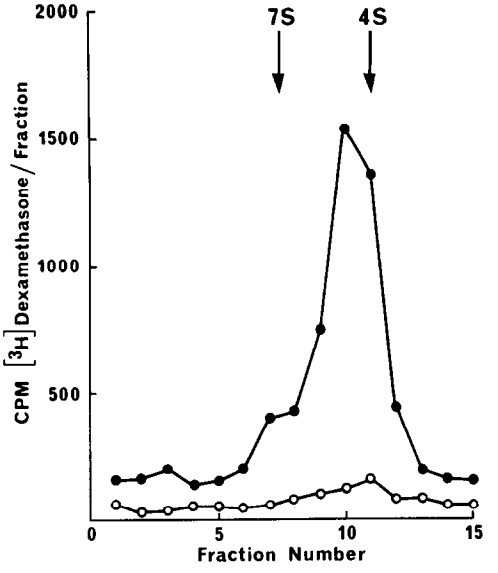

Fig. 2. Specific binding of [^{3}H]-dexamethasone to cytosol fractions obtained after centrifugation in sucrose gradients in the presence of cortisol. Cytosol was incubated for 2 h at 0°C with 1×10^{-7} M of non-labeled cortisol and centrifuged for 7 h at 290,000 *g* in sucrose gradients prepared in Tris–EDTA buffer containing the same concentration of the hormone. Following fractionation, each gradient fraction was treated with charcoal to remove excess cortisol and incubated for 2 h at 0°C with 2×10^{-8} M [^{3}H]-dexamethasone alone (●——●) or together with 2×10^{-6} M dexamethasone (○——○). Specific binding of [^{3}H]-dexamethasone was then estimated by gel filtration.

ever, the presence of excess cortisol in the studies shown in Fig. 2 may stabilize the 7 S cortisol-protein complexes[1]. Thus the inability of [^{3}H]-dexamethasone to bind to cytosol fractions in the 7 S region of the gradient (Fig. 2) may be due to a slow rate of exchange of cortisol bound to 7 S proteins with [^{3}H]-dexamethasone at 0°C. Alternatively, the interaction of cortisol with 7 S proteins may induce conformational changes resulting in alteration of their aggregation properties in solutions of low ionic strength. This could lead to a change in the sedimentation coefficient of the cortisol-protein complexes from 7 S to 4 S.

To test the above possibilities, cytosol was incubated for 2 h at 0°C with 2×10^{-8} M [^{3}H]-cortisol and centrifuged in sucrose gradients prepared in buffer containing the same concentration of the labeled hormone. In parallel runs an excess nonlabeled cortisol or dexamethasone was also added in both cytosol and sucrose solutions used to prepare the gradients. After fractionation, specific binding of [^{3}H]-cortisol in each gradient fraction was measured by the charcoal assay and by gel filtration. Neither of these methods detected specific binding of [^{3}H]-cortisol to cytosol fractions in the 7 S region of the gradient. The only complexes observed sedimented at 4 S and their formation was completely inhibited by cortisol and partially by dexamethasone (not shown). Thus if 7 S [^{3}H]-cortisol-protein complexes were present after centrifugation of the gradients, they could not be detected by the charcoal assay or by gel filtration. Since the data shown in Table 1 also indicate that these methods do not measure quantitatively cortisol-receptor complexes, the interaction of [^{3}H]-cortisol with cytosol was examined by a third method. In the latter method we took advantage of an earlier observation that specific uptake of cortisol by isolated lung nuclei requires the presence of cytosol and apparently involves transfer of the cytoplasmic cortisol-receptor complexes into the nucleus[4]. After fractionation of cytosol in sucrose gradients by the procedure outlined above, each gradient fraction was incubated with purified lung nuclei at 25°C. The nuclei were reisolated by centrifugation, washed, and assayed for specifically bound [^{3}H]-cortisol[4]. As shown in Fig. 3, specific

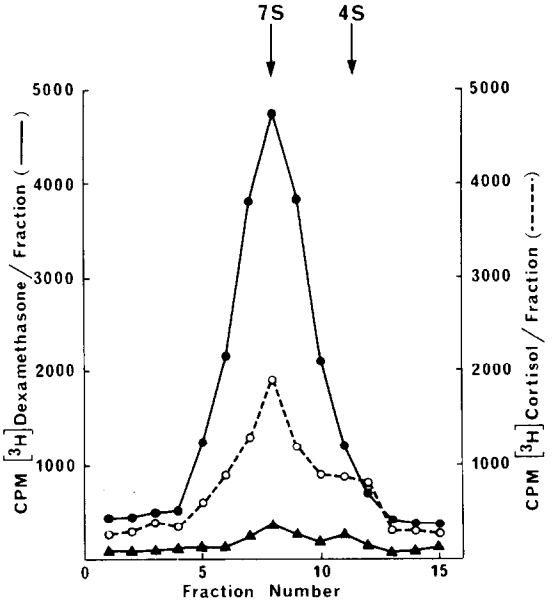

Fig. 3. Effect of cytosol fractions separated by sucrose gradient centrifugation on the specific uptake of glucocorticoids by isolated lung nuclei. Aliquots of cytosol were incubated for 2 h at 0°C with 2×10^{-8} M [^{3}H]-dexamethasone (●——●), 2×10^{-8} M [^{3}H]-cortisol (○- - - -○) or 1×10^{-7} M non-labeled cortisol (▲——▲) and layered on 5–20% sucrose gradients prepared in Tris–EDTA buffer containing the corresponding hormone at the same concentration and 0.001 M $MgCl_2$. The gradients were centrifuged for 7 h at 290,000 *g* and fractionated. In the case of samples centrifuged in the presence of cortisol (▲——▲), each gradient fraction was treated with charcoal to remove excess hormone and was further incubated for 2 h at 0°C with 2×10^{-8} M [^{3}H]-dexamethasone. In all cases, each gradient fraction was incubated for 30 min at 25°C with an equivalent amount of purified nuclei (200 μg DNA). Following the incubation, the nuclei were reisolated by centrifugation, washed three times and assayed for radioactivity and DNA [4]. In parallel experiments, nuclei were incubated with cytosol fractions containing the same labeled hormone and a large amount of the corresponding nonlabeled steroid to estimate non-specific nuclear uptake. The amount of radioactivity retained specifically by the nuclei was then plotted against the number of cytosol fraction isolated by sucrose gradient centrifugation.

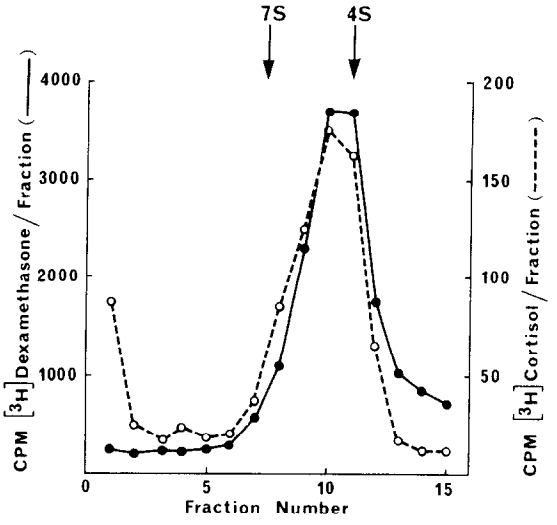

Fig. 4. Sucrose density gradient patterns of nuclear extracts from fetal rabbit lung. Fetal lung slices were incubated for 2 h at 37°C in Eagle's HeLa medium containing 2.5×10^{-8} M [^{3}H]-dexamethasone or 1×10^{-7} M [^{3}H]-cortisol. Purified nuclei were prepared and extracted with Tris–EDTA buffer containing 0.4 M KCl. Aliquots of the nuclear extracts were applied on 5–20% sucrose gradients prepared in Tris–EDTA buffer containing 2×10^{-8} M [^{3}H]-dexamethasone or [^{3}H]-cortisol and centrifuged for 7 h at 290,000 *g*. Following fractionation, bound labeled hormone in each gradient fraction was estimated by gel filtration.

nuclear uptake of [^{3}H]-cortisol was enhanced predominantly by cytosol fractions sedimenting at 7 S and only to a small extent by fractions sedimenting at 4 S. Similarly, only cytosol fractions sedimenting at 7 S enhanced the specific uptake of [^{3}H]-dexamethasone by isolated lung nuclei (Fig. 3). These results demonstrate indirectly a specific interaction of cortisol with 7 S components of lung cytosol.

Figure 3 also shows that following centrifugation of cytosol in cortisol-containing gradients, and treatment of gradient fractions by the exchange assay described in Fig. 2, specific nuclear uptake of [^{3}H]-dexamethasone is not enhanced significantly by either 7 S- or 4 S- sedimenting cytosol fractions. This supports the conclusion that the 4 S proteins do not promote nuclear transfer of glucocorticoids. It also indicates that cortisol bound to the 7 S proteins is very slowly exchanged with [^{3}H]-dexamethasone at 0°C, although significant exchange occurs with cortisol bound to 4 S proteins under the same assay conditions (Fig. 2).

Nuclear cortisol- and dexamethasone-protein complexes. [^{3}H]-Dexamethasone-protein complexes extracted from nuclei after incubation of fetal lung slices with the labeled hormone at 37°C sediment at about 4 S in the presence or absence of 0.4 M KCl. Nuclear [^{3}H]-cortisol-protein complexes, although detectable by gel filtration, dissociate rapidly and could not be characterized by sucrose density gradient centrifugation[2]. Partial stabilization of these complexes has been achieved by centrifugation in sucrose gradients containing an excess of [^{3}H]-cortisol. After fractionation, bound [^{3}H]-cortisol in each gradient fraction was estimated by gel filtration. Using this method the nuclear [^{3}H]-cortisol-protein complexes have been shown to sediment at about 4 S in gradients of low oinic strength (Fig. 4).

DISCUSSION

The results presented in this report demonstrate that fetal rabbit lung cytosol contains at least two proteins, sedimenting at 4 S and 7 S, having a binding affinity for both cortisol and dexamethasone. Stabilization of these proteins, facilitating their detection, is ligand-specific. All methods used, including charcoal adsorption, gel filtration and sucrose density gradient centrifugation, detect the 7 S protein when complexed with dexamethasone but fail to detect it when complexed with cortisol. However, formation of 7 S cortisol–protein complexes is indicated by the ability of cytosol fractions sedimenting at 7 S to enhance specific uptake of cortisol by isolated lung nuclei. Conversely, the 4 S protein is detected when complexed with cortisol but not with dexamethasone. Fractionation of cytosol in cortisol-containing sucrose gradients stabilizes the 4 S protein so that its interaction with dexamethasone can be demonstrated by exchange of bound cortisol with [^{3}H]-dexamethasone.

The relationship between the 4 S and 7 S proteins is not clear. Since only the 7 S protein appears to promote specific nuclear uptake of both natural and synthetic glucocorticoids, it probably represents the physiologically important glucocorticoid receptor of fetal lung. The 4 S protein may be an inactive form of the same receptor or it may represent a distinct receptor acting at some extranuclear site. It is also possible that the 4 S protein may be a cortisol-metabolizing enzyme.

The properties of glucocorticoid-binding proteins described here may not be unique to the fetal lung. Specific proteins with a binding affinity for both natural and synthetic glucocorticoids but exhibiting a ligand-specific sedimentation behavior in sucrose gradients of low ionic strength have been reported to be present in cytosol fractions of a number of rat tissues such as liver[8] thymus[9] and muscle[10]. We have previously suggested[1] that the differences in the sedimentation properties of cortisol- and dexamethasone-protein complexes may be due to differences in conformational changes induced by each steroid resulting in different aggregation states of the same receptor. The present observations do not support this explanation. They rather indicate the presence of two proteins sedimenting at 4 S and 7 S which are differentially stabilized by cortisol and dexamethasone. As a result, methods most commonly used in steroid binding studies can detect only the 7 S protein with dexamethasone as ligand and only the 4 S pro-

tein with cortisol as the ligand. Since most available information indicates that the 7 S protein may be the physiologically important glucocorticoid receptor in target cells, its detection may be completely missed or underestimated when cortisol is used as tracer. On the other hand, the use of dexamethasone as a steroid ligand may prevent detection of other labile binding proteins. Thus a more complete picture of specific glucocorticoid–cell interactions may be obtained by the use of both natural and synthetic labeled glucocorticoids as complexing agents.

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