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Biochemistry

An antiserum to the rat liver glucocorticoid receptor

(immunoglobulin G/DNA-cellulose chromatography/immunoaffinity chromatography/antibodies)

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ABSTRACT **A rabbit immunized with a highly purified preparation of rat liver [^{3}H]triamcinolone–receptor complex developed antibodies to the receptor. Although precipitating reactions were not detected, complexes formed between IgG and the receptor could be detected by *Staphylococcus aureus* protein A-Sepharose and gel permeation chromatography. IgG was purified and covalently immobilized on Sepharose CL-4B; this affinity matrix adsorbed the ligand-free receptor and both activated and nonactivated forms of the [^{3}H]triamcinolone–receptor complex. Rat liver cytosol proteins adsorbed by control and immune immunoglobulin-Sepharoses were eluted with 0.1 M acetic acid and analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. A protein with molecular weight 78,000 was the major species eluted from immune immunoglobulin-Sepharose, and it was not present in eluates from control columns. Rat transcortin, glucocorticoid binder IB, and an estrogen-binding protein from rat liver were not adsorbed by immune IgG-Sepharose. Mouse and hamster liver glucocorticoid receptors showed only limited adsorption. Thus, the antiserum does not crossreact with other major glucocorticoid-binding proteins and demonstrates species specificity.**

Glucocorticoids bind to protein receptors in the cytosol of target cells. Radioactive steroids have been used to define the properties of the receptor and to trace its subcellular distribution. However, the glucocorticoid-binding site can be inactivated during purification (1, 2). Mutant cells resistant to the cytolytic actions of glucocorticoids often lack detectable glucocorticoid binding (3, 4). Furthermore, the receptor may be covalently modified during the process of nuclear uptake (5). In these situations, methods are needed for direct detection and isolation of the receptor. Antibodies to the glucocorticoid receptor would greatly aid the analysis of such altered receptors.

This report describes the properties of an antiserum produced to a highly purified preparation of rat liver [^{3}H]triamcinolone–receptor complex. IgG was purified from this serum and covalently immobilized on Sepharose CL-4B. The IgG-Sepharose is an effective and specific adsorbent of the rat liver glucocorticoid receptor and does not react with other rat glucocorticoid-binding proteins such as transcortin. In addition, this antiserum demonstrates species specificity.

MATERIALS AND METHODS

Cytosol Preparation. Adrenalectomized male and oophorectomized female Sprague–Dawley rats were obtained from Taconic Farms (Germantown, PA). Male mice (C57BL/6) were obtained from Charles River (Wilmington, MA) and male Syrian golden hamsters (LaK:LVG/SYR) were obtained from Lakeview Hamster Colony (Newfield, NJ). Livers were removed from these animals and cytosols were prepared by using previously described methods (6). The standard buffer (HEDG) used for these experiments contained 10 mM Hepes (pH 7.6), 1 mM EDTA, 0.5 mM dithiothreitol, and 10% (vol/vol) glycerol; NaCl was added at concentrations indicated in text. Radioactive and unlabeled steroids were added to homogenates before the high-speed centrifugation step (100,000 × *g*, 1 hr). [^{3}H]Triamcinolone acetonide (20 Ci/mmol), [^{3}H]estradiol (110 Ci/mmol), and [^{3}H]corticosterone (53 Ci/mmol) were obtained from New England Nuclear (1 Ci = 3.7×10^{10} becquerels).

Immunization Protocol. The activated form of the [^{3}H]-triamcinolone–receptor complex was purified approximately 10,000-fold (to specific activity of 6500 pmol of [^{3}H]triamcinolone per mg of protein) by using previously described conditions for two-stage DNA-cellulose chromatography (6). The purified receptor preparation (obtained from 20 ml of crude liver cytosol) in 3–5 ml of HEDG buffer containing 0.45 M NaCl was mixed with an equal volume of complete Freund's adjuvant (Calbiochem, Irvine, CA). The emulsified material was injected at multiple sites into the gluteal muscles of two male New Zealand rabbits. With freshly prepared antigen, injections were repeated 2 and 10 weeks after the initial injection. Each rabbit received approximately 100 pmol of [^{3}H]triamcinolone–receptor complex per injection. If one assumes a molecular weight of 80,000 and a single steroid binding site per receptor, each rabbit received approximately 8 μg of glucocorticoid receptor per injection. Blood was drawn from a marginal ear vein; serum was separated and stored at −20°C.

Immunoaffinity Chromatography. IgG was purified from serum by the method of Goding (7), using staphylococcal protein A-Sepharose (obtained from Pharmacia). The 0.1 M acetic acid eluate containing purified IgG was dialyzed against 0.5 M NaCl/0.1 M $NaHCO_3$, pH 9.0 (coupling buffer) for 18 hr at 4°C. CNBr-activated Sepharose (1 g) obtained from Pharmacia was swollen and washed in 1 mM HCl and then washed with coupling buffer. Three milligrams of purified IgG in 5 ml of coupling buffer was mixed with the activated Sepharose and the suspension was rotated end-over-end for 2 hr at 20°C. Unbound protein was removed by washing with coupling buffer, and remaining active groups were allowed to react with 0.2 M glycine, pH 8.5, for 2 hr. The gel was washed with three cycles of 0.1 M acetic acid followed by coupling buffer. The gel was then washed with HEDG buffer containing 0.5 M NaCl and stored in this buffer in the presence of 0.02% sodium azide. As judged by measurement of protein in supernatants of the coupling mixture, 80–85% of the IgG was coupled by this procedure. The gel was equilibrated in HEDG buffer containing 0.5 M NaCl and then packed into a column (1.0 cm inside diameter) with a bed volume of 3 ml. Cytosol samples were adjusted to NaCl concentration of 0.5 M by addition of HEDG buffer containing 2.5 M NaCl. Samples (5–20 ml) were applied to the column by gravity flow. The column was then

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Abbreviations: triamcinolone, 9α-fluoro-11β,16α,17,21-tetrahydroxy-pregna-1,4-diene-3,20-dione; HEDG buffer, Hepes/EDTA/dithiothreitol/glycerol buffer.

washed with 50 ml of HEDG buffer (0.5 M NaCl). The column was eluted with 10 ml of 0.1 M acetic acid to remove bound protein and radioactivity. The column was washed with HEDG buffer containing 100 mM Hepes and then with HEDG (0.5 M NaCl). Immune IgG-Sepharose for the experiments described in this communication was prepared from serum obtained 3 months after the final injection of the rabbit.

Other Chromatographic Methods. Gel permeation chromatography was done with Sephacryl S-300 superfine (Pharmacia). The gel was equilibrated in HEDG buffer (0.5 M NaCl), and the columns (0.9 cm inside diameter × 50 cm) were prepared. The column was calibrated with proteins of known Stokes radius (obtained from Pharmacia). Cytosol samples were adjusted when necessary to 0.5 M NaCl and then applied to the column and chromatographed by descending flow (5 ml/hr).

Protein A-Sepharose (Pharmacia) was equilibrated in HEDG buffer containing 0.1 M NaCl. Columns (0.7 cm inside diameter) were prepared containing 0.5-ml bed volume of protein A-Sepharose. Samples were applied to the column, and the columns were then washed with 10 ml of HEDG containing 0.1 M NaCl. [^{3}H]Triamcinolone and protein bound to the columns were eluted with 2 ml of 0.1 M acetic acid. The eluted samples were neutralized with NaOH. Radioactivities were measured in Aquasol (New England Nuclear, Boston, MA). Tritium in this and other procedures was measured by using a Tracor Analytic Mark III spectrometer; counting efficiency was 40–50%.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gels (7.5% acrylamide/0.002% *N,N'*-methylenebisacrylamide) containing 0.1% sodium dodecyl sulfate were prepared by the method of Laemmli (8), omitting the stacking gel. Proteins were precipitated by 10% trichloroacetic acid at 4°C for 2 hr. The precipitates were washed with ethanol, dried, and then dissolved in 0.075 M Tris·HCl (pH 9.0), containing 1% sodium dodecyl sulfate/1% dithiothreitol/10% (vol/vol) glycerol. Samples were heated for 1 min at 100°C. Electrophoresis was performed in a slab gel apparatus (Bio-Rad). Gels were stained for 1 hr in 50% methanol/7% acetic acid (vol/vol) containing 0.02% Coomassie blue G250. The gels were then destained in 5% methanol/7% acetic acid. Molecular weight standards were obtained from Pharmacia.

RESULTS

Detection of Antibodies to the Rat Liver Glucocorticoid Receptor. Sera from the immunized rabbits did not cause precipitation of the [^{3}H]triamcinolone–receptor complex and did not produce precipitin bands (Ouchterlony immunodiffusion) against rat liver cytosol. Two methods were used to detect nonprecipitating immune complexes. Staphylococcal protein A was used to detect the formation of complexes between the glucocorticoid receptor and IgG molecules (7). Gel permeation chromatography was also used to demonstrate the formation of such complexes.

Protein A interacts with the Fc portion of the IgG molecule and does not affect the antigen binding sites. IgG–antigen complexes are therefore adsorbed by protein A-Sepharose; IgG and antigen can be eluted by dilute acids or high concentrations of chaotropic salts (7). In the experiment shown in Table 1, serum and cytosol containing the [^{3}H]triamcinolone–receptor complex were mixed and incubated for 2 hr at 4°C. The sample was then chromatographed on a small column of protein A-Sepharose and [^{3}H]triamcinolone remaining on the column was eluted with 0.1 M acetic acid. Under the conditions of this experiment, immune serum from rabbit R-100 (obtained 3 months after the last immunization) resulted in retention of approximately 35% of the [^{3}H]triamcinolone–receptor complex.

Table 1. Effect of serum on binding of [^{3}H]triamcinolone–receptor complex to protein A-Sepharose

Serum	Total ^{3}H bound, dpm
R-519 nonimmune	2,190
R-100 preimmune	2,470
R-100 immune (3 mo)	52,200
(5 mo)	41,200
(10 mo)	29,830
R-100 immune (3 mo) 1:5	13,100
1:10	3,300
1:50	3,400

Cytosol was prepared from rat liver in HEDG (0.1 M NaCl) containing 50 nM [^{3}H]triamcinolone. Cytosol (0.50 ml) and serum (0.050 ml) were mixed and incubated for 2 hr at 4°C and then chromatographed on a column of protein A-Sepharose. Serum dilutions were made in HEDG (0.1 M NaCl). The [^{3}H]triamcinolone–receptor complex content was 161,000 dpm/0.5 ml as determined by gel permeation chromatography on Sephacryl S-300.

In other experiments, equivalence (defined as adsorption of >80% of the [^{3}H]triamcinolone–receptor complex) was reached by using higher ratios of antiserum to cytosol. Sera obtained from later times resulted in decreasing amounts of [^{3}H]triamcinolone retention. Preimmune serum from this rabbit and control sera from other rabbits did not result in retention of [^{3}H]triamcinolone. Protein A was required, because no adsorption of the receptor complex took place to Sepharose or glycine-blocked CNBr-activated Sepharose (not shown).

Corroborative evidence for the formation of antibody–receptor complex was provided by gel permeation chromatography. Chromatography on Sephacryl S-300 was done in HEDG buffer containing 0.5 M NaCl. The [^{3}H]triamcinolone–receptor complex has a Stokes radius of approximately 50–55 Å; this value is similar to that obtained by Wrange and Gustafsson (9) for the [^{3}H]triamcinolone–receptor complex from rat liver. Incubation of immune serum and cytosol for 2 hr at 4°C resulted in a shift in the elution profile of the [^{3}H]-triamcinolone–receptor complex (Fig. 1). As would be expected on the basis of the experiment with protein A-Sepharose (Table 1), some of the [^{3}H]triamcinolone–receptor complex was not affected by the quantity of serum used in this experiment and eluted at the normal position.

Immunoaffinity Chromatography of the Rat Liver Glucocorticoid Receptor. The previous experiments were consistent with the hypothesis that immune serum contained IgG that formed a complex with the rat liver glucocorticoid receptor. In order to get direct evidence for such antibodies, IgG was purified to homogeneity from immune and nonimmune sera and covalently immobilized on CNBr-activated Sepharose CL-4B. As shown in Fig. 2, immune IgG-Sepharose adsorbed 80% of the [^{3}H]triamcinolone–receptor complex contained in 7 ml of crude cytosol. The unadsorbed [^{3}H]triamcinolone–receptor complex eluted in the normal position (50–55 Å) from the Sephacryl S-300 column. Control experiments demonstrated that glycine-blocked CNBr-Sepharose and nonimmune IgG-Sepharose (Fig. 3) did not adsorb the [^{3}H]triamcinolone–receptor complex.

In order to minimize nonspecific or ionic interactions between cytosol protein and the affinity matrix, all chromatography was performed at high ionic strength (0.5 M NaCl). Under these conditions, adsorption of protein to the column was low and carrier protein had to be added to the acetic acid eluates to produce quantitative precipitation and recovery for sodium dodecyl sulfate/polyacrylamide gel electrophoresis. As shown in Fig. 4, a major protein band (M_r 78,000) was

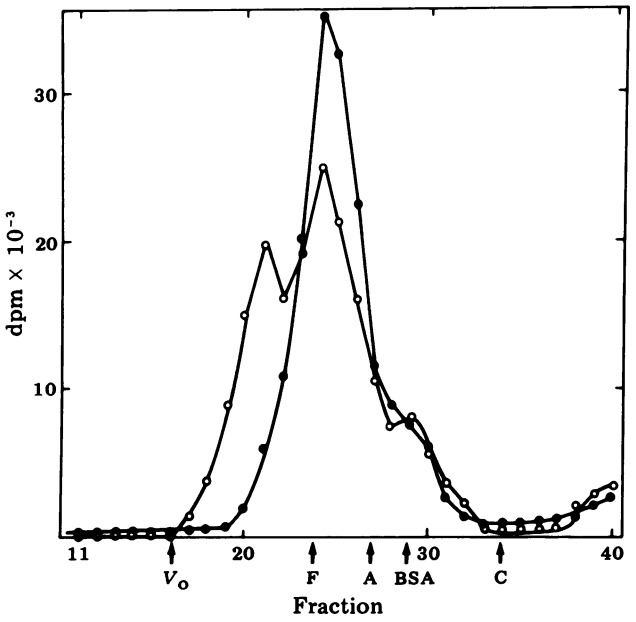

FIG. 1. Gel permeation chromatography of the rat liver [^{3}H]triamcinolone–receptor complex: Effect of immune and control sera. Cytosol was prepared in HEDG buffer containing 50 nM [^{3}H]triamcinolone. Cytosol (0.4 ml) and 0.1 ml of immune (○) or control (●) serum were mixed and incubated for 2 hr at 4°C. After the NaCl concentration was increased to 0.5 M by addition of 2.5 M NaCl in HEDG buffer, 0.4 ml was chromatographed on a column of Sephacryl S-300. In this and all subsequent figures, fractions (0.8 ml) were collected and radioactivity was determined (expressed as total dpm per fraction). V_o, void volume determined with blue dextran; F, ferritin (Stokes radius 65 Å); A, aldolase (51 Å); BSA, bovine serum albumin (37 Å); C, cytochrome *c* (17 Å).

present in eluates from the immune column but was not detected in eluates from the nonimmune IgG-Sepharose column. Densitometric scans of the gels and comparison with a known quantity of albumin indicate that the M_r 78,000 band may account for a total of 6.5 μg of protein from the 10 ml of cytosol (250 mg of protein) applied to the column. This amount of

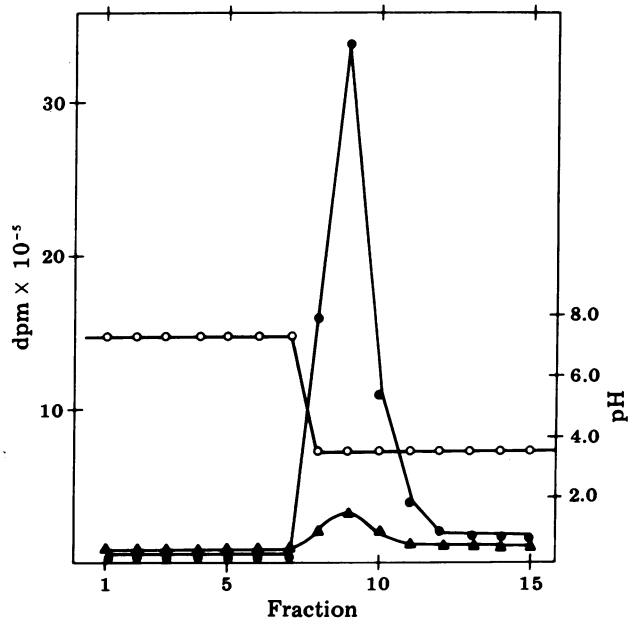

FIG. 3. Chromatography of rat liver [^{3}H]triamcinolone–receptor complex on IgG-Sepharoses. Cytosol was prepared in HEDG containing 0.1 M NaCl and 50 nM [^{3}H]triamcinolone. After adjustment to 0.5 M NaCl, samples (20 ml) were applied to 3-ml bed volume columns of immune and nonimmune IgG-Sepharose. The columns were washed with 50 ml of HEDG buffer (0.5 M NaCl) and then eluted with 0.1 M acetic acid. ●, Immune IgG-Sepharose; ▲, nonimmune IgG-Sepharose; ○, pH of fractions, marking position of buffer shift. Only fractions from the 0.1 M acetic acid eluate are shown in the figure.

cytosol contained 75 pmol of [^{3}H]triamcinolone–receptor complex; assuming M_r 78,000 and a single steroid-binding site per molecule, one would expect approximately 5 μg of receptor protein in this sample.

The pI of the receptor is altered during the process of thermal "activation" of the DNA-binding site. Various studies also indicate that the receptor undergoes a conformational change when it binds to glucocorticoids. Because these conformational

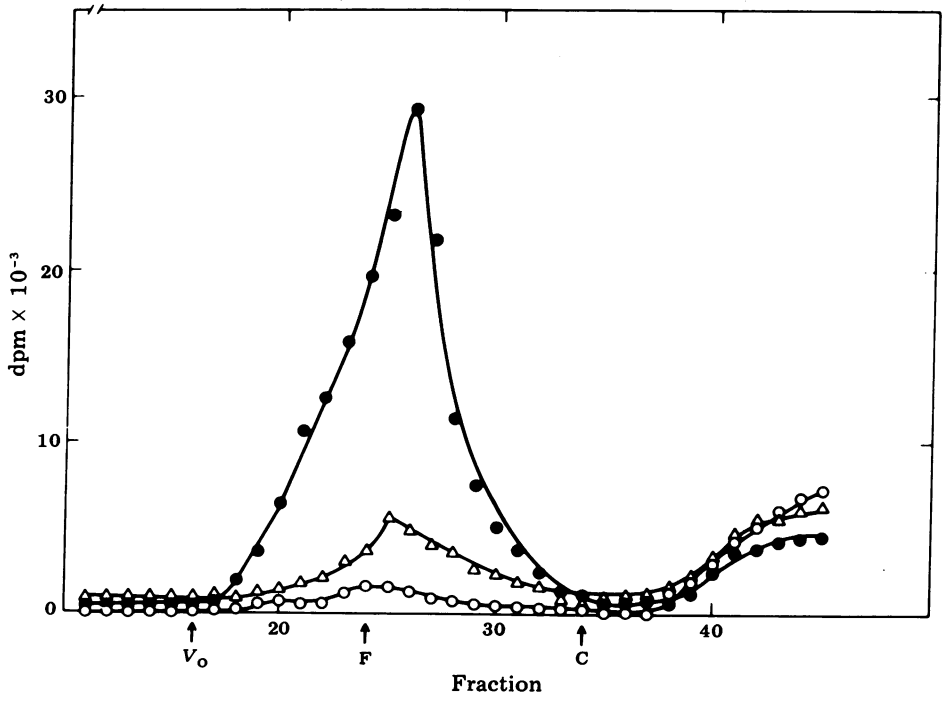

FIG. 2. Adsorption of [^{3}H]triamcinolone-receptor complex from rat liver by immune IgG-Sepharose. Cytosol was prepared in HEDG buffer containing 0.1 M NaCl and 50 nM [^{3}H]triamcinolone (●) or 50 nM [^{3}H]triamcinolone plus 5 μM triamcinolone (○). The NaCl concentration was adjusted to 0.5 M by addition of 2.5 M NaCl; 0.50-ml samples were chromatographed on columns of Sephacryl S-300. Cytosol (7 ml) labeled with 50 nM [^{3}H]triamcinolone (△) was passed through the column of immune IgS-Sepharose, and fractions were collected; 0.50 ml was chromatographed on Sephacryl S-300, V_o, blue dextran; F, ferritin (65 Å); C, cytochrome *c* (17 Å).

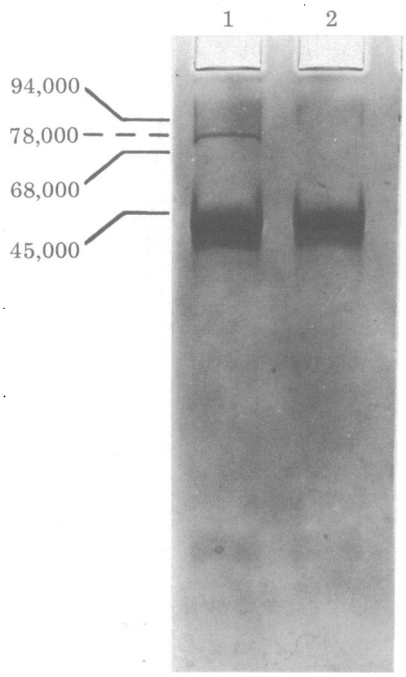

FIG. 4. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of proteins adsorbed by immune and nonimmune IgG-Sepharose. Rat liver cytosols (10 ml) were chromatographed as described for Fig. 3. Fractions from the acid eluates (2.4 ml total volume) were pooled. After addition of 300 μg of ovalbumin as carrier, proteins were precipitated by trichloroacetic acid (10% wt/vol) at 4°C for 2 hr. The precipitates were washed with ethanol, dried, and then dissolved in 300 μl of 0.75 M Tris·HCl (pH 9.0) containing 1% sodium dodecyl sulfate/10% glycerol/1% dithiothreitol. Samples were heated for 1 min at 100°C; 10-μl samples were then subjected to electrophoresis. Lane 1, eluate from immune IgG-Sepharose; lane 2, eluate from nonimmune IgG-Sepharose. Molecular weight standards: Phosphorylase A (94,000); bovine serum albumin (68,000); ovalbumin (45,000). Densitometric scans were taken to determine apparent M_r (78,000) of the band in lane 1.

changes might alter the immunoreactivity of the receptor, it was important to determine if ligand-free receptor and both activated and nonactivated forms of the [^{3}H]triamcinolone–receptor complex were adsorbed by immune IgG-Sepharose. In order to measure adsorption of the ligand-free receptor, cytosol prepared without steroid was passed through the immune IgG-Sepharose column and the fractions were then incubated with [^{3}H]triamcinolone to form the steroid–receptor complex. Gel permeation chromatography then was used to measure [^{3}H]triamcinolone–receptor complex in control (unchromatographed) and chromatographed cytosol; 15% of the control amount of receptor complex was recovered in the wash-through fractions. Although these data indicated significant adsorption of the ligand-free receptor, it was also important to measure losses related to inactivation of the steroid-binding site (2). Therefore, cytosol was chromatographed on nonimmune IgG-Sepharose; 67% of the initial steroid–receptor complex was recovered in the wash-through of this column. Thus, losses from chromatography alone did not account for the adsorption observed on the immune IgG-Sepharose column. Although the cytosol preparations used in the experiments shown in Figs. 1–4 were not heat treated, 0.5 M NaCl would be expected to convert the [^{3}H]triamcinolone–receptor complex to its activated form. However, we have also used a buffer (20 mM potassium phosphate, pH 6.8) that stabilizes the nonactivated form of the [^{3}H]triamcinolone–receptor complex. We have found that both nonactivated and heat-activated forms of the [^{3}H]triamcinolone–receptor complex were adsorbed equally well by the immune IgG-Sepharose column (10).

Table 2. Specificity of adsorption of steroid-binding proteins by immune IgG-Sepharose

Exp.	Protein	^{3}H, dpm × 10^{-4}* Applied†	Adsorbed	% adsorbed
A	Rat transcortin	197	5	2.5
B	Glucocorticoid receptor			
	Rat liver	282	219	78
	Mouse liver	68	6	9
	Hamster liver	96	17	19
C	Rat liver estradiol-binding protein	119	1	1

(Exp. A) Serum from adrenalectomized rats was diluted 1:10 in HEDG buffer containing 0.5 M NaCl and then incubated with [^{3}H]-corticosterone (75 nM) in the presence or absence of 7.5 μM corticosterone for 2 hr at 4°C. Samples (0.5 ml) were chromatographed on immune IgG-Sepharose. (Exp. B) Liver cytosols were prepared and incubated with 50 nM [^{3}H]triamcinolone in the presence or absence of 5 μM triamcinolone for 1 hr at 4°C, and samples (7 ml) were chromatographed. (Exp. C) Rat liver cytosol was incubated with 10 nM [^{3}H]estradiol in the presence or absence of 1 μM estradiol for 1 hr at 4°C, and samples (7 ml) were chromatographed.

* Tabulated values were corrected for nonspecific adsorption by subtraction of dpm adsorbed in the presence of 100-fold excess unlabeled steroid.

† Macromolecule-bound steroid determined by gel filtration.

Specificity of Immunoadsorption. Although the above experiments indicated a high degree of molecular specificity of the antibody, it was important to determine if other glucocorticoid-binding proteins and other steroid-binding proteins in liver reacted with the antibody. Transcortin, the serum binding protein for glucocorticoids, was labeled with [^{3}H]corticosterone and chromatographed on immune IgG-Sepharose. As shown in Table 2, less than 2.5% of the applied specifically bound [^{3}H]corticosterone was adsorbed by the column. Glucocorticoid binder IB also does not react with the immune IgG-Sepharose (10). Rat liver is a target organ for estrogens and contains an estrogen-binding protein that is a putative estrogen receptor (11). An estrogen-binding protein was detected (Stokes radius ≈40 Å) in rat liver cytosol, but it was not adsorbed by immune IgG-Sepharose (Table 2).

Crossreactivity with Liver Glucocorticoid Receptors from Other Rodent Species. Liver cytosols from two other rodent species were used as sources of [^{3}H]triamcinolone–receptor complex. When equal amounts of cytosol were chromatographed on the immune IgG-Sepharose column, 18% of receptor from Syrian hamster liver and 9% of receptor from mouse liver were adsorbed (Table 2), whereas 80% of rat liver receptor was adsorbed.

DISCUSSION

Antibodies to the glucocorticoid receptor are detected by three independent methods. *Staphylococcus aureus* protein A is a highly specific ligand for the major subgroups of rabbit IgG and does not react with other rabbit immunoglobulins (12). Thus, the serum-dependent adsorption of the [^{3}H]triamcinolone–receptor complex by protein A-Sepharose is strong evidence for the presence of anti-receptor antibodies. Gel permeation chromatography confirms the presence of serum components that bind the [^{3}H]triamcinolone–receptor complex. Finally, highly purified IgG was covalently immobilized on Sepharose CL-4B, and this affinity matrix adsorbs the receptor.

The receptor preparation used as antigen in these experiments was estimated to be 10–30% pure glucocorticoid receptor (6). Wrange *et al.* (13) have recently used a similar procedure

(based on DNA-cellulose chromatography of the receptor complex in its nonactivated and activated forms) and have attained purification to 85% of homogeneity. These investigators give substantial evidence that the major glucocorticoid receptor from rat liver has a M_r of 89,000 on sodium dodecyl sulfate/polyacrylamide gels. Another protein, accounting for 15% of the protein in the receptor preparation, has a M_r of 75,000. A M_r of 78,000 for the glucocorticoid receptor has been calculated from gel permeation and velocity sedimentation data (14). In contrast, Govindan and Sekeris (15) have used a derivative of 11-deoxycorticosterone for purification of the rat liver glucocorticoid receptor. These investigators isolated a protein with M_r 45,000; the conditions used for this purification appear to favor proteolysis (16), and it is possible that this protein is the 37-Å, M_r 44,000 proteolytic fragment described by Wrange and Gustafsson. Govindan and Sekeris have prepared antibodies to the purified receptor, and these antibodies clearly react with the glucocorticoid receptor in crude cytosol preparations (15).

As shown in Figs. 1 and 2, the Stokes radius of the glucocorticoid receptor is 50–55 Å. This represents the nonaggregated form of the receptor (9); hence, it is likely that the antibodies react directly with the receptor and not with proteins in an aggregate. Because the antigen used was not pure receptor, it is possible that some antibodies are directed against proteins other than the glucocorticoid receptor. The M_r 78,000 protein band demonstrated in Fig. 4 may contain the denatured glucocorticoid receptor; this protein is similar in size to the minor component in the receptor preparations of Wrange *et al.* (13). As noted by Wrange *et al.* (13), this component may be a proteolytic product of the major M_r 89,000 form of the receptor, but this possibility has to be proved rigorously by further experimentation.

It is informative to compare the properties of the antiserum described in the current report with the antiserum to the estrogen receptor prepared by Greene *et al.* (17). These investigators also used partially purified receptor preparation as antigen. The resultant antibodies did not cause precipitation of the estrogen receptor. Although the initial antibody preparation reacted with estrogen receptor from several species, Greene *et al.* (18) have recently reported the production of species-specific antibodies to the estrogen receptor in cloned hybridoma cells. Fox (19) has found that nonspecific stimulation of the immune system can result in serum factors that form complexes with the estrogen receptor. These factors, which copurify with IgG, react only with the 5S "activated" form of the estrogen receptor complex and show no species specificity. In contrast, the antiserum described in this report reacts with the glucocorticoid receptor in its nonactivated and activated forms and demonstrates species specificity. Furthermore, we have tested sera from rabbits and mice immunized with various antigens and have not detected anti-glucocorticoid receptor antibodies.

In the current experiments, immunological crossreactivity was measured by determining the adsorption of a steroid-binding protein by the immunoaffinity column. Thus, 80% or more of an applied load of rat liver glucocorticoid receptor is adsorbed by the column. The capacity of the 3-ml immunoaffinity column is "saturated" with cytosol loads above 200 pmol of receptor (20 ml of cytosol) and the relative adsorption decreases. In the experiment shown in Table 2, equal amounts of liver cytosol from other rodents were applied to the column; the relative adsorption of [^{3}H]triamcinolone–receptor complex was greatly decreased. This finding probably indicates that only a small proportion of the antibodies recognize antigenic determinants present on mouse and hamster liver receptors. By using immunoadsorption techniques, it may be possible to purify antibodies that do not crossreact with mouse glucocorticoid receptor. The species specificity is a unique and interesting property of the antiserum described in this report. Potential uses for this particular property of the antiserum include study of the expression of receptor in interspecies somatic cell hybrids and identification of the chromosome that carries the structural gene for the glucocorticoid receptor.

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