Glucocorticoid Receptor Phosphorylation, Transformation, and DNA Binding*  

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Glucocorticoid receptors were isolated by immunoadsorption from cytosol of L cells that were cultured for 18 h in the presence of \(^{32}P\)orthophosphate, and the phosphorylation state of the receptor was examined before and after transformation to the DNA-binding state. Temperature-mediated transformation of the glucocorticoid receptor under cell-free conditions results in no change in receptor size or degree of phosphorylation. When cytosol containing transformed receptors is incubated with DNA-cellulose, 30–50% of the receptors are able to bind to DNA and the remainder do not bind to DNA. Both the heated receptors that bind to DNA and the receptors that do not bind to DNA are phosphorylated to the same degree.

When intact cells containing \(^{32}P\)-labeled receptors are incubated for 2 h at 0 °C with triamcinolone acetate and then for 20 min at 37 °C in the presence of the hormone, 80% of the receptor becomes tightly associated with the nucleus in a manner that is both temperature-dependent and ligand-dependent. Approximately 80% of the nuclear-bound receptor is extracted with 0.4 M NaCl. Both the cytosolic receptor from cells incubated at 0 °C and the salt-extracted nuclear receptor from cells incubated at 37 °C have been resolved by immunoadsorption to protein A-Sepharose with the BuGR1 monoclonal antibody and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by immunoblotting and autoradiography of the immunoblots. In addition, direct measurements of the amounts of \(^{32}P\) contained per unit of receptor protein were performed for receptors transformed both in the intact cell and in cell-free lysates. The results demonstrate that the untransformed receptor and the nuclear-bound transformed receptor are labeled with \(^{32}P\) to the same extent.

From their early studies on the relationship between glucocorticoid binding capacity and ATP content in intact rat thymocytes (1, 2), Munck and his co-workers (3) proposed that the glucocorticoid receptor might be a phosphoprotein and that the steroid binding activity of the receptor and its recovery in cytosol or nuclear preparations after cell rupture might be regulated by a phosphorylation-dephosphorylation mechanism. Subsequently, it was demonstrated that glucocorticoid receptors in intact cells can be labeled with \(^{32}P\), thus establishing that the receptor is a naturally phosphorylated protein (4–7). It is also clear that the progesterone receptor is phosphorylated in intact chick oviduct cells (8–10), suggesting that phosphorylation may be a common feature of steroid receptors.

Although there is some indirect evidence suggesting that phosphorylation may play a role in determining glucocorticoid binding activity in cytosol preparations (11–14), there is no direct evidence that the phosphorylation state of either glucocorticoid or progesterone receptors affects their steroid binding activity. Recently, Aurrichio and his co-workers (15) have published that estrogen receptors purified from calf uterus must be phosphorylated on a tyrosine residue in order to bind steroid. It may prove to be very difficult to determine the relationship between the phosphorylation state of glucocorticoid or progesterone receptors and their steroid binding capacity because the large M, (300,000–350,000) untransformed 8 S form of these receptors exists as a heteromeric complex containing at least two different phosphoproteins (5, 7, 9, 16). In each case, a phosphoprotein that contains the steroid binding site is associated with a 90-kDa nonsteroid-binding phosphoprotein that is clearly different from the receptor. This receptor-associated phosphoprotein has been identified as the 90-kDa heat shock protein (17–19).

It has been shown that during transformation to the DNA-binding state the 90-kDa heat shock protein dissociates from the receptor (7, 17). This dissociation is accompanied by a reduction in the size of the receptor from an apparent molecular mass of 300–350 kDa to about 100 kDa (20–22). On the basis of indirect observations made in both intact cells and in cytosols, several laboratories have suggested that transformation of the receptor to the DNA-binding form may require dephosphorylation (14, 23–25). There are examples where the DNA-binding activity of DNA-binding proteins of prokaryotes is affected by phosphorylation (e.g. Refs. 26 and 27), and the techniques are now available for asking whether the DNA-binding activity of the transformed 100-kDa glucocorticoid receptor is regulated in this manner.

The only studies that have examined the relationship between steroid receptor phosphorylation and DNA binding have been performed with progesterone receptors, and they have yielded seemingly conflicting results. Logeat et al. (28) isolated progesterone receptors located in cytosolic and nu...
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Clear fractions prepared from rabbit uterine slices incubated with \(^{32}\text{P}\). They concluded that the receptor recovered from the nucleus had undergone a hormone-dependent increase in phosphorylation. In contrast, Garcia et al. (10) were unable to detect \(^{32}\text{P}\) in progesterone receptor recovered from the nuclear fraction of chick oviduct cells cultured in the presence of \(^{32}\text{P}\)orthophosphate, although radioactivity could be readily detected in cytosolic receptor. In this paper, we examine the phosphorylation state of L cell glucocorticoid receptors that have been transformed and bound to DNA under cell-free conditions and receptors recovered from cytosolic and nuclear fractions after ligand-dependent transformation in intact cells. Since the submission of this work, Mendel et al. (29) have published that cytosolic glucocorticoid-receptor complexes are not dephosphorylated during transformation in intact WEHI-7 cells.

**EXPERIMENTAL PROCEDURES**

**RESULTS AND DISCUSSION**

*Separation of the 90-kDa Phosphoprotein from the 100-kDa Phosphoprotein—* The experiment of Fig. 1 (Miniprint) is presented to show the relationship between the \([\text{H}]\) dexamethasone\(^{-}\)mesylate-labeled and the \(^{32}\text{P}\)-labeled components of L cell cytosol that are adsorbed to protein A-Sepharose in an immune-specific manner by antireceptor serum. As recently reported (5, 7), the predominant species specifically labeled by \([\text{H}]\)dexamethasone mesylate in mouse cytosol migrates at 100 kDa (lane E), whereas two \(^{32}\text{P}\)-labeled species migrating in this region are selectively adsorbed (lane F), one migrating at 100 kDa and another at 90 kDa.

We have reported (17) that molybdate, which stabilizes steroid receptors in their 300–350-kDa untransformed state (20–22), must be present during washing of the protein A-Sepharose pellet for the 90-kDa receptor-associated phosphoprotein to be visualized. In our published work, we have routinely washed the protein A-Sepharose pellet with buffer containing sodium chloride and Triton X-100. If the salt washes are omitted, the 90-kDa protein is retained in the immunoadsorbed pellet even when molybdate is not present (Fig. 2, lane 1). Washing the immunoadsorbed pellet with salt in the absence of molybdate results in loss of the 90-kDa phosphoprotein band (lane 2), and the salt-induced loss is inhibited by molybdate (lane 3). Thus, molybdate is only required to see the 90-kDa phosphoprotein when the receptor complex is exposed to conditions of high salt which promote dissociation. As shown in lane 4 of Fig. 2, receptors that have been transformed by warming prior to immunoadsorption and then washed in the presence of molybdate have lost the 90-kDa protein. Lane 5 shows that the AC88 monoclonal antibody against the 90-kDa heat shock protein causes the immunoadsorption of a 90-kDa phosphoprotein band that migrates in position identical to that of the receptor-associated phosphoprotein. Because we are interested in defining any possible changes in phosphorylation of the steroid-binding protein related to its transformation and DNA binding, in all subsequently reported experiments immunoadsorbed pellets were washed with salt in the absence of molybdate to eliminate the possibility of confusing dissociation of the 90-kDa protein with a dephosphorylation of the receptor.

Since salt above isotonic concentrations is known to transform either steroid-bound or unbound receptors to the DNA-binding state (39), we considered the possibility that salt treatment of the immunoadsorbed receptor could of itself result in changes in the phosphorylation state of the receptor protein. The ratio of \(^{32}\text{P}\) label to receptor protein was determined for untransformed and transformed receptors that were immunoadsorbed to protein A-Sepharose and washed with various combinations of salt and molybdate. As shown in Table II (Miniprint), the salt washes do not change receptor phosphate levels.

It can be noted in Fig. 1 (lane F) that the polyclonal antiserum causes the immunoadsorption of two additional \(^{32}\text{P}\)-labeled bands at 170–180 kDa and 35 kDa which are not present when cytosol is incubated with preimmune serum (lane G). The 35-kDa band is not present when the receptor is immunoadsorbed with the BuGR1 monoclonal antibody, and this band does not appear to be related to the receptor. As shown in Fig. 3 (Miniprint), the band at Mr 170 kDa is adsorbed in an immune-specific manner by the BuGR1 monoclonal antibody, and this band does not appear to be related to the receptor.
antibody or that the epitope is not recognized under the denaturing immunoblots conditions. It could be argued that the 170-kDa phosphoprotein is another cytosolic component that associates with the 100-kDa steroid binding protein. However, the 170-kDa phosphorylated band is recovered regardless of whether the receptor is transformed or untransformed (see Figs. 2 and 5). It is also recovered after immunoadsorption of $^{32}$P-labeled cytosol prepared from L cells incubated with glucocorticoid at 37 °C (see Fig. 8). This cytosol is largely depleted of receptor because it has associated with the cell nucleus. Thus, it is unlikely that the 170-kDa band represents a receptor-associated protein. A final possibility is that, as this protein is not recovered after immunoadsorption of DNA-cellulose-extracted receptor (Fig. 7), it is a major cytosolic contaminant that is incompletely removed under the wash conditions employed.

Evidence That Dephosphorylation of the 100-kDa Steroid-binding Protein Is Not Required for Transformation—If the receptor undergoes any change in phosphorylation state during transformation under cell-free conditions, the transformed state of the receptor could have a slightly different $M_r$ from the untransformed receptor, as has been shown for rabbit and chicken progesterone receptors (28, 40). We have carefully looked for a change in receptor size by labeling it in a site-specific manner with $[^3H]$dexamethasone mesylate and then transforming it to the DNA binding state. As shown in Table III (Miniprint), L cell receptors that are labeled with $[^3H]$dexamethasone mesylate can be transformed by heating. We consistently observe, however, that a smaller percentage of L cell receptors labeled with $[^3H]$dexamethasone mesylate bind to DNA after heat transformation than receptors in the same cytosol that are bound with $[^3H]$triamcinolone acetonide. It is clear from the experiment shown in Fig. 4 (Miniprint) that $[^3H]$dexamethasone mesylate-labeled receptors that have been transformed by heating cytosol at 25 °C or by precipitation with 40% ammonium sulfate have exactly the same $M_r$ as the untransformed receptor.

We have performed several experiments in which we have examined $^{32}$P-labeled receptor before and after transformation in cytosol preparations. The results of a typical experiment are shown in Fig. 5 (Miniprint). The phosphorylated receptor band is seen migrating just above the 97-kDa marker in lanes A, C, and E. There is often a small but visible decrease in the intensity of $^{32}$P labeling of the receptor heated at 25 °C (lanes C and E) as compared to untransformed receptor maintained at 0 °C (lane A). This is accompanied by the generation of a cleavage product at about 50 kDa that is indicated by the arrow in the figure. The minor change that occurs is not related to receptor transformation as it occurs both when the receptors are bound with steroid (lane C) and when they are not (lane E). Temperature-mediated transformation of L cell receptors to the DNA-binding state is a ligand-dependent event, and any change in phosphorylation that is related to transformation should also occur in a ligand-dependent manner.

Demonstration That Phosphorylated Receptor Binds to DNA—As only a portion (30–50%) of the receptors in heated L cell cytosol binds to DNA-cellulose, it could be argued that receptors submitted to the transformation procedures used in Figs. 4 and 5 exist as a mixed population containing both phosphorylated and dephosphorylated forms and that only the dephosphorylated form is capable of binding to DNA. Again, we have examined the size of the receptor that binds to DNA by using $[^3H]$dexamethasone mesylate as the label. As shown in Fig. 6 (Miniprint) the DNA-bound receptor is the same size regardless of whether transformation was caused by salt (ammonium sulfate) at 0 °C, a method that is unlikely to favor dephosphorylation or by warming at 25 °C, a condition that may favor dephosphorylation.

We have performed several experiments in which we have examined two populations of steroid-bound heated receptors in cytosol prepared from $^{32}$P-labeled L cells. The two populations are the heated receptors that bind to DNA-cellulose and those that do not. The results of a typical experiment are shown in Fig. 7. In this experiment, $^{32}$P-labeled cytosol containing heated receptor was mixed at 0 °C with DNA-cellulose. The mixture was then centrifuged, and receptors that did not bind to DNA-cellulose were immunoadsorbed from the supernatant with the BuGR1 antibody. In other experiments with $[^3H]$triamcinolone acetonide-labeled L cell cytosol......

![Fig. 7. The DNA-binding form of the receptor is not in a dephosphorylated state.](image)

$^{32}$P-Labeled L cell cytosol (4 ml) was bound with 50 nM nonradioactive triamcinolone acetonide for 2 h on ice, and the steroid-receptor complexes were then transformed by heating at 25 °C for 1 h. The transformed receptors were allowed to bind to DNA-cellulose for 45 min at 0 °C; the DNA-cellulose suspension was centrifuged, and the supernatant containing receptor that did not bind to DNA was separated from the pellet containing DNA-bound receptors. Aliquots of supernatant (lanes A–F) were immunoadsorbed with BuGR1 monoclonal antibody (lanes B–E) or with nonimmune mouse IgG (lane A) as described under "Experimental Procedures." The DNA-bound receptor was extracted with NaCl, and the extract was divided into two aliquots. One aliquot was immunoadsorbed with BuGR1 monoclonal antibody (lane F) and the other with nonimmune mouse IgG (lane G). The proteins in all conditions were then resolved by SDS-polyacrylamide gel electrophoresis and immunoblotting with BuGR1 monoclonal antibody (Panel A) followed by direct autoradiography of the immunoblot (Panel B). Lane A, 0.3 ml of non-DNA-binding receptor incubated with nonimmune mouse IgG. Lanes B–E, 0.3 ml (B), 0.2 ml (C), 0.1 ml (D), or 0.05 ml (E) of non-DNA-binding receptor was incubated with BuGR1 monoclonal antibody. Lane F, salt-released DNA-bound receptor incubated with BuGR1. Lane G, salt-released DNA-bound receptor incubated with nonimmune mouse IgG.
sol, we have found that receptors that do not bind to DNA-cellulose during this first binding assay will not bind to DNA if they are incubated with DNA-cellulose again, even if they have been warmed a second time (data not shown). Thus, these steroid-receptor complexes are intrinsically unable to bind to DNA. After washing the DNA-cellulose pellet, receptors were released from DNA with 0.4 M NaCl and immunoadsorbed with the BuGR1 antibody. We have found that receptors released with salt are immunoadsorbed better with this antibody than receptors that have been released by pyridoxal 5'-phosphate. The immunoadsorbed material was submitted to SDS-polyacrylamide gel electrophoresis and immunoblotted using BuGR1 as the probe antibody (Fig. 7, Panel A). It is clear from the immunoblot that, within the limits of the resolution of this technique, temperature-transformed receptors that bind to DNA are the same size as receptors that do not. An autoradiogram made directly from the immunoblot is shown in Panel B of Fig. 7. The 32P-labeled band migrating in the region of the 97-kDa marker in lane F of Panel B superimposes on the receptor immunoblot shown in lane F of Panel A. Thus, the DNA-bound receptor is clearly phosphorylated. There is also a 32P-labeled band at about 50 kDa that is not visualized as a BuGR1-reactive band in the immunoblot of Panel A, and its identity is unknown.

We have directly measured the amount of 32P radioactivity in the untransformed receptor and in the DNA-binding and non-DNA-binding forms of the heated receptor. These data are presented in Table IV. Although the specific activity of 32P labeling varies with each lot of 32P cytosol, it is nonetheless clear that the extent of receptor phosphorylation as indicated by the 32P to 125I ratio does not change with transformation, and after heating, both the DNA-binding and non-DNA-binding forms of the receptor contain the same amount of phosphate.

Smith and Harmon (41) have resolved the [3H]-dexamethasone mesylate-labeled receptor from IM-9 human lymphocyte cytosol into two and possibly three isoforms using a two-dimensional gel procedure in which separation in the first dimension was achieved with nonequilibrium pH gradient electrophoresis. It was speculated that this charge heterogeneity could reflect different degrees of receptor phosphorylation. Since then, the same laboratory has reported that transformed and untransformed [3H]-dexamethasone mesylate-labeled IM-9 receptors exhibit the same pattern of charge heterogeneity, indicating that no change in the number of receptor phosphate groups occurs during transformation (42).

We have previously reported that the 32P-labeled glucocorticoid receptor of L cells could be resolved into two species using non-denaturing isoelectric focusing to achieve the first dimension (4). It subsequently became clear, however, that we were examining two isoelectric species of the 90-kDa heat shock protein that had copurified with the receptor (5). We have tried to resolve the 32P-labeled immunoadsorbed receptor from L cells using the same technique as Smith and Harmon and have not been able to resolve the 32P signal into discrete isoelectric species. Indeed, very little of the receptor enters the gel.

There clearly must be some difference in the structure of transformed receptor that binds to DNA and receptor that does not; yet, as seen in Fig. 7 and demonstrated in Table IV, the receptors are the same size and they are both phosphorylated to an equal degree. As the heated receptor does not bind to DNA when it is reheated, it seems likely that the difference between the heated receptor that does not bind to DNA and that which does must reflect a covalent modification of the protein. Since this difference clearly does not reflect a phosphorylated versus a dephosphorylated state, the covalent modification must take another form, such as a change in receptor redox state which would not be reflected by a change in size on denaturing reducing gel electrophoresis.

Phosphorylation State of the Receptor After Transformation in Intact L Cells—Although no major changes in receptor phosphorylation have been detected with transformation and DNA binding under cell-free conditions, there is a possibility that a change in phosphorylation state occurs in intact cells and is required for association of the receptor with the cell nucleus. Accordingly, we have set up an intact cell system that permits examination of the 32P-labeled receptors before and after they have undergone the ligand-dependent and temperature-dependent change that causes them to be recovered in tight association with the cell nucleus.

The experiments of Table V (Miniprint) demonstrate that incubation of intact L cell suspensions for 20 min at 37°C results in the majority of the receptor being recovered in the "nuclear" pellet after cell rupture and low speed centrifugation. If cells are incubated for 10 min at 37°C, about 40% of the receptor is located in the nuclear fraction, and maximum nuclear association is achieved by 20 min. The slow time course reflects the fact that it takes several minutes for the cells and medium to warm up. The change in affinity of the receptor for the cell nucleus is clearly both temperature-dependent and ligand-dependent (Table V). As shown in Table VI (Miniprint) about 80% of the receptor that is tightly associated with the nucleus is extracted by salt. More than half of the receptor that remains associated with the nuclear fraction after salt extraction is released by digestion with micrococcal endonuclease. With our current techniques, we can examine the phosphorylation state of only the salt-extractable nuclear receptor.

In the experiment shown in Fig. 8, receptors were isolated by immunoadsorption from both cytosol and nuclear salt extracts of 32P-labeled L cells containing steroid-bound receptors that were incubated at 0 or 37°C for 20 min. Receptors were resolved by SDS-polyacrylamide gel electrophoresis, and a crude estimate of the relative amounts of receptor can be obtained by comparing the intensity of the immunoblots (Panel A). It can be seen that at 37°C most of the receptor is recovered from the nuclear salt extract (lane 7) and that the predominant species of cytoplasmic receptor (lanes 1 and 3) is the same size as the nuclear receptor. The phosphorylated

### Table IV

| Receptor state          | Condition | 32P | 125I | 32P/125I |
|-------------------------|-----------|-----|------|----------|
| Experiment 1            |           |     |      |          |
| Untransformed           | Total cytosol | 453 | 59,696 | 0.008    |
| Transformed             | Total cytosol | 342 | 51,165 | 0.007    |
| Transformed             | Non-DNA-bound | 153 | 21,392 | 0.007    |
| Transformed             | DNA-bound | 401 | 50,065 | 0.008    |
| Experiment 2            |           |     |      |          |
| Untransformed           | Total cytosol | 3,558 | 132,092 | 0.027    |
| Transformed             | Total cytosol | 3,159 | 136,221 | 0.023    |
| Transformed             | Non-DNA-bound | 1,677 | 67,276 | 0.025    |
| Transformed             | DNA-bound | ND* | ND | ND       |

* ND, not determined.
band shown in lane 7 of Panel B superimposes on the immunoblot of the salt-extracted nuclear receptor shown in lane 7 of Panel A. Thus, this experiment shows that the salt-extractable receptor that becomes located in the nucleus in a temperature- and ligand-dependent manner is phosphorylated. The extent of receptor phosphorylation was determined in similar experiments (Table VII) in which both the amount of receptor protein and receptor-specific $^{32}$P were assayed. The data presented in Table VII demonstrate that no increase or decrease in the extent of receptor phosphorylation occurs with transformation in intact cells.

In contrast to the experiment of Fig. 8 where cells were prelabeled for many hours with $^{32}$P orthophosphate and then exposed to steroid, the experiments of Logeat et al. (28), which demonstrated "hormone-dependent" increase in $^{32}$P labeling of progesterone receptor recovered from rabbit uterine cell nuclei, utilized a short-term $^{32}$P labeling protocol that might more readily identify phosphorylation of sites on the receptor that are subject to a more rapid turnover of phosphate. Accordingly, in the experiment of Fig. 9, intact L cells were incubated for 1 h at 37°C with $^{32}$P orthophosphate in the presence or absence of triamcinolone acetonide in order to detect changes in rapid turnover phosphorylation that might be missed with the long-term labeling procedure. Again, with this experimental protocol, which mimics that of Logeat et al. (28) and Sullivan et al. (40), we see no change in either receptor size or degree of phosphorylation when the receptor undergoes the ligand-dependent shift to a high affinity association with the nuclear fraction. Taken together, our results and those of Mendel et al. (29) suggest that, in contrast to progesterone receptors, transformation of glucocorticoid receptors does not result in the exposure of a new phosphorylation site.

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291, 000; beta- galactosidase, 110, 000; phosphorylase b, 94, 000; trypsinogen 
alpha- 1, 66, 000; and ovalbumin, 45, 000. For RNA gel electrophoresis, 
according to the manufacturer's directions before drying.

Phosphorylation and dephosphorylation of the glucocorticoid receptor were monitored by SDS-PAGE. The receptor was isolated from each sample as described in the manufacturer's instructions for the immunochemically 

Gel filtration of phosphorylated and dephosphorylated receptor was achieved by eluting the receptor from a column of Sepharose 4B at pH 7.0 in 0.1 M Tris-HCl buffer containing 1 M NaCl.

Addition of Akt to the phosphorylation reaction resulted in a significant increase in the phosphorylation of the glucocorticoid receptor. This effect was dose-dependent, with a maximum at an Akt concentration of 100 nM.

The glucocorticoid receptor was phosphorylated at serines 211 and 214, as well as at threonine 219, in response to the glucocorticoid dexamethasone.

Table II

| Addition | Specific activity (cpm/mg) |
|----------|---------------------------|
| None     | 123                      |
| Akt 10 nM| 345                      |
| Akt 100 nM| 783                     |

Table III

| Condition | Specific binding (cpm/mg) | Control binding (cpm/mg) | % of total binding |
|-----------|---------------------------|--------------------------|-------------------|
| Untransformed | 25,000                    | 2,500                    | 92                |
| Transformed | 37,200                    | 4,500                    | 88                |

Table IV

| Condition | Specific activity (cpm/mg) |
|-----------|---------------------------|
| Untransformed | 25,000                    |
| Transformed | 37,200                    |

Table V

| Condition | Specific activity (cpm/mg) |
|-----------|---------------------------|
| Untransformed | 25,000                    |
| Transformed | 37,200                    |

Table VI

| Condition | Specific activity (cpm/mg) |
|-----------|---------------------------|
| Untransformed | 25,000                    |
| Transformed | 37,200                    |
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Figure 1. Immuno-specific adsorption of L-cells glucocorticoid receptors covalently labeled with [%]desamethasone mesylate at 205. Lane A and B, 120 ul aliquots of L-cell cytosol were incubated for 3 h at 0°C with 30 pm [%]desamethasone 21-mesylate in the presence (lane A) or absence (lane B) of a 1000-fold excess of nonradioactive desamethasone. After incubation with steroid, samples were extracted with charcoal, and reacted by SDS-Polyacrylamide gel electrophoresis and autoradiography. Lane C, sample bound in the presence of competing desamethasone and incubated with hormone antiserum. Lane D and E, samples bound with [%]desamethasone mesylate in the absence of competing steroid and incubated with preimmune (D) or immune serum (E). For lanes F and G, 120 ul aliquots of L-cell cytosol were incubated with immune or preimmune serum and reacted by SDS-Polyacrylamide gel electrophoresis and autoradiography. Lane H, sample precipitated with charcoal and all the solutions used to wash the immunoadsorbed protein-A-Sepharose pellets.

Figure 2. [%]Desamethasone mesylate bound transformed receptors after binding to DNA-cells. Receptors in L-cell cytosol were bound with [%]desamethasone mesylate, extracted with charcoal, and reacted with five steroid, and 300 ul aliquots of cytosol were transformed by heating at 25°C for 15 min or precipitation with 50% ammonium sulfate. Each aliquot was then reacted with [%]desamethasone mesylate bound transformed receptors and binded to DNA-cells. Lane A, sample incubated in the presence of competing nonradioactive desamethasone and reacted with immune serum; lane B, transformed receptor plus immune serum. Lane C, transformed receptor plus preimmune serum. Lane D, heat transformed receptor plus immune serum. Lane E, heat transformed receptor plus preimmune serum. Lane F, cystosol first heated at 37°C for 15 min and then reacted with [%]desamethasone mesylate bound transformed receptors and reacted with immune serum; lane G, immune serum. Lane H, ammonium sulfate transformed receptor plus immune serum.