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Interaction of Glucocorticoids with Rat Liver Nuclei. I. Role of the Cytosol Proteins†

Mohammed Kalimi, Miguel Beato,‡ and Philip Feigelson*

ABSTRACT: Addition of liver cytosol from adrenalectomized rats to isolated liver nuclei from the same animals increases many-fold the nuclear uptake of [^{3}H]dexamethasone *in vitro*. This effect is attributable to the cytosol proteins and is less evident with liver nuclei derived from nonadrenalectomized rats. The manifestation of the cytosol effect on nuclear glucocorticoid uptake requires incubation of the cytosol with the steroid at 20° and is dependent on the concentration of $MgCl_2$ and the ionic strength. Scatchard analysis indicates that this cytosol effect is due to the generation within the nuclei of high affinity glucocorticoid binding sites which do not bind the steroid in the absence of the cytosol proteins. We therefore assume that these new sites represent nuclear acceptor sites for the receptor–dexamethasone complex. At high levels of steroid, liver nuclei become saturated with respect to cytosol at relative ratio of cytosol to nucleus within the physiologic range, with half-saturation of the nuclei reached at concentrations of receptor–dexamethasone complexes of 1–3 × 10^{-9} M. Liver nuclei incorporate [^{3}H]dexamethasone in the presence of cytosol from kidney or spleen proportionally to the concentration of dexamethasone binding protein in the cytosol of these tissues. Nuclei prepared from kidney or spleen show only a fraction of the affinity of liver nuclei for the receptor–dexamethasone complex. Thus, tissue-specific differences exist with respect to nuclear receptor–steroid binding.

Purified liver nuclei from intact rats are able to incorporate glucocorticoids *in vitro*, but this ability is progressively lost following adrenalectomy (Beato *et al.*, 1969b, 1973a). Since injection of cortisol to adrenalectomized rats results in a depletion of the glucocorticoid receptor from the cytosol accompanied by the appearance of increased steroid binding within the nucleus, we presume that the cytosol receptor protein participates in this process. Further support for this possibility derives from experiments in which rat liver cytosol proteins were shown to enhance the capacity of isolated nuclei to incorporate [^{3}H]cortisol *in vitro* and to facilitate the response of the nuclei to glucocorticoids with increased RNA synthesis (Beato *et al.*, 1970).

In this paper we will present further studies on the role of the cytosol proteins in the interaction of glucocorticoids with purified liver nuclei *in vitro* with respect to: (a) its influence upon the rates of association and dissociation of the steroid with the nucleus; (b) the temperature dependence of the cytosol-mediated transfer of the steroid into the nucleus; (c) the influence of Mg^{2+} and ionic strength upon this process; and (d) the role of tissue specificity upon steroid binding to the nucleus. In the following article, further studies concerning the nature and functional properties of the cytosol protein responsible for the transfer of the steroid into the nucleus as well as the nuclear acceptor site will be presented (Beato *et al.*, 1973b).

Materials and Methods

[1,2,4-^{3}H]Dexamethasone (specific activity 31 Ci/mmol) was obtained from New England Nuclear, Inc., and its radiochemical purity was periodically checked by thin layer chromatography (Beato *et al.*, 1969a). Nonlabeled dexamethasone was obtained from Sigma. The Sephadex gels and Dextran-500 were from Pharmacia. Activated charcoal was purchased from Mallinckrodt. Male Sprague-Dawley rats weighing 150–200 g were used. When indicated, the animals were adrenalectomized bilaterally 4–5 days before the beginning of the experiments and maintained on standard Purina Chow and 0.9% NaCl solution *ad libitum*.

The preparation of liver cytosol and nuclei has been described (Beato *et al.*, 1969a,b).

† From the Institute of Cancer Research and the Department of Biochemistry, College of Physicans and Surgeons, Columbia University, New York, New York 10032. *Received March 23, 1973.* These studies were supported in part by Grants CA 02332 and CA 05011 from the National Institutes of Health. P. F. is an established investigator of the Health Research Council of the City of New York (I-104).

‡ Present address: Department of Biochemistry, University of Marburg, D355 Marburg, West Germany.

TABLE I: Effect of Cytosol on the Uptake of [^{3}H]Dexamethasone by Isolated Liver Nuclei of Intact or Adrenalectomized Rats.[a]

Source of Nuclei	Cytosol[b]	[^{3}H]Dexamethasone Incorpd (dpm/mg of Nuclear Protein)
Intact rats	−	650 ± 70
Intact rats	+	820 ± 63
Adrenalectomized	−	210 ± 29
Adrenalectomized	+	605 ± 75

[a] Intact and 4-day adrenalectomized rats of the same age were used for the preparation of liver nuclei. The steroid transfer assay was performed as indicated under Materials and Methods. The final concentration of [^{3}H]dexamethasone was 2×10^{-8} M, and the concentration of nonradioactive dexamethasone, to correct for nonspecific binding, was 2×10^{-5} M. The cytosol was preincubated with [^{3}H]dexamethasone at 20° for 20 min and the incubation of the nuclei with the cytosol was at 20° for 20 min. After reisolation of the nuclei the specifically bound radioactivity and the protein content were measured. The values represent the mean and standard deviation, calculated from three experiments. [b] The liver cytosol was prepared from 4-day adrenalectomized rats.

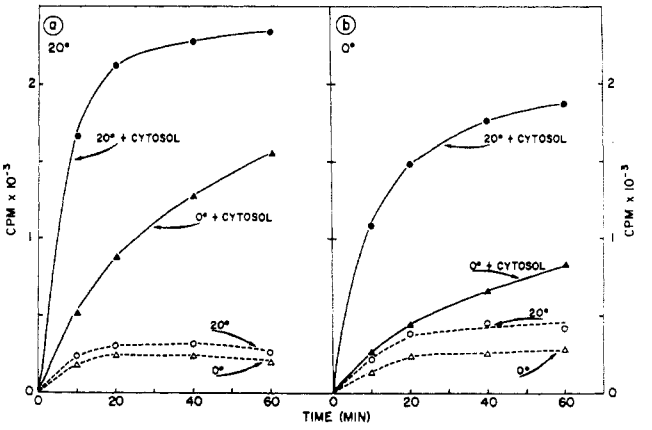

FIGURE 1: Temperature dependence of the incorporation of [^{3}H]-dexamethasone by isolated nuclei; effect of cytosol. Cytosol and nuclei were prepared from the livers of 5-day adrenalectomized rats. The nuclear transfer experiment was performed as described under Materials and Methods. The final concentration of [^{3}H]-dexamethasone was 2×10^{-8} M: (a) transfer assay at 20°; preincubation of the cytosol with steroid at the indicated temperature; (b) transfer assay at 0°; preincubation of the cytosol with steroid at the indicated temperature. Nuclei preincubated with dexamethasone in the absence of cytosol at 0° (△- - -△) or at 20° (○- - - -○), or in the presence of cytosol at 0° (▲—▲) or at 20° (●—●). The values are taken from a typical experiment but essentially the same pattern was observed in two additional experiments.

Quantitative evaluation of the binding of [^{3}H]dexamethasone to its cytosol receptor was accomplished by separating bound and free steroid employing dextran-coated charcoal as previously described (Beato and Feigelson, 1972). When the effect of cytosol proteins on [^{3}H]dexamethasone binding to isolated nuclei was studied (the "transfer" assay), cytosol from adrenalectomized rats (8 mg of protein/ml) was preincubated at 20° for 20 min with saturating concentrations of the labeled steroid alone or in the presence of a 1000-fold excess of nonradioactive dexamethasone.

Purified nuclei, resuspended to a final concentration of 10^8 nuclei/ml in 50 mM Tris-HCl (pH 7.55) containing 250 mM sucrose, 25 mM KCl, 3 mM $MgCl_2$, 1 mM mercaptoethanol, and 1 mM Na_2EDTA (homogenization buffer), were then added. At the end of the incubation period, aliquots were rapidly diluted in 10 vol of ice-cold homogenization buffer, layered over 10 vol of 5% Dextran-500 in the same buffer, and centrifuged at 3000g for 15 min at 0°. The supernatant was discarded and the nuclear pellet resuspended in 0.5 ml of homogenization buffer and used for radioactivity measurements and protein determinations. For the determination of radioactivity 0.5-ml aliquots were added to 10 ml of Bray's solution and shaken vigorously at room temperature before counting. Proteins were determined according to Lowry *et al.* (1951), using bovine serum albumin as standard. The concentration of nuclei was determined microscopically using a homocytometer; the A_{260}/A_{280} ratio of the nuclear pellet obtained after centrifugation through Dextran-500 was also measured.

In some experiments, the nuclear pellet obtained after centrifugation of nuclei through 5% Dextran-500 as described above was extracted with 0.5 ml of homogenization buffer containing 0.5 M NaCl for 30 min at 0°. The concentration of protein-bound radioactivity was then determined in the nuclear extracts using the charcoal technique (Beato and Feigelson, 1972). Although 40–50% of the dexamethasone incorporated into the nuclei can be extracted with 0.5 M NaCl, only 10–15% of the extracted radioactivity behaved as protein bound in the charcoal assay (Beato *et al.*, 1973a). For the determination of the concentration of steroid binding sites in the cytosol or nuclear preparations, incubations with ten or more different concentrations of steroid were continued until equilibrium was reached and the data analyzed according to Scatchard (1949).

The rate of dissociation of nuclear bound dexamethasone was determined after incubation of the nuclei for 60 min at 20° with saturating concentrations of [^{3}H]dexamethasone alone or preincubated with cytosol. The reisolated nuclei were then incubated at 20° in homogenization buffer (50 mM Tris-HCl (pH 7.55), containing 250 mM sucrose, 25 mM KCl, 3 mM $MgCl_2$, 1 mM Na_2EDTA, and 1 mM mercaptoethanol) containing 10^{-5} M nonradioactive dexamethasone. At the indicated time intervals thereafter, aliquots were taken for determination of nuclear bound radioactivity.

For the studies on the tissue specificity of the dexamethasone binding, nuclei and cytosol were prepared from the kidney, spleen, and thymus of adrenalectomized rats, following the same procedure used for the liver. The organs were weighed, minced, and homogenized in 2 vol of homogenization buffer. The nuclei were prepared using the technique previously described (Beato *et al.*, 1969b), and the cytosol was obtained by centrifugation of the 3000g supernatant at 240,000g for 30 min. The concentration of protein was adjusted to 8 mg/ml in all cytosol preparations (Lowry *et al.*, 1951), and the transfer assay was performed as described above. Measurements of the absorbancy at 260 nm, after dissolving in 1 N NaOH–1 M NaCl, were used to quantitate the reisolated nuclear pellet.

Results

Effect of Liver Cytosol on Nuclear Uptake of [^{3}H]Dexamethasone in Vitro. The effect of the liver cytosol of adrenalec-

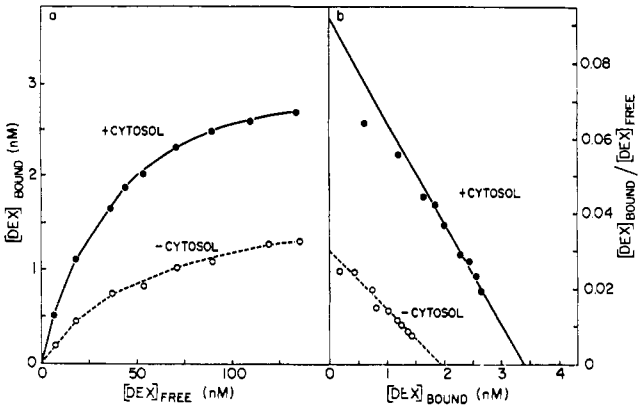

FIGURE 2: Effect of cytosol on the nuclear uptake of [^{3}H]dexamethasone at equilibrium. Cytosol and nuclei were prepared from the livers of 4-day adrenalectomized rats. Aliquots of the purified nuclei were incubated at 20° for 60 min with various concentrations of [^{3}H]dexamethasone, alone (O- - -O) or preincubated with cytosol (●—●), and the amount of specifically bound dexamethasone in the nuclear pellet was measured: (a) linear plot of the concentration of nuclear bound dexamethasone against the concentration of free dexamethasone; (b) representation of the data according to Scatchard (1949).

tomized rats on the incorporation of [^{3}H]dexamethasone into isolated liver nuclei from intact and adrenalectomized rats is depicted in Table I. As previously reported (Beato *et al.*, 1973a), the incorporation of glucocorticoids into nuclei from adrenalectomized rats is decreased compared with the intact counterparts. The addition of the liver cytosol fraction to nuclei from intact rats results in a 27% increase in the nuclear glucocorticoid uptake. When the same cytosol fraction is added to liver nuclei from adrenalectomized rats, a threefold higher incorporation of [^{3}H]dexamethasone is observed. The experiments presented in Table I were performed with unfractionated liver cytosol from adrenalectomized rats, but essentially the same results are obtained when the macromolecular components of the cytosol (Sephadex G-25 excluded) are used for the transfer experiment. Digestion of the cytosol with Pronase or trypsin prevented its stimulatory effect on [^{3}H]dexamethasone uptake by liver nuclei, whereas treatment with pancreatic ribonuclease was without effect. Furthermore, similar results were obtained when liver nuclei from adrenalectomized rats purified through hypertonic sucrose (Beato *et al.*, 1969b) were used instead of the nuclei treated with detergent.

The effect of the cytosol proteins on the incorporation of [^{3}H]dexamethasone by isolated liver nuclei is temperature dependent (Figure 1). Incubation of liver nuclei at 0° with cytosol, which had been preincubated with [^{3}H]dexamethasone at 0°, leads to only a slight increase in the nuclear steroid uptake in comparison with the control, wherein nuclei were incubated with dexamethasone in the absence of cytosol (Figure 1b). Preincubation of the cytosol with the steroid at 20°, however, results in a significant enhancement of nuclear steroid uptake upon subsequent incubation at 0° (Figure 1b). Optimal cytosol enhancement of nuclear steroid binding is observed when the nuclei are incubated at 20° with cytosol which had been preincubated with [^{3}H]dexamethasone at 20° (Figure 1a).

The rate of dissociation of the nuclear bound [^{3}H]dexamethasone is reduced by the presence of cytosol in the preincubation medium, and the dissociation reaction follows apparent first-order kinetics. The dexamethasone bound to the nuclei, preincubated in the absence of cytosol, dissociates

TABLE II: Factors Affecting the Nuclear Uptake of [^{3}H]-Dexamethasone *in Vitro* in the Absence and Presence of Hepatic Cytosol.[a]

Additions	Concn	[^{3}H]Dexamethasone Incorpd (dpm/mg of Protein)	
		Buffer	Cytosol
None		230 ± 25	635 ± 72
$MgCl_2$	10 mM	480 ± 86	568 ± 95
ATP	10 mM	198 ± 32	650 ± 108
p-Chloromercuriphenylsulfonic acid	1 mM	25 ± 12	178 ± 106
Mercuriacetate	1 mM	7 ± 5	13 ± 11
N-Ethylmaleimide	1 mM	10 ± 6	290 ± 82
3′,5′-cAMP	10 nM	225 ± 81	648 ± 102
3′,5′-cAMP	1.0 μM	245 ± 32	629 ± 57
3′,5′-cAMP	10 μM	261 ± 46	592 ± 31
Glucose	3 mM	229 ± 13	586 ± 92
2,4-Dinitrophenol	0.5 mM	205 ± 22	578 ± 86
KCN	0.5 mM	209 ± 31	608 ± 30

[a] Liver nuclei from 4-day adrenalectomized rats were used to study the specific incorporation of [^{3}H]dexamethasone in the absence or presence of the homologous cytosol. Additions were made after preincubation of the cytosol with [^{3}H]-dexamethasone at 20° for 20 min.

with a half-time of 31 min (range 27–39 in 3 determinations), whereas in nuclei labeled in the presence of the cytosol, the rate of dissociation of the nuclear bound radioactivity is 75% slower ($t_{1/2}$ = 57 min, range 49–61 in 3 determinations).

Thus, the addition of cytoplasmic receptor to nuclei from adrenalectomized rats increases the amount of [^{3}H]dexamethasone bound to the nucleus (Table I and Figure 1) and significantly decreases its rate of exchange with free dexamethasone. This slower rate of dissociation should be reflected at equilibrium by a higher affinity of the steroid for the nuclei when the cytosol is present. To study binding at equilibrium, the nuclei were incubated at 20° for 60 min, with different concentrations of dexamethasone, and the amount of specifically bound steroid was plotted according to Scatchard (1949). The concentration of dexamethasone binding sites as well as the apparent association constant were estimated (Figure 2). The equilibrium association constant, K_a, of the steroid for the nuclei in buffer is 1.6×10^7 M^{-1} (range 1.2–2.1 in 4 determinations) whereas in the presence of cytosol proteins it is 2.8×10^7 M^{-1} (range 2.6–3.5 in 5 determinations), thus confirming the tighter nuclear binding of the steroid caused by the cytosol. The intersection with the abscissa in Figure 2b also shows that the concentration of nuclear dexamethasone binding sites is markedly increased when cytosol is present during the incubation: from 5900 sites per nucleus in the absence of cytosol (range 4100–6900 in 3 determinations) it rises to 10,000 (range 8,000–14,000) when the cytosol proteins are present. This difference is even more pronounced when nuclei from rats adrenalectomized for longer than 1 week were used. As will be seen below, the number of about 4000 "new" binding sites per nucleus observed in the presence of cytosol compares well with the value calculated from the

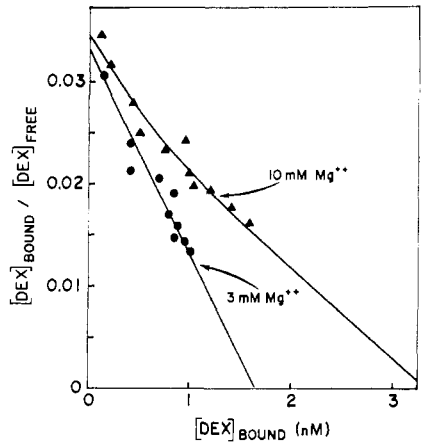

FIGURE 3: Effect of Mg^{2+} concentration on the uptake of [^{3}H]-dexamethasone by isolated nuclei. Liver nuclei were prepared from 4-day adrenalectomized rats in homogenization buffer containing either the usual 3 mM $MgCl_2$ (●) or 10 mM $MgCl_2$ (▲). The purified nuclei were then incubated at 20° for 60 min with various concentrations of [^{3}H]dexamethasone, alone or after addition of a 1000-fold excess of nonradioactive dexamethasone. The amount of specifically bound steroid in the reisolated nuclei was calculated as described under Materials and Methods, and the binding data are plotted according to Scatchard (1949).

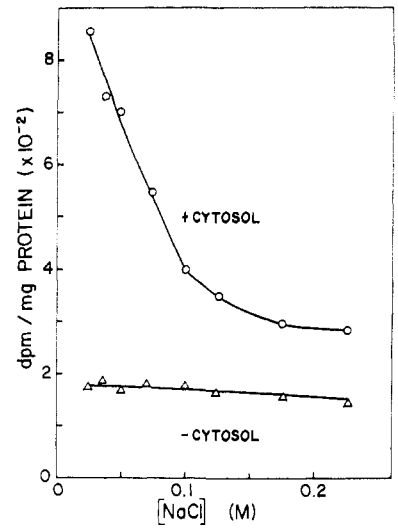

FIGURE 5: Effect of NaCl concentration and cytosol on the uptake of [^{3}H]dexamethasone by isolated liver nuclei. Liver nuclei and cytosol were prepared from 5-day adrenalectomized rats. The nuclei were resuspended in homogenization buffer and the cytosol was incubated at 20° for 20 min with [^{3}H]dexamethasone (final concentration 5×10^{-8} M). Increasing concentrations of NaCl in buffer were added to the incubated cytosol (○) and to control incubations containing [^{3}H]dexamethasone in buffer (△). Aliquots were then added to the nuclei and the amount of specifically bound radioactivity in the nuclear pellet was determined after incubation at 20° for 40 min. The values in the abscissa represent the final concentration of NaCl in the incubation mixture after addition of the nuclei. Each point is the average of two experiments performed in duplicate.

saturation of the nuclei with the receptor–dexamethasone complex (Figure 6).

Parameters Influencing the Cytosol Enhancement of the Nuclear Uptake of [^{3}H]Dexamethasone in Vitro. As shown in Table II, the concentration of magnesium ions in the incubation mixture is of relevance for the manifestation of the cytosol effect on steroid transfer to the nucleus. Increasing the Mg^{2+} concentration from the usual 3 to 10 mM results in a higher uptake of [^{3}H]dexamethasone by the nuclei in the absence of the cytosol, whereas in the presence of cytosol the nuclear uptake of steroid does not further increase. The consequence is that at 10 mM Mg^{2+} the cytosol-mediated increase in the uptake of dexamethasone by the nuclei is much less evident than at 3 mM Mg^{2+}. Scatchard analysis of the nuclear binding data obtained in the absence of the cytosol, at different concentrations of Mg^{2+}, shows that the rise in dexamethasone uptake into the nuclei at 10 mM Mg^{2+} is due to the appearance of a set of steroid binding sites with lower affinity than those manifested at 3 mM Mg^{2+} (Figure 3). While at 3 mM Mg^{2+} the Scatchard plot of the nuclear binding data shows linearity, at 10 mM Mg^{2+} nonlinearity is observed. Extrapolation of the shallower slope line to the abscissa indicates the presence, in 10 mM Mg^{2+}, of a high concentration in the nucleus of lower affinity dexamethasone binding sites.

The effect of Mg^{2+} is not the consequence of changes in the cytosol receptor, but rather is due to intranuclear modifications. This is deduced from crossing experiments in which nuclei prepared at either 3 or 10 mM Mg^{2+} were incubated with buffer or cytosol containing 3 or 10 mM Mg^{2+} (Figure 4). It is obvious that when the nuclei are prepared in medium containing 3 mM Mg^{2+}, the cytosol effect is preserved independently of the Mg^{2+} concentration in the cytosol preparation. Nuclei prepared in 10 mM Mg^{2+} bind more [^{3}H]dexamethasone and show poor further cytosol-mediated enhancement of steroid binding with either 3 or 10 mM Mg^{2+} in the cytosol preparations.

The incorporation of [^{3}H]dexamethasone into the nuclei in the absence of cytosol as well as the cytosol-mediated effect on steroid transfer are markedly inhibited by the addition to the incubation mixture of sulfhydryl blocking reagents (Table II). This finding was expected in view of earlier studies demonstrating that the binding of dexamethasone to the cytosol receptor and the *in vitro* uptake of [^{3}H]cortisol by

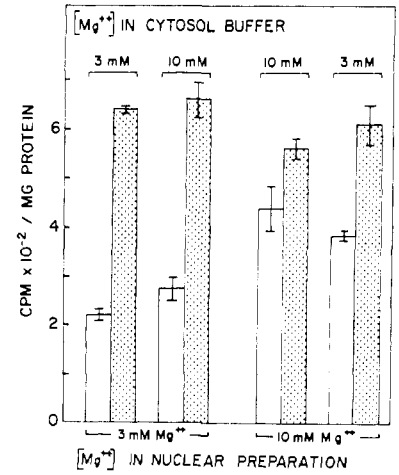

FIGURE 4: Effect of Mg^{2+} concentration and cytosol on the uptake of [^{3}H]dexamethasone by isolated liver nuclei. Liver nuclei and cytosol were isolated from 4-day adrenalectomized rats in a buffer containing 3 or 10 mM $MgCl_2$. Nuclei suspended in buffer containing 3 mM $MgCl_2$ were incubated with 2×10^{-8} M [^{3}H]dexamethasone in the presence of buffer (open bars) or cytosol (dotted bars) containing either 3 or 10 mM $MgCl_2$. A similar set of assays was performed with nuclei prepared and suspended in buffer containing 10 mM $MgCl_2$. After incubation at 20° for 20 min, the amount of specifically bound radioactivity in the nuclear pellet was determined. The values are the mean and standard deviation of two experiments performed in duplicate.

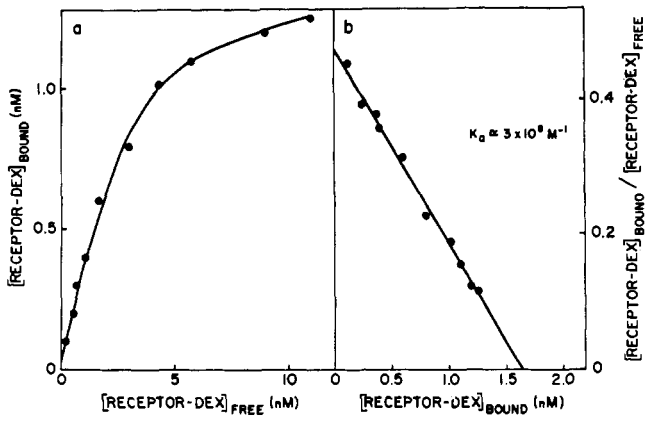

FIGURE 6: Saturation of isolated liver nuclei with the receptor–dexamethasone complex. Liver nuclei and cytosol were prepared from 4-day adrenalectomized rats. The cytosol was incubated at 20° for 20 min with 5×10^{-8} M [^{3}H]dexamethasone alone or after adding 5×10^{-5} M nonradioactive dexamethasone. Increasing amounts of cytosol were then added to the nuclei and the volume was kept constant by adding the necessary amount of buffer containing 5×10^{-8} M [^{3}H]dexamethasone alone or with additional 5×10^{-5} M nonradioactive dexamethasone. After incubation at 20° for 40 min, the concentration of dexamethasone specifically bound to the nuclear pellet was determined. A blank without added cytosol was subtracted from all values. The concentration of receptor sites in the corresponding cytosol was determined by running a saturation curve of the cytosol at 20° for 2 hr (Beato and Feigelson, 1972): (a) linear plot of the concentration of the nuclear bound receptor–dexamethasone complex against the concentration of free receptor–dexamethasone complex in the medium; (b) representation of the data according to Scatchard (1949).

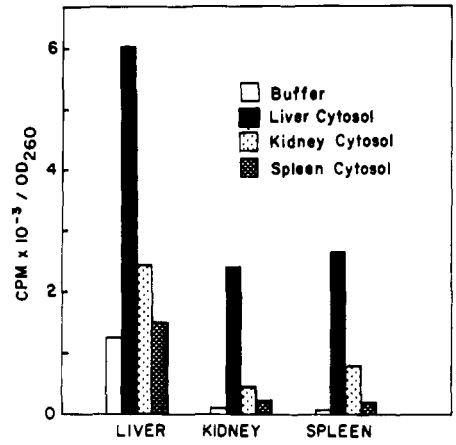

FIGURE 7: Uptake of [^{3}H]dexamethasone alone or complexed to the cytosol of liver, kidney, or spleen by isolated nuclei from these organs. Cytosol and nuclei were prepared from liver, kidney, and spleen of 5-day adrenalectomized rats. The isolated nuclei of each organ were incubated for 20 min at 20° with [^{3}H]dexamethasone (2×10^{-8} M) which had been preincubated with buffer, or the cytosol from liver, kidney, or spleen. Appropriate subtractions were made from assays where 2×10^{-5} M nonradioactive dexamethasone was added. The values are average of two experiments performed in duplicate.

isolated nuclei of intact rats are both inhibited by mercurial reagents (Beato *et al.*, 1969b; Koblinsky *et al.*, 1972).

The presence of ATP and an ATP regenerating system does not change significantly the pattern of steroid uptake by isolated nuclei alone or in the presence of cytosol (Table II). No effect is observed either when KCN, 2,4-dinitrophenol, or glucose was added. 3′,5′-cAMP, at concentrations ranging from 10^{-8} to 10^{-6} M, has no marked effect on the nuclear uptake of [^{3}H]dexamethasone. At the unphysiologic concentration of 10^{-5} M 3′,5′-cAMP the incorporation of steroid alone into the nuclei is slightly increased, and the further cytosol enhancement is less evident.

The influence of salt concentration on the incorporation of [^{3}H]dexamethasone into liver nuclei is shown in Figure 5. As the concentration of NaCl or KCl rises above 0.025 M (the concentration usually present in the homogenization buffer) the incorporation of radioactivity into nuclei incubated without cytosol is only slightly affected. However, the cytosol-mediated effect decreases sharply up to a NaCl concentration of about 0.1 M. Above this concentration NaCl or KCl have little effect on the enhancement of nuclear steroid binding observed in the presence of cytosol; even at the highest salt concentration tested, 0.225 M, about twice as much [^{3}H]dexamethasone is incorporated in the nuclei incubated in the presence of cytosol.

Affinity of Liver Nuclei for the Dexamethasone–Receptor Complex. Experiments were directed to study the saturability and affinity of isolated nuclei for the cytoplasmic receptor–[^{3}H]dexamethasone complex. Varying amounts of cytosol, preincubated at 20° with a saturating concentration of [^{3}H]-dexamethasone, were added to a nuclear suspension and the final concentration of [^{3}H]dexamethasone was brought to 5×10^{-8} M. After incubation at 20° for 40 min, the amount of nuclear bound radioactivity was determined and the values observed in the absence of cytosol were subtracted. A saturation curve of the nucleus for the specific receptor–dexamethasone complex was obtained, which approached plateau at a concentration of receptor–dexamethasone complex of about 8–10 mM (Figure 6a). This value roughly corresponds to the physiological concentration of cytosol receptor (Beato and Feigelson, 1972). A Scatchard plot of the data shows an apparent association constant of the receptor–dexamethasone complex for the nuclei of about $3\text{–}4 \times 10^8\ M^{-1}$ (Figure 6b).

The concentration of receptor–dexamethasone binding sites is 1.5 mM, corresponding to 4000–5000 molecules per nucleus. This number compares well with the values observed in Figure 2b when the difference between the concentration of nuclear steroid binding sites in the presence and in the absence of cytosol was estimated.

Tissue Specificity of the Cytosol-Mediated Transfer of the Steroid to the Nucleus. As previously reported the cytosol receptor for glucocorticoid hormones is rather tissue specific (Beato and Feigelson, 1972). Besides the liver, only kidney and thymus show a specific dexamethasone binding protein in the cytosol fraction. Moreover, the binding of [^{3}H]dexamethasone to isolated nuclei of organs from intact rat is also tissue specific (Beato *et al.*, 1973a). Experiments were directed to study the ability of nuclei from various tissues to bind cytoplasmic receptor–dexamethasone complexes from homologous and heterologous organs (Figure 7). Nuclei prepared from livers of 5-day adrenalectomized rats incorporate little dexamethasone; addition of liver cytosol of the same animals enhances fivefold the nuclear binding of [^{3}H]dexamethasone. Kidney cytosol was about one-fourth as efficient as liver cytosol in enhancing dexamethasone uptake by liver nuclei, and the spleen cytosol did not significantly enhance the nuclear uptake of the steroid. Kidney and spleen nuclei are unable to incorporate dexamethasone in the absence of cytosol, but these heterologous nuclei bind the liver cytosol receptor–dexamethasone complex rather efficiently, reaching values which are about half those observed with liver nuclei. Kidney cytosol is much less efficient in promoting the incorporation of steroid by either kidney or spleen nuclei, and spleen cytosol has vir-

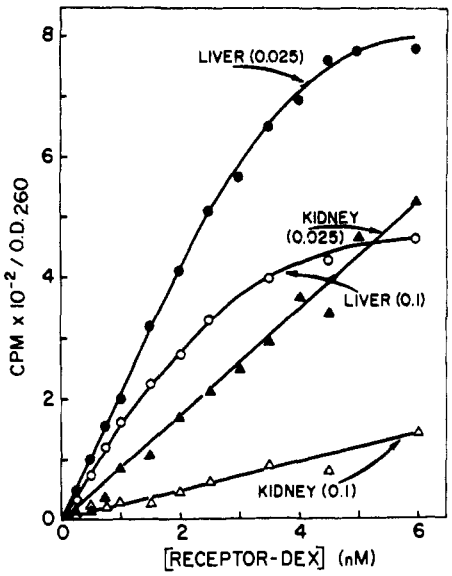

FIGURE 8: Effect of salt concentration on the binding of receptor–dexamethasone complex from liver cytosol to nuclei from liver and kidney. Liver cytosol and nuclei, as well as kidney nuclei, were prepared from 5-day adrenalectomized rats. The liver cytosol was incubated at 20° for 20 min with 5×10^{-8} M [^{3}H]dexamethasone alone or after addition of 5×10^{-5} M nonradioactive dexamethasone. To bring the salt concentration to the indicated values, 0.1 vol of either homogenization buffer (containing NaCl instead of KCl) or 2 M NaCl in buffer was added to the cytosol at the end of the incubation. Increasing amounts of the cytosol were then incubated with equal aliquots of a suspension of liver or kidney nuclei. The volume was kept constant by adding the necessary amount of either homogenization buffer or 0.2 M NaCl in buffer, both containing 5×10^{-8} M [^{3}H]dexamethasone alone or additionated with nonradioactive dexamethasone. After incubation at 20° for 40 min the concentration of specifically bound dexamethasone was determined in the reisolated nuclear pellets. The values obtained in blank assays without added cytosol and with the appropriate concentration of NaCl were subtracted. The concentration of receptor in the liver cytosol was determined by running a saturation curve with [^{3}H]dexamethasone at 20° for 20 hr (Beato and Feigelson, 1972).

tually no effect. Therefore, both the ability of cytosol to increase the incorporation of [^{3}H]dexamethasone by isolated nuclei, as well as the capacity of the nuclei to accept the receptor–dexamethasone complex, appear to be partially tissue specific. Saturation experiments showed that while the liver nuclei approach saturation at a concentration of the receptor–dexamethasone complex of about 5 nM, both at 0.025 and 0.1 M NaCl, the binding to the kidney nuclei was a linear function of the receptor–dexamethasone concentration (Figure 8). Furthermore, the affinity of the receptor–dexamethasone complex for liver nuclei was considerably higher than its affinity for kidney nuclei, and this difference was more evident at near physiologic ionic strength.

Discussion

The data presented in this paper confirm previous observations that a protein factor from liver cytosol enhances the incorporation of glucocorticoids by isolated liver nuclei *in vitro* (Beato *et al.*, 1970). The fact that this effect is magnified when the liver nuclei used are derived from adrenalectomized rats indicates that in liver nuclei of intact rats, due to the presence of endogenous steroid, some of the nuclear steroid binding sites are occupied.

Calculation of the number of molecules of dexamethasone specifically bound to the liver nuclei of adrenalectomized rats in the absence and in the presence of cytosol (Figure 2) shows that between 4000 and 8000 additional nuclear binding sites become evident when the cytosol is present during the *in vitro* incubation. This value is only slightly lower than that found when cortisol is injected to adrenalectomized rats *in vivo* and the number of "new" binding sites subsequently determined in these liver nuclei *in vitro* (Beato *et al.*, 1973a). It may therefore be possible that the enhanced uptake of [^{3}H]dexamethasone observed in the *in vitro* incubation of nuclei with the cytosol receptor–steroid complex mimics and corresponds to the appearance of "new" nuclear binding sites occurring after cortisol injection *in vivo*.

The observation that the cytosol effect on the transfer of glucocorticoids to the nucleus *in vitro* is time and temperature dependent is reminiscent of earlier findings with estradiol and uterine nuclei *in vitro* (Jensen *et al.*, 1968; Shyamala and Gorski, 1969) and may reflect a general feature in hormone–receptor interaction with the target cell nuclei. Preincubation experiments indicate that once the temperature-dependent transformation of the receptor–dexamethasone complex is accomplished in the cytosol, the further transfer of the complex into the cell nucleus takes place at 0° as well as at 20°. The necessity of the steroid for receptor transformation has been challenged by DeSombre *et al.* (1972), and recent work by Puca *et al.* (1972) indicates that a special protein factor may be catalytically involved in the transformation of the estradiol receptor required for intranuclear migration of the hormone.

The dependency on the NaCl concentration of the cytosol-mediated enhancement in [^{3}H]dexamethasone binding to the nuclei is an interesting observation. Spelsberg *et al.* (1971) have reported that the interaction of the progesterone receptor of the chicken oviduct with homologous chromatin shows a sharp optimum at near physiological ionic strength, and then declines in a manner similar to that shown in Figure 5. This behavior suggests that ionic forces contribute significantly and stabilize the interaction between the [^{3}H]dexamethasone–receptor complex and the nucleus. However, at relatively high ionic strength, a clear cytosol-mediated enhancement of glucocorticoid binding to the nucleus is retained indicating that forces other than ionic are involved as well. Furthermore, at near physiologic ionic strength, the interaction of the cytosol with the nucleus appears to be more tissue specific than at low ionic strength (Figure 8).

A puzzling observation is the large number of molecules of the receptor–dexamethasone complex (4000–6000) required to saturate the nuclear acceptor sites. Similar findings have been reported for other steroid target tissues (Jensen and DeSombre, 1972; Baxter *et al.*, 1972). It is possible that only a minor fraction of the receptor–steroid molecules moving into the nucleus is actually involved in meaningful interaction with the genome, and that our present method cannot distinguish between these interactions and a larger set of physiologically meaningless receptor binding sites in the chromatin. However, if we assume that all the molecules of receptor–steroid complex reaching the nucleus are necessary for maximal hormonal effects, a stoichiometric model in which a steroid molecule acts upon one gene appears rather unrealistic, since the number of gene products actually known to be under glucocorticoid control is certainly at least one order of magnitude smaller. A plausible explanation will be to consider that the receptor–steroid complex interacts with nonstructural repeated sequences of the DNA present in relatively high number and corresponding to evolutionary related glucocorticoid responsive genes of the different target tissues. In a particular

organ, for instance the liver, only a fraction of these interactions would lead to actual regulation of genes, for the rest of potentially responsive genes are permanently repressed as a consequence of cell differentiation. This latest assumption is supported by the lack of an absolute tissue specificity of the nuclear acceptor function. In this model, the receptor–dexamethasone complex will act as a positive regulator of gene activity in a manner similar to the regulation of the transcription of the *lac* operon in *E. coli* by the cAMP-binding protein (Zubay *et al.*, 1970; De Crombrugghe *et al.*, 1971; Nissley *et al.*, 1971). The final expression of the gene's information will depend on additional negative controls which may or may not be under hormonal regulation.

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