J. steroid Biochem. Vol. 21, No. 6, pp. 633–637, 1984
Printed in Great Britain.

0022-4731/84 $3.00 + 0.00

MURINE GLUCOCORTICOID RECEPTORS AND THE *H-2* LOCUS—A REAPPRAISAL

SHARON L. LIU, JOSEPH F. GRIPPO*, ROBERT P. ERICKSON and WILLIAM B. PRATT*†
Departments of Human Genetics and *Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109, U.S.A.

(*Received* 30 *April* 1984)

Summary—It has been demonstrated that susceptibility to glucocorticoid-induced formation of cleft palate is regulated by the mouse histocompatibility complex (*H-2*). This has encouraged us to examine *H-2* effects on glucocorticoid binding in tissues of adult animals which would provide sufficient material with which to study the biochemical mechanism of the *H-2* effect. Although it has been reported that cytosol prepared from lungs of adult mice with a high susceptibility to steroid-induced cleft palate formation have a higher level of glucocorticoid binding than lung cytosol prepared from a low-susceptibility strain, we are unable to demonstrate any influence of *H-2* on binding capacity in this tissue from adult animals when glucocorticoid receptors are assayed in the presence of receptor reducing and stabilizing agents that maximize binding capacity.

Cytosol prepared from rat liver contains an endogenous receptor-reducing system composed of NADPH and thioredoxin. It has also been reported that the murine *H-2* complex contains a gene(s) that regulates the level of a modifier(s) in fetal hepatic cytosol that affects the binding of glucocorticoids to the receptor. Of two known low molecular weight modifiers that could account for this effect, we have previously established that the heat-stable, steroid receptor "modulator" is not regulated by the *H-2* complex. In the present work we have assayed thioredoxin, a second potential modifier, in liver cytosols prepared from adults of two pairs of two *H-2* congenic mouse strains. Our results show that the amount of thioredoxin is the same in all four mouse strains and that it is not regulated by the *H-2* locus.

At this time, we are unable to identify a system in adult mice in which the widely reported regulation of glucocorticoid binding by the mouse histocompatibility locus can be submitted to definitive biochemical study.

INTRODUCTION

Several years ago, it was established that various strains of hamsters exhibit different degrees of susceptibility to glucocorticoid-induced cleft palate [1]. In mice, the different susceptibilities to glucocorticoid-induced cleft palate are partially controlled by the *H-2* locus, the major histocompatibility locus in this species [2, 3]. Subsequently, it was reported [4] that facial mesenchyme cells cultured from fetuses of a mouse strain that is highly susceptible to glucocorticoid-induced cleft palate (A/J) have twice the specific glucocorticoid binding capacity as cells from a low susceptibility strain (C57BL/6J). Higher levels of specific glucocorticoid binding capacity have also been measured in fetal palatal tissue obtained from highly susceptible strains when compared to palatal tissue from less susceptible strains [5–7]. Studies of binding capacity in congenic mouse strains that differ primarily at the *H-2* locus have led to the proposal that glucocorticoid binding capacity, like glucocorticoid-induction of cleft palate, is regulated in part by the *H-2* haplotype [5–7].

†To whom correspondence should be addressed.
Address correspondence to: W. B. Pratt, Department of Pharmacology, 6448, Medical Science Bldg I, The University of Michigan Medical School, Ann Arbor, MI 48109-0010, U.S.A.

As mouse strains with defined *H-2* haplotypes could prove to be powerful experimental tools with which to investigate the factors that control specific glucocorticoid binding capacity, we examined the effect of the *H-2* locus on steroid binding capacity in mouse liver [8]. Mouse liver was chosen because it is a large organ that is a well characterized target of glucocorticoid action and because *H-2* is expressed in liver [9]. Specific glucocorticoid binding capacity was assayed in liver cytosols prepared from four mouse strains: A/J (high susceptibility), C57BL/6J (low susceptibility), and the two *H-2* congenic lines A.BY and B10.A. We found that the level of specific glucocorticoid binding activity in liver cytosol is not mediated by the *H-2* locus [8]. Katsumata *et al.* [7] have directly compared the glucocorticoid binding capacity of fetal palate and maternal liver in four mouse strains and found that the *H-2* locus affects total specific glucocorticoid binding capacity in the former but not in the latter.

Although the total glucocorticoid binding capacity in liver is not regulated by *H-2*, there is evidence that the *H-2* complex contains a gene(s) that regulates the level of a modifier(s) in hepatic cytosol that affects the binding of glucocorticoids to the receptor. The evidence was obtained by Katsumata *et al.*[10] who found that plots of dexamethasone binding versus concentration of fetal liver cytosol were linear for

C57BL/10 and B10.A(5R) mice whereas similar binding plots for strains B10.A and B10.A(2R) were not. There are two low molecular weight modifiers that could account for this *H-2* effect in liver. One is a heat-stable molecule that stabilizes the glucocorticoid binding capacity of cytosol preparations. This stabilizing factor was originally reported by Cake *et al.*[11] and has been called the "modulator" [12, 13]. We have developed an assay to quantitate this receptor stabilizing factor [14] and have reported that the level of its activity in mouse liver cytosol is not regulated by the *H-2* locus [15]. A second modifier is a cytosolic system that activates[1] receptors from a nonsteroid-binding to a steroid-binding state [16–19]. We have recently shown that this receptor activating system is a NADPH-dependent, thioredoxin-mediated reducing system that maintains the glucocorticoid receptor in an appropriate steroid-binding conformation [19]. In the work to be reported in this paper we have examined whether this receptor reducing system is regulated by the *H-2* locus and could account for the *H-2* effects on glucocorticoid binding that have been reported in both liver and other tissues.

MATERIALS AND METHODS

Materials

[6,7-^{3}H]Triamcinolone acetonide[2] (31.4 Ci/mmol) was obtained from New England Nuclear, Boston, Massachusetts. Nonradioactive dexamethasone and bovine insulin were from Sigma Chemical Co., St Louis, Missouri. Adult A/J (A background, *H-2*^{a}), A.BY/SnJ (A background, *H-2*^{b}), C57BL/6J (B background, *H-2*^{b}), and B10.A/SgSnJ (B background, *H-2*^{a}) mice were purchased originally from Jackson Laboratory and are bred and maintained in our mouse colony. All mice used in this study were adult males. Purified *Escherichia coli* thioredoxin was generously provided by Dr Vincent Pigiet in the Department of Biochemistry, Johns Hopkins University.

Methods

Preparation of cytosols. Mice were killed by decapitation and tissues were removed immediately and placed in ice cold Earle's balanced salt solution. Livers and lungs were lightly blotted to remove excess liquid, weighed, and transferred to a prechilled homogenizing vessel. All tissues were homogenized on ice with a Teflon pestle in 1.5 ml of 10 mM Hepes buffer (pH 7.35) per gram of tissue. For some experiments the homogenizing buffer also contained 10 mM sodium molybdate, or 10 mM dithiothreitol, or both sodium molybdate and dithiothreitol. The homogenates were centrifuged at 27,000 *g* for 20 min and the resulting supernatants were recentrifuged at 100,000 *g* for 2 h. The 100,000 *g* supernatants are referred to as cytosol preparations and were used for all of the binding assays in this work. In some cases, cytosols were stored at −70°C until used.

Incubation conditions and steroid binding assay. Each 100 μl assay contained 45 μl of cytosol, 45 μl of Hepes buffer pH 7.35 (or buffer with additions of dithiothreitol or molybdate or both as noted in the legends to the tables), and 50 nM [^{3}H]triamcinolone acetonide in the presence or absence of a 1,000-fold excess of nonradioactive dexamethasone or vehicle. All samples were incubated overnight at 0°C prior to addition of 0.15 ml of a suspension containing 1% charcoal (w/v) and 0.2% Dextran (w/v) in 10 mM Hepes buffer, pH 7.35, to absorb free steroid. The charcoal–cytosol mixture was incubated for 10 min at 4°C and centrifuged at 12,000 *g* for 5 min. Bound steroid was assayed by liquid scintillation counting of the supernatant fluid. The specific binding capacity represents the average of duplicate assays made in the absence of dexamethasone minus the nonspecific binding values and is expressed as fmoles per mg cytosol protein. It should be noted that the values for specific binding reported in this paper are somewhat lower than the values reported in our previous study of glucocorticoid binding in mouse hepatic cytosol [8]. All of the calculations in the previous study contain a constant error that had been introduced into the calculating factor that was used to convert from counts per minute of steroid bound to picomoles of steroid bound. The concentration of [^{3}H]triamcinolone acetonide used to assay glucocorticoid binding capacity in this study (50 nM) is several times higher than the concentration required to occupy all specific binding sites and is a rapid and valid assay for the total binding activity of cytosol [20, 21].

Assay for thioredoxin. Thioredoxin was assayed by the insulin disulfide reduction method of Holmgren [22]. Portions of the 100,000 *g* liver supernatants were boiled for 2 min and centrifuged at 12,000 *g* for 5 min. The boiled supernatants were lyophilized to dryness, dissolved in 1 ml of distilled water, and passed through a column of Sephadex G-25 (2.5 × 94 cm) using an elution buffer of 10 mM Hepes, pH 7.35. The fractions containing the macromolecular material (determined by A_{280}) were pooled and concentrated again by lyophilization. The lyophilized material was redissolved in an amount of distilled water equivalent to one third of the volume

[1]The term "activation" will be used throughout this paper to describe the process whereby the receptor is converted from a nonbinding form to a form that binds steroids. We use the term "transformation" to describe the process whereby the steroid-bound receptor is converted to a form that binds to nuclei, DNA-cellulose, etc.

[2]The trivial names for steroids and abbreviations used are: triamcinolone acetonide, 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20,dione 16,17-acetonide; dexamethasone, 9α-fluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione; Hepes, 4-(2-hydroxyethyl)-1-piperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol.

Table 1. Glucocorticoid binding capacity in cytosols prepared from livers and lungs of A/J and C57BL/6J mice

Homogenizing buffer	Specific glucocorticoid binding capacity (fmol/mg cytosol protein)					
	Liver			Lung		
	A/J	C57BL/6J	P	A/J	C57BL/6J	P
Hepes alone	295 ± 28 (10)	218 ± 32 (10)	N.S.	17 ± 8 (7)	1 ± 1 (7)	N.S.
plus DTT	506 ± 46 (10)	323 ± 35 (10)	0.01	226 ± 30 (10)	294 ± 38 (10)	N.S.
plus Molybdate	330 ± 41 (10)	208 ± 43 (10)	N.S.	15 ± 9 (10)	13 ± 4 (10)	N.S.
plus DTT and Molybdate	562 ± 65 (9)	437 ± 20 (9)	N.S.	260 ± 27 (10)	348 ± 50 (11)	N.S.

Liver or lungs from the two strains of mice were homogenized in 10 mM Hepes buffer, in buffer plus 10 mM dithiothreitol (DTT), buffer plus 10 mM sodium molybdate, or in buffer plus DTT and molybdate. Aliquots of each cytosol were incubated with [^{3}H]triamcinolone acetonide and specific binding was determined as described under Methods. Each value represents the mean and standard error for the number of cytosol preparations noted in parentheses. *P* values were determined by Student's *t*-test (two tailed). N.S. = not significant, $P > 0.05$.

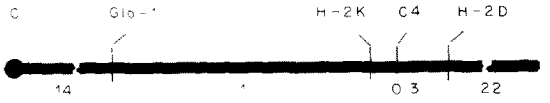
of the original boiled supernatant. An aliquot (50–100 μl) of each test sample was incubated in a final volume of 0.6 ml in a 1 ml cuvette at room temperature with 500 μl of bovine insulin (1 mg/ml), 8 μl of 50 mM dithiothreitol, and the appropriate volume of 100 mM phosphate buffer, pH 7.0, containing 2 mM EDTA. Measurements were performed at A_{650} every 5–10 min. Rates of insulin reduction were expressed as the change in turbidity with time observed between 0.02 and 1.0 A_{650} above background. The nonenzymatic reduction of insulin by dithiothreitol was recorded in a control cuvette without thioredoxin and this value (0.008 A_{650} nm $\times$ min^{-1}) was subtracted from that recorded with each test sample to yield the rate of insulin reduction due to thioredoxin. A standard curve for the assay was prepared with purified *Escherichia coli* thioredoxin and the amount of thioredoxin is expressed as nmoles per mg of original cytosol protein.

RESULTS AND DISCUSSION

The steroid binding capacity of glucocorticoid receptors in cytosol preparations is considerably more labile than that of other steroid receptors. The rapidity of receptor inactivation varies according to the tissue from which the cytosol is prepared. In the rat, for example, receptors in liver cytosol are quite stable, whereas receptors in thymocyte cytosol are relatively labile, and those in lung cytosol are completely inactivated during the time of cytosol preparation [16–19]. To obtain maximum glucocorticoid binding capacity in cytosols from most rat tissues, it is necessary to have both a sulfhydryl reducing agent, such as dithiothreitol, and a receptor stabilizing agent, such as molybdate, present during tissue homogenization and centrifugation [23]. If cytosols are prepared in the absence of molybdate, then one assays only the binding capacity remaining at the time of steroid addition. In particularly labile cytosols, such as cytosol prepared from lung tissue, the number of receptor sites that are assayed may represent only a small fraction of the total possible number of binding sites in the preparation. When one is trying to establish strain-specific or tissue-specific differences in the number of glucocorticoid receptors, it is clearly important that assays be carried out under conditions that maximize binding capacity.

The experiment of Table 1 shows the effect of adding receptor-reducing and stabilizing agents to the buffers used for homogenizing livers and lungs from two mouse strains. These two organs were chosen for this study because: (1) They provide enough tissue mass to permit biochemical characterization of any observed strain differences in binding; (2) *H-2* regulation of glucocorticoid binding has been reported in fetal mouse hepatic cytosol [10]; and (3) It has recently been reported that glucocorticoid receptor levels are significantly higher in cytosols prepared from lungs of adult mice with the *H-2*^{a} haplotype (B10.A) than in a strain with the *H-2*^{b} haplotype (strain B10, which differs from B10.A within the *H-2* complex only) [24]. In both of these cases, glucocorticoid binding capacity was assayed in cytosols lacking reducing agents. If the *H-2* haplotype determines a real 2-fold difference in the number of receptors in lung cytosols, then this difference should also be observed when glucocorticoid binding capacity is maximized by the presence of dithiothreitol and molybdate. In the experiments presented in Table 1 we have assayed the binding in cytosols prepared from lungs of A/J mice which have the *H-2*^{a} haplotype and from C57BL/6J which have the *H-2*^{b} haplotype (Fig. 1). It is clear that no significant difference was found in binding capacity between the two strains, either in the absence or presence of additions.

The relatively low levels of specific glucocorticoid binding that have been reported [24] in lung cytosols prepared from B10.A and B10 mice (62 and 31 fmol/mg protein, respectively) probably do not represent the total binding capacity of the tissue. The glucocorticoid binding levels that we have observed in lung cytosol in the absence of reducing and stabilizing agents are so low that comparisons between strains would be meaningless. We have, however, prepared lung cytosols from the two congenic lines A.BY and B10.A using buffer containing dithiothreitol and molybdate. A.BY has the *H-2*^{b} region of the C57 strain placed on the A genetic background, while B10.A has the C57 background but the A/J *H-2*^{a} region. As shown in Table 2, there is no



Strain	Alleles at loci			
	Glo-1	H-2K	C4	H-2D
A/J	a	k	d	d
B10.A	a	k	d	d
C57BL/6	a	b	b	b
A.BYSn	a	b	b	b

Fig. 1. Partial genetic map of genes on chromosome 17 in the H-2 region of four congenic mouse strains. Data shown in Fig. 1 has been compiled from Klein *et al.* [28]. The mouse strains A/J and B10.A are designated H-2 haplotype a, while strains C57BL/6 and A.BYSn are designated H-2 haplotype b. Beneath the chromosome diagram, the letters a, b, k and d represent alleles for the following genes: glyoxylase-1 (Glo-1), complement component 4 (C4), histocompatibility-2K (H-2K) and histocompatibility-2D (H-2D). The numbers shown are approximate distances between the indicated loci in centimorgans. The centromere is indicated by the symbol (c).

relationship between *H-2* haplotype and specific binding capacity under these assay conditions. It is possible that the *H-2* related difference reported for lung cytosols in the absence of reducing and stabilizing agents reflects a genetically-determined difference in endogenous receptor-reducing capacity. Such a difference could explain both the difference in binding capacity reported in mouse lung cytosols [24] and the existence of an *H-2* regulated modifier of glucocorticoid binding reported in mouse liver cytosols [10].

Rat liver cytosol contains an endogenous, heat-stable glucocorticoid receptor-reducing system composed of NADPH and thioredoxin [19]. the glucocorticoid binding capacity of cytosol prepared from different rat tissues in the absence of dithiothreitol varies according to the activity of the

Table 2. Relationship of specific glucocorticoid-binding capacity of lung cytosols to *H-2* and genetic background

Background	Haplotype H-2[a]	Haplotype H-2[b]
	A/J	A.BY
A	260 ± 27	380 ± 59
	(10)	(6)
	B10.A	C57BL/6J
B	390 ± 51	348 ± 50
	(6)	(11)

Lungs obtained from four strains of mice were homogenized in 10 mM Hepes buffer, pH 7.35, with 10 mM dithiothreitol and 10 mM sodium molybdate. Aliquots of each cytosol preparation were incubated with [^{3}H]triamcinolone acetonide to determine the specific binding capacity as described under Methods. Glucocorticoid binding data is presented as fmoles steroid bound per mg of liver cytosol protein. Each value represents the mean and standard error for the number of cytosol preparations noted in parentheses. Analysis of variance revealed no significant difference among the groups ($F = 1.27$, $F_{0.95} = 2.93$, $F_{0.75} = 1.45$).

Table 3. Relationship of thioredoxin concentration in liver cytosols to *H-2* and genetic backgorund

Background	Haplotype H-2[a]	Haplotype H-2[b]
	A/J	A.BY
A	1.37 ± 0.29	1.70 ± 0.30
	(9)	(2)
	B10.A	C57BL/6J
B	1.94 ± 0.06	1.43 ± 0.14
	(2)	(8)

Thioredoxin levels were determined by the insulin disulfide reduction assay described in detail under Methods. The thioredoxin levels are expressed in nmoles per mg of liver cytosol protein, and the values represent the mean and standard error for the number of cytosol preparations noted in parentheses. Analysis of variance revealed no significant difference among the groups ($F = 0.50$, $F_{0.95} = 3.20$, $F_{0.75} = 1.50$).

endogenous reducing system [19]. Of four rat tissues that we have examined (liver, lung, spleen, thymus), the highest binding capacity, the highest endogenous receptor-activating activity, and the highest NADPH and thioredoxin levels were found in liver cytosol, whereas the lowest levels of all four activities were found in cytosol from lung [19]. As shown in Table 1, the glucocorticoid binding capacity of mouse liver cytosol is not highly sensitive to the addition of dithiothreitol. The relative stability of liver cytosol binding capacity is reflected in its substantial thioredoxin content. It is clear from the data of Table 3, however, that the thioredoxin content is similar in cytosols prepared from two pairs of two congenic mouse strains and that variations in the *H-2* locus do not regulate thioredoxin content. The levels of thioredoxin in mouse lung are not presented because they were below the sensitivity of the assay. The level of thioredoxin we have assayed in rat lung cytosol is somewhat higher than that of mouse but it is still only about 15% of that assayed in rat liver [19].

At this time, we feel that it is appropriate to reappraise the relationship between glucocorticoid binding capacity and the mouse histocompatibility locus. Several observations can be made that are useful to investigators who are interested in studying the biochemical mechanisms that determine the steroid-binding conformation, the stability, and the amount of glucocorticoid receptors. First, several laboratories have reported a higher glucocorticoid binding capacity in palatal cells of fetal mice exhibiting a high susceptibility to glucocorticoid-induced cleft palate (A/J) than in strains with low susceptibility (C57BL/6J and others) [4–7]. In one comprehensive study of triamcinolone acetonide binding in tissue from whole heads or palate-containing head sections of fetal mice, however, no correlation could be established between glucocorticoid binding capacity and cleft palate susceptibility [25]. Differences between laboratories may reflect the time at which fetal tissue was obtained. It has been reported that differences in glucocorticoid binding activity between A/J and C57BL/6J embryos which can be observed on day 11

have disappeared by day 13 or 14 of gestation [16]. Any differences in glucocorticoid binding capacity that are regulated by the *H-2* locus are not determined by expression of the structural gene for the glucocorticoid receptor protein, as that gene is located on chromosome 18, not with the *H-2* locus on chromosome 17 [27]. If the *H-2* complex contains a gene(s) that regulates the level of a modifier(s) that affects the binding of glucocorticoid to its receptor in liver cytosol [10], then that modifier is not either the steroid receptor "modulator" [15] or thioredoxin (Table 3). When specific glucocorticoid binding capacity is measured in the presence of reducing equivalents, there is no *H-2*-determined difference in binding in adult mouse lung cytosols (Tables 1 and 2). No *H-2* related difference in glucocorticoid binding capacity has been observed in cytosols prepared from mouse liver [7, 8]. Thus, there does not seem to be an adult mouse tissue where there is both clear *H-2* regulation of glucocorticoid binding capacity and sufficient tissue mass to permit the biochemical characterization of a strain difference.

Acknowledgements—This work was supported by NIH Grants DE06108 (R.P.E.) and AM31573 (W.B.P.).

REFERENCES

1. Shah R. M. and Travill A. A.: Morphogenesis of the secondary palate in normal and hydrocortisone-treated hamsters. *Teratology* **13** (1976) 71–84.
2. Bonner J. J. and Slavkin H. C.: Cleft palate susceptibility linked to histocompatibility-2 (*H-2*) in the mouse. *Immunogenetics* **2** (1975) 213–218.
3. Biddle F. G. and Fraser F. C.: Cortisone-induced cleft palate in the mouse. A search for the genetic control of the embryonic response trait. *Genetics* **85** (1977) 289–302.
4. Salomon D. S. and Pratt R. M.: Glucocorticoid receptors in murine embryonic facial mesenchyme cells. *Nature* **264** (1976) 174–177.
5. Goldman A. S., Katsumata M., Yaffe S. J. and Gasser D. L.: Palatal cytosol cortisol-binding protein associated with cleft palate susceptibility and *H-2* genotype. *Nature* **265** (1977) 643–645.
6. Salomon D. S. and Pratt R. M.: Involvement of glucocorticoids in the development of the secondary palate. *Differentiation* **13** (1979) 141–154.
7. Katsumata M., Baker M. K., Goldman A. S. and Gasser D. L.: Influence of *H-2*-linked genes on glucocorticoid receptors in the fetal mouse palate. *Immunogenetics* **13** (1981) 319–325.
8. Butley M. S., Erickson R. P. and Pratt W. B.: Hepatic glucocorticoid receptors and the *H-2* locus. *Nature* **275** (1978) 136–138.
9. Edidin M.: The tissue distribution and cellular location of transplantation antigens. In *Transplantation Antigens.* (Edited by B. D. Kahan and R. A. Reisfeld). Academic Press, New York (1979) pp. 125–140.
10. Katsumata M., Baker M. K. and Goldman A. S.: An *H-2* linked difference in the binding of dexamethasone to murine hepatic cytosol receptor. *Biochim. biophys. Acta* **676** (1981) 245–256.
11. Cake M. H., Goidl J. A., Parchman L. G. and Litwack G.: Involvement of a low molecular weight component(s) in the mechanism of action of the glucocorticoid receptor. *Biochem. biophys. Res. Commun.* **71** (1976) 45–52.
12. Goidl J. A., Cake M. H., Dolan K. P., Parchman L. G. and Litwack G.: Activation of the rat liver glucocorticoid-receptor complex. *Biochemistry* **16** (1977) 2125–2130.
13. Schmidt T. J. and Litwack G.: Activation of the glucocorticoid-receptor complex. *Physiol. Rev.* **62** (1982) 1131–1192.
14. Leach K. L., Grippo J. F., Housley P. R., Dahmer M. K., Salive M. E. and Pratt W. B.: Characteristics of an endogenous glucocorticoid receptor stabilizing factor. *J. biol. Chem.* **257** (1982) 381–388.
15. Leach K. L., Erickson R. P. and Pratt W. B.: The endogenous heat-stable glucocorticoid receptor stabilizing factor and the *H-2* locus. *J. steroid Biochem.* **17** (1982) 121–123.
16. Granberg J. P. and Ballard P. L.: The role of sulfhydryl groups in the binding of glucocorticoids by cytoplasmic receptors of lung and other mammalian tissues. *Endocrinology* **100** (1977) 1160–1168.
17. Sando J. J., Nielsen C. J. and Pratt W. B.: Reactivation of thymocyte glucocorticoid receptors in a cell-free system. *J. biol. Chem.* **252** (1977) 7579–7582.
18. Sando J. J., Hammond N. D., Stratford C. A. and Pratt W. B.: Activation of thymocyte glucocorticoid receptors to the steroid binding form. *J. biol. Chem.* **254** (1979) 4779–4789.
19. Grippo J. F., Tienrungroj W., Dahmer M. K., Housley P. R. and Pratt W. B.: Evidence that the endogenous heat-stable glucocorticoid receptor activating factor is thioredoxin. *J. biol. Chem.* **258** (1983) 13658–13664.
20. Pratt W. B., Kaine J. L. and Pratt D. V.: The kinetics of glucocorticoid binding to the soluble specific binding protein of mouse fibroblasts. *J. biol. Chem.* **250** (1975) 4584–4591.
21. Nielsen C. J., Sando J. J., Vogel W. M. and Pratt W. B.: Glucocorticoid receptor inactivation under cell-free conditions. *J. biol. Chem.* **252** (1977) 7568–7578.
22. Holmgren A.: Thioredoxin catalyzes the reduction of insulin disulfides by dithiothreitol and dihydrolipoamide. *J. biol. Chem.* **254** (1979) 9627–9632.
23. Dahmer M. K., Housley P. R. and Pratt W. B.: Effects of molybdate and endogenous inhibitors on steroid receptor inactivation, transformation and translocation. *A. Rev. Physiol.* **46** (1984) 67–81.
24. Gupta C. and Goldman A.: *H-2* histocompatibility region: Influence on the murine glucocorticoid receptor and its response. *Science* **216** (1982) 994–996.
25. Hackney J. F.: A glucocorticoid receptor in fetal mouse: Its relationship to cleft palate formation. *Teratology* **21** (1980) 39–51.
26. Salomon D. S., Zubairi Y. and Thompson E. B.: Ontogeny and biochemical properties of glucocorticoid receptors in mid-gestation mouse embryos. *J. steroid Biochem.* **9** (1978) 95–107.
27. Franke U. and Gehring U.: Chromosome assignment of a murine glucocorticoid receptor gene (GR 1-1) using intraspecies somatic cell hybrids. *Cell* **22** (1980) 657–664.
28. Klein J., Figueroa F. and Klein D: H-2 haplotypes, genes, and antigens: second listing. *Immunogenetics* **16** (1982) 285–317.