millennia by their location in a well-sealed environment and by concealment between the glumes of a whole spikelet. As a result of the transfer of the fragile material from the excavated site and handling in the laboratory, several specimens disintegrated, exposing the fungi and leading to more detailed examination.

According to some commentators (*6*), rust is mentioned in the Hebrew Bible (*7*) as a crop-damaging disease called *yeraqon*. This Hebrew word, referring to some phenomenon characterized by the color green or orange, also appears in the Mishnah to describe a kind of spreading plague of wheat (*8*). From the Biblical and the Mishnaic contexts, it is reasonable to suggest that *yeraqon* was the ancient term for infestations of *Puccinia graminis* f. sp. *tritici*, for when this rust strikes in a given year, its effects may be devastating and widespread. The Greeks also knew it in classical times (*9*). Other wheat diseases, although more common, are usually less destructive to the annual crop.

Archeological fungal remnants are found only rarely. These include sclerotia of species such as *Claviceps purpurea* or *Cenococcum geophilum*, or the hard fruiting bodies of *Polyporus* (*10*). In addition, some exceptional findings of microscopic fungi have been reported: *Mycosphaerella*-infested *Lolium perenne* grass from ancient Egypt more than 3000 years old (*11*); the hyphae of a fungus that attacked darnel grains in Egypt about 2000 B.C. (*12*); wine and beer yeast cells from the 11th Egyptian dynasty (*13*); and spores of several species of *Ustilago*-attacking barley found in the stomach of Grauballe man from the first centuries A.D. in Denmark (*14*).

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References and Notes

1. A. Mazar and G. L. Kelm, *Isr. Explor. J.* **29**, 241 (1979).
2. M. Kislev, *Isr. J. Bot.* **28**, 95 (1980).
3. W. M. Bowden, *Can. J. Bot.* **37**, 657 (1959).
4. A. Dinoor of the Hebrew University of Jerusalem confirmed the identification.
5. M. Hopf, *Ber. Dtsch. Bot. Ges.* **68**, 191 (1955).
6. A. J. Einhorn, *Torat Avodat Ha'adama* (Gordizki, London, 1910), p. 211; I. Löw, *Die Flora der Juden* (Löwit, Vienna, 1924), vol. 1, p. 40.
7. Deut. 28:22; 1 Kings 8:37.
8. *Taanith*, chapter 3:5.
9. Theophrastus, *Enquiry into Plants* (Loeb Classical Library, Harvard Univ. Press, Cambridge, Mass.), chap. 10, p. 1.
10. E. Neuweiler, *Vjschr. Naturf. Ges. Zürich* **80**, 98 (1935); K. H. Knörzer, *Bonner Jahrb.* **171**, 9 (1971); G. Seehann, in *Berichte über die Ausgrabungen in Haithabu*, K. Schietzel, Ed. (Wachholz, Neumünster, 1977), pp. 120–140.
11. V. Laurent-Täckholm, *Sven. Bot. Tidskr.* **34**, 141 (1940).
12. G. Lindau, *Sber. Preuss. Akad. Wiss.* **92**, 1031 (1904).
13. J. Grüss, *Tagesztg. Brau.* **27**, 275 (1929); *ibid.*, p. 517; *ibid.*, p. 679.
14. H. Helbaek, *Kuml* **1958**, 83 (1959).
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H-2 Histocompatibility Region: Influence on the Murine Glucocorticoid Receptor and Its Response

Abstract. *The influence of the* H-2 *histocompatibility complex on glucocorticoid receptor levels, and the biochemical response of glucocorticoid action measured as the degree of inhibition of prostaglandin production, has been studied in the mouse thymus and lung. The B10A* (H-2^a) *strain of mice has significantly higher glucocorticoid receptor levels and a significantly greater biochemical response to glucocorticoid than the B10* (H-2^b) *strain, which differs from B10A within the* H-2 *complex only. Thus, the anti-inflammatory hormone response of glucocorticoids is correlated to hormone receptor level, both of which are influenced by the* H-2 *locus.*

Susceptibility to cortisone-induced cleft palate in the mouse is regulated in part by two genes acting by complementation within the *H-2* histocompatibility complex on chromosome 17 (*1–6*). The same genetic region also appears to influence the level of glucocorticoid receptors in mouse embryonic palatal cells (*7, 8*). *H-2*–linked genes cannot be demonstrated to regulate receptor levels in the liver (*8–10*); this is in agreement with observations of tissue-specific variation in cortisol receptors in rats (*11*). Recently, it has been reported that *H-2* regulates the degree of glucocorticoid thymolytic responses in the mouse (*12, 13*). Thus, it is possible that *H-2* regulates glucocorticoid receptor levels in the thymus. Since both the hormonal action (*14, 15*) and teratogenic action (*16, 17*) of glucocorticoids involve inhibition of arachidonic acid release and of subsequent prostaglandin and thromboxane production, we have sought to examine whether glucocorticoid receptor levels and the degree of inhibition of prostaglandin production may be influenced by *H-2* in thymocytes of the B10A and B10 mouse strains. The B10 and B10A strains are genetically similar, except for the *H-2* complex and closely linked genes on chromosome 17, where B10A is *H-2*^{a} and B10 is *H-2*^{b}. We now report that *H-2*–linked genes influence glucocorticoid receptor levels in the thymus as well as in the lung. We also demonstrate that, in thymocytes, glucocorticoid receptor levels correspond to the amount of inhibition of prostaglandin and thromboxane production by glucocorticoids.

Dexamethasone was used for most of the experiments, since dexamethasone is tightly bound to the receptor and does not bind to transcortin or ligandin (*18, 19*). Moreover, dexamethasone has a very high potency as a glucocorticoid (*20*). We used the method of Katsumata *et al.* (*8*) to look for a glucocorticoid-binding receptor protein in thymocytes and lung cytosol. The receptor level and degree of inhibition of prostaglandin production by glucocorticoid were studied in the B10A congenic strain and its inbred partner B10, and inhibition of prostaglandin synthesis was studied by use of a radioimmunoassay procedure.

We found that the [^{3}H]dexamethasone binding sites in thymocytes (Fig. 1) and lung (Fig. 2) are saturable. A Scatchard analysis of the binding activity indicated a single class of binding molecules in both lung and thymus. The dissociation constant, K_d, of the glucocorticoid receptor protein in thymocytes is significantly higher in the B10A than in the B10 receptor protein ($K_d = 6.3 \times 10^{-8} \pm 2.6 \times 10^{-8} M$ in B10A, and $3.4 \times 10^{-8} \pm 0.9 \times 10^{-8} M$ in B10), but no significant difference in the K_d value was noticed in the lung cytosol of the two strains (Table 1).

The receptor content in the lungs and in the thymocytes was significantly higher in the B10A strain than in the B10 strain (Table 1), and this was true for both males and females. Although there was no difference in the receptor content of male and female thymuses, we found a significantly higher receptor content in lung cytosols from females than from males in both strains.

We also determined the receptor level in the thymocyte nuclear fraction in order to examine whether the strain difference in receptor content could be observed in the receptors that translocated to the nucleus. The results showed that the nuclei of the B10A mice had a significantly higher receptor content than did those of the B10 mice (Table 1).

In the second series of experiments, we investigated glucocorticoid-induced suppression of prostaglandin production (Table 2). Dexamethasone (5 to 100 μM)

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inhibited prostaglandin production in a dose-dependent fashion (data not shown). We analyzed four prostaglandins: (i) 6-keto–prostaglandin $F_{1\alpha}$ (6-keto–$PGF_{1\alpha}$), the stable metabolite of prostacyclin; (ii) thromboxane B_2 (TXB_2), the stable metabolite of thromboxane A_2; (iii) prostaglandin E_2 (PGE_2); and (iv) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). A significantly higher degree of inhibition by dexamethasone was observed in B10A thymocytes than in B10 thymocytes in the production of each of the four prostaglandins at each concentration of dexamethasone, with the exception of PGE_2 at the lower concentration (Table 2). A higher degree of inhibition of 6-keto–$PGF_{1\alpha}$, TXB_2, and $PGF_{2\alpha}$ was also observed in lung homogenates and spleen cells of B10A as compared to B10 (not shown).

Glucocorticoids produce their biological action by binding to cytosolic receptors. The steroid-receptor complex is then translocated to the nucleus where it induces a nondialyzable protein called macrocortin (*14*) or lipomodulin (*15*), which depresses prostaglandin and thromboxane production by inhibition of phospholipase A_2. Our study shows that glucocorticoid receptor levels correspond to the anti-inflammatory hormonal action of inhibition of prostaglandin and thromboxane synthesis from arachidonic acid in thymocytes and that this level is influenced by the *H-2* histocompatibility

Table 1. Glucocorticoid binding by lung cytosol and thymocytes of B10 and B10A mice. The mice were decapitated, and the thymus and lungs were removed and placed in ice-cold RPMI medium. The thymus was grated on a steel wire gauge in the presence of medium, and a cell suspension (1×10^7 to 2×10^7 cells per milliliter) was used for determination of glucocorticoid receptor level by the method of Katsumata *et al.* (*8*). Cell suspension (2 ml) was added in a series of tubes containing 5 to 100 n*M* [^{3}H]dexamethasone with or without 10 μ*M* unlabeled dexamethasone and incubated for 1 hour at 37°C, with occasional shaking. At the end of the incubation period, 4 ml of medium was added to each tube, and the tubes were centrifuged at 800*g*. The pellet was washed twice with 2 ml of 50 m*M* tris buffer containing 120 m*M* NaCl and 1 m*M* $CaCl_2$, *p*H 7.7, and centrifuged. The resulting pellet was homogenized in 1 ml of buffer. Portions of the homogenate were transferred for the measurement of radioactivity, protein, and DNA. The rest of the homogenate was centrifuged at 800*g* for 10 minutes for preparation of a nuclear pellet and washed twice with 2 ml of buffer. The nuclear pellet was suspended in 1 ml of buffer and used for the determination of radioactivity, protein, and DNA. The specific binding of dexamethasone was determined by subtracting the binding in the presence of 10 μ*M* unlabeled dexamethasone from the binding found in its absence. Scatchard plots were drawn for both whole cell binding and nuclear binding of dexamethasone binding sites, and dissociation constants were obtained from the Scatchard plot in each determination. The lungs were homogenized 1:2 (weight to volume) in the buffer, and the cytosol was prepared by centrifuging the homogenate at 100,000*g*. The supernatant (0.2 ml) was incubated with 10 to 100 n*M* [^{3}H]dexamethasone in the presence or absence of 10 μ*M* unlabeled dexamethasone at 4°C for 2 hours. At the end of the incubation period, free dexamethasone was removed with 0.2 ml of 0.5 percent charcoal containing 0.005 percent dextran in the buffer. After a 10-minute wait at 4°C, the tubes were centrifuged at 1000*g* for 10 minutes. The supernatant was used for the determination of radioactivity and protein concentration. Specific binding of dexamethasone, the concentration of binding sites, and the dissociation constants were determined as described above. The data represent means ± standard deviations (S.D.) of four to six sets of experiments for each sex and each tissue.

Strain	Sex	Preparation	Binding sites (pmole)			K_d	
			Thymus		Lung		
			Per milligram of protein	Per milligram of DNA	Per milligram of protein	Thymus ($\times 10^{-8} M$)	Lung ($\times 10^{-9} M$)
B10A	Male	Whole cell	4.16 ± 1.14	7.28 ± 1.28		6.31 ± 2.67	
		Nucleus	3.80 ± 0.84	3.47 ± 1.11		5.85 ± 1.95	
		Cytosol			0.062 ± 0.006		2.60 ± 0.80
	Female	Whole cell	4.87 ± 0.41	6.53 ± 1.28		6.46 ± 1.65	
		Nucleus	3.89 ± 0.82	5.45 ± 1.97		4.64 ± 1.50	
		Cytosol			0.143 ± 0.022		4.53 ± 1.42
B10	Male	Whole cell	2.33 ± 0.93*	3.17 ± 0.95†		3.41 ± 0.92‡	
		Nucleus	1.54 ± 0.76‡	1.39 ± 0.61†		1.67 ± 1.05†	
		Cytosol			0.031 ± 0.005†		1.80 ± 0.70
	Female	Whole cell	2.13 ± 0.71†	2.82 ± 0.81‡		3.15 ± 1.02‡	
		Nucleus	2.18 ± 0.63‡	2.53 ± 0.32‡		1.89 ± 0.55‡	
		Cytosol			0.073 ± 0.008‡		3.10 ± 1.73

*$P < .001$. †$P < .005$. ‡$P < .01$. The probabilities are associated with Student's *t*-test of the differences between corresponding values in B10A and B10.

Table 2. Inhibition of prostaglandin production by dexamethasone in B10 and B10A thymocytes. The thymocytes from B10 and B10A male mice were prepared in 0.15*M* phosphate buffer containing 0.9 percent NaCl and 0.1 percent gelatin with a procedure similar to that described in Table 1. Two milliliters of thymocyte preparation (1×10^7 to 2×10^7 cells) were incubated in the presence of zymosan (250 μg/ml) to stimulate prostaglandin production (*26*) with or without dexamethasone (20 and 100 μ*M*) at 37°C for 3 hours. The reaction was stopped by adding 10 ml of ethyl acetate. Prostaglandins in the incubation mixture were extracted with ethyl acetate and dried under N_2. The dried residue in the tubes was dissolved in 1 ml of ethanol, and portions were used for prostaglandin estimation with a radioimmunoassay procedure (*27*). The antibodies used for 6-keto–$PGF_{1\alpha}$, TXB_2, and $PGF_{2\alpha}$ were specific, whereas the antibody used for testing PGE_2 also cross-reacts with PGE_1, PGA_1, and PGA_2. Percent inhibition was determined by dividing the amount of product made in the absence of dexamethasone minus the amount of product made in the presence of dexamethasone by the amount of product made in the absence of dexamethasone and multiplying the result by 100. Data represent means ± S.D. from 24 determinations for each prostaglandin or thromboxane and for each thymus.

Product	Control production (pg/10^6 cell)		Percent of inhibition			
			With 20 μ*M* dexamethasone		With 100 μ*M* dexamethasone	
	B10A	B10	B10A	B10	B10A	B10
6-Keto–$PGF_{1\alpha}$	103.3 ± 42.1	91.3 ± 57.2	42.7 ± 13.0	21.2 ± 21.3*	72.2 ± 11.3	41.3 ± 16.2†
TXB_2	67.4 ± 54.2	53.2 ± 54.2	12.9 ± 8.7	3.1 ± 3.5†	40.5 ± 14.8	29.9 ± 15.8‡
$PGF_{2\alpha}$	59.9 ± 24.6	62.4 ± 41.5	12.4 ± 5.4	7.3 ± 5.6‡	31.8 ± 14.5	4.9 ± 4.6†
PGE_2	428.5 ± 249.5	414.0 ± 231.6	7.7 ± 12.1	8.2 ± 11.6§	14.8 ± 12.8	8.3 ± 7.5‡

*$P < .001$. †$P < .00001$. ‡$P < .05$. The probabilities are associated with Student's *t*-test of the differences between corresponding values in B10A and B10. §Not significant.

complex. These findings are consistent with the increased thymolytic responses of glucocorticoid produced in the B10A strain (*12, 13*). Although the biochemical pathway of growth arrest is quite complex, it may eventually be shown that inhibition of prostaglandins, thromboxanes, or other arachidonic acid metabolic products are involved in the thymolytic effect of glucocorticoids. The present findings may also be related to those concerning susceptibility to cortisone-induced cleft palate (*1–7*). Glucocorticoid receptor levels also correspond to the glucocorticoid responses of palatal clefting, and this is also influenced by the *H-2* complex (*7, 8*). Although it cannot be said with certainty that the *H-2*–linked increased receptor level accounts for the susceptibility phenotype, glucocorticoid action in palatal clefting also depends on the arachidonic acid pathway inasmuch as teratogenicity can be considerably reversed by exogenous arachidonic acid (*16, 17*).

The dissociation constants of the receptor-dexamethasone complex in the thymocytes and nuclei of each sex of the B10 strain are significantly lower than those of the B10A strain. This could be due to an influence of *H-2* on the thymus receptor binding site or on the presence of modifiers of binding in the thymus. There is evidence that *H-2* influences the presence of low molecular weight modifiers in the liver that affect binding of dexamethasone to its receptor (*9*). The locus for the major glucocorticoid receptor is on chromosome 18 (*21*). Therefore, the *H-2* region on chromosome 17 either codes for a different receptor or has an indirect effect on the major receptor.

H-2–linked differences in receptor content have been reported for other hormones, such as estrogen (*22*), insulin (*23*), and glucagon (*24*). Androgen level is also controlled by the *H-2*–linked complex (*25*). These results suggest that the *H-2* complex influences a variety of hormone actions.

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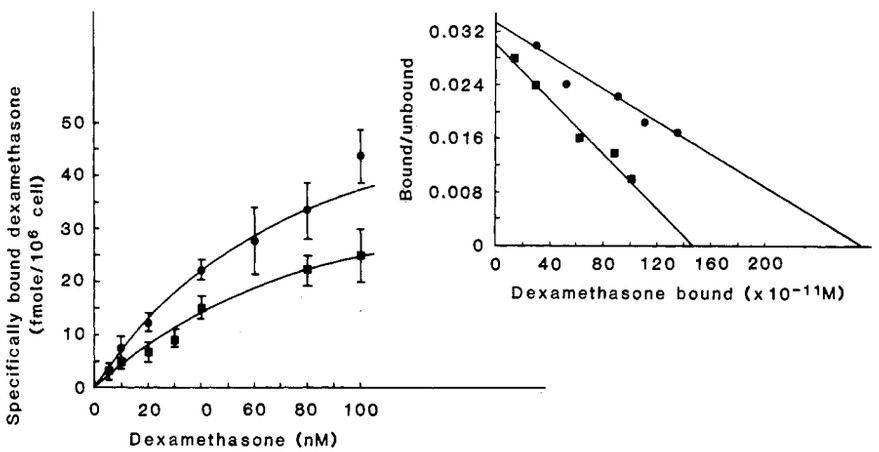

Fig. 1. Glucocorticoid-binding activity in thymocytes of (■) B10 and (●) B10A male mice. Details of the experiment are described in the legend of Table 1. Data represent means ± S.D. from six sets of experiments for each strain of mice. The inset shows a Scatchard plot of the binding data. For B10A, the number of binding sites was 67.5 fmole of [^{3}H]dexamethasone per 10^6 cells, and $K_d = 8.2 \times 10^{-8}M$. For B10, the number of binding sites was 35.5 fmole of [^{3}H]dexamethasone per 10^6 cells, and $K_d = 4.6 \times 10^{-8}M$.

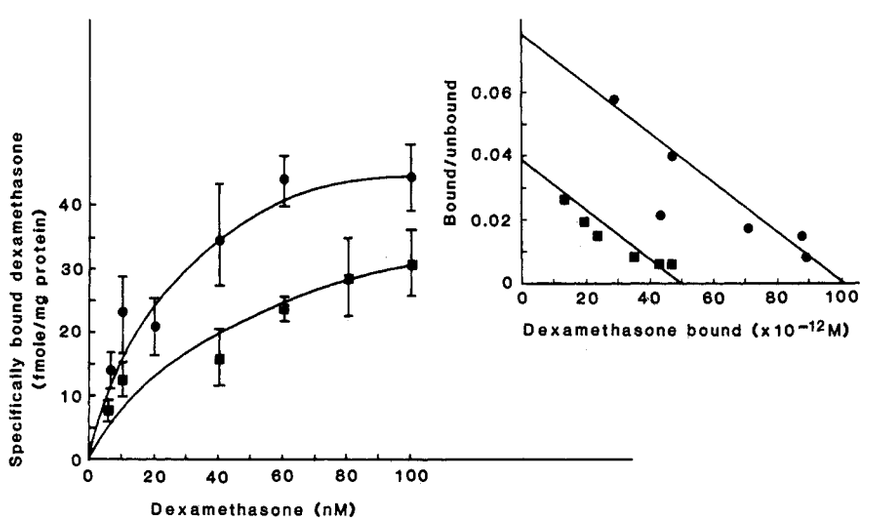

Fig. 2. Glucocorticoid-binding activity in lung cytosol of (■) B10 and (●) B10A male mice. Details of the experiment are described in the legend of Table 1. Data represent means ± S.D. from four sets of experiments for each strain of mice. The inset shows a Scatchard plot of the binding data. For B10A, the number of binding sites was 66.6 fmole per milligram of protein, and $K_d = 1.3 \times 10^{-8}M$. For B10, the number of binding sites was 33.3 fmole per milligram of protein, and $K_d = 1.0 \times 10^{-8}M$.

References and Notes

1. J. J. Bonner and H. C. Slavkin, *Immunogenetics* **2**, 213 (1975).
2. F. G. Biddle and F. C. Fraser, *Genetics* **85**, 289 (1977).
3. M. L. Tyan and K. K. Miller, *Proc. Soc. Exp. Biol. Med.* **158**, 618 (1978).
4. D. L. Gasser, L. Mele, A. S. Goldman, *Seventh International Convocation of Immunology, Niagara Falls* (Karger, Basel, 1981), p. 320.
5. D. L. Gasser, L. Mele, D. D. Lees, A. S. Goldman, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3147 (1981).
6. M. Melnick, T. Jaskoll, H. C. Slavkin, *Immunogenetics* **13**, 443 (1981).
7. A. S. Goldman, M. Katsumata, S. J. Yaffe, D. L. Gasser, *Nature (London)* **265**, 643 (1977).
8. M. Katsumata, M. K. Baker, A. S. Goldman, D. L. Gasser, *Immunogenetics* **13**, 319 (1981).
9. M. Katsumata, M. K. Baker, A. S. Goldman, *Biochim. Biophys. Acta* **676**, 245 (1981).
10. M. S. Butley, R. P. Erickson, W. B. Pratt, *Nature (London)* **275**, 136 (1978).
11. R. D. Markovic, H. J. Eisen, L. G. Parchman, C. A. Barnett, G. Litwack, *Biochemistry* **19**, 4556 (1980).
12. M. Pla, J. Zakany, J. Fachet, *Folia Biol. (Prague)* **22**, 49 (1976).
13. M. L. Tyan, *Immunogenetics* **8**, 177 (1979).
14. G. J. Blackwell, R. Carnuccio, M. DiRosa, R. J. Flower, L. Parente, P. Persico, *Nature (London)* **287**, 147 (1980).
15. F. Hirata *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3190 (1981).
16. G. G. Tzortzatou, A. S. Goldman, W. C. Boutwell, *Proc. Soc. Exp. Biol. Med.* **166**, 321 (1981).
17. A. S. Goldman, R. Herold, R. Piddington, *Teratology* **23**, 6 (1981).
18. G. Litwack, R. Filler, S. A. Rosenfield, N. Lichtash, C. A. Lishman, S. Singer, *J. Biol. Chem.* **248**, 7481 (1973).
19. G. G. Rousseau, J. D. Baxter, G. M. Tomkins, *J. Mol. Biol.* **67**, 99 (1972).
20. R. J. Flower and G. J. Blackwell, *Nature (London)* **278**, 456 (1979).
21. V. Francke and V. Gehring, *Am. J. Hum. Genet.* **32**, 155A (1980).
22. D. A. Palumbo and A. O. Vladutiu, *Experientia* **35**, 1103 (1979).
23. D. Meruelo and M. Edidin, *Contemp. Top. Immunobiol.* **9**, 231 (1980).
24. W. Lafuse, and M. Edidin, *Biochemistry* **197**, 49 (1980).
25. P. Ivany, *Proc. R. Soc. London Ser. B* **202**, 117 (1978).
26. J. L. Humes, R. J. Bonney, L. Pelus, M. E. Dahlgren, S. J. Sadowski, F. A. Kuehl, Jr., P. Davies, *Nature (London)* **269**, 149 (1977).
27. E. Granstrom and H. Kindaht, in *Advances in Prostaglandin and Thromboxane Research*, J. Frohlich, Ed. (Raven, New York 1978), vol. 5, p. 119.
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