Antibody Titers and Plasma Glucocorticoid Concentrations Near Weaning in Steer and Heifer Calves

F. C. GWAZDAUSKAS, W. B. GROSS, T. L. BIBB AND M. L. MCGILLIARD*

INTRODUCTION

The involvement of adrenal glucocorticoids and immunologic competence has been under investigation in laboratory animals for many years. As early as 1936, Selye (16) reported that injury to rats or stressful stimuli could cause adrenal enlargement and thymic involution. More recent experimentation demonstrated that glucocorticoids are involved with depletion of cortical thymic lymphocytes and with depressions in germinal centre formation and lymphoid cell proliferation in the spleen of mice (4, 9, 14). In mice, the period of maximum sensitivity to glucocorticoid action on loss of spleenic mass and reduction of antibody production was between 24 and six hr prior to sheep red blood cell (SRBC) injection (8). Studies in which laboratory mice are subjected to crowding reveal that this stress leads to reduced antibody production following injections of SRBC and a decreased capability of the laboratory animals to resist parasitic invasion (2, 3).

Although the literature abounds with data on the effects of stress on antibody production in laboratory animals (2, 3, 4, 8, 9, 12, 16), there is a relative paucity of information delineating stress involvement with humoral or cell mediated immunity in domestic animals. Research with chickens has demonstrated that stress and elevated blood glucocorticoid concentrations are involved with both humoral and cell mediated immunity (5, 6, 15, 19, 20). Thaxton *et al* (19) found no decline in antibody titers to SRBC when chickens were exposed to heat stress. However, adrenocorticotropin (ACTH) injections between 24 and nine hr prior to antigen injection reduced antibody titers.

The objective of this study was to evaluate relationships of antibody production to porcine red blood cells (PRBC), equine red blood cells (HRBC) and plasma glucocorticoid concentrations near the stressful time of weaning in beef calves.

MATERIALS AND METHODS

Sixty-three Shorthorn steer calves and 62 Shorthorn heifer calves between six and eight months of age were used in the study. Calves were weaned from dams at one time as routine management of the university herd. All calves received 1 ml of 5% cells (10^9 cells) of either PRBC or HRBC intravenously in a reversal trial one week prior to weaning. The red blood cells were obtained from healthy animals. Erythrocytes were washed twice with saline and used as a suspension in saline. In the 1974 study the second antigen was injected (the opposite of the first administered) on the day of weaning, whereas in 1975, the second antigen was given the day after weaning.

Heparinized blood samples were collected via vena puncture at the time of antigen injection and seven days following weaning. Antibody titers against PRBC or HRBC antigen were determined by the tube agglutination method on blood samples collected seven days after the antigen injection. Serial serum dilutions were incubated at 37°C with 0.5% red blood cells (RBC) overnight. A group of six tubes was used and the titer of a sample was designated as the last tube in the serial to exhibit agglutination. Titer was expressed in $\log_2$ of the reciprocal of the highest dilution to give agglutination. These values were analyzed statistically. Concentrations of total plasma glucocorticoid were determined on all samples following organic solvent extraction and quantification by competitive protein binding radioassay (7, 13). Statistical evaluation of the data was by univariate regression analyses (17).

RESULTS

Effects on antibody titers

Antibody titer production in calves was significantly affected by the year in which they received antigen ($P < 0.01$), their sex classification ($P < 0.05$) and the period ($P < 0.01$) of injection of the antigen, i.e. 1) antigen injection preweaning and 2) antigen injection near weaning (Table I). The coefficient of multiple correlation (R^2) for antibody titer in the model in Table I was 0.28 and the coefficient of variation (CV) was 44.1%. Means and standard errors of antibody titers for both antigens were 5.13 ± 0.22 ($\bar{x} \pm$ SE) $\log_2$ in 1974 and 4.02 ± 0.18 $\log_2$ in 1975. Steer calves responded with a greater antibody production than heifers to either antigen. Mean antibody titer for steers was 4.85 ± 0.22 $\log_2$ compared to 4.31

*Department of Dairy Science (Gwazdauskas and McGilliard) and Department of Veterinary Science (Gross and Bibb), Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061. Supported by grant #8488571 from the Virginia Agricultural Foundation.

TABLE I

ANALYSIS OF VARIANCE FOR ANTIBODY TITER ON ALL DATA

Source	df	MS
Year	1	74.5[b]
Sex[a]	1	20.5[c]
Antigen	1	11.2
Sex[a] × Antigen	1	0.7
Period	1	221.2[b]
Antigen × Period	1	16.8[c]
Year × Period	1	24.0[c]
Sex[a] × Period	1	0.7
Error	241	4.1

[a]Sex denotes classification for steers and heifers.
[b]$P < 0.01$.
[c]$P < 0.05$.

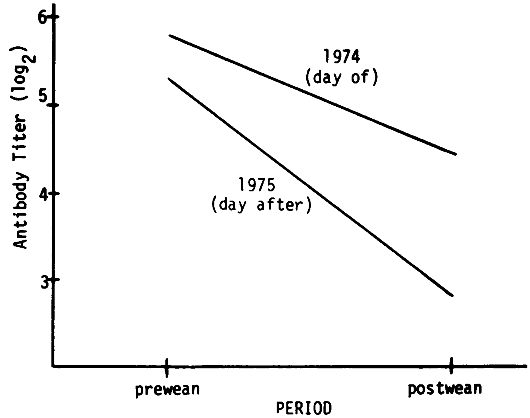

FIGURE 2. Year × period interaction for antibody titer ($\log_2$).

± 0.19 $\log_2$ for heifers ($P < 0.05$). The stress of weaning was associated with a significant ($P < 0.01$) 1.9 $\log_2$ titer decline in antibody production. When either antigen (HRBC or PRBC) was injected one week prior to weaning, antibody titers averaged 5.52 ± 0.20 $\log_2$. However, when the antigen was administered either the day of or the day after weaning, antibody titers were only 3.64 ± 0.19 $\log_2$.

Interactions between antigen and period and between year and period are best illustrated by Figures 1 and 2. The responses to PRBC and HRBC in the first period were comparable (5.5 ± 0.2 and 5.6 ± 0.3 $\log_2$). However, there was a significantly lower response in antibody production when HRBC were given near weaning (3.1 ± 0.3 vs. 4.2 ± 0.3 $\log_2$). The reduction in antibody titer from period one to period two was greater in 1975. The initial antibody titer in 1974 was 5.8 ± 0.3 compared to 5.3 ± 0.2 $\log_2$ for 1975. The interaction is apparent because of the depressed antibody response to the second antigen in 1975 (4.5 ± 0.3 vs. 2.8 ± 0.2 $\log_2$).

Hormonal – antibody titer relationships

Concentrations of total plasma glucocorticoids were determined on blood samples collected during the second year of the study. The regression analysis (Table II) revealed sex classification differences associated with final glucocorticoid concentrations ($P < 0.01$) and that initial glucocorticoid concentration (corticoid 1) and the crossproduct of glucocorticoid concentrations (corticoid 1.2) affected antibody titer differences ($P < 0.05$).

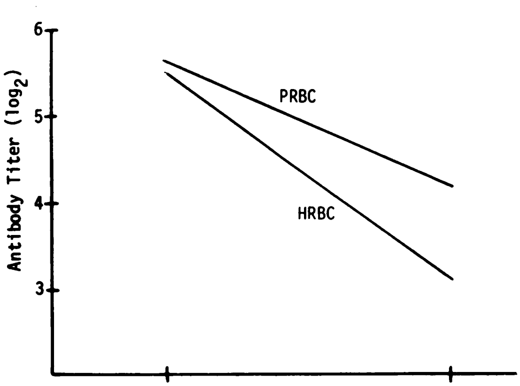

FIGURE 1. Antigen × period interaction for antibody titer ($\log_2$).

Least squares means for glucocorticoid concentrations taken when the second antibody titer was determined were 33.1 ng/ml for steers and 15.9 ng/ml for heifers.

Each 1 ng/ml increase in the initial glucocorticoid concentration was associated with an increase in antibody titer difference of 0.09 $\log_2$ units. Figure 3 depicts antibody titer differences in response to glucocorticoid concentrations in plasma when blood was collected at seven days following the initial antigen injection and seven days after the second antigen injection. When glucocorticoids were low at the time antigens were injected their antibody titer differences were low. If glucocorticoids were elevated at the first antigen injection and remained low for the second antigen injection, a large antibody titer difference was found. The same situation holds for low glucocorticoid concentrations at the first antigen injection and elevated steroid at the second injection. However, if glucocorticoids were elevated when either antigen was injected, the difference in antibody titers reverses such that at 68 ng/ml glucorcorticoid at each injection the second antibody titer was actually greater than it was following administration of the first antigen.

DISCUSSION

Antibody production was lower during the second year of the study. Many factors may be involved with the difference. Climatic conditions and environmental stress across years may account for part of the difference. A major portion of the year variability may be due to the year by

TABLE II

MEAN SQUARES FOR SOURCES OF VARIATION IN CORTICOID CONCENTRATIONS AND ANTIBODY TITER DIFFERENCE

Source	df	Final Corticoid MS	Antibody Titer Difference MS
Sex[a]	1	5572.5[e]	0.6
Antigen	1	13.5	1.2
Corticoid 1[b]	1	1.0	25.4[f]
Corticoid 2[c]	1	0.5	7.3
Corticoid 1.2[d]	1	221.7	23.6[f]
Sex[a] × Antigen	1	293.3	0.6
Error	59	131.9	4.8

[a]Sex denotes classification for steers and heifers.
[b]Initial glucocorticoid concentration at first antigen injection.
[c]Glucocorticoid concentration at second antigen injection.
[d]Crossproduct of b and c.
[e]$P < 0.01$.
[f]$P < 0.05$.

period interaction. Part of this effect is probably due to the time of the second antigen administration. In 1975 the second antigen was given the day after weaning. The amount of time away from the dam prior to antigen injection probably influenced antibody titer production. It appears that the duration of stress prior to antigen injection influences the antibody response (5, 8, 20). This is supported by ACTH and corticoid experimentation which demonstrated that elevated blood corticoid concentrations must be maintained for at least six hours prior to antigen injection in order to diminish antibody titer production (5, 8, 19).

Steers produced significantly higher antibody titers than heifers. Differences in antibody titer response may be due to circulating sex steroids in the heifers compared to the lack of sex steroids in the steers. Immunosuppressive activity of progesterone and estrogen has been demonstrated in mice and rats (1, 9, 11). Testosterone propionate has been reported to depress bacterial antigen response in chickens (10). A number of heifers appeared to be cycling during the time of antigen injections.

The stress of weaning (period) was associated with a significant 1.9 $\log_2$ titer decline in antibody production. This observation supports research with laboratory animals showing that stress lowers the ability of animals to produce antibodies to various antigens (2, 3, 9).

The significant antigen by period interaction (Figure 1) suggests that there was a differential response to the type of antigen injected. It appears that the PRBC antibody production was not as inhibited as was HRBC antibody production the day of or day after weaning. This response may be related to antigenicity of the RBC due to surface area. HRBC have appropriately 74% of the surface area of PRBC (84 sq μ vs. 113 sq μ) (18). Also, there may be antigenic factors present in one species of RBC and not in the other species. McCarthy and Dutton (12) recently reported a differential response to SRBC injected into mice. They distinguished a discriminator and nondiscriminator response which was not related to the age or sex of the sheep or age of the RBC after bleeding.

Although a single glucocorticoid measurement had no effect on antibody titer, a significant relationship exists between glucocorticoid concentrations at the time of each antigen injection and the difference in antibody titer to each antigen (Figure 3). These data support previous research with laboratory animals demonstrating a detrimental effect of elevated corticoid concentrations on development of antibody titers. In our study, calves which had low plasma steroid concentrations at the time of either injection developed antibody titers of the same magnitude following either injection. The same basic condition prevailed for calves with high glucocorticoid concentrations at each of the antigen injections. However, if glucocorticoid concentrations were high at one injection and low at the time of the other antigen injection, there was a large difference in antibody titers to each antigen.

Our findings suggest that the stress of weaning and high glucocorticoid concentrations at the time of antigen injections are detrimental to antibody formation. Consideration should be given to active immunization of calves to minimize stress in order to maximize antibody production for protection against organisms producing disease.

SUMMARY

Differences in antibody titer production to porcine red blood cells or horse red blood cells in beef calves were significantly affected by year, sex of the calf and time of antigen injection prior

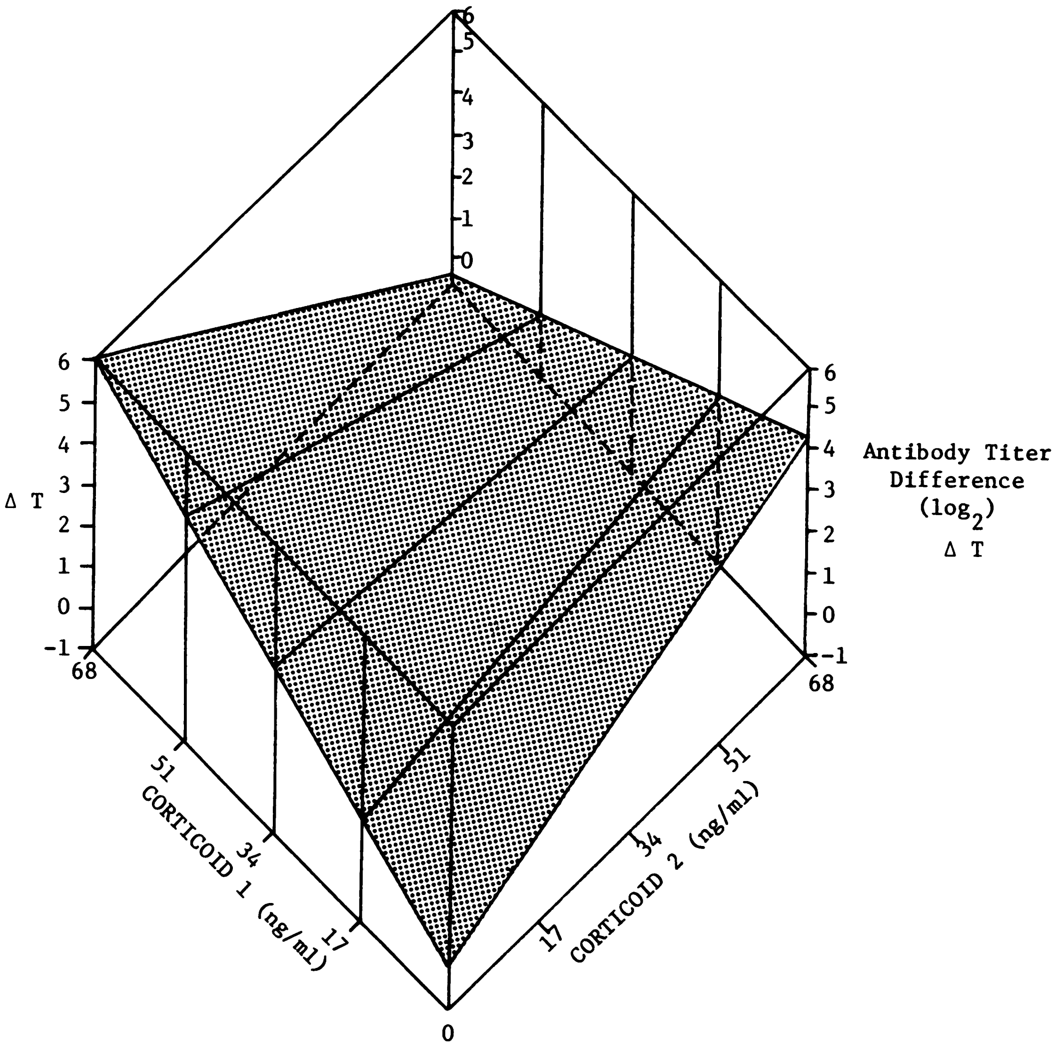

FIGURE 3. Response of antibody titer difference to initial glucocorticoid concentration (corticoid 1) and glucocorticoid concentration (corticoid 2) at the second antigen injection.

to weaning. Steers produced higher antibody titers than heifers to either antigen. Antibody production was greater when antigen was given one week prior to weaning than when antigen was administered the day of or day after weaning. Differences in antibody production were detected in response to the type of antigen given near the time of weaning. Plasma glucocorticoid concentrations at the time of antigen injection were related to antibody titer differences. It appears that stress and high glucocorticoid concentrations depress immune systems of the animal.

RÉSUMÉ

Les auteurs ont enregistré des différences appréciables dans l'élaboration d'anticorps à l'endroit d'hématies de porc ou de cheval, chez des veaux de boucherie, selon l'année, le sexe du veau et le moment de l'injection de l'antigène avant le sevrage. Les bouvillons produisirent plus d'anticorps que les taures à l'endroit des deux antigènes. La production d'anticorps atteignit un niveau plus élevé quand l'injection de l'antigène eut lieu une semaine avant le sevrage, comparativement à une injection donnée le jour même ou le lendemain du sevrage. On décela des différences dans la production d'anticorps en réponse au type d'antigène injecté à l'approche du sevrage. La teneur du plasma en glucocorticoïdes, lors de l'injection de l'antigène, s'avéra reliée aux différences dans la production d'anticorps. Il semble que le stress et une forte concentration de glucocorticoïdes dépriment le système immunitaire d'un animal.

REFERENCES

1. ANDRIOLE, V. T. and G. L. COHN. Estrogen and the pathogenesis of hematogenous pyelonephritis in the rat. Clin. Res. 11: 408. 1963.

2. BRAYTON, A. R. and P. F. BRAIN. Studies on the effects of differential housing on some measures of disease resistance in male and female laboratory mice. J. Endocr. 61: xlviii. 1974.
3. BRAYTON, A. R. and P. F. BRAIN. Effects of differential housing and glucocorticoid administration on immune responses to sheep red blood cells in albino TO strain mice. J. Endocr. 64:4P. 1975.
4. GISLER, R. H., A. E. BUSSARD, J. C. MAZIE and R. HESS. Hormonal regulation of the immune response. I. Induction of an immune response *in vitro* with lymphoid cells from mice exposed to acute systemic stress. Cell Immun. 2: 634–645. 1971.
5. GROSS, W. B. Effect of social stress on the occurrence of Marek's disease in chickens. Am. J. vet. Res. 33: 2275–2279. 1972.
6. GROSS, W. B. Immune response to *Escherichia coli*. Am. J. vet. Res. 36: 568–571. 1975.
7. GWAZDAUSKAS, F. C., W. W. THATCHER and C. J. WILCOX. Physiological, environmental and hormonal factors at insemination which may affect conception. J. Dairy Sci. 56: 873–877. 1973.
8. HELLIG, H. and J. F. WALDEK. Studies on the effect of cortisol on the primary immune response to sheep erythrocytes *in vitro* by mouse spleenic cells. Onderstepoort J. vet. Res. 41: 29–37. 1974.
9. HELLIG, H. R. and W. H. GERNEKE. A histological study of the effect of cortisol and some sex steroids on the immune response to sheep erythrocytes by the mouse. Onderstepoort J. vet. Res. 42: 53–62. 1975.
10. HIROTA, Y., T. SUZUKI, Y. CHAZONO and Y. BITO. Humoral immune response characteristics of testosterone-propionate-treated chickens. Immunology 30: 341–348. 1976.
11. MARKHAM, R. B., A. WHITE and A. L. GOLDSTEIN. Selective immunosuppressive activity of steroids in mice inoculated with the Moloney sarcoma virus. Proc. Soc. exp. Med. Biol. 148: 190–193. 1975.
12. MCCARTHY, M. M. and R. H. DUTTON. The humoral response of mouse spleen cells to two types of sheep erythrocytes. I. Genetic control of response to H and L SRBC. J. Immun. 115: 1316–1321. 1975.
13. MURPHY, B. E. P. Some studies of the protein binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein binding radioassay. J. clin. Endocr. 27: 973–990. 1967.
14. NORTH, R. J. The action of cortisone acetate on cell-mediated immunity to infection: Histogenesis of the lymphoid cell response and selective elimination of committed lymphocytes. Cell. Immun. 3: 501–515. 1972.
15. SATO, K. and B. GLICK. Antibody and cell mediated immunity in corticosteroid-treated chicks. Poult. Sci. 49: 982–986. 1970.
16. SELYE, H. Thymus and adrenals in the response of the organism to injuries and intoxications. Br. J. exp. Path. 17: 234–248. 1936.
17. SNEDECOR, G. W. and W. G. COCHRAN. Statistical Methods. 6th Edition. Ames: Iowa State University Press. 1969.
18. SWENSON, M. J. Duke's Physiology of Domestic Animals. 8th Edition. Ithaca: Cornell University Press. 1970.
19. THAXTON, P., C. R. SADLIER and B. GLICK. Immune response of chickens following heat exposure or injections of ACTH. Poult. Sci. 47: 264–266. 1968.
20. YOUNG, P. S., P. THAXTON and G. W. MORGAN. *In vitro* reactivity of chicken hemagglutinins in the presence of corticosterone. Poult. Sci. 54: 1322–1324. 1975.