Bienn. Symp. North. Wild Sheep
and Goat Counc. 7:76-91.

EFFECT OF CHRONIC STRESS ON IMMUNE SYSTEM FUNCTION OF ROCKY MOUNTAIN BIGHORN SHEEP

E. LEE BELDEN, Department of Veterinary Sciences and Molecular Biology,University of Wyoming, Laramie, WY 82070

ELIZABETH S. WILLIAMS, Department of Veterinary Sciences, University of Wyoming, Laramie, WY 82070

E. TOM THORNE, Wyoming Game and Fish Department, Research Laboratory, Box 3312 University Station, Laramie, WY 82071

HENRY J. HARLOW, Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82070

KAREN WHITE, Department of Molecular Biology, University of Wyoming, Laramie, WY 82071

SANDRA L. ANDERSON, Wyoming Game and Fish Department, Research Laboratory, Box 3312 University Station, Laramie, WY 82071

Abstract: Decreased immunocompetence due to stress has been implicated in the susceptibility of bighorn sheep (Ovis canadensis canadensis) to bronchopneumonia induced by a variety of pathogens. In a 34-day preliminary study, features of the immune system of domestic sheep (O. aries) stressed by confinement and noise were studied. During a 150-day experiment, we investigated the effect of chronic stress on immune system function of (1) wild-caught bighorn ewes, which we assumed to be stressed by recent capture, a novel environment, and repeated handling, and (2) tame bighorn ewes, which we assumed experienced minimal stress due to handling. Plasma cortisol was also measured. Lymphocyte blastogenesis was depressed below baseline and plasma cortisol increased during the trial in domestic sheep. In the bighorn sheep study, lymphocyte blastogenesis was depressed during the first 35 days but then returned to baseline. During most of the study, lymphocyte blastogenesis and leukocyte numbers of wild and tame bighorns were similar even though plasma cortisol was significantly higher in the wild animals. Alterations of lymphocyte blastogenesis and leukocyte numbers were associated with lambing, adrenocorticotropic hormone treatment, and acute confinement. Results suggest that approximately the first month of protracted exposure to stressors is the most detrimental to immune system function and that portions of this system can adapt to chronic stress even with elevated levels of plasma cortisol.

Stress, defined as the need for an individual to make abnormal or extreme adjustments in physiology or behavior to cope with adverse aspects of its environment (Fraser et al. 1975), is thought to be an important co-

factor in precipitating bronchopneumonia, and perhaps other diseases, in bighorn sheep (Forrester 1971, Thorne 1971, Hudson 1973, Thorne 1982, Spraker 1984, Bailey 1986). The paradigm is "shipping fever" of cattle which involves transport stresses and, often, contact with viral or bacterial pathogens (Hoerlein and Marsh 1957, Hambdy et al. 1963, Kelley 1988). The proposed mechanism for this interaction is through stress induced elevated circulating glucocorticoids which inhibit the immune system, predisposing an individual to infectious disease (Munch et al. 1984, Roth 1985, Kelley 1988, Griffin 1989). The physiologic effects of stress have been measured by elevations of plasma, serum, urine or fecal glucocorticoids, in a variety of animals, including domestic and bighorn sheep (Fulkerson and Jamieson 1982, Harlow et al. 1987b, Spraker et al. 1984, Miller et al. 1990, Spraker and Adrian 1990). Effects of stress on immune system function have not been studied extensively in ruminants, though recently there has been increased interest in stress in these animals (Moberg 1985, Roth 1985, Kelley 1988). Observations of stress-related epizootics of bronchopneumonia in wild bighorns and occurrences of similar disease in recently captured, presumably highly stressed, bighorn sheep (Hudson 1973, Thorne 1982) prompted us to study immune system function in chronically stressed domestic and bighorn sheep.

Lymphocyte blastogenesis tests are commonly used in many species to assess function of portions of the immune system (Outteridge 1985). Briefly, these tests measure the nonspecific responsiveness of T and B lymphocytes. These cells play a central role in animal immune systems. T lymphocytes (T cells) are particularly important in cell-mediated immunity and in regulating of other portions of the immune system. B lymphocytes (B cells) are the primary cells involved in humoral immunity or antibody production. Though lymphocyte blastogenesis tests do not measure all functions of the immune system they provide valuable information about the function of major segments of this system.

This study was partially funded by the Foundation for North American Wild Sheep, the Wyoming Game and Fish Department, and the Department of Veterinary Sciences, University of Wyoming. We thank H. Dawson, B. Meyer, and C. Engstrom for animal handling and care. We also acknowledge M. Miller, M. Reis, and C. Clote for blood collection and hematology.

MATERIALS AND METHODS

This study was conducted concurrently with investigations of the effects of acute and chronic stress on adrenal responsiveness and cardiac frequency in domestic and bighorn sheep (Harlow et al. 1987a,b).

Animals

Five adult mixed-breed domestic sheep were used during preliminary studies (Harlow et al. 1987a). They were acclimated to halter restraint and confined separately in adjoining 1.5 x 3 m stalls. Indwelling jugular catheters were surgically implanted, threaded through tubing secured at the halter rope and passed through a concrete block wall into an adjoining room where blood was collected. The sheep were stressed for 34 days by exposure to short bursts of loud noise occurring at random intervals (between 15 and 160 sec) during day and night.

A group of chronically stressed bighorn sheep was established by bringing 5 pregnant ewes from Whiskey Basin near DuBois, Wyoming into captivity at the Sybille Wildlife Research and Conservation Education Unit near Laramie, Wyoming in late April. Only 4 animals were sampled after day 35 of captivity. Four pregnant bighorn ewes, hand-raised at Sybille, were used as unstressed control animals.

During the study, all bighorns had lambs; lambs were removed from the wild ewes for hand-raising, those from the tame ewes were allowed to remain with their dams. Birth dates were 4, 5, 11 June and 2 July for the wild bighorns and 12 June (2 lambs), 9 and 16 July for the tame bighorns. One tame ewe developed mild clinical signs of pneumonia during late May and again in early July and was treated with antibiotics. The other tame and wild bighorns remained clinically healthy. As part of the study of heart rate and stress (Harlow et al. 1987b), the bighorns were subjected to adrenocorticotropic hormone (ACTH) treatment once each during June and July and to acute confinement stress within a handling crate for 3 hrs with repeated blood sampling during August.

Hematology and Lymphocyte Blastogenesis

Blood from domestic sheep was periodically collected through catheters during the noise stress study. Blood was collected during late April-September from manually restrained bighorns by venipuncture within 15 min; the order of bleeding was variable. Samples were obtained from 9:00 - 11:00 AM when cortisol levels are lowest during the circadian cycle in desert bighorn sheep (O. canadensis cremnobates) (Turner 1984). Blood was collected directly into syringes containing 40 U/ml sodium heparin (Elkins-Sinn, Inc., Cherry Hill, NJ 08034) and processed within 8 hr. Blood was diluted 1:2 in Hank's balanced salt solution (HBSS), layered on ficoll/hypaque type 400 (Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178), centrifuged 30 min at 1200 x g, and the mononuclear cell layer harvested. Mononuclear cells were rinsed 3 times in HBSS and diluted in RPMI 1640 plus 5% fetal bovine serum to contain 4×10^6 viable cells/ml. Mitogen assays were performed in 96 well microtiter plates with triplicate wells set for each of 3 mitogens and a triplicate without mitogens serving as an unstimulated control. Each well contained 100 ul of cell suspension plus 100 ul of mitogen diluted in culture medium, or, in control wells, 100 ul of additional culture medium. The T lymphocyte mitogens phytohemagglutinin (PHA-M, Difco Laboratories, P.O. Box 1058, Detroit, MI 48232) and concanavalin A (con A, Pharmacia Fine Chemicals, Box 175, 575104, Uppsala, Sweden) were used at a 1:80 dilution and at 5 ug/ml respectively. Bacterial lipopolysaccharide (LPS, E. coli 0127:B8, Sigma Chemical Co.) was used at 100 ug/ml as a B lymphocyte mitogen.

Plates were incubated at 37 C in a humidified, 5% CO_2 atmosphere. At 48 hr of incubation, 0.5 uCi tritiated thymidine (specific activity 2.0 Ci/mmole; DuPont Co., NEN Products BRML-Chandler Mill, Wilmington, DE 19898) was added to each well. After an additional 18 hr incubation, cells were harvested onto glass fiber filters with a multisample cell harvester. Filters were dried and placed in scintillation vials with 3 ml scintillation fluid. Each vial was counted in a scintillation counter 3 times for 1 min and average counts per minute recorded for each

triplicate. Results were expressed as mitogen stimulated average counts per minute minus unstimulated background counts per minute.

Plasma cortisol levels were determined by radioimmunoassay (Harlow 1987a,b). Routine complete blood counts and differential counts were conducted on heparinized blood samples (Jain 1986). Results are reported as absolute cell numbers.

Differences between means were tested using paired t tests of samples at day 0 and at various poststress sampling intervals. P values < 0.05 were considered significant.

RESULTS

Domestic Sheep

Mean plasma cortisol values increased significantly during confinement and noise stress (Fig. 1) except when the noise generator failed (Harlow et al. 1987a). Mean total leukocyte counts remained fairly stable during the trial, but mean lymphocyte and eosinophil numbers declined, though not significantly (Fig. 2). Lymphocyte blastogenesis responses (Fig. 3) to con A and PHA were similar and significantly depressed below prestress values by day 5. By the end of the 34-day trial, mean responses were reduced approximately 45 % below baseline; these differences were not statistically significant due to considerable individual variation. By 34 days, however, mean responses to LPS were depressed approximately 90 % below baseline.

Bighorn Sheep

Mean plasma cortisol was significantly elevated in captive wild bighorn sheep as compared to tame bighorns (Fig. 4) during most of the 150-day trial (Harlow et al. 1987b). Peak mean cortisol values occurred around the time of lambing in both groups of bighorns.

Mean total leukocyte counts in the 2 groups of bighorns were similar over time, ranging from about 6,000-12,000/ul (Fig. 5). Counts varied greatly near the end of the trial in wild bighorns, associated with confinement stress trials. Mean total lymphocyte counts in the 2 groups of bighorns were also similar over time with significant increases in the mean count for each group occurring during 1 sampling near the time of lambing (Fig. 6). Again, considerable fluctuation in mean lymphocyte numbers occurred in the wild bighorns near the end of the study, in association with confinement stress. Mean absolute numbers of circulating eosinophils increased during June and July following lambing in both groups and then declined back to baseline near the end of the study. However, the increase was not statistically significant due to considerable individual variation (Fig. 7).

Lymphocyte blastogenesis varied considerably over time (Fig. 8), with much of the fluctuation occurring around the time of lambing. Mean responses to T cell mitogens were significantly depressed in wild-caught bighorns during the first month of captivity (Fig. 9), and then rebounded to starting values or higher during the second month. Mean responses to LPS were slightly depressed, but not significantly, in the wild bighorns during the first month. Mean response to PHA, but not to con A, was significantly depressed

near the end of the study in the wild bighorns. During the study, there was declining responsiveness of lymphocytes from both groups of bighorns to LPS (Fig. 10).

Increases in mean B cell responses, but not mean T cell responses, occurred about 1 mo prior to lambing in both groups of bighorns. This was followed by a decline in B cell responses immediately prior to lambing and a return to near normal values after lambing (Fig. 11).

DISCUSSION

Increased plasma cortisol values in the noise stressed domestic sheep were associated with decreases in absolute numbers of circulating lymphocytes and eosinophils and little alteration in the total leukocyte count. This combination of hematologic changes characterizes a "stress leukogram" (Duncan and Prasse 1977, Jain 1986) that has been described in a variety of species. Significant depression of both T and B lymphocyte responses during the 34 days suggests that these animals were immunosuppressed during this period. The functional significance of the apparent immunosuppression is not clear because the animals were not challenged with exogenous infectious agents; however, this model of chronic stress may be useful in future controlled studies of immunocompetence.

Increased mean plasma cortisol of wild bighorn sheep as compared to tame bighorn sheep indicated that the wild animals were stressed by captivity and handling. Changes in lymphocyte blastogenesis and hematologic parameters in the wild bighorns during the first month of captivity were very similar to the responses of the stressed domestic sheep (Figs. 3,9). Mean absolute numbers of lymphocytes was depressed and responses of T lymphocytes declined. This suggests there may have been immunosuppression during this month, possibly predisposing these animals to disease. Although all our wild bighorns remained clinically normal, out-breaks of disease have been reported in recently captured bighorns (Parks et al. 1972, Hudson 1973, Thorne 1982) suggesting this apparent immunosuppression is functionally significant. Immunosuppression during the first month of captivity has been investigated in bighorns using PHA stimulated mononuclear cell cultures (Hudson 1973), and in red deer (Cervus elaphus) using con A, PHA and pokeweed mitogen, a B and T cell mitogen (Griffin 1989). In both of these cases, lymphocyte responses were significantly depressed during 2-4 weeks following capture.

Decreased resistance to disease associated with chronic stress is most often associated with infections and disease due to opportunistic pathogens rather than to more virulent primary pathogens (Griffin 1989). The predisposition to disease caused by these less virulent agents in animals compromised by stress has been suggested in the "shipping fever" example in domestic animals related to infection by Pasteurella spp. (Hoerlein and Marsh 1957). Pasteurellosis is one of the most important diseases of bighorn sheep (Thorne 1982). We know that many bighorns carry Pasteurella spp. in tonsil and upper respiratory tract (Thorne 1982, Dunbar 1990). The effects of chronic stress, with elevated circulating cortisol levels and declining responsiveness of lymphocytes could be important in the pathogenesis of some forms of pasteurellosis in bighorn sheep.

Our study in wild bighorns extends previous observations (Hudson 1973) by

showing a rebound in T lymphocyte responsiveness during the second month of captivity. Surprisingly, this rebound occurred in the face of elevated plasma cortisol levels. Presumably this rebound is a reflection of adaptation of portions of the immune system to chronic stress. The ability of the immune system to adjust to chronic stress has been documented in rats conditioned to stressors. In these animals, there was an initial depression of lymphocyte response to con A followed by a rebound in the presence of elevated corticosteroids (Croiset et al. 1987). These findings provide evidence that factors other than corticosteroids effect regulation of the immune system under stress. In addition, Keller et al. (1983) showed that immunosuppression associated with stress may occur in the absence of elevated corticosteroids in adrenalectomized rats, again suggesting a multiplicity of factors affecting regulation of the immune system (Moberg 1985, Griffin 1989).

It is reassuring to document that portions of the immune system of bighorns can adjust to chronic stress, at least under controlled conditions. We do not know how the responses of bighorns compare to other species. There is abundant evidence for individual and species variation in response to stress (Moberg 1985).

Mechanisms of adaptation to chronic stress are not known; however, recent studies suggest the effects of cortisol on lymphocytes may be modified. Lymphocytes and monocytes have receptors for corticosteroids on cell membranes (Kelley 1985, Golub and Gershwin 1985). A possible mechanism for immune system adaptation to prolonged elevated levels of cortisol is a T cell derived lymphokine (glucosteriod response-modifying factor) that blocks the suppressive effects of corticosteroids on T cell helper activity for antibody synthesis (Fairchild et al. 1984). Prolonged elevated levels of circulating corticosteroids could affect receptor densities and sensitivities of these cells.

The trend of declining responses of lymphocytes to LPS is more problematic. B cells are central to the humoral arm of the immune system which is known to be very important in defense against bacterial pathogens (Bellanti 1978), including Pasteurella spp. (Confer et al. 1989). This decline in responsiveness occurred in both the tame and wild bighorns in association with increases of plasma cortisol in both groups. Increased cortisol in the tame bighorns could have been due to the stress of repeated handling and blood collection. The decline in B lymphocyte responses could have been due elevated cortisol levels, however, it is also possible that changes in unmeasured factors were affecting responses of these cells. The hormonal changes associated with parturition and lactation could influence function of these cells. A similar pattern of fairly stable T cell function and declining B cell function during chronic stress simulated with repeated ACTH injections for 41 days has been described in captive bighorns (Miller 1988).

There was considerable fluctuation in lymphocyte blastogenesis and hematologic responses around the time of lambing in both groups of bighorns. The trend of increasing eosinophil numbers during this time may have reflected visceral migration of larvae of Protostrongylus stilesi across the placenta in late pregnancy (Hibler et al. 1972, 1974). Visceral migration of nematode larvae is associated with peripheral eosinophilia (Duncan and Prasse 1977, Jain 1986). It is also of note that eosinophilia occurred when plasma

cortisol values were high.

Fluctuations in B cell responses, but relatively stable T cell function, occurred around the time of lambing. These changes could be explained by the need to assure natural passive transfer of immunity to the lamb via antibodies in colostrum. The ewe must mount an adequate B cell response to infectious agents in the environment prior to lambing to provide those antibodies. However, this hyperreactivity of the humoral immune response would not be desirable at the time of lambing because of the increased likelihood of alloantigen response to paternal antigens at parturition. Hence, a decline in responsiveness just prior to lambing might be desirable. The stresses of late gestation and parturition may cause additional decrease in B cell responses with a rebound to full competency as these factors are removed.

We suggest researchers be very careful when interpreting the immune competence of individual bighorns from a single measure of plasma cortisol. Normal T cell blastogenesis may occur with elevated cortisol levels though B cell responses may be depressed. Conversely, suppressed lymphocyte function may occur at normal cortisol levels.

This study provides preliminary information on the function of the immune system of bighorn sheep exposed to stress. The immune system is extremely complex and variable among individuals. Many factors, including inputs from the endocrine and nervous systems determine an animal's response to stress. We only investigated only a few aspects of this system. Because of the apparent importance of stress and disease in bighorn sheep, and our need to understand these processes to effectively manage wild sheep populations, additional studies of the effects of stress on the immune system are needed.

LITERATURE CITED

Bailey, J. A. 1986. The increase and die-off of Waterton Canyon bighorn sheep: biology, management and dismanagement. Bienn. Symp. North. Wild Sheep and Goat Counc. 5:325-340.

Bellanti, J. A. 1978. Immunology II. W. B. Saunders, Philadelphia, PA. 813 pp.

Confer, A. W., K. R. Simons, R. J. Panciera, A. J. Mort and D. A. Mosier. 1989. Serum antibody response to carbohydrate antigens of Pasteurella haemolytica serotype 1: relation to experimentally induced bovine pneumonic pasteurellosis. Am. J. Vet. Res. 50:98-105.

Croiset, G., H. D. Veldhuis, R. E. Ballieux, D. De Wied and C. J. Heijnen. 1987. The impact of mild emotional stress induced by passive avoidance procedure on immune reactivity. Ann. N. Y. Acad. Sci. 496:477-484.

Dunbar, M. R., A. C. S. Ward and G. Power. 1990. Isolation of Pasteurella haemolytica from tonsillar biopsies of Rocky Mountain bighorn sheep. J. Wildl. Diseases 26:210-213.

Duncan, J. R. and K. W. Prasse. 1977. Veterinary laboratory medicine. Iowa State University Press, Ames. 243 pp.

Fairchild, S. S., K. Shannon, E. Kwan and R. I. Mishell. 1984. T cell-derived glucosteriod response-modifying factor ($GRMF_T$): a unique lymphokine made by normal T lymphocytes and a T cell hybridoma. J. Immunol. 132:821-827

Forrester, D. J. 1971. Bighorn sheep lungworm pneumonia complex. pp. 158-173 in J. W. Davis and R. C. Anderson (Eds.), Parasitic diseases of wild mammals. Iowa St. Univ. Press, Ames. 364 pp.

Fraser, D., J. S. D. Ritchie and A. F. Fraser. 1975. The term "stress" in a veterinary context. Br. Vet. J. 131:653-662.

Fulkerson, W. J. and P. J. Jamieson. 1982. Pattern of cortisol release in sheep following administration of synthetic ACTH or imposition of various stressor agents. Aust. J. Biol. Sci. 35:215-222.

Golub, M. S. and M. E. Gershwin. 1985. Stress-induced immunomodulation: what is it, if it is? pp. 177-192. in G. P. Moberg (Ed.), Animal stress. Am. Physiol. Soc., Waverly Press, Bethesda, MD. 324 pp.

Griffin, J. F. T. 1989. Stress and immunity: a unifying concept. Vet. Immunol. and Immunopathol. 20:263-312.

Hambdy, A. H., A. I. Trapp and C. Gale. 1963. Experimental transmission of shipping fever in calves. Am. J. Vet. Res. 24:287-294.

Harlow, H. J., E. T. Thorne, E. S. Williams, E. L. Belden and W. A. Gern. 1987a. Adrenal responsiveness in domestic sheep (Ovis aries) to acute and chronic stress as predicted by remote monitoring of cardiac frequency.

Can. J. Zool. 65:2021-2027.

———, E. T. Thorne, E. S. Williams, E. L. Belden and H. A. Gern. 1987b.Cardiac frequency: a potential predictor of blood cortisol levels during acute and chronic stress exposure in Rocky Mountain bighorn sheep (Ovis canadensis canadensis). Can. J. Zool. 65:2028-2034.

Hibler, C. P., R. E. Lange and C. J. Metzger. 1972. Transplacental transmission of Protostrongylus sp. in bighorn sheep. J. Wildl. Diseases 8:389.

———, C. J. Metzger, T. R. Spraker and R. E. Lange. 1974. Further observations on Protostrongylus sp. infection by transplacental transmission in bighorn sheep. J. Wildl. Diseases 10:39-41.

Hoerlein, A. B. and G. L. Marsh. 1957. Studies on the epizootiology of shipping fever in calves. J. Am. Vet. Med. Assoc. 131:123-127.

Hudson, R. J. 1973. Stress and in vitro lymphocyte stimulation by phytohemagglutinin in Rocky Mountain bighorn sheep. Can. J. Zool. 51:479-482.

Jain, N. C. 1986. Schalm's veterinary hematology, 4th edition. Lea and Febiger, Philadelphia. 1221 pp.

Keller, S. E., J. M. Weiss, S. J. Schleifer, N. E. Miller and M. Stein. 1983. Stress-induced suppression of immunity in adrenalectomized rats. Science 22:1301-1304.

Kelley, K. W. 1985. Immunological consequences of changing environmental stimuli. pp.193-223 in G. P. Moberg (Ed.), Animal stress. Am. Physiol. Soc., Waverly Press, Bethesda, MD. 324 pp.

———. 1988. Cross-talk between the immune and endocrine systems. J. Anim. Sci. 66:2095-2108.

Miller, M. W. 1988. Experiments toward detecting and managing stress in Rocky Mountain bighorn sheep (Ovis canadensis canadensis). PhD. Thesis, Colorado St. Univ., Fort Collins. 106 pp.

———, N. T. Hobbs and M. C. Sousa. 1991. Detecting stress responses in Rocky Mountain bighorn sheep (Ovis canadensis canadensis): reliability of cortisol concentrations in urine and feces. Can. J. Zool. 69: (in press).

Moberg, G. P. (Ed.). 1985. Animal stress. Am. Physiol. Soc., Waverly Press, Bethesda, MD. 324 pp.

Munch, A., P. M. Guyer and N. J. Holbrook. 1984. Physiologic function of glucocorticoids in stress and their relation to pharmacologic action. Endocr. Rev. 5:25-44.

Outteridge, P. M. 1985. Veterinary immunology. Academic Press, London. 280 pp.

Parks, J. B., G. Post, T. Thorne and P. Nash. 1972. Parainfluenza 3 virus infection in Rocky Mountain bighorn sheep. J. Am. Vet. Med. Assoc. 161:669-672.

Roth, J. A. 1985. Cortisol as a mediator of stress-associated immunosuppression in cattle. pp. 225-243 in G. P. Moberg (Ed.), Animal stress. Am. Physiol. Soc., Waverly Press, Bethesda, MD. 324 pp.

Spraker, T. R. and W. J. Adrian. 1990. A proposed health/stress panel to evaluate and monitor the health status of free-ranging bighorn sheep. Bienn. Symp. North. Wild Sheep and Goat Counc. 7: (in press).

————, C. P. Hibler, G. G. Schoonveld and W. S. Adney. 1984. Pathologic changes and microorganisms found in bighorn sheep during a stress-related dieoff. J. Wildl. Diseases 20:319-327.

Thorne, E. T. 1971. A die-off due to pneumonia in a semi-captive herd of Rocky Mountain bighorn sheep. pp. 92-97 in E. Decker (Ed.), Transactions of the North American Wild Sheep Conference, Fort Collins, CO. 187 pp.

————. 1982. Pasteurellosis. pp. 72-77. in E. T. Thorne, N. Kingston, W. R. Jolley and R. C. Bergstrom (Eds.), Diseases of wildlife in Wyoming. 2nd edition. Wyoming Game and Fish Depart., Cheyenne. 353 pp.

Turner, J. C. 1984. Diurnal periodicity of plasma cortisol and corticosterone in desert bighorn sheep demonstrated by radioimmunoassay. Can. J. Zool. 62:2659-2665.

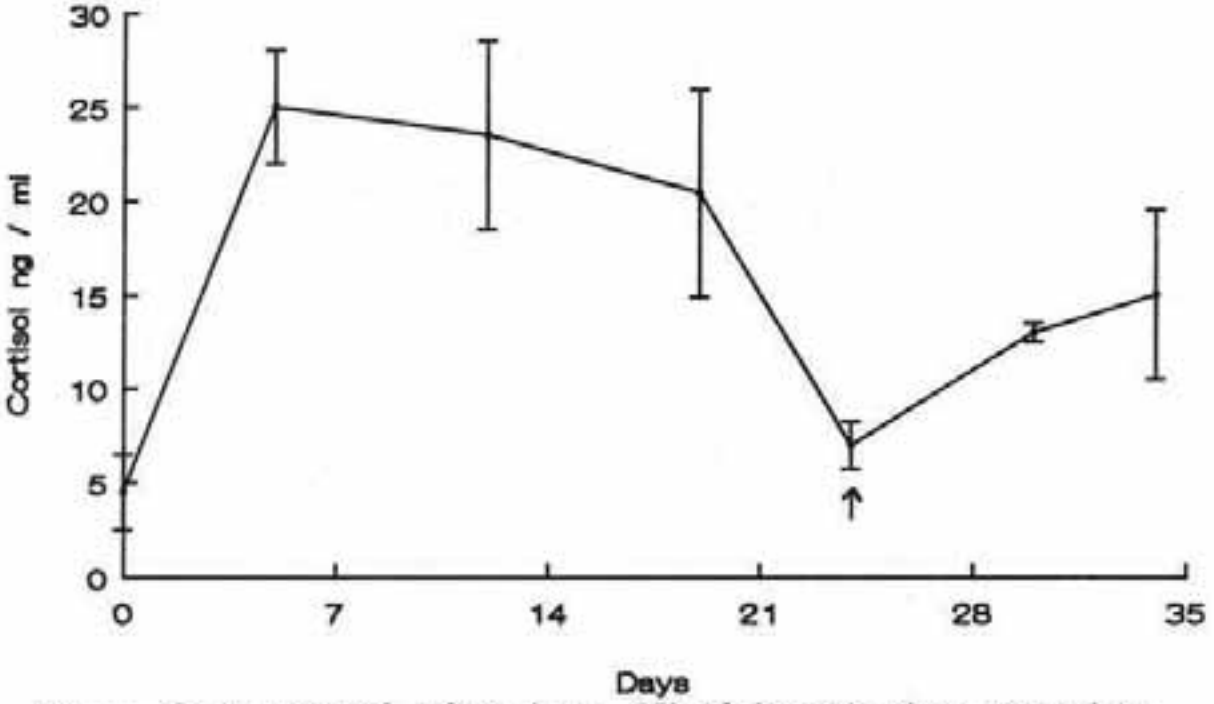

Fig. 1. Plasma cortisol values (mean, SE) of domestic sheep exposed to noise stress. The noise generator failed on days 23 and 24 (arrow).

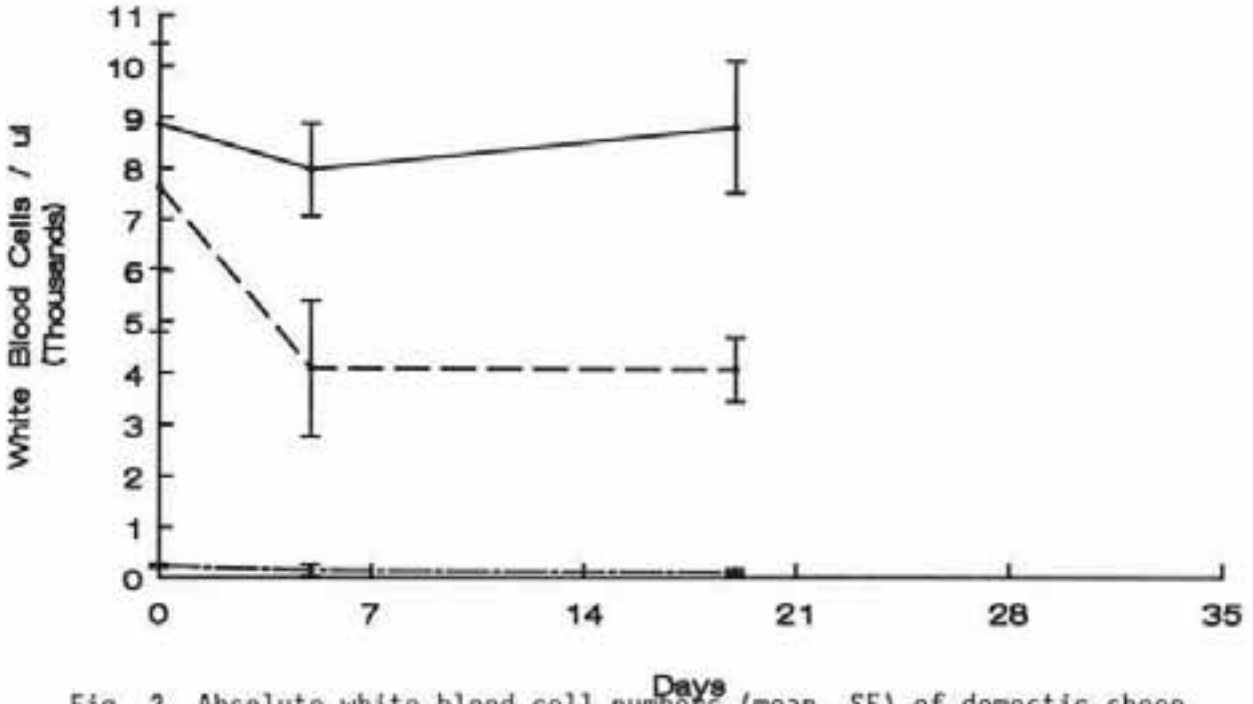

Fig. 2. Absolute white blood cell numbers (mean, SE) of domestic sheep exposed to noise stress. —— = total leukocytes/ul; - - - = lymphocytes/ul; ---= eosinophils/ul.

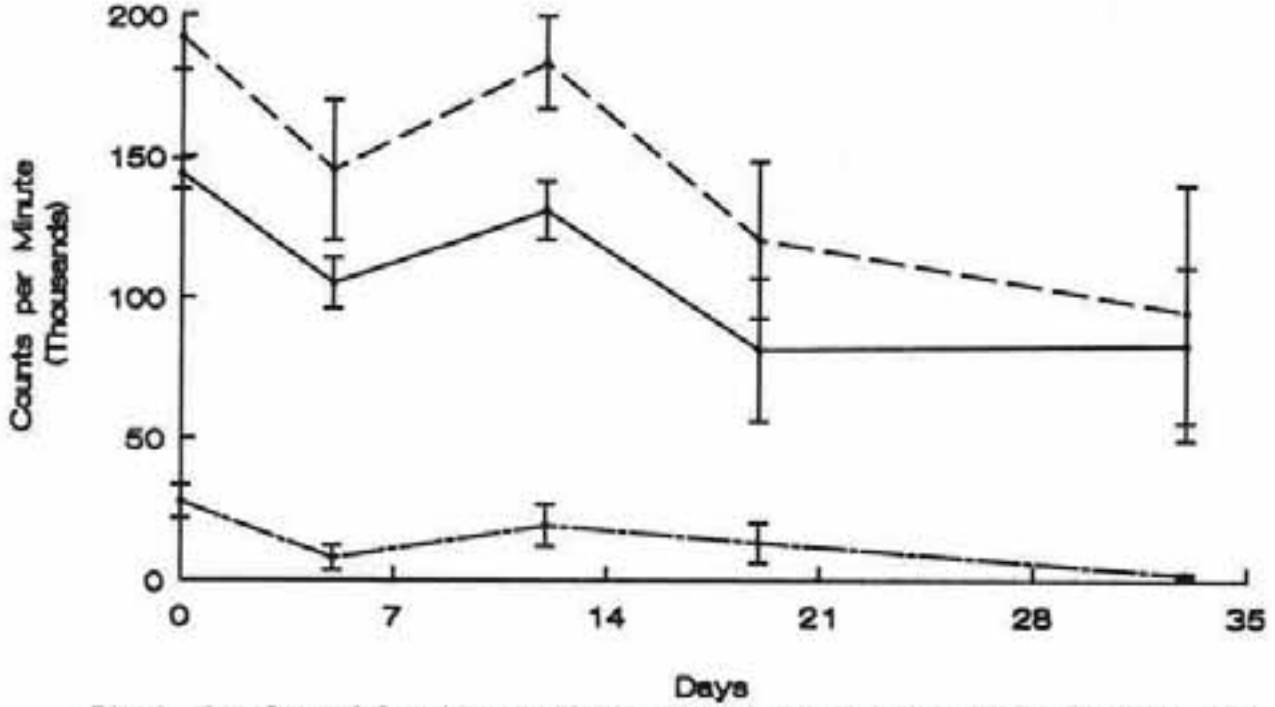

Fig.3. Results of lymphocyte blastogenesis tests (mean counts/minute, SE) from domestic sheep exposed to noise stress. ---- con A stimulation; —— = PHA stimulation; —·— = LPS stimulation.

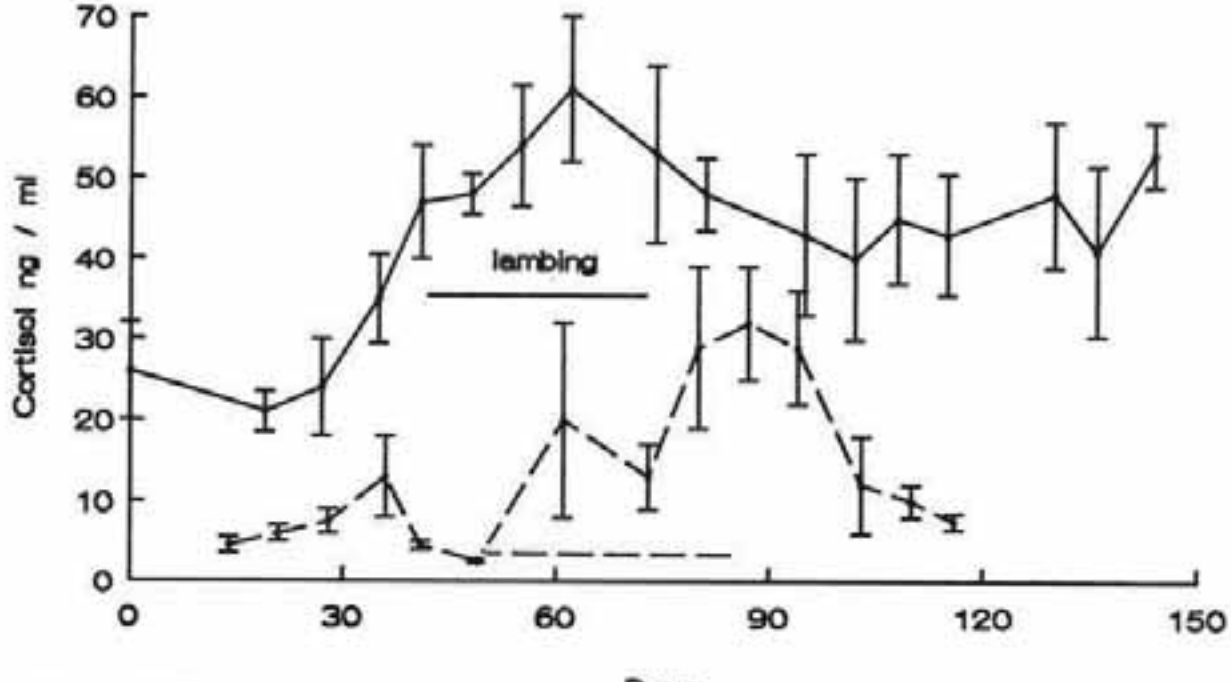

Fig. 4. Plasma cortisol values (mean, SE) of recently captured bighorn sheep and tame bighorn sheep. —— = recently captured bighorn sheep; ---- = tame bighorn sheep.

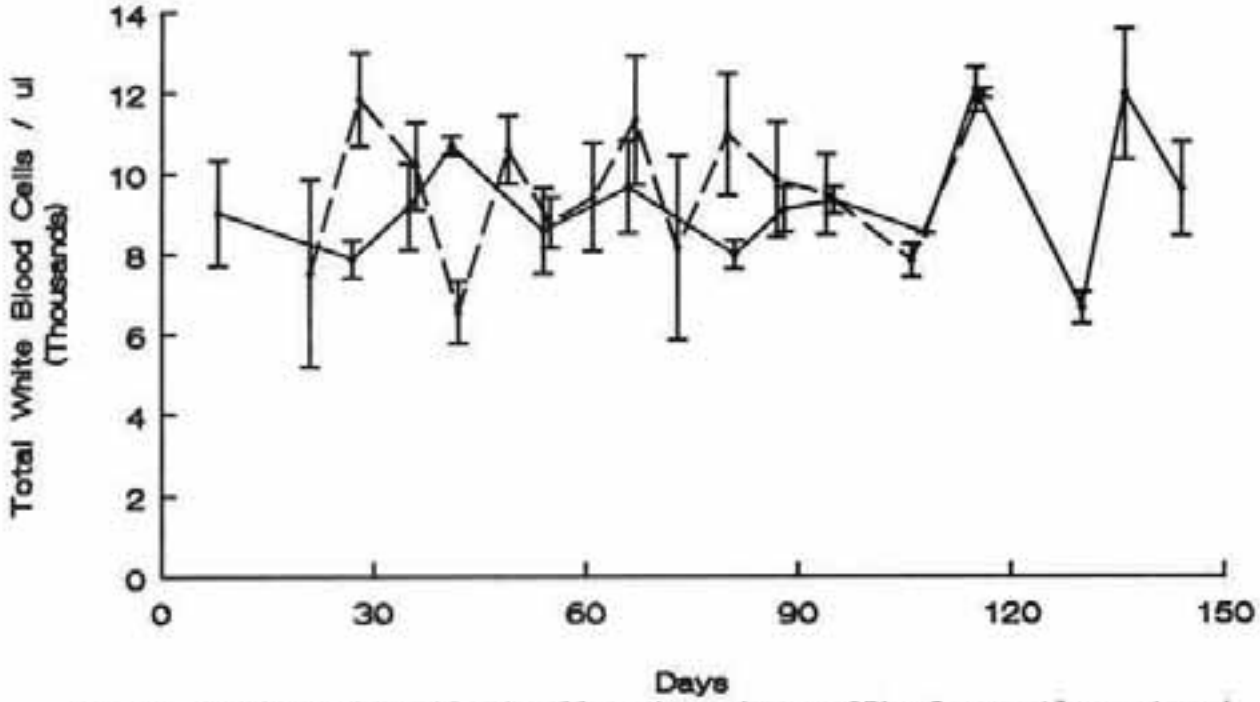

Fig. 5. Absolute white blood cell numbers (mean, SE) of recently captured bighorn sheep and tame bighorn sheep. —— = recently captured bighorn sheep; — — — = tame bighorn sheep.

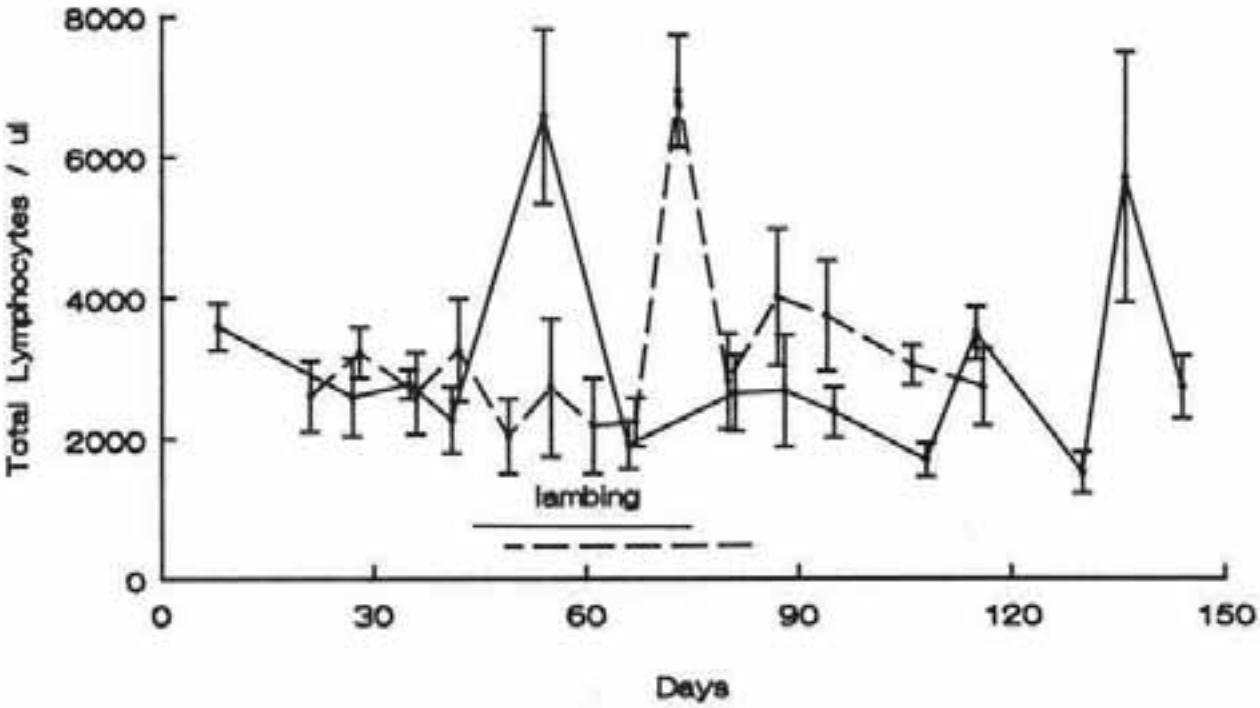

Fig. 6. Absolute lymphocyte numbers (mean, SE) of recently captured bighorn sheep and tame bighorn sheep. —— = recently captured bighorn sheep; — — = tame bighorn sheep.

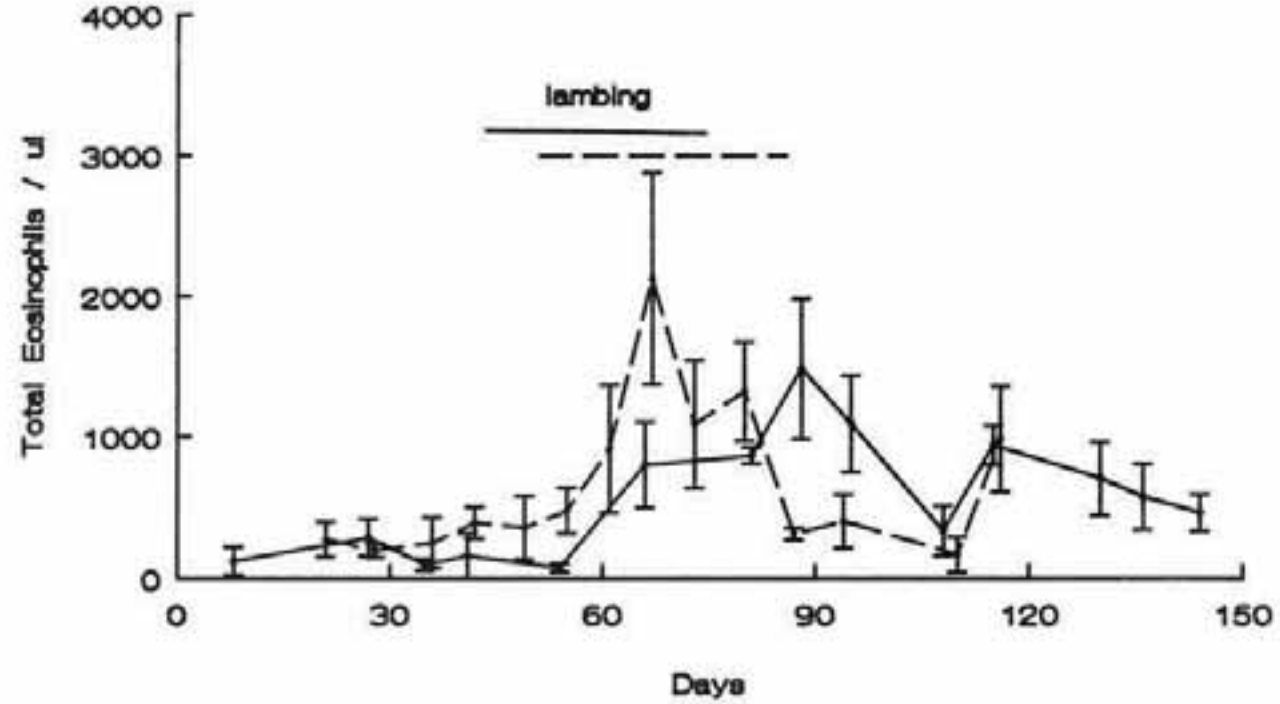

Fig. 7. Absolute eosinophil numbers (mean, SE) of recently captured bighorn sheep and tame bighorn sheep. —— = recently captured bighorn sheep; – – – = tame bighorn sheep.

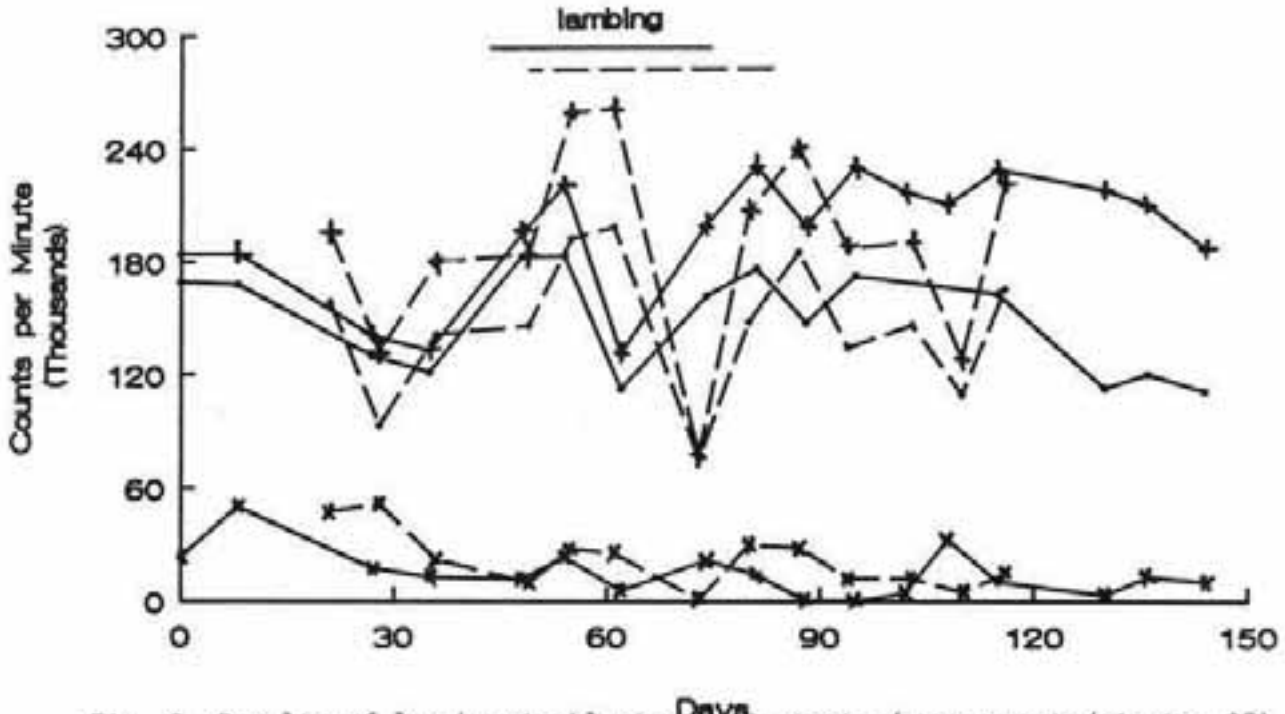

Fig. 8. Results of lymphocyte blastogenesis tests (mean counts/minute, SE) from recently captured bighorn sheep and tame bighorn sheep. —— = recently captured bighorn sheep; – – – = tame bighorn sheep; + = con A stimulation; • = PHA stimulation; × = LPS stimulation.

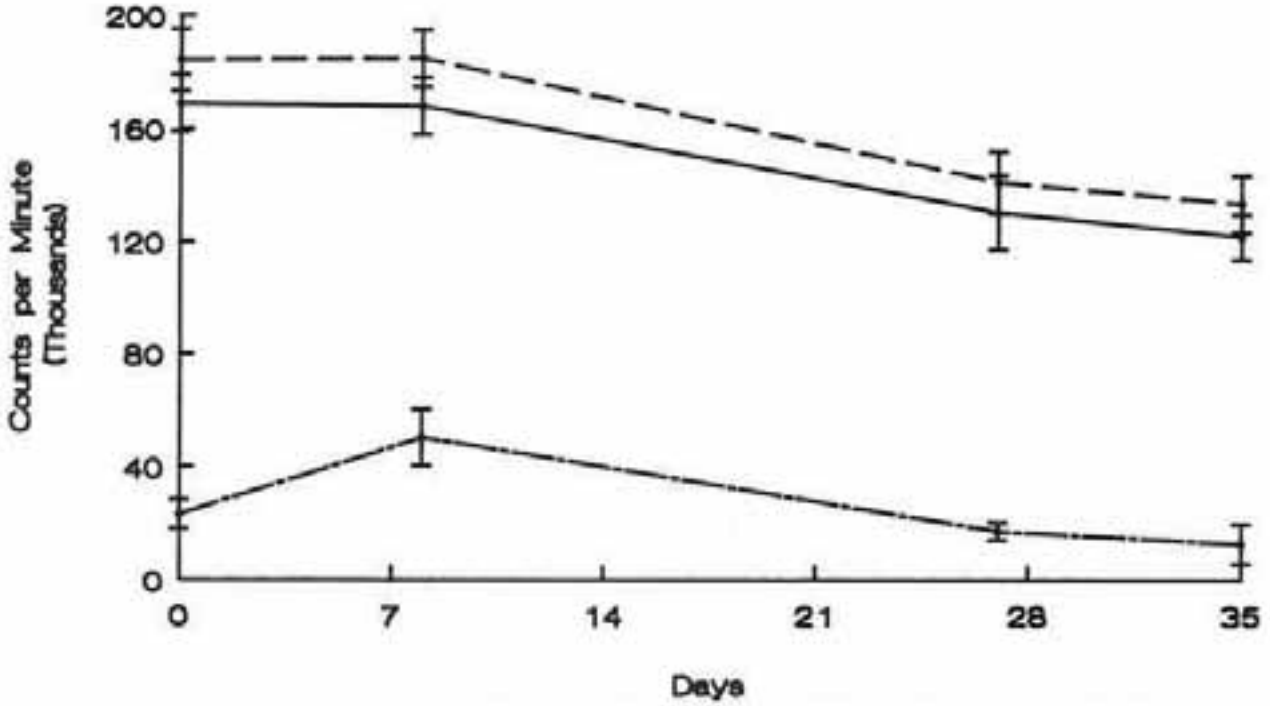

Fig. 9. Results of lymphocyte blastogenesis tests (mean counts/minute, SE) from recently captured bighorn sheep.– – – = con A stimulated; ——— = PHA stimulated;—·—·— = LPS stimulated.

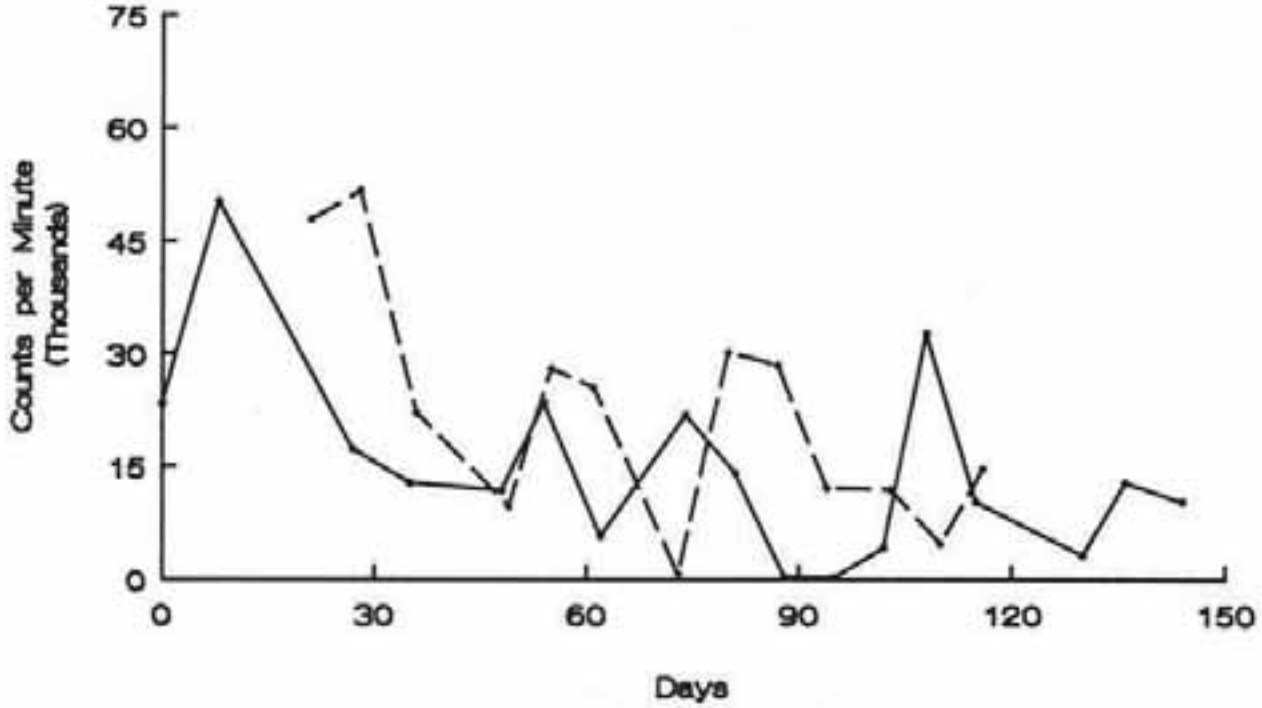

Fig. 10. Results of lymphocyte blastogenesis tests (mean counts/minute, SE) stimulated with LPS from recently captured bighorn sheep and tame bighorn sheep. ——— = recently captured bighorn sheep;– – – = tame bighorn sheep.

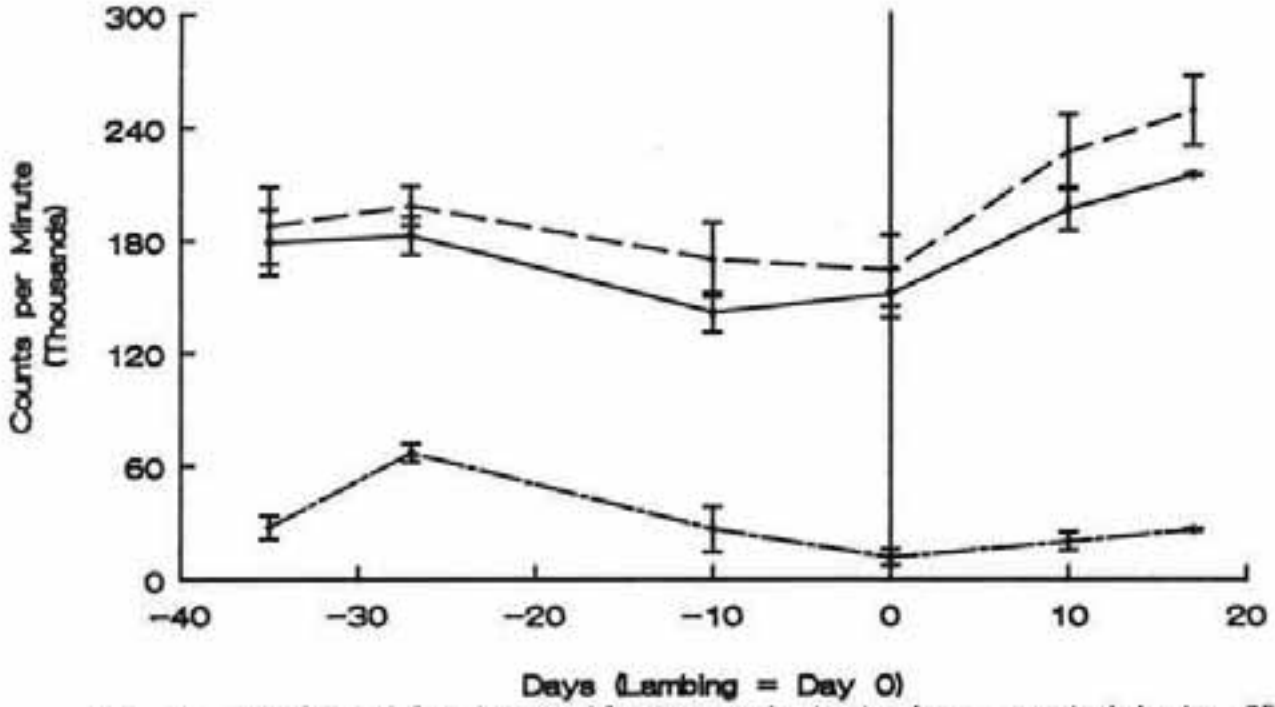

Fig. 11. Results of lymphocyte blastogenesis tests (mean counts/minute, SE) from bighorn sheep around the time of parturition. — — = con A stimulated; —— = PHA stimulated; — — = LPS stimulated.