CORTISOL CONCENTRATIONS IN MALE ALASKAN MOOSE (*Alces a. gigas*) AFTER EXOGENOUS ACTH ADMINISTRATION

George A. Bubenik[1], Charles C. Schwartz[2] and John Carnes[2]
[1]Department of Zoology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1; [2]Alaska Department of Fish & Game, Soldotna, Alaska 99669 USA.

ABSTRACT: Blood levels of cortisol were determined in five yearling Alaskan moose after an exogenous administration of 40 I.U. of ACTH. A rapid elevation of cortisol concentration (over 15µ g/100 mL) within 60 min. of ACTH injection demonstrated an unexpectedly high level of adrenocortical response to a simulated stress. The results in moose are compared to several other deer species.

Endocrine investigations of reindeer (*Rangifer tarandus tarandus*) and moose (*Alces alces*) are rare. Stress-induced variations of serum corticoid levels in moose were investigated by Franzmann and *et al.* (1975) and in reindeer by Rehbinder and Edqvist (1981) and Wiklund *et al.* (1993). Winter, summer and fall concentrations of triiodothyronine (T_3), thyroxine T_4) and cortisol in moose were reported by Nilssen *et al.* (1985) and seasonal changes of T_3, T_4 and growth hormone were published by Ryg (1982). However, concentrations or seasonal profiles of many other hormones in moose remain undetermined.

In several other cervids, the response to stress has been quantified by measuring plasma concentrations of cortisol after an exogenous administration of the pituitary adrenocorticotropin (ACTH) (Seal *et al.* 1982, Smith and Bubenik 1990, Bubenik *et al.* 1991, Bubenik and Bartos 1993). The time course of the elevation of cortisol in response to ACTH may be used as an indicator of stress adaptations which each species developed in response to their particular environment (Bubenik and Reyes-Toledo 1994). Such measurements were performed in a wide variety of wild and domesticated mammals (Friend *et al.* 1977, Fulkerson and Jamieson 1982, Seal *et al.* 1982).

To expand our knowledge of stress adaptations of cervids and to compare these data to other deer species, we examined the time course of cortisol concentrations in Alaskan moose after an exogenous administration of ACTH.

MATERIAL AND METHODS

Five tame, male, yearling Alaskan moose (*A. alces gigas*) born and raised by their mothers at the Kenai Moose Research Center (MRC) at Soldotna, Alaska, were tranquilized with a 2:1 mixture of xylazine hydrochloride (Anased - Lloyd Lab. Shenandoah, Iowa, USA) and ketamine hydrochloride (Ketaset - Aveco Co. Fort Dodge, Iowa, USA), using pressurized darts shot from a blow pipe. The dosages of the immobilizing mixture varied between 3 and 3.5 mg/kg. The induction times were between 5 and 10 minutes. Once anaesthetised, a teflon cannula (Criticon, Cathlon, gauge 18 - Mississauga, Ontario, Canada) was implanted into the jugular vein and secured with a suture. Moose were then sequentially sampled (5 ccm each time) for 4 hr. All experiments started between 0900 and 1000 hr. The first experiment [the treatment with 40 International Units (I.U.) of ACTH] was performed on April 27, the second experiment (control with 0 I.U.) on May 18, 1993. The reverse order of dosages was used

to minimise the effect of unfamiliar stress on the baseline concentrations of cortisol.

After the first 3 samples, taken 10 min apart into pre-heparinized syringes, either 0 or 40 I.U. of porcine ACTH (ACTHAR Gel, Armour Pharmaceutical, Kankakee, Ill, USA), diluted in 5 ml of saline, were administered intramuscularly (i.m.) After ACTH, three additional blood samples were taken 10 min. apart, two samples 30 min. apart and the final two samples at 60 min intervals. During sampling, animals were maintained in a semi-conscious stage, mostly in a sternal recumbence, by an infrequent administration of small doses (100 mg) of xylazine. In the previous study (Bubenik and Bartos 1993), these maintenance doses did not influence the cortisol secretion in any statistically significant way. Details of the sampling procedure were published in previous papers (Smith and Bubenik 1990; Bubenik and Bartos, 1993). After completion of the sampling all moose received 12 mg of yohimbine HCl and antibiotics.

Blood was immediately centrifuged and plasma frozen until laboratory analysis of cortisol levels by a commercial RIA kit (Joldan Bioclinical Inc., Scarborough, Ontario).

All assays were performed in duplicates. The intra- and interassay coefficients of variance were 5.2 and 13.4%; the recovery averaged 93.5%. The sensitivity of the assay was found to be less than 0.14 g/100 mL.

To measure cortisol response to ACTH, the area under the curve after ACTH (40 I.U.) was compared with the response after 0 I.U. The cut-off points for the calculations was chosen at the 90 min interval, the period of maximal cortisol elevation.

For statistical analysis we used SAS General Linear Models Procedure, where classes were ACTH doses, Treatment (Pre-treatment vs Post-treatment), Time and Individual Animals.

RESULTS

The model (Fig.1) proved significant variation $F_{(17,79)}=12.37$, $P<0.0001$. All classes appeared influential. For ACTH doses F $(1,79)= 17.43$, $P<0.0001$, for Treatment $F_{(1,79)}=62.21$, $P<0.0001$, for Time $F_{(6.79)}=14.51$, $P<0.0001$, and for Individual Animal $F_{(8,79)}=2.03$, $P<0.06$).

Cortisol values (Mean ± Standard Error) increased after time application in both groups (for 0 I.U. from 2.96 ± 0.29 to 4.21 ± 0.44;
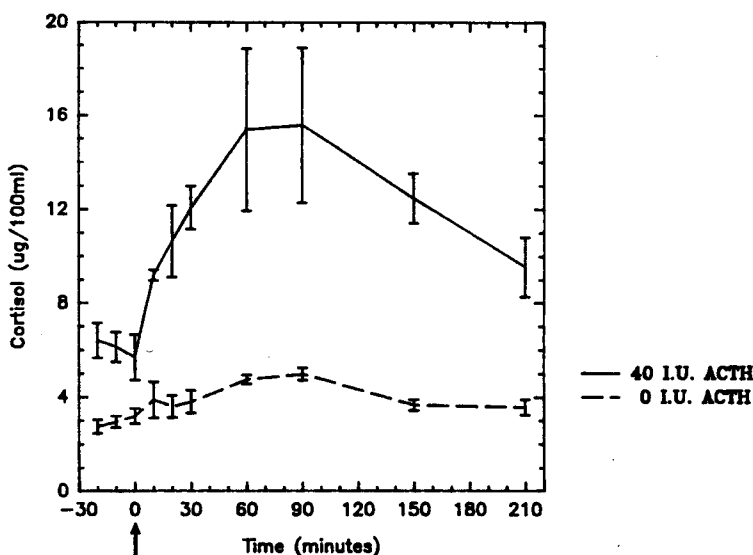

Fig. 1. Average cortisol levels (± S.E.) in plasma of five yearling moose sampled before and after ACTH administration (arrow).

P<0.01; for 40 I.U. from 6.06 ± 0.78 to 12.57 ± 0.19, P<0.0001). The cortisol concentrations rose rapidly within the first 60 minutes and peak levels (15.4 and 15.6µg/100 mL), were achieved 60 and 90 min, respectively, after the injection of ACTH. While pre-treatment values did not differ significantly between the two groups (P<0.92), the post-treatment values were markedly higher in the 40 I.U. group when compared to 0 I.U. treatment (12.57 ± 0.19 vs 4.21 ± 0.44, P<0.0001).

DISCUSSION

Elevation of cortisol concentrations during stress is a response essential for the survival of animals (McEwen et al. 1986). A reproducible stress is difficult to administer; therefore simulated stress responses (by i.m. administration of pituitary ACTH) have been investigated in various cervids such as rusa deer (Cervus rusa timorensis), roe deer (Capreolus capreolus), red deer (Cervus elaphus), fallow deer (Dama dama), axis (Axis axis), white-tailed deer (Odocoileus virginianus) and pudu (Pudu puda) (Bubenik et al. 1991, Bubenik and Bartos - 1993, Bubenik and Reyes-Toledo 1993, Seal et al. 1982, Smith and Bubenik 1991, Sempere & Bubenik - unpublished, Van Mourik and Stelmasiak, 1984). To compare the stress responses among deer species and relate it to their respective habitats and behavior, boreal cervids such as reindeer and moose should also be investigated. Seasonal cortisol concentrations have not been determined in moose, but were reported in reindeer. Plasma cortisol concentrations in male reindeer did not differ between summer and winter in one study (Ringberg et al. 1978). In contrast, Nilssen et al. (1985) reported higher cortisol concentrations in summer than in fall and winter. In response to stress, cortisol concentrations rose rapidly in reindeer (Rehbinder and Edqvist 1981; Wiklund et al. 1993) as well as in the moose (Franzmann et al. 1975).

Because of a large variation in individual concentrations (Bubenik et al. 1977), seasonal variation of cortisol in white-tailed deer (Bubenik et al.1975, 1983), pudu (Bubenik et al. - unpublished data) or reindeer (Bubenik et al. - unpublished data) was mostly non-significant. However, Bubenik and Leatherland (1984) observed significantly higher values of cortisol during the rut in a group of white-tailed bucks which behaviorally appeared much calmer than a comparable group of excitable males.

Whereas the basal values of glucocorticoids in cervids are relatively low, compared to some other mammals (Brown et al. 1971, Taylor et al. 1976) the ACTH-induced elevations can reach over 20-30µg/100 mL. The relatively high pretreatment concentrations (approximately 6µg/100 ml) in our 40 I.U. experiment (Fig.1) was most likely caused by stress due to confinement. It appears that the young animals were not entirely habituated to their smaller holding pens into which they were moved a few days before the experiment. During the immobilization (done by darts) they tried to escape and became agitated. During the second experiment (control) performed three weeks later, the moose were much calmer and their pretreatment levels (3-4µg/100 mL) were more similar to the basal concentrations found in red deer, fallow deer (Bubenik and Bartos 1993), white-tailed deer (Smith and Bubenik 1990) and reindeer (Rehbinder and Edquist 1981, Wiklund et al. 1993).

The smallest increase of cortisol concentrations after ACTH was observed in small, solitary, reclusive deer, such as the roe-deer (2.1µg/100 mL) (Sempere and Bubenik - unpublished data and the pudu (2.6µg/ 100 mL) (Bubenik and Reyes-Toledo 1994). These species escape their predators by hiding in a thick forest understory. In gregarious deer, which outrun their predators, average levels were higher: approximately 8µg/100mL in fallow deer and 10µg/100 mL in red deer (Bubenik and Bartos 1993) and 12µg/100 mL

in rusa deer (van Mourik and Stelmasiak 1984). Finally, the highest average elevation after an ACTH administration were reported in the most easily excitable and flighty deer: 19µg/100 mL in axis deer (Bubenik *et al.* 1991) and over 20µg/100 mL in white-tailed deer (Smith and Bubenik 1990).

The relatively high peak concentrations of cortisol (over 16µg/100 mL) in moose detected after ACTH injection is surprising because it is higher than values in red deer or fallow deer. Moose is considered a non-gregarious, non-excitable cervid. As such it should theoretically exhibit lower cortisol levels during the stress response than the more gregarious and flighty fallow deer.

Perhaps, the response to stress of arctic cervids living in harsh climatic conditions, requires a higher cortisol secretion than that of fallow deer originating in the moderate Mediterranean climate. Another explanation for the high cortisol concentrations after stress would be the requirement of large arctic mammals to mobilize energy reserves to fight off predators such as the bear (*Ursus arctos*) and the wolf (*Canis lupus*), the animals which they usually can not outrun. However, to support any of these hypotheses requires further studies, including the investigation of the cortisol response to ACTH in reindeer.

ACKNOWLEDGEMENTS

REFERENCES
BROWN, G.M., SCHALCH, D. and REICHLIN, S. 1971. Hypothalamic mediation of growth hormone and adrenal stress response in the squirrel mon-

key. Endocrinology 89: 649-703.

BUBENIK, G.A. and J.F. LEATHERLAND. 1984. Seasonal levels of cortisol and thyroid hormones in intact and castrated mature male white-tailed deer. Can.J.Zool. 62:783-787.

_____, and L. BARTOS, 1993. Cortisol levels in red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) after an acute ACTH administration. Can.J.Zool. 71:2258-2261.

_____, and E. REYES-TOLEDO. 1994. Plasma levels of cortisol, testosterone and growth hormone in pudu (*Pudu puda* Molina) after ACTH administration. Comp.Biochem.Physiol. 107A:523-527.

_____, A.B. BUBENIK, G.M. BROWN, A. TRENKLE, and D.A. WILSON. 1975. Growth hormone and cortisol levels in the annual cycle of white-tailed deer (*Odocoileus virginianus*). Can.J. Physiol.Pharmacol. 53:787-792.

_____, A.B. BUBENIK, A. TRENKLE, A. SIREK, D.A. WILSON, and G.M. BROWN. 1977. Short-term changes in plasma concentration of cortisol, growth hormone and insulin during the annual cycle of a male white-tailed deer (*Odocoileus virginianus*). Comp. Biochem.Physiol. 58A:387-391.

_____, A.B. BUBENIK, D. SCHAMS, and J.F. LEATHERLAND. 1983. Circadian and circannual rhythms of LH, FSH, testosterone, prolactin, cortisol, T_3, and T_4 in plasma of mature male white-tailed deer. Comp.Biochem.Physiol. 76A:37-45.

_____, R.D. BROWN, and D. SCHAMS. 1991. Antler cycle and endocrine parameters in male chital (*Axis axis*) deer: Seasonal levels of LH, FSH, testosterone and prolactin and results of GnRH and ACTH challenge tests. Comp. Biochem.Physiol. 76A:37-75.

FRANZMANN, A.W., A. FLYNN, and P.D. ARNESON. 1975. Serum corticoid lev-

els relative to handling stress in Alaskan moose. Can.J.Zool. 53:1424-1426.

FRIEND, T.H., POLAN, C.E., GWAZDAUKAS, F.C., and HEALD, C.W. 1977. Adrenal glucocorticoid response to exogenous adrenocorticotropin mediated by social density and social discruption in lactating cows. J. Dairy Sci. 60: 1958-1963.

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

HASTINGS, B.E., D.E. ABBOTT, L.M. GEORGE, and S.G. STADLER. 1992, Stress factors influencing plasma cortisol levels and adrenal weights in Chinese water deer (*Hydropotes inermis*). Res.Vet.Sci. 53:375-380.

McEWEN, B.S., E.R. DE KLOET, and W. ROSTENE. 1986. Adrenal steroid receptors and actions in the nervous system. Physiol.Rev. 66:1121-1188.

NILSSEN, K.J., K. BYE, J.A. SUNDSFJORD, and A.S. BLIX. 1985. Seasonal changes in T_3, FT_4, and cortisol in free-ranging Svalbard reindeer (*Rangifer tarandus platyrhynchus*). Gen.Comp.Endocrinol. 59:210-213.

REHBINDER, C., and L-E. EDQVIST. 1981. Influence of stress on some blood constituens in reindeer (*Rangiferi tarandus L.*). Acta vet.scand. 22:480-492.

RINGBERG LUND-LARSEN, T., J. KOFSTAD, and A. AAKVAAG. 1978. Seasonal changes of aldosterone, cortisol and inorganic ions in the reindeer (*Rangifer tarandus*). Comp.Biochem.Physiol. 60A:383-386, 1978.

RYG, M. 1982. Seasonal changes in weight gain, growth hormone, and thyroid hormones in intact and castrated male moose (*Alces alces alces*). Can.J.Zool. 60:2941-2946.

SEAL, U.S., L.J. VERME, J.J. OZOGA, and E.D. PLOTKA. 1982. Metabolic and endocrine responses of white-tailed deer to increasing population density. J.Wild. Manage. 47:451-462.

SMITH, J.H. and G.A. BUBENIK. 1990. Plasma concentrations of glucocorticoids in white-tailed deer; the effect of acute ACTH and dexamethasone administration. Can.J.Zool. 68:2123-2129.

TAYLOR, A.N., LORENZ, R.J., TURNER, B.B., RONNEKLEIV, O.K., CASADY, R.L. and BRANCH, B.J. 1976. Factors influencing pituitary-adrenal rhythmicity: its ontogeny and circadian variations in stress responsiveness. Psychoneuroendocrinology 1: 291-301.

VAN MOURIK, S. and T. STELMASIAK. 1984. Adrenal response to ACTH in Rusa deer (*Cervus rusa timorensis*). Comp.Biochem.Physiol. 79A:581-584.

WIKLUND E., P.J. GODDARD, and C. REHBINDER. 1993. Remote blood collection in reindeer (*Rangifer tarandus tarandus L.*): a preliminary study. Rangifer 14:29-32.

ZOMBORSZKY, Z., J. HAFNER, and T. FEHER. 1993. Values of serum-cortisol concentration - studies on the stress-sensibility and adaptability of intensively bred red deer. (in Hungarian). Magyar Allatorvosok Lapja, pp.91-95, February 1993.