

FACTORS AFFECTING CORTICOSTEROID CONCENTRATIONS IN YELLOW-BELLIED MARMOTS

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Abstract—1. Bound and total corticosteroid concentrations of yellow-bellied marmots (*Marmota flaviventris*) were lowest in May after emergence from hibernation and peaked in August prior to immersion.
2. Total corticosteroids were affected by age but not by sex or reproductive status.
3. There was no consistent relationship between measures of population density and concentrations of corticosteroids; when a significant relationship occurred, only 22–34% of the variation was explained.
4. Social status and social behavior were the major factors affecting corticosteroid concentrations.

INTRODUCTION

The adrenal cortex secretes glucocorticoids that control carbohydrate and mineral metabolism (Jørgensen, 1982). The relative concentrations of cortisol, corticosterone, and cortisone are species dependent, but all respond to stress (Ferguson, 1985). A major stress postulated to increase adrenocortical response is population density (Christian and Davis, 1964). Briefly, the hypothesis states that increased population density leads to increased social interactions that cause physiological changes, including hypertrophy of the adrenal glands and increased activity of the adrenal cortex. Increased adrenocortical activity, in turn, inhibits reproductive function and increases mortality indirectly through lowered resistance to disease or other adverse environmental conditions. The increase in mortality and decrease in reproduction cause a population decline; thus, the stress response was postulated to be a density-dependent mechanism that regulates population size.

The evidence that the adrenocortical response functions as a population-regulating mechanism is inconclusive (see review by Flowerdew, 1987) and most mammalian population biologists conclude that there is little evidence from field studies to support the stress hypothesis (Cockburn, 1988).

However, there is evidence that stress reactions occur in natural populations. Brown rats (*Rattus norvegicus*) from a saturated population evidenced classic stress responses (Andrews *et al.*, 1972) and free plasma corticosteroids of male *Antechinus stuartii* increased sharply during mating; subsequently all males died within 2 weeks (Lee *et al.*, 1977). Laboratory studies identified other stressors, e.g. confinement elevated plasma levels of cortisol in Mongolian gerbils, *Meriones unguiculatus* (Fenske, 1984).

This study investigates the factors associated with plasma levels of adrenocorticoids in the social yellow-bellied marmot, *Marmota flaviventris*.

MATERIALS AND METHODS

Yellow-bellied marmot populations were studied since 1962 in the upper East River Valley, Gunnison County, Colorado, USA. Each year, all animals living at four major

study sites were trapped, weighed, sexed and marked with a non-toxic fur dye for visual identification. Animals never previously trapped were provided with an individually numbered monel metal tag inserted in each ear for permanent identification. Animals were observed in the morning and late afternoon when marmots are most active (Armitage, 1962). Individuals were located regularly and their positions recorded as grid coordinates from a map of the study site. Location data were used to determine the home range of each individual and to calculate home range overlap between individuals. All social interactions were recorded as either amicable or agonistic (Johns and Armitage, 1979). Animals at an additional 12 sites were regularly trapped and their demographic data recorded, but behavior was not observed.

Marmots are clumped on favourable habitats in the montane to alpine environments where rock outcroppings or talus provide burrow sites near grass-forb meadows where marmots forage. However, where marmot habitat patches are small, marmots may live singly (Svendsen, 1974). Adult females form social groups of one to five closely-related females that continue through time as matrilineal (Armitage, 1984). Adult females may be associated with young (animals in their first summer of life) and yearlings (animals 1 year old) of both sexes). Males are territorial (Armitage, 1974) and form harems of one to six females by associating with one or more matrilineal groups. Virtually all male yearlings and about half of the female yearlings disperse; about half of the female yearlings become resident in their natal population (Armitage, 1986a).

Marmots display considerable individuality in their behavior that could affect their response to stress. Therefore, in 1971 and 1972, animals were submitted to mirror-image stimulation as a measure of their behavioral phenotypes (Svendsen and Armitage, 1973; Svendsen, 1974).

Blood samples were taken when free-ranging animals were live-trapped between May and August of 1965, 1969, 1971 and 1972. The samples were collected into Vacutainers from the femoral vein of a hind leg. Samples were centrifuged to separate plasma from cells and the plasma frozen until analysis. Concentrations of corticosteroid were determined by fluorometric measurement (Guillemin *et al.*, 1959). Because acute stress increases unbound corticosteroids and chronic stress increases bound steroids, both bound and unbound corticosteroids were measured (Knigge and Hoar, 1963). For most samples, duplicate determinations were made and the concentration reported is the mean of the two determinations. All values are reported as $\mu\text{g}/100\text{ ml}$ of plasma.

RESULTS

Reproductive status

For the analysis of the relationship between reproductive status and adrenocorticoid concentration, samples collected in August were not included. Most litters were weaned by mid July (Armitage *et al.*, 1976); therefore, all females are non-reproductive by August.

The mean concentration of unbound corticosteroids of reproductive females ($\bar{x} = 2.11 \pm 0.26$) did not differ significantly from that of nonreproductive females ($\bar{x} = 1.97 \pm 0.23$; $t = 0.4$, $P = 0.7$). Similarly, the mean concentration of bound corticosteroid of reproductive females ($\bar{x} = 1.30 \pm 0.19$) did not differ from that of nonreproductive females ($\bar{x} = 1.55 \pm 0.26$; $t = 0.8$, $P = 0.4$). Because reproductive status did not significantly affect corticosteroid concentration, all females were lumped together in subsequent analyses.

Age, sex and season

The concentration of total corticosteroids varied from 1.15 to 10.25 and was correlated with the concentration of both bound ($r = 0.76$, $P < 0.01$) and unbound ($r = 0.76$, $P < 0.01$) corticosteroids. The concentrations of bound and unbound corticosteroids were weakly but significantly correlated ($r = 0.21$, $0.05 > P > 0.01$). Therefore, in the analyses that follow, data will be reported as the concentrations of total and bound corticosteroids; the concentrations of unbound corticosteroids can be determined by difference.

A nested ANOVA revealed that concentrations of adrenocorticoids were significantly affected by age ($P < 0.001$). Therefore, a more detailed ANOVA was performed to determine the nature of the age effects. The concentration of total corticosteroids was significantly affected by age ($F = 7.2$, $df = 127$, $P < 0.001$). The mean (\pm SE) concentration for adults ($n = 59$) was 3.76 ± 0.25 ; for yearlings, 4.05 ± 0.37 ; and for young, 5.52 ± 0.43 . The mean concentration of total corticosteroids was significantly greater in young than in either adults or yearlings; the concentrations of total corticosteroids did not differ between adults and yearlings. This age effect was a consequence of the significant relationship between age and the concentration of unbound corticosteroids ($F = 14.7$, $df = 125$, $P < 0.0001$). The concentration of unbound corticosteroids in young (3.61 ± 0.35) was greater than that in yearlings (2.39 ± 0.20) and adults (1.98 ± 0.13); the values for yearlings and adults did not differ significantly. The concentration of bound corticosteroid was unaffected by age ($F = 0.3$, $df = 58$, $P = 0.7$). Values were 1.92 ± 0.21 for young; 1.64 ± 0.25 for yearlings, and 1.78 ± 0.20 for adults. The overall mean concentration was 1.77 ± 0.13 .

Because concentrations of corticosteroids did not differ between yearlings and adults, the values from these two age groups were combined for the analysis of the effects of sex and season. The nested ANOVA did not detect any effects of sex ($P > 0.5$); therefore, the sexes were combined in the analysis of the effects of season.

Season significantly affected the concentrations of total ($F = 4.7$, $df = 3$, $P = 0.004$) and of bound

($F = 3.04$, $df = 3$, $P = 0.03$) corticosteroid. The mean concentrations of bound and total corticosteroid in adults and yearlings were significantly lower in May, shortly after emergence from hibernation (Fig. 1) than in June. Although concentrations increased in July and August, the mean values did not differ significantly from June levels. The late July/early August values of young were strikingly similar to the July values for adults/yearlings (Fig. 1); however, the mean concentration of total corticosteroids in the late August/early September samples from young was about 32% greater than that of the adult/yearling group and significantly greater than the late July/early August levels in young ($F = 2.15$, $df = 29$, $P = 0.04$). Bound corticosteroids in young did not increase significantly ($F = 0.88$, $df = 29$, $P = 0.39$); thus the increase in total corticosteroids in young resulted from a marked increase in unbound corticosteroids about 2–4 weeks prior to emergence in hibernation.

Density

Two measures were used to determine the effects of population density on corticosteroid concentrations. For the first measure, adult females were divided into two groups; one group consisted of those females living with one or more other adult females and the other group consisted of those females who were not associated with any other adult female. The mean concentration of unbound corticosteroid was 1.88 ± 0.23 for females in groups and 2.43 ± 0.31 for females living solitarily. These differences were not statistically significant ($t = 1.4$, $df = 26$, $P = 0.17$). The mean concentration of bound corticosteroid was 1.70 ± 0.27 for females in groups and 1.52 ± 0.19 for females living solitarily. The means did not differ significantly ($t = 0.5$, $df = 26$, $P = 0.6$).

The second measure compared the concentrations of corticosteroids with the density of adult females (Table 1). The concentrations of corticosteroids were ranked from high to low and the density of adult females was ranked from high to low. Correlation

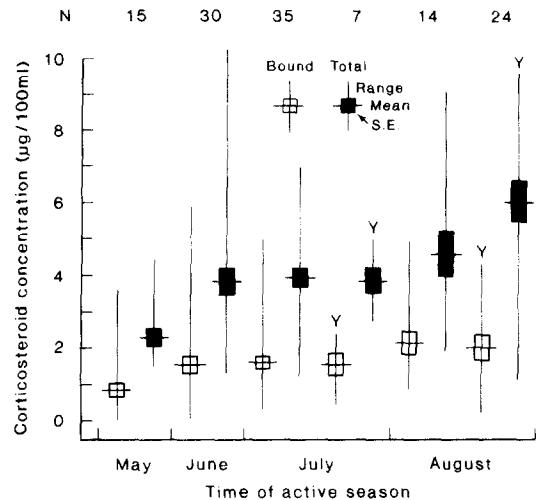


Fig. 1. Seasonal changes in the concentration of bound (open bars) and total (solid bars) corticosteroid in young and adult yellow-bellied marmots. Y designates values from young. All values are mean, SE and range.

Table 1. Corticosteroid concentrations ($\mu\text{g}/100\text{ ml}$) of adult female yellow-bellied marmots and associated behavioral and demographic parameters. Females are identified by their left ear-tag number. Behavioral phenotype was determined by mirror-image stimulation

Female	Corticosteroid concentration		Number of other female adult residents	Home-range overlap (%)		Behavioral phenotype	Number of social interactions	
	Total	Bound		Kin	Non-kin		Amicable	Agonistic
643	5.12	2.92	1	—	70	—	1	3
834	4.54	1.07	2	80	—	Avoider	0	0
774	3.87	0.37	1	100	—	Social	1	0
643	3.75	2.10	1	—	80	—	0	0
976	3.54	2.28	1	45	—	Aggressive	7	5
940	3.43	1.67	2	80	—	—	0	0
866	3.20	2.05	1	—	90	—	0	0
683	3.14	1.46	1	80	—	Social	3	1
870	3.10	1.27	0	0	0	Aggressive	—	—
68	2.55	1.75	5	100	50	—	0	6
52	1.25	0.43	5	100	10	—	0	0

between ranks was tested with the Spearman rank correlation. Rankings for the concentration of total, bound and unbound corticosteroids were not correlated significantly with the ranking for density of adult females (r_s varied from -0.28 to -0.33 , $n = 11$, $P > 0.05$).

Similarly, the concentrations of corticosteroids of female yearlings was compared with the total density of adults and yearlings of both sexes (Table 2). Concentrations of corticosteroids and density of residents were ranked as for adults. Total corticosteroids were positively correlated with density ($r_s = 0.49$); but the correlation was not quite statistically significant (r_s for $P = 0.05$ is 0.51). Given that the yearlings were sampled in different colonies in different years, the high correlation suggests possible biological significance; however, even if biologically significant, the relationship explains only 22% of the variation between ranks. Although the concentrations of bound and unbound corticosteroids were correlated with density, neither relationship approached significance ($r_s = 0.25$ and 0.3 , respectively). If the ranking of total corticosteroids is compared with the ranking of adult female density or yearling density, no significant correlation occurs ($r_s = 0.17$ and 0.49 , respectively), although the correlation with yearling density approaches significance. However, total density is significantly correlated with the density of yearlings ($r_s = 0.90$, $P < 0.01$). Because these intercorrelations could affect the correlations between density and

total corticosteroids, the Kendall partial correlation coefficients were determined. The partial correlation between total corticosteroids and total density of all yearlings and adults was not significant ($P = 0.44$), whereas the partial correlation between the density of yearling females and total corticosteroids approached significance ($P = 0.17$). Technically, there is no test of significance for the Kendall partial correlation (Siegel, 1956); however, the P values presented were calculated for the Kendall rank correlation and indicate the likelihood of statistical significance.

Neither the concentration of total, unbound, nor bound corticosteroid of male yearlings (Table 3) was correlated with total density of residents or with the density of yearlings (r_s ranged from -0.17 to 0.55 , $P > 0.05$, $n = 8$).

For adult males, the total residents were calculated as all yearlings and adults with whom a resident male could interact (Table 4). Two males whose residence was either uncertain or transient were eliminated from the analysis ($n = 10$). The total number of residents was positively and significantly correlated with the concentration of total ($r_s = 0.62$, $P < 0.05$) and of unbound ($r_s = 0.58$, $P = 0.05$), but not of bound ($r_s = 0.27$, $P > 0.05$) corticosteroid. Although statistically significant, the correlations with density of residents explain only 38% and 34% of the variation in ranks with total and unbound corticosteroids, respectively. The significant correlations are strongly affected by the way in which peripheral male

Table 2. Corticosteroid concentrations ($\mu\text{g}/100\text{ ml}$) of yearling female yellow-bellied marmots and associated behavioral and demographic parameters. Yearling females are identified by their left ear-tag number. Behavioral phenotypes were determined by mirror-image stimulation. Total density was determined as the sum of female adults plus yearlings plus one adult male (except 1050, 1032 and 1076 which were associated with two adult males)

Female	Corticosteroid concentration		Number of other residents		Home-range overlap (%)			Behavioral phenotype	Amicable/agonistic interactions	
	Total	Bound	Female adults	Yearlings (both sexes)	Adults	Yearlings	Kin		Non-kin	Adults
1050	5.60	1.68	4	4	Disappeared	Late June	—	—	0	0
1076	4.50	1.14	4	4	14	77	0	14	0/1	1/0
377	4.50	1.7	2	3	12	76	12	0	1/9	7/4
843	3.83	2.46	2	4	46	67	80	10	4/3	4/1
834	3.80	2.02	2	4	55	77	80	35	2/4	9/2
793	3.73	2.28	2	3	15	63	15	0	0/2	8/1
774	3.57	0.97	2	4	63	65	80	60	3/2	4/0
1082	3.18	0.62	4	4	8	77	0	8	Social	0/1
301	2.87	0.88	5	6	21	31	50	15	—	—
1177	2.67	1.03	4	1	29	46	35	14	Social	4/5
1156	2.45	0.39	2	2	48	90	86	10	Avoider	5/0
1166	2.38	0.15	2	2	40	90	72	0	Avoider	6/0

Table 3. Corticosteroid concentrations ($\mu\text{g}/100\text{ ml}$) of yearling male yellow-bellied marmots and associated behavioral and demographic parameters. Yearling males are identified by their left ear-tag number. Behavioral phenotypes were determined by mirror-image stimulation

Male	Corticosteroid concentration		Number of other residents		Home-range overlap (%)				Behavioral phenotype	Amicable/agonistic interactions	
	Total	Bound	Adults	Yearlings	Adults	Yearlings	Kin	Non-kin		Adults	Yearlings
874	8.15	2.8	5	3	46	50	0	50		0/4	1/0
1118	7.48	4.42	6	4	20	77	40	11	Aggressive	0/5	0
1051	4.46	3.64	5	1	25	44	36	8	Avoider	9/4	2/0
221	4.40	1.35	6	6	17	25	10	16		1/0	0
1128	3.95	2.15	3	0	100	10	20	80		0/2	0
1162	3.72	0.84	3	2	46	90	90	0	Avoider	8/3	6/0
1	3.36	2.07	5	1	26	44	39	5	Aggressive	8/7	2/0
756	3.28	1.53	3	4	60	80	90	40		1/1	0

401 and new resident male 505 were treated. It was assumed that because each male was newly resident, it could have explored the entire habitat site and potentially contacted all of the residents at their respective sites. However, if only those residents in the area where the two males eventually settled are considered, the density rankings change such that density and total corticosteroid are not significantly correlated ($r_s = 0.31$, $P > 0.05$) but density and unbound corticosteroid are significantly correlated ($r_s = 0.68$, $P < 0.05$). Bound corticosteroid and density become negatively but not significantly related ($r_s = -0.18$, $P > 0.05$). Interestingly, the two rankings of density were highly correlated ($r_s = 0.96$, $P < 0.01$). This relationship illustrates that even small changes in rank can markedly affect the significance of correlations and that large numerical changes may affect rankings only slightly. Thus, one must be cautious in the biological interpretation of rank-related relationships.

Behavioral phenotype

Several lines of evidence support a relationship between concentrations of adrenocorticoids and behavioral phenotypes as determined from a factor analysis of behavioral scores derived from mirror-image stimulation. Three factors accounted for 88% of the variance (Svendsen and Armitage, 1973). Animals could be categorized according to the factor on which their scores had the highest loading. These categories were aggressive (Axis I), social (Axis III) and avoider (Axis II).

At one site, five yearling females and no adult females formed the population. The concentration of bound corticosteroid ranged from 0.4 to 1.05 and was closely related to the factor scores of the marmots on Axis I ($r_s = 0.8$, $P > 0.05$). Although the correlation is not statistically significant, the high r_s associated with the small sample size suggests a significant biological relationship.

The concentration of bound corticosteroid of six yearling females from several sites also is related to their behavioral phenotypes. The highest values (1.14–1.68) occurred in aggressive animals, the social phenotypes were associated with intermediate values (0.62–1.03) and avoider yearlings had the lowest values (0.15–0.39) (Table 2). A rank correlation between the concentration of bound corticosteroid and the factor scores of the six yearlings on the aggressive factor (Axis I) was highly significant ($r_s = 0.94$, $P = 0.01$).

Behavioral phenotypes were available for five adult females (Table 1). No relationship between corticosteroids and behavioral phenotype is evident. However, evidence suggests that behavioral phenotype affects the concentration of corticosteroids. The concentration of bound corticosteroids is correlated with the factor scores of the adult females on Axis I ($r_s = 0.71$, $P > 0.05$). The concentration of both total and bound corticosteroids is correlated with the factor scores on Axis II (avoider) ($r_s = 0.48$, $P > 0.05$) and the concentration of bound corticosteroid is correlated with the factor scores on Axis III (social) ($r_s = 0.54$, $P > 0.05$). Thus, from 23 to 50% of the variation among ranks is explained by these corre-

Table 4. Corticosteroid concentrations ($\mu\text{g}/100\text{ ml}$) of adult male yellow-bellied marmots and associated behavioral parameters. Adult males are identified by their left ear-tag number. Behavioral phenotypes were determined by mirror-image stimulation. For social status, R = territorial resident; P = peripheral, living at the edge or just beyond the territory of a resident; T = transient, passing through a colony and not establishing residency in the habitat patch. Total residents includes all yearlings and adults of both sexes and is a measure of density

Male	Corticosteroid concentration		Social status	Behavioral phenotype	Total residents	Other adult males present	Encounters with other males	
	Total	Bound					Adults	Yearlings
1079	10.25	5.90	?	Avoider	—	?	—	—
505	6.98	4.97	R (New)	Aggressive	11	1R, 1T, 1P	0	0
401	6.70	5.15	P		10	1R	6	0
968	4.21	0.59	R	Aggressive	6	0	0	3
355	3.93	2.21	R		2	1T	1	5
1084	3.05	1.50	P	Avoider	11	2R, 1P	0	0
1173	2.96	0.48	R	Social	4	1P	3	0
216	2.55	1.80	R		4	2T	0	4
767	2.30	1.24	T		—	1R	1	1
401	2.09	0.23	R		4	0	0	5
5	1.84	0.28	R	Aggressive	5	1T	2	0
817	1.33	1.28	R		2	1T	—	—

lations; however, much of the variation remains unexplained and will be described in the next section.

There is no obvious relationship between corticosteroid concentrations and behavioral phenotypes of yearling males; the sample size is too small for rank analysis. For adult males, there is no significant relationship between behavioral phenotype and concentrations of bound or total corticosteroids (r_s varied from 0.08 to -0.31).

Social behavior

The possible influence of social behavior on the concentration of corticosteroids was assessed in two ways. First, the number of amicable and agonistic interactions was determined for each animal for whom those data were available. Second, home-range overlap with kin and unrelated animals was determined. In general, marmots behave amicably with kin and agonistically with non-kin (Armitage and Johns, 1982; Armitage, 1986a). Adult females may suppress reproduction of other females living in close proximity to them (Armitage, 1986a) and generally space is shared with kin (Armitage, 1984). As competition among females increases, matrilineal groups divide and space is partitioned so that overlap of home ranges is reduced, but this process may take several years (Armitage, 1984, 1986a, 1989). Thus, home-range overlap, especially with non-kin, was considered a stress that could affect levels of blood corticosteroids.

Among adult females, female 643 (in two different years) and 866 had high home-range overlap with non-kin and had three of the four highest concentrations of bound corticosteroid (Table 1). Female 976, who had the third highest concentration of bound corticosteroid, had a high number of agonistic interactions. The lowest concentrations of bound corticosteroids occurred in females 52 and 774; both females shared space primarily with kin and had no agonistic interactions. By contrast, female 68, whose home range overlapped that of female 52, had numerous agonistic interactions, had more home-range overlap with non-kin and had four times the concentration of bound corticosteroids than that of female 52 (Table 1). It is not possible to account for all the variation of corticosteroid concentration among females, as behavioral phenotype also affects those concentrations.

Among female yearlings, sisters 1156 and 1166 had the lowest concentrations of both total and bound corticosteroids. Their home-range overlap was primarily with each other and other kin and their social interactions were entirely amicable (Table 2). However, there is no consistent relationship between the concentration of either total or bound corticosteroid and overlap of home range with adults or yearlings or with kin or nonkin (r_s varied from 0.08 to -0.34 , $P > 0.05$). Those yearling females for whom social interactions were available were divided into two groups, those with a high proportion of amicable interactions and those with a high proportion of agonistic interactions. The mean bound corticosteroid in the group with predominantly amicable behavior was 0.76; the mean was 1.90 for the group with predominantly agonistic behavior. The difference between the two groups, tested by the

Mann-Whitney U test (Siegel, 1956), was highly significant ($P = 0.008$).

Among yearling males, there was no relationship between the concentrations of total or bound corticosteroid and home-range overlap with adults or yearlings (Table 3, r_s varied from -0.16 to -0.41 , $P > 0.05$). However, the concentration of total corticosteroids was negatively correlated with home-range overlap with kin ($r_s = 0.61$, $0.1 > P > 0.05$) and positively related with home-range overlap with non-kin ($r_s = 0.24$, $P > 0.05$). The same, non-significant relationship occurred between the concentration of bound corticosteroid and home-range overlap with kin and non-kin. The yearling males were divided into two groups, those with a low proportion of agonistic interactions and those with a high proportion. The mean concentration of bound corticosteroid of the four yearling males in the low agonistic behavior group was 1.98 and for those in the high agonistic behavior group, 2.73. The difference between the two groups was not statistically significant (Mann-Whitney U test, $P = 0.17$).

The possible influence of social behavior on the corticosteroid concentrations of adult males is illustrated by male 401. When he was a peripheral male he encountered the defence of the resident male that was continuously present and who defeated 401 in all their encounters. In that situation, male 401 had the highest concentration of bound corticosteroids and the second highest concentration of total corticosteroids for those adult males for whom behavioral data were available (Table 4). However, when male 401 became the resident, territorial male and had social encounters only with yearling males, his corticosteroid concentrations were among the lowest.

Because adult, resident males are territorial and regularly patrol their territories and actively attempt to drive away nearby (peripheral) males and resident yearling males and rebuff intruding males (Armitage, 1974), it was assumed that social stress would be proportional to the presence of competing males plus social encounters with males. Because males are dominant to females and there is no evidence of males:female conflict (Armitage, 1974, 1986a), female residents were not included in the analysis. Each male received a behavioral score that reflected potential and observed social conflict. Points were awarded for each other adult male present: 10 points for another resident, five points for each peripheral male, and two points for each transient male (Table 4). The heaviest weighting was given to the resident male as another resident would be continuously present within the perceptual range of the subject male whereas a peripheral male, although present throughout the summer, would usually be distant and probably out of the perceptual range of the subject male most of the time. The lowest score was awarded to the transient male that was usually present for only a few days. Four points were awarded for each social interaction with an adult male, and two points for each yearling male. Few points were awarded for an encounter as they were usually brief (an acute stress), whereas the presence of a male could be viewed as a long-term conflict (a chronic stress). A low score was given to an encounter with a yearling because adult males are

always dominant to yearlings (Armitage, 1974). The points were totalled for each male to provide a conflict score and the scores were rank ordered. The rank order of conflict scores was significantly correlated with the concentration of total corticosteroids ($r_s = 0.66$, $P < 0.05$) and of bound corticosteroids ($r_s = 0.62$, $P < 0.05$).

DISCUSSION

There was no relationship between reproductive status of adult female yellow-bellied marmots and concentrations of corticosteroids. This result contrasts with the high levels of cortisol during gestation in female New Zealand rabbits (Kriesten and Murawski, 1988). Because most of these samples were collected in June and July during lactation or early post-lactation, a reproductive effect may have been missed. However, samples collected in May, when female marmots are pregnant, had significantly lower concentrations of corticosteroids than samples collected later in the active season (Fig. 1) and a comparison of May samples of reproductive and non-reproductive females does not provide any evidence for a reproductive effect. Quite possibly, the numerous other factors affecting the concentration of corticosteroids masks any effect of pregnancy or lactation. A controlled, laboratory study of confined animals is required to confirm the lack of an effect of reproductive condition.

The seasonal effect with lowest concentrations occurring shortly after emergence from hibernation is similar to the pattern of fluctuation of cortisol in male European ground squirrels, *Citellus citellus*; in euthermic animals, lowest levels occurred in May after emergence from hibernation (Shivatcheva *et al.*, 1988). The low levels in May are probably a consequence of the low levels during hibernation (Shivatcheva *et al.*, 1988, and references cited therein), but it is not clear why levels are high in late summer prior to immergence. The seasonal trends reported here contrast with the absence of seasonal trends of cortisol in male axis deer (Bubenik and Brown, 1989) and white-tailed deer, *Odocoileus virginianus* (Chao and Brown, 1984).

The positive relationship between total corticosteroids and age occurred because of the high levels of unbound adrenocorticoids in young in late August/early September (Fig. 1). Thus, the effect of age may be primarily a seasonal effect, but does not explain why only young had such high values prior to immergence. It is unlikely that the difference between young and older animals is a sampling error. Although most adults hibernate by late August or early September when young are still active, enough adult animals were sampled in August to detect a strong trend toward higher levels, if such a trend were present. If an increase in glucocorticoids is essential for preparation for hibernation (Shivatcheva *et al.*, 1988), perhaps the preparation is more stringent in young that are preparing for their first hibernation. Another possible factor is that young may respond to cold stress in late summer when the standard operating temperature often is lower than the lower critical temperature (Melcher *et al.*, 1990).

Population density and social strife often increase adrenocortical activity (Christian, 1980) and presumably limit population growth. Although adrenal mass may be a useful indicator of adrenocortical responses, it was not considered in this study. A sample size adequate to demonstrate significant effects of social behavior and/or density on adrenocortical activity would have required the extirpation of the marmot population. Despite the problems of acute responses of corticosteroids to handling or the pulsed release of corticosteroids into the blood stream (Florant and Weitzman, 1980), sampling plasma levels of corticosteroids is the only realistic procedure for most studies of free-ranging, wild animals. The major reason that bound corticosteroids were emphasized in this study was not only to detect chronic responses to stress, but also to try to avoid acute responses due to trapping, handling and bleeding.

Neither adrenal mass nor measures of physiological responses consistently correlate with population density in natural populations (see review by Christian, 1980 and Flowerdew, 1987). For example, eosinophil concentrations were significantly correlated with population density of California voles (*Microtus californicus*) from May to October, but not over the time period of February–September. Eosinophil levels declined significantly when the population decreased not when the population increased, as would be expected if density acted directly on the pituitary–adrenal axis (Houlihan, 1963). Among white-tailed deer fed *ad libitum* in an enclosure, there was no relationship between population density and adrenal weights or serum cortisol levels (Seal *et al.*, 1983). In this study, there was no consistent relationship between density and concentration of corticosteroids. There was no significant difference in corticosteroid concentrations of solitary and grouped marmots; no density relationships were detected for adult females or for yearling males.

Several correlations between measures of corticosteroid concentration of yearling females and measures of population density approached statistical significance, i.e. total corticosteroids and density of residents and total corticosteroids and density of yearlings. The relationships probably are not biologically significant. Little conflict occurred among yearlings or between yearling females and adult females (Armitage, 1986b) except when the yearling females were non-kin (Armitage, 1986a). Furthermore, rates of amicable and agonistic behavior between adults and yearling females were independent of measures of population density (Armitage, 1975). Finally, in an experimental population of six female yearlings from three litters, only three of 161 social interactions were agonistic (Armitage, 1986c).

The only significant relationship between density and corticosteroid concentration was the correlation between both bound and total corticosteroids of adult males with the total density of residents. These positive relationships must be viewed with care. Because a large number of correlation coefficients were calculated, some significant correlations can be expected by chance. Also, the various tests of density, social behavior, and behavioral phenotype are not independent. The same animals were used repeatedly in these analyses and such practice could lead to

spurious correlations. Because adult males are dominant to adult females and yearlings (Armitage, 1974), it is not obvious why the density of residents should be stressful. Social behavior of adult males is predominantly agonistic; only with adult females is the behavior predominantly amicable. The agonistic behavior with yearling and adult females consists primarily of alerts or flight on the part of the females; the more aggressive chase by the adult males occurred in only 26% of the agonistic interactions and in only 12.6% of all social interactions (Armitage, 1974). However, the resident male regularly approaches animals within his territory. Possibly, the greater number of residents requires more patrolling and approaching animals to determine if they are intruders. Thus, the need to approach more animals could be stressful and result in the observed correlations. However, a large number of residents is frequently associated with numerous males (Table 4), such that the relationship between density may be affected by the social interactions and/or presence of intruder males.

The overlap in home ranges was used as an index of possible social stress, on the assumption that the greater the overlap the greater the potential for conflict. Overlap of home range increases with increasing population density (Armitage, 1975) and this increased sharing of space occurs primarily among close kin (Armitage, 1984, 1989). Thus, higher overlap in home range may be associated with increased social tolerance, but the social tolerance is not without competition (Armitage, 1986a, 1989); what determines the intensity of competition is not fully understood, but it is affected by age, individual behavioral phenotypes, population density, and familiarity (Armitage, 1977, 1986, 1989). Thus, it is not surprising that overlap of home ranges was only weakly associated with corticosteroid concentrations. For yearling males, total corticosteroid was negatively related to overlap of home range with kin and positively related to overlap with the home range of non-kin. These relationships are consistent with the general pattern of social tolerance of kin by yellow-bellied marmots. There was some suggestion that overlap of home ranges and social behavior affected corticosteroid concentrations of adult females.

Social status and social behavior were the major factors affecting concentrations of corticosteroids. The behavioral phenotype of yearling females was correlated with the concentration of bound corticosteroid and there was some evidence for an effect of behavioral phenotype on corticosteroid concentration of adult females. The concentration of bound corticosteroid was higher in female yearlings with a high proportion of agonistic behavior and the conflict score of adult males was positively correlated with the concentrations of total and bound corticosteroid. The effects of social behavior and social status on corticosteroid concentrations probably explains why there was no independent effect of sex on hormonal levels.

If density and/or social stress regulate population density by increasing corticosteroid concentrations, there should be some indication that either reproduction or survival are related to corticosteroid concentrations. It was previously noted that reproductive status was not related to corticosteroid con-

centrations. The relationship between survival and corticosteroid concentrations was tested by the Mann-Whitney U test for adult males and adult and yearling females. Yearling males could not be tested because they disperse and their survivorship is unknown. There was no relationship between survival of adult females and the concentration of bound ($P = 0.45$) or of total ($P = 0.27$) corticosteroid. Among adult males, total corticosteroids were higher ($\bar{x} = 4.14$) in those that survived than in those that did not ($\bar{x} = 2.69$); this relationship approached significance ($P = 0.18$). There was no relationship between survival and bound corticosteroids ($P = 0.54$). Yearling females were divided into two groups: (1) those females that were recovered as 2-year-olds in their natal colony and (2) those that dispersed or failed to survive their hibernation in their natal colony. Total corticosteroids were higher in yearlings that survived as residents ($\bar{x} = 4.0$) than those that died or dispersed ($\bar{x} = 3.2$). This difference approached statistical significance ($P = 0.066$). There was no relationship between survival and the concentration of bound corticosteroids ($P = 0.4$). Interestingly, the concentration of total corticosteroids was higher in the surviving yearling females and adult males and was also higher in the adult females that survived. This pattern suggests that high concentrations of corticosteroids enhance survival rather than increase mortality by causing the animals to be more susceptible to disease or other environmental hazards. The indication that the stress response increased survival suggests that the stress response is adaptive and enables yellow-bellied marmots to cope with social stress. This interpretation is similar to that which suggests that high plasma glucocorticoids enable male *Antechinus* to participate successfully in intense competition for mates, even though all males subsequently die (Lee and Cockburn, 1985a,b).

This analysis indicates that differences in corticosteroid concentration among individual yellow-bellied marmots primarily results from social stress interacting with an animal's behavioral phenotype. The quantification of the contribution of the various social stressors requires an extensive analysis of corticosteroid concentrations in animals of known age, sex, and relatedness and for whom rates of social interactions and patterns of space use can be determined.

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