PLASMA CORTICOSTEROIDS AS AN INDEX OF STRESS IN CAPTIVE FERAL HORSES

In studies involving captive feral horses, capture techniques and handling procedures are such that some degree of stress is usually induced. Stress, in this study, is defined as any noxious stimuli not normally encountered by the horses and which cause an elevation of adrenal cortical steroids. Such stimuli can include trauma, disease, emotional crisis, or acute anxiety and severe exercise. The pathway by which glucocorticoids and most notably cortisol are elevated involves, in the following sequence, (1) excitation of the amygdaloid nuclei and the reticular activating system, (2) stimulation of the ventral hypothalamus-median eminence, (3) release of corticotropin releasing hormone (CRH), (4) secretion of ACTH by the anterior pituitary, and (5) secretion of cortisol and other glucocorticoids by the adrenal cortex (Liddle 1974). The response of this pathway to any of the stimuli mentioned above is rapid.

The implications of this induced stress, as defined above, can be serious from the standpoint of altering physiological parameters that might be studied and also from the standpoint of criticism by animal protection societies. Clearly, concern for the animals requires a consideration of induced stress. Such studies involving feral equids therefore should provide some quantitative measure of physiological stress in order to evaluate and improve handling procedures, and to validate the normality of other physiological parameters.

During 4 years, we examined the endocrinology of 2 herds of feral horses (Pryor Mountain National Wild Horse Range, Montana, and Cedar Mountain, Utah). Most of the studies involved measurements of 17-beta-hydroxyandrogens (Kirkpatrick et al. 1976, 1977*b*). Plasma corticosteroids were examined as a possible physiological index of handling-induced stress. In this report, the effects of 3 handling and blood collection procedures on plasma corticosteroid concentrations are presented.

METHODS AND MATERIALS

A total of 137 different feral horses was captured and 317 blood collections were carried out between 1973 and 1976. Collections were made during every month of the year. Feral horses were captured by directing them from horseback into runways created by natural barriers and from these into corrals. Each captured horse band (3–5 animals) was kept in a

Table 1. Plasma corticosteroid levels in feral horses, as a function of blood collection technique.

Blood collection technique	Horses (N)	Blood samples (N)	Corticoid level, ng/ml (x̄ ± SE)
Heading/heeling	121	223	50 ± 2.20
Jugular venipuncture	13	63	49 ± 1.92
Indwelling catheter	3	31	56 ± 1.51

separate corral with its own water supply for several days before blood collection. The animals were put on a ration of high quality alfalfa hay. At the time of blood collection, individual animals were ushered into a large corral (ca. 5,000 m²) and roped and restrained.

The collection techniques included (1) jugular venipuncture from standing, restrained horses (13 different horses, 63 samples); (2) jugular venipuncture from horses brought down by roping, i.e., heading and heeling (121 different horses, 223 samples); and (3) indwelling catheters in standing restrained horses (3 different horses, 31 samples—approximately 10 for each horse at hourly intervals). Method 1 utilized the more docile horses. These were restrained by 2 halter ropes to posts on opposite sides. This allowed some movement by the animals without danger of injuring themselves. The horses were approached on foot and standard jugular venipuncture was carried out.

Method 2 (heading and heeling) involved roping by head and hind legs and stretching the horse out until it fell. Again, standard jugular venipuncture was carried out. The 3rd method involved placing a PE-10 indwelling jugular catheter in the horse (while restrained on the ground) and then securing him as described in method 1. The catheter, approximately 3 m long, was filled with heparinized saline, and the attached collection syringe was positioned at maximum distance from the horse.

Blood was collected in heparinized Vacutainers and centrifuged. The plasma was frozen immediately on dry ice. Total corticosteroids were extracted from duplicate 0.1-ml plasma samples with 2 ml of reagent grade ethanol, and quantitated by the competitive-protein-binding assay as described by Murphy (1967). Reliability for the assay was reported by Kirkpatrick et al. (1977*a*). Student's *t*-test was used to evaluate differences.

RESULTS

Of the 137 feral horses sampled, 135 possessed corticosteroid levels between 24 and 62 ng/ml plasma (mean = 51 ± 8.7 [SD] ng/ml). Two horses had levels that were higher ($P < 0.05$) than the mean for the 135 animals. Both of them had been restrained and bled by the 1st method. One animal was a 2½-year-old male that had been recently ejected from his band by the harem stud and had suffered numerous and severe injuries, including a 35-cm cut on the neck. Plasma corticosteroid concentration in this horse was 85 ng/ml. The 2nd horse with significantly elevated plasma corticosteroid levels (87 ng/ml) was a 6-year-old male that never adjusted to the restraint and handling procedures of method 1. Both animals showed clinical signs of shock and the 6-year-old was too weak to stand on its own.

Comparison of plasma corticosteroid levels as a function of collection technique is given in Table 1. No differences in plasma corticosteroid levels were observed with respect to the 3 handling procedures ($P < 0.05$).

DISCUSSION

The use of plasma corticosteroid levels as an index of physiological stress ap-

pears to be valid and sound. James et al. (1970) demonstrated significant plasma cortisol increases in domestic horses during surgery, and Hoffsis and Murdick (1970) demonstrated significantly higher plasma corticosteroid levels in domestic horses suffering from shock, colic, and fractures. Franzmann et al. (1975) showed correlations between plasma corticosteroids and excitability in captive moose (*Alces alces gigas*). In this latter study, rectal temperature was used as an independent variable for comparison with corticosteroid values.

Kirkpatrick et al. (1977*a*) reported a diurnal fluctuation in corticosteroid levels in relatively docile feral horses, with a 24-hour average corticosteroid level of 40.2 ± 6.7 ng/ml and a range of 27 to 51 ng/ml. Both the range and the mean compare favorably with values for unstressed domestic horses (Hoffsis and Murdick 1970, James et al. 1970). In that diurnal study however, the mean plasma corticosteroid value for the hours 0800 through 1600 was 44.3 ± 4.1 ng/ml. With the exception of the 2 obviously stressed horses discussed earlier, the 137 feral horses that were captured had an average plasma corticosteroid value of 51 ± 8.7 ng/ml and all samples were collected between 0800 and 1600.

The mean plasma corticosteroid value for the 3 handling procedures taken collectively does not differ ($P < 0.05$) from the 0800–1600 values in the diurnal study, nor do the mean values for the 3 handling procedures differ ($P < 0.05$) from one another. However, the mean plasma corticosteroid value for method 3, the indwelling catheter procedure, was higher ($P < 0.05$) than the mean daytime value in the diurnal study. This was probably the result of anxiety produced by placement of the catheter.

During the heading and heeling procedure the horses run at full speed until they are roped. The exercise is sharp and vigorous and is reflected in rapid heavy breathing. In spite of this, the mean plasma corticosteroid value for this method represents only a 12% increase over the "resting" value of the diurnal study. These results provide a moderate contrast to the study of James et al. (1970) where strenous exercise (1.8 km uphill canter) resulted in a 30% increase in plasma cortisol in racehorses.

The strong similarities between the mean plasma corticosteroid levels for methods 1 and 2 and values for physiologically unstressed horses, both feral and domestic, bring up an important consideration. Present management policies of the North American wild horse populations necessitates accurate aging and identification (lip tatooing in our case) in order to obtain population dynamics data. There has been criticism by humane societies regarding the heading and heeling techniques used on feral horses. At the same time, there has been general advocacy for using chutes to handle these animals. In our experience, chutes have provided little opportunity to age and mark the horses, and have inflicted numerous wounds. On the basis of the data presented, it appears that heading and heeling is both an effective and safe procedure for feral horses, if implemented properly. Use of plasma corticosteroid levels to evaluate physiological stress has additional possibilities. Various tranquilizer compounds (with the exception of those known to suppress the pituitary-adrenal system) and delivery systems, as well as handling procedures for other wild or exotic species, might well be evaluated in terms of induced physiological stress by means of this simple, accurate, and inexpensive method.

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