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## ELECTROPHORETIC PROTEIN ANALYSIS IN THE CONDITIONED CAPTIVE WILD COYOTE, *Canis latrans*<sup>[1]</sup>

E. K. GOERING, C. S. CARD, D. F. BROBST and N. L. GATES

*Abstract:* Total protein, albumin and serum protein values were determined on 19 male and 14 female captive, vaccinated, wild coyotes. Male coyotes had significantly higher total protein, alpha 1 and alpha 2 globulin levels than female coyotes. Captive, wild coyotes had lower values for total protein, albumin and beta globulins, and higher values for alpha 2 and gamma globulins than similar values for laboratory dogs.

Albumin values determined by bromcresol green were slightly higher than values derived by electrophoresis. This difference was non-significant.

### INTRODUCTION

The purpose of this study was to evaluate the absolute and relative values of total serum protein fractions in conditioned captive wild coyotes. This study was prompted by the need for alternative methods of predator control. Current chemosterilant research involves the use of chemical sterilants which may alter normal physiological values of treated animals. However, before a complete evaluation of the changes produced by these chemosterilants can be made, it is essential to determine normal values for the research animals. Many of these values are not available in publication form. This investigation, which describes several normal values for coyotes, will contribute to future research efforts using the conditioned captive wild coyote.

### MATERIALS AND METHODS

Nineteen adult male and 14 adult female coyotes were captured with the use of snowmobiles for this study. They were captured between December, 1974, and March, 1975, within a 170 km radius of the U.S. Sheep Experiment Station, Dubois, Idaho. Average altitude of this area is 1.7 km. A minimum of 3 months in captivity was allowed for acclimation of the coyotes to the new environment; *i.e.*, in a modern kennel of standard design, provided with water and a commercial dog food,<sup>[2]</sup> *ad libitum*. The coyotes were vaccinated after entry into the kennel with a commercial canine vaccine.<sup>[3]</sup> The coyotes appeared clinically normal throughout this investigation.

Serum evaluations were performed on peripheral blood samples collected from the jugular vein in Vacutainer tubes

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[2] Friskies Sauce Cubes, Carnation Company, Los Angeles, California 90036, USA.

[3] Enduracell d-h-1, Norden Laboratories, Lincoln, Nebraska 68500, USA.

using a 20 gauge needle,<sup>[4]</sup> from unanesthetized male and female coyotes fasted for a minimum of 8 hrs. Serum was separated by centrifugation within one hr after collection.

Electrophoresis was carried out on a microzone electrophoretic cell using cellulose acetate strips with sodium barbital buffer (pH 8.6).<sup>[5]</sup> The method utilized was a modification of Nerenberg's.<sup>6</sup> Ponceau-S dye was used as the protein staining agent and the electrophoretic patterns were quantitated on an integrating densitometer. Calculations of the percentages of protein fractions were made from the densitometer tracings obtained. Total protein values were determined using the biuret procedure after a calibration curve was prepared from an albumin standard<sup>[6]</sup> and normal sera.<sup>[7]</sup> The bromcresol green dye method was used to measure albumin. The albumin value obtained from dogs using the hydroxyazobenzene ben-

zoic acid (HABA) assay are depressed when compared to values obtained by other procedures.<sup>3</sup> To avoid this possibility, duplicate albumin values were derived using the bromcresol green (BCG) assay and electrophoresis. Results were analyzed by the standard student T-test (unpaired).<sup>8</sup> Significance was assumed for  $P < (0.05)$ .

## RESULTS

The results of this study, summarized in Table 1, are expressed as the mean value, standard deviation and observed range for the various parameters. Albumin values were determined by the bromcresol green and electrophoresis techniques while globulins which are listed as alpha 1, alpha 2, beta, and gamma globulin were determined as electrophoretic absolute (grams/100 mls) and relative (percentages) values.

TABLE 1. Serum Protein Electrophoresis Values Determined on 19 Male and 14 Female Conditioned Captive Wild Coyotes.

Evaluation Method	Mean	Std. Dev.	Percent	Observed Range
Total Protein (biuret)	5.4 gr/100 ml ± .45		100.0%	4.5-6.1 gr/100 ml
Albumin (bromcresol green)*	2.8 gr/100 ml ± .30		52.0%	2.2-3.3 gr/100 ml
Albumin (electrophoresis)*	2.6 gr/100 ml ± .31		48.6%	2.1-3.3 gr/100 ml
Globulins				
Alpha 1 Indirect (electrophoresis)	0.4 gr/100 ml ± .06		6.5%	0.2-0.5 gr/100 ml
Alpha 2 Indirect (electrophoresis)	0.7 gr/100 ml ± .17		13.3%	0.5-1.1 gr/100 ml
Beta Indirect (electrophoresis)	0.6 gr/100 ml ± .20		12.0%	0.4-1.4 gr/100 ml
Gamma Indirect (electrophoresis)	1.1 gr/100 ml ± .18		19.6%	0.8-1.4 gr/100 ml

\* Albumin values determined by bromcresol green did not differ significantly from those derived by electrophoresis.

[4] Becton, Dickinson, and Co., Rutherford, New Jersey 07070, USA.

[5] Beckman Instruments, Inc., Maywood, Illinois 60153, USA.

[6] American Monitor Corp., Indianapolis, Indiana 46200, USA.

[7] Metrix Normal Serum, Armour Pharmaceutical Co., Chicago, Illinois 60600, USA.

The mean total protein value found in 19 male and 14 female coyotes was  $5.35 \pm .45$  gm/100 ml, with an observed range of 4.5 to 6.1 mg/100 ml. The mean albumin values, determined by the bromocresol green (BCG) dye method, were  $2.8 \pm .30$  gm/100 ml with an observed range of 2.2 to 3.3 gm/100 ml making up 52% of the protein fractions. The same values obtained by electrophoresis were  $2.6 \pm .31$  gm/100 ml, with an observed range of 2.1 to 3.3 gm/100 ml. The latter values made up 48.6% of the total protein fraction, and are similar to those obtained using the bromocresol green dye method (Table 1).

Electrophoretic values for globulin fractions are listed in Table 1, while Table 2 compares the mean value, standard deviation and observed range found in the 19 male and 14 female coyotes using the Student t-test evaluation.<sup>7</sup> The values for albumin and beta and gamma globulins for females were not significantly different from the male values, however, total protein and alpha 1 globulin values in the females were significantly lower than in the males. Because

of the similarity of observed ranges and the extreme sensitivity of the paired t-test, this difference may have a limited diagnostic significance.

A comparison between mean female and male coyote values obtained in this study, and mean values for female and male dogs previously determined,<sup>9</sup> are listed in Table 3. Mean total protein values for both coyote sexes were lower than values for the dog. The difference in values is greater between female coyotes compared to female dogs than between male coyotes and male dogs. Mean values for albumin and alpha 1 and beta globulin followed a similar pattern in both coyote sexes when compared to the dog (Table 3).

The mean values for alpha 2 and gamma globulin of the coyotes were higher than comparable values in dogs. The gamma globulin level of coyotes was nearly double the value for domestic animals.<sup>8</sup> Male and female coyote values followed a similar trend (Table 4) when compared with two different populations of dogs.

TABLE 3. Comparison of Protein Fractions of Male and Female Coyotes and Male and Female Dogs.

	19 Male Coyotes	53 Male <sup>9</sup> Dogs	14 Female Coyotes	47 Female Dogs
Total Protein (gm/100 ml)	5.5	6.1	5.1	6.2
%	100%	100%	100%	100%
Albumin (gm/100 ml)**	2.6	3.5	2.6	3.6
%	47%	57%	50%	57%
Alpha 1 (gm/100 ml)**	0.4	0.5	0.3	0.5
%	7%	7%	6%	9%
Alpha 2 (gm/100 ml)**	0.8	0.5	0.6	0.5
%	15%	8%	12%	8%
Beta (gm/100 ml)	0.6	1.1	0.65	1.1
%	11%	18%	13%	17%
Gamma (gm/100 ml)**	1.1	0.6	1.0	0.5
%	20%	9%	19%	9%

\* Determined by biuret technique in both dogs and coyotes.

\*\*Determined by serum electrophoresis in both dogs and coyotes.

TABLE 2. Conditioned Captive Wild Coyotes Serum Protein Electrophoresis Values—Sex Comparison (grams protein/100 ml).

Evaluation #	Males (19) Mean (gm/100 ml)	Std. Dev.	Observed Range	Females (14) Mean	Std. Dev.	Observed Range	"T" Test Value
Total Protein (biuret)	5.5	.40	4.5-6.1	5.1	.43	4.5-5.8	*2.276
Albumin (bromoresol green)**	2.8	.32	2.2-3.3	2.7	.26	2.2-3.1	1.365
Albumin (electrophoresis)**	2.6	.34	2.1-3.3	2.6	.27	2.2-3.0	0.500
Alpha 1 (electrophoresis)	0.4	.06	0.3-1.1	0.3	.06	0.2-0.4	*2.607
Alpha 2 (electrophoresis)	0.8	.17	0.6-1.1	0.6	.14	0.4-1.0	*2.495
Beta (electrophoresis)	0.6	.16	0.4-0.9	0.6	.26	0.4-1.4	0.531
Gamma (electrophoresis)	1.1	.15	0.8-1.3	1.0	.20	0.8-1.4	0.930

\* "T" test value was 2.042 for 33 samples and 31 degrees of freedom, (unpaired).  $P < (0.05)$ .

\*\*Albumin values determined by bromoresol green did not differ significantly from values derived by electrophoresis in either male or female coyote groups (t-statistic of 2.021 and 2.293 respectively).

# Evaluation given in Gm/100 ml).

TABLE 4. Protein Fraction Comparison Between Conditioned Captive Wild Coyotes and Dogs.

Evaluations	Value	Biuret Method			
		Total Protein	Albumin	Alpha 1 Globulin	Alpha 2 Globulin
Coyotes (33) *	Relative Value	100%	48.6%	6.5%	13.3%
	Absolute Value (gm/100 ml)	5.4	2.6	0.35	0.7
Laboratory Dog <sup>o</sup> (43)	Relative Value	100%	38%	10%	12%
	Absolute Value (gm/100 ml)	6.3	2.4	0.63	0.75
Beagle Dog <sup>o</sup> (53)	Relative Value	100%	53.8%	4.4%	8.9%
	Absolute Value (gm/100 ml)	6.6	3.6	.29	.59
					Gamma Globulin
					Beta Globulin
					12.0%
					0.64
					19.6%
					1.05
					14.4%
					.91
					13.0%
					.86

\* Number of animals in trial.

## DISCUSSION

The coyotes used in this investigation were wild animals and confinement undoubtedly resulted in stress. During a period of stress comparable to that which a caged coyote may encounter, glucocorticoids are secreted continuously to aid the individual in adaptation to the stress. This adaptational mechanism is essential for survival during the innumerable stress periods that many wild species must withstand. Hypophysectomized or adrenalectomized animals treated with only maintenance doses of glucocorticoids die when exposed to the same stress a normal animal would survive.<sup>3</sup> When studies on wild confined animal species are carried out using restraint techniques, workers can attempt to minimize the stress during procedural activities and hope that reactions to the stress are uniform throughout the experimental population.

The difference in values of total protein and albumin between wild coyotes and dogs was consistently observed in this investigation (Table 3 and 4). Whether this is a true interspecies difference or is dependent on dietary factors existing between wild coyotes and dogs was not established. However, the coyotes had been fed, *ad libitum*, a commercial dog food meeting minimal requirements for dogs for approximately three months

prior to sampling. Wild wolf pups (*Canis lupus*) had similar total protein values<sup>4</sup> but the authors did not speculate on the difference between these values and those of beagle dogs.

Bossack *et al.*<sup>2</sup> demonstrated increased alpha 2 globulin values in dogs treated with continuous high dosages of cortisone with a concomitant decrease in the beta globulin values while Bjorneboe *et al.*<sup>1</sup> demonstrated that cortisone produced a small rise in albumin and alpha 2 globulin and a decrease in alpha 1, beta and gamma globulins. This steroid-induced response could explain the moderate decrease in alpha 1 globulins and the increase in alpha 2 globulins observed in this comparison between coyotes and dogs (Table 3 and 4). This response would tend to decrease the extreme differences in beta globulin values that were noted between coyotes and dogs in this study, and increase the differences noted in the gamma globulin levels. However, the elevation of gamma globulin levels in the plasma of coyotes could also be a postvaccinal phenomena following the routine vaccination with several commercial vaccines at the time of capture (distemper, hepatitis, and leptospirosis). The increased gamma globulin levels could also be caused in part by close confinement and the subsequent increase in number and types of pathogenic agents in the new environment.

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