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VITREOUS HUMOR ANALYSIS FOR SELECTED BIOCHEMICAL PARAMETERS FROM CERVIDS IN IDAHO

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ABSTRACT: Vitreous humor and liver samples were collected from hunter-harvested elk (*Cervus elaphus*) and mule deer (*Odocoileus hemionus*) in Idaho (USA). Concentrations of calcium, chloride, potassium, sodium, urea nitrogen and selenium were determined and evaluated according to species, age, gender, geographic location, and time elapsed following death. Vitreous humor analysis yielded reliable biochemical information for ≤96 hr subsequent to the death of the animal. Vitreous potassium concentration changes over time could be used to estimate the time that elapsed following death.

Key words: Biochemical analyses, *Cervus elaphus*, elk, mule deer, *Odocoileus hemionus*, vitreous humor.

INTRODUCTION

Establishing biochemical base-line data for wildlife over an extended geographical area is logistically difficult and usually economically prohibitive. However, such information is often essential in wildlife disease investigations, and health and forage management programs. Thus, to facilitate the collection of biochemical information, postmortem analysis of vitreous humor has been proposed as an alternative to serum testing in some situations.

The eyeball is anatomically isolated and well protected. Therefore, vitreous humor may remain relatively stable chemically despite putrefactive changes in other parts of the body after death (Coe, 1972). In man, vitreous humor has been used with varying degrees of success in the estimation of the time of death, detection of electrolyte and carbohydrate disturbances, diagnosis of drowning, and in the identification of selected toxic substances such as ethyl alcohol (Coe, 1969; Jaffe, 1962; Leahy and Farber, 1967; Sturner, 1972). Postmortem chemical analysis of ocular fluids also has been investigated on a limited basis as a diagnostic aid in dogs, cattle, hogs and horses (Crowell and Duncan, 1974; Lane and Lincoln, 1985; Lincoln and Lane, 1985; McLaughlin and McLaughlin, 1987; Cantor et al., 1989; Drolet et al., 1990). No studies involving wildlife have been reported.

The purpose of the present study was to obtain useable biochemical base-line data for the primary big game species in Idaho (USA), the Rocky Mountain elk (*Cervus elaphus*) and mule deer (*Odocoileus hemionus*). Further objectives were to (1) determine if there were useful correlations between chemical concentrations in vitreous humor and serum, (2) monitor vitreous chemical concentrations over time after death and determine if any changes are predictable, (3) investigate possible chemical concentration differences between hunting regions within Idaho, and (4) determine if there were differences in chemical concentrations between species, gender and age of animals evaluated.

MATERIALS AND METHODS

The state of Idaho is divided into 99 hunting units in 7 regions (Fig. 1). Biological samples were obtained from wildlife from 5 of the 7 regions (Regions 3 to 7; 42°00′ to 46°00′N to 111°00′ to 117°00′W) covering an area of approximately 80% of the state. During the course of the study, samples (vitreous humor, liver or serum) were obtained from 967 animals (330 elk and 637 mule deer).

Vitreous humor samples were collected during the annual fall big game hunting seasons of 1993, 1994 and 1995. Selected conservation officers of the Idaho Department of Fish and Game headquartered in Boise (Idaho, USA) were supplied quantities of individual collection kits. Each kit consisted of a self-sealing plastic bag containing a 6-ml syringe fitted with an 18-ga 4.5 cm needle, a 3-ml draw vacuum

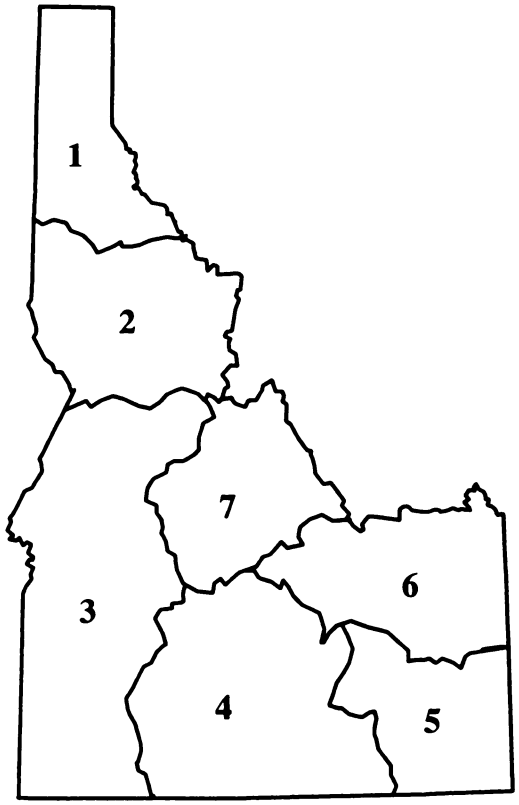

FIGURE 1. The state of Idaho (USA) divided into seven hunting regions.

blood tube (Vacutainer, Becton Dickinson, Rutherford, New Jersey, USA), and a data sheet. Information recorded included species sampled, gender, estimated age of animal (based on dentition), hunting unit, date of kill and date of sample collection. As hunters presented their harvested game at hunting check stations throughout the state, officers collected vitreous samples as per the technique described by Lincoln and Lane (1985). In the 1994 sampling, officers also obtained approximately 5 cm^3 sections of liver, if available, for quantifying of selenium (Se) concentrations.

All samples were immediately refrigerated in chilled chest coolers and transported within 24 hr to the Caine Veterinary Teaching and Research Center, (Caldwell, Idaho, USA). Upon arrival, samples were stored at 4 C until evaluated.

Clinical chemical analysis of vitreous humor was conducted with the same procedures and equipment as for serum. Concentrations of the electrolytes; ionized calcium (Ca^{2+}), chloride (Cl), potassium (K), and sodium (Na) were determined with a blood gas/electrolyte analyzer, Model 850 (Ciba Corning Diagnostics Corp., Norwood, Massachusetts, USA). Vitreous urea nitrogen (VUN) values were determined using commercial quantitative determination kits (RefLab, Medical Analysis Systems, Inc., Camarillo, California, USA). Results for VUN evaluations were read via a digital readout spectrophotometer (Strasar 3, Gilford Instrument, Oberlin, Ohio, USA). Selenium concentrations from liver samples were obtained by the Analytical Sciences Laboratory (University of Idaho, Moscow, Idaho, USA) using standard vapor generation inductively coupled plasma spectrometry methods.

Data from each group set were analyzed using descriptive statistics of means, standard error of the mean (SE), and minimum and maximum ranges computed by the STATISTIX Version 4.1® computer program (Analytical Software, Tallahassee, Florida, USA). Each group set was tested for normality using the Wilk-Shapiro Statistic and found to have coefficients in excess of 0.80. Two-way ANOVA was used to identify the degree of agreement between like samples. Differences in means were compared using Fisher's least square difference.

RESULTS

Very few differences were found in vitreous humor biochemical concentrations between elk and mule deer, the genders, and ages of animals at the time of sampling (Table 1). Vitreous concentrations of Ca, Cl, and Na remained stable for 96 hr after death (Table 2). Both K and VUN concentrations significantly increased with post mortem (PM) interval increases.

Elk and mule deer biochemical data from 5 of the 7 geographic hunting regions in Idaho confirmed that few differences were found (Table 3). Rough correlations between PM vitreous biochemical concentrations from both elk and mule deer, and elk serum concentrations were identified (Table 4).

DISCUSSION

From the data presented in Table 1 it was concluded that for field surveys or other studies in which precise measurements are not required, pooling of vitreous samples from both elk and mule deer, both sexes and all ages to facilitate data management is acceptable. Such data could

TABLE 1. Vitreous humor biochemical concentrations (mean ± SE) from elk and mule deer in Idaho sampled within 24 hr after death separated by gender and age in years.

Species	Sex	Age	n[a]	Ca^{2+} mg/dl	Cl mEq/L	K mEq/L	Na mEq/L	VUN mg/dl
E[b]	♂	<1	21	3.48 ± 0.12[d]	121.13 ± 1.41[d]	10.77 ± 0.66[d]	137.93 ± 1.43[d]	11.70 ± 1.93[d]
		1–3	124	3.73 ± 0.08[d]	126.08 ± 1.14[de]	9.97 ± 0.27[d]	143.25 ± 1.35[de]	17.75 ± 1.23[d]
		>3	10	3.08 ± 0.35[de]	133.60 ± 4.86[de]	9.72 ± 0.92[d]	154.52 ± 7.36[de]	16.20 ± 3.40[d]
	♀	<1	12	3.23 ± 0.34[d]	121.62 ± 1.89[d]	12.01 ± 1.38[d]	134.97 ± 1.20[d]	8.67 ± 2.33[d]
		1–3	28	3.47 ± 0.19[d]	123.14 ± 2.27[de]	10.72 ± 0.88[d]	137.27 ± 2.61[d]	10.33 ± 1.53[d]
		>3	3	2.95 ± 0.45[de]	134.73 ± 5.63[de]	9.33 ± 0.97[d]	152.90 ± 8.89[de]	12.50 ± 8.50[d]
E total	♂♀		198	3.59 ± 0.13[d]	125.38 ± 1.63[de]	9.86 ± 0.51[d]	142.05 ± 1.95[de]	15.35 ± 1.63[d]
MD[c]	♂	<1	138	2.70 ± 0.06[e]	123.50 ± 0.88[d]	10.54 ± 0.28[d]	142.78 ± 1.11[de]	13.64 ± 0.73[d]
		1–3	221	3.37 ± 0.06[d]	128.41 ± 0.83[e]	10.57 ± 0.25[d]	144.87 ± 0.99[e]	14.67 ± 0.69[d]
		>3	10	2.89 ± 0.35[de]	129.60 ± 2.15[e]	10.95 ± 1.21[d]	148.27 ± 3.79[de]	12.00 ± 2.62[d]
	♀	<1	13	3.07 ± 0.21[de]	122.77 ± 3.15[de]	12.21 ± 0.97[d]	139.20 ± 3.49[de]	13.15 ± 1.86[d]
		1–3	27	3.38 ± 0.16[d]	128.19 ± 2.14[de]	10.11 ± 0.79[d]	143.46 ± 1.86[de]	13.33 ± 1.23[d]
		>3	6	2.57 ± 0.34[de]	127.67 ± 3.41[de]	8.98 ± 0.96[d]	147.12 ± 4.93[de]	9.00 ± 2.03[d]
MD total	♂♀		415	3.18 ± 0.08[d]	126.60 ± 1.07[e]	10.57 ± 0.35[d]	144.02 ± 1.28[e]	14.05 ± 0.84[d]
E and MD Totals			613	3.22 ± 0.05[d]	125.61 ± 0.68[e]	10.34 ± 0.40[d]	143.25 ± 0.72[e]	14.56 ± 0.53[d]

[a] n = sample size.
[b] E = elk.
[c] MD = mule deer.
[d,e] Means in the same column with different superscript letters differ ($P < 0.05$, mean ± 2 SE).

TABLE 2. Vitreous biochemical concentrations and liver selenium levels (mean ± SE) from elk and mule deer (all ages and sexes) in Idaho sampled at different times following death.

Hours since death	n[a]	(mg/dl) Ca^{2+}	(mEq/L) Cl	(mEq/L) K	(mEq/L) Na	(μg/g) Se[b]	(mg/dl) VUN
<24	613	3.22 ± 0.05[c]	125.61 ± 0.68[c]	10.34 ± 0.40[c]	143.25 ± 0.72[c]	0.86 ± 0.41[c] (263)	14.56 ± 0.53[c]
24–48	290	3.30 ± 0.07[c]	127.31 ± 0.86[c]	12.56 ± 0.24[d]	144.53 ± 1.04[c]	0.26 ± 0.06[c] (164)	16.96 ± 0.90[cd]
48–96	64	3.13 ± 0.11[c]	127.45 ± 1.67[c]	17.47 ± 0.53[e]	143.91 ± 2.04[c]	0.38 ± 0.19[c] (18)	21.55 ± 2.05[d]

[a] n = number of animals tested.
[b] Numbers in parentheses = quantity of liver samples tested/group.
[c,d,e] Means in the same column with different superscript letters differ ($P < 0.05$, mean ± 2 SE).

provide rough standards of biochemical concentrations to estimate concentrations in other elk and mule deer populations.

Perhaps the potentially greatest factors of concern in evaluating the data generated in the present study were time between death and sample collection, and the temperatures to which the samples were subjected during that interval. The periods from death until vitreous sampling were measured in 24 hr blocks of time. Because so few variations were found in biochemical concentrations between species at 24 hr after death (Table 1) vitreous samples from both elk and mule deer were pooled in further evaluations of the effects of time on biochemical concentrations.

While Ca, Cl, and Na concentrations remained constant for 96 hr after death, K and VUN levels increased over PM time. However, those concentration elevations were predictable such that rising linear relationships were noted with each over time (Fig. 2). Lincoln and Lane (1985) reported similar findings in bovine vitreous studies. Further, increases in K concentrations in ocular samples have been used in forensic medicine to estimate PM interval (Adelson et al, 1963; Coe 1972). Thus, data from the present study showed that PM vitreous K levels can also be used to roughly estimate the time of death up to 96 hr PM of elk and mule deer. A similar statement might be made for VUN except that the standard error increases with each 24 hr PM interval to such a degree that confidence in any estimations is lost (Fig. 2).

Some ocular fluid electrolyte concentrations (Ca, K, Na, and phosphorus) were reported to increase over time and elevations in temperature in cattle (McLaughlin and McLaughlin, 1987), dogs (Schoning and Strafuss, 1980), horses (Cantor et al., 1989), and swine (Drolet et al., 1990). Conversely urea nitrogen and creatinine concentrations were found to remain stable for 24 to 48 hr PM, in some cases at temperatures up to 37 C (Coe, 1969; Schoning and Strafuss, 1981, Lane and Lincoln, 1985; Palmer et al., 1985; McLaughlin and McLaughlin, 1987).

It was estimated that the temperature range for the periods between when animals in the present study were harvested and when vitreous samples were collected was −5 C to 30 C. However, because of the nature of the study it was impossible to determine specific temperatures for each sample source. Thus, although reportedly important in evaluating biochemical concentrations most accurately, temperature variations were not factored into the present data.

Both elk and mule deer were sampled from geographic hunting regions 3 through 7 (Fig. 1) or from an area of approximately 116,750 km^2. Elevation varied from about 500 meters to over 2,500 meters and the vegetation types included desert sagebrush up through alpine conifer

TABLE 3. Vitreous humor biochemical concentrations and liver selenium levels (mean ± SE) from elk and mule deer (all ages and both sexes) in Idaho sampled within 96 hr after death separated by species and hunting regions.

Region	Species	n[a]	Ca^{2+} mg/dl	Cl mEq/L	K mEq/L	Na mEq/L	Se[b] µg/g	VUN mg/dl
3	E[c]	122	3.62 ± 0.07[e]	123.14 ± 1.10[e]	10.71 ± 0.32[efg]	139.49 ± 1.26[e]	0.71 ± 0.41[e] (74)	14.45 ± 1.42[e]
	MD[d]	261	3.45 ± 0.07[eg]	128.03 ± 0.90[f]	11.90 ± 0.29[f]	143.59 ± 1.02[ef]	0.69 ± 0.48[e] (119)	15.18 ± 0.90[e]
4	E	4	3.52 ± 0.09[eg]	125.14 ± 3.98[ef]	10.47 ± 0.93[efg]	144.51 ± 3.24[ef]	ND[h]	ND
	MD	8	3.26 ± 0.11[eg]	126.89 ± 1.67[ef]	9.91 ± 1.11[efg]	141.16 ± 2.05[ef]	ND	ND
5	E	7	3.96 ± 0.26[e]	123.00 ± 4.86[ef]	9.80 ± 1.75[efg]	143.68 ± 7.37[ef]	0.45 ± 0.28[e] (5)	21.00 ± 5.21[e]
	MD	178	2.56 ± 0.07[f]	125.61 ± 1.00[ef]	9.82 ± 0.30[eg]	146.01 ± 1.19[f]	0.36 ± 0.03[e] (69)	15.27 ± 1.12[e]
6	E	16	3.48 ± 0.16[eg]	129.89 ± 1.90[f]	10.50 ± 0.66[efg]	141.11 ± 2.00[ef]	ND	ND
	MD	6	3.30 ± 0.19[eg]	127.18 ± 3.04[ef]	10.72 ± 0.86[efg]	142.19 ± 3.47[ef]	ND	ND
7	E	181	3.23 ± 0.08[g]	126.63 ± 1.28[ef]	10.07 ± 0.40[eg]	143.68 ± 1.47[ef]	0.75 ± 0.52[e] (106)	14.77 ± 0.94[e]
	MD	184	3.20 ± 0.09[g]	126.98 ± 1.33[ef]	10.15 ± 0.41[eg]	144.11 ± 1.53[ef]	0.79 ± 0.57[e] (72)	13.98 ± 0.83[e]
Totals		967	3.23 ± 0.04[g]	126.28 ± 0.51[ef]	11.12 ± 0.16[efg]	143.64 ± 0.56[ef]	0.68 ± 0.28[e] (445)	15.70 ± 0.46[e]

[a] n = sample size.
[b] Numbers in parentheses are number of liver samples tested per category.
[c] E = elk.
[d] MD = mule deer.
[h] ND = not done.
[e,f,g] Means in the same column with different superscript letters differ ($P < 0.05$, mean ± 2 SE).

TABLE 4. Comparison between electrolyte and urea nitrogen concentrations in vitreous humor sampled within 24 hr after death of elk and mule deer, and in antemortem elk serum.

Species	S[a]/V[b]	n[c]	Ca^{2+} mg/dl	Cl mEq/L	K mEq/L	Na mEq/L	UN[d] mg/dl
E[e]	V	198	3.59 ± 0.13[h]	125.38 ± 1.63[h]	9.86 ± 0.51[h]	142.05 ± 1.95[h]	15.35 ± 1.63[h]
E	S[j]	39	4.57 ± 0.45[h]	NA[g]	5.50 ± 0.65[i]	148.00 ± 8.60[h]	26.34 ± 7.22[h]
MD[f]	V	415	3.18 ± 0.08[i]	126.60 ± 1.07[h]	10.57 ± 0.35[h]	144.02 ± 1.28[h]	14.47 ± 1.10[h]

[a] S = serum.
[b] V = vitreous.
[c] n = number of animals sampled.
[d] UN = urea nitrogen.
[e] E = elk.
[f] MD = mule deer.
[g] NA = not available.
[h,i] Means in the same column with different superscript letters differ ($P < 0.05$, mean ± 2 SE).
[j] Data from Kitchen (1978).

forests. Comparing the biochemical data from the separate hunting regions confirmed that few differences were found (Table 3). Although the differences noted were statistically significant, they were judged not to be clinically or diagnostically significant.

As a stated objective of the present study it was hoped that a working correlation between PM vitreous biochemical concentrations and normal serum concentrations could be identified. No serum samples were available from the animals providing vitreous samples in this study to allow the best comparisons. Unfortunately, few published surveys of serum biochemical concentrations of free-ranging North American cervids exist. The data in Table 4 provide a comparison between biochemical concentrations in vitreous humor sampled ≥24 hr PM of elk and mule deer, and those concentrations in elk serum. The serum data were reported by Kitchen (1978). Caution must always be exercised with all comparisons of data from different sources. Still, vitreous Ca, Na and UN concentrations were essentially the same as those reported in elk serum. Potassium concentration differences were predictable, as previously stated. Therefore, it appears that rough estimates of antemortem serum concentrations of Ca, K, Na and UN might be made from vitreous humor analyses. Such information will prove useful to wildlife management and health personnel in evaluating possible deviations to normal concentrations.

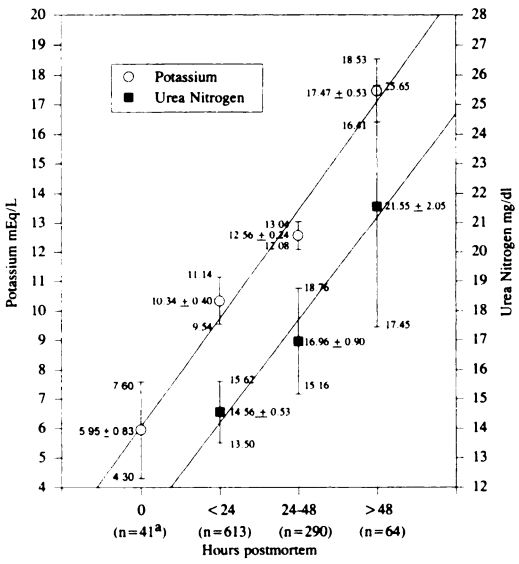

FIGURE 2. Antemortem serum potassium and postmortem, time-dependent vitreous potassium and urea nitrogen concentrations from combined elk and mule deer. (Mean ± 2 SE) a = Data from Kitchen (1978).

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