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Time of Death Estimation in Blacktail Deer by Temperature and Aqueous Humor Glucose

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ABSTRACT: Aqueous humor glucose and body temperature are utilized to estimate the postmortem interval. Sequential sampling of a 15-deer control study provided sufficient data to establish a 95% prediction interval for the first 8 h. Statistical analysis of 187-deer field study shows 94.7% to be inside the prediction interval.

KEYWORDS: pathology and biology, aqueous humor, deer, glucose, eye fluid

The need to determine the time of death (TOD) in deer within the first several hours is a significant problem. A game officer may contact a hunter late at night or during the early morning hours possessing a deer carcass. Intuition and some circumstantial evidence may suggest the deer was obtained after the legal shooting hours. Direct physical evidence from body temperature and eye fluid glucose can aid in the prosecution of poachers, or can exonerate persons trapped by unfortunate or prejudicial circumstances.

Several scientists [*1-3*] have studied glycolysis in the peripheral blood and in vitreous humor (eye fluid) of humans. During the 1950s and 1960s several comparisons were made between antemortem and postmortem specimens. More recently, Coe [*4*] demonstrated the postmortem changes in vitreous glucose occurs quite rapidly, but were of limited value in diagnosing deaths caused by hypoglycemia.

In 1979, Schoning and Strafuss [*5*] tested vitreous humor glucose in 60 mongrel dogs and found that the glucose was affected by time but not by the outside environmental temperature between 4 and 37°C. Determination of TOD by body temperature is also of value and has a high correlation to the eye fluid glucose. Kistner and members of the Oregon State Police Game Division [*6*] obtained sequential body temperatures from 30 freshly killed deer. Graphical illustrations of temperature versus time revealed the body weight (muscle mass in the rump) had a direct effect on the rate of cooling. Larger deer cooled slightly slower than smaller deer.

The purpose of this study is to combine body temperature and eye fluid glucose data to provide a time of death in deer carcasses with 95% confidence. The total number of deer studied was 202 (15 in a controlled study and 187 in a field study).

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Materials and Methods

For purposes incident to a range management impact study, 15 deer were killed. The animals were obtained in three range management areas near Alsea, OR. The animals were shot in the neck, field dressed, and weighed. Physical measurements were obtained and an examination for disease was made by a wildlife veterinarian. All deer were free from significant physiological problems that might influence the body temperature of eye fluid glucose. The outside temperature varied from 3.2 to 17°C.

The field study was conducted at the McDonald Forest Check Station—a unique opportunity where hunters must check in and out of a 32-km (20-miles) special hunting area. It is an either sex hunt for blacktailed deer. Successful hunters were required to "punch" a special tag which was provided to each hunter for recording the TOD to the nearest 15 min. Each deer collected is weighed, examined for disease, and a record of age and sex obtained. The method of transportation, area of wound, rump temperature, and eye fluids were also collected. The deer varied in weight from 16 to 68 kg (36 to 150 lb). Methods of transportation varied considerably from open bed pickups, pickups with canopies, and vans and automobile trunks. Outside temperatures varied from 6 to 20°C. Animals that were skinned, totally dismembered, or suffered injury to the eyes or the eye sockets were not sampled.

Approximately 0.5 mL of aqueous humor was removed from an eye with a needle and syringe. The clear, colorless fluid is placed in an evacuated tube containing sodium fluoride as a preservative. In the control study, samples were obtained at 1, 3, and 6 h after death. Also at those intervals, an incision was made into the rump. A thermometer was inserted until contact was made with the femur, before the temperature was obtained.

The aqueous humor specimens were centrifuged and the clear supernatant removed. An occult blood test was performed as an aid to rule out physical damage to the eyes. Fifty microlitres of fluid, control, and standards were added to 1 mL of 6% *o*-toluidine in appropriately labeled 13- by 75-mm test tubes. The mixture was incubated at 100°C for 10 min, cooled, and read in a spectrophotometer at 630 nm. All tests were performed in duplicate. Low optical density measurements prevented an accurate reading below 5 mg/100 mg.

Statistical Methods

The hours-since-death variable Y is estimated from measurements of glucose (variable X_1) and body temperature (variable X_2) by a standard linear regression model in these two variables. The data used to effect the estimation of the regression coefficients were the 26 observations in the control study previously defined. The fitted least squares regression equation for the mean time since death is $Y = 17.1 - 0.0704\,X_1 - 0.333\,X_2$ and the estimated standard deviation about the regression is $s = 0.7853$ on 23 degrees of freedom. The coefficient of determination is $R^2 = 0.879$.

The problem at hand is to use these data and computations to predict the value of the variable Y (hours since death) by using measurements on X_1 (glucose) and X_2 (body temperature). Standard regression analysis theory [*1*] yields the 95% prediction intervals as given in Table 1 and Fig. 1. For example, if a deer carcass has $X_1 = 59$ (glucose level) and $X_2 = 37.5$ (body temperature in °C) then from the table corresponding to $X_2 = 37.5$, we read opposite $X_1 =$ 59, that the lower prediction limit (LPRED) $= -1.53$, the upper prediction limit (UPRED) $=$ 2.117, and the estimated mean is 0.318. The interpretation is that deer carcasses with $X_1 = 59$ and $X_2 = 37.5$ average about 0.318 h since death but that the time of death is predicted to be between 0 and 2.17 h. The confidence of this prediction is 95%.

A separate table was prepared for each 0.5°C increment. The range of temperatures for the computations runs from 26.5 to 40°C (80 to 104°F). The data presented in the table is the result of a mathematical computation which, in some instances, produces variable combinations that may be considered to be physiologically unacceptable. For example, one would not expect to see a body temperature of 26.5°C and an aqueous humor glucose of 60 mg/100 mg.

TABLE 1—*Temperature at 37.8°C (100°F).*

Glucose, mg/100 mg	*Y*-PRED (Mean)	LPRED (Lower)	UPRED (Upper)
5	4.15700	2.18081	6.13319
6	4.08590	2.12265	6.04915
7	4.01480	2.06416	5.96544
8	3.94370	2.00535	5.88205
9	3.87260	1.94620	5.79900
10	3.80150	1.88672	5.71628
11	3.73040	1.82689	5.63391
12	3.65930	1.76670	5.55190
13	3.58820	1.70617	5.47023
14	3.51710	1.64526	5.38894
15	3.44600	1.58399	5.30801
16	3.37490	1.52234	5.22746
17	3.30380	1.46031	5.14729
18	3.23270	1.39790	5.06750
19	3.16160	1.33509	4.98811
20	3.09050	1.27189	4.90911
21	3.01940	1.20828	4.83052
22	2.94830	1.14426	4.75234
23	2.87720	1.07983	4.67457
24	2.80610	1.01498	4.59722
25	2.73500	0.94972	4.52028
26	2.66390	0.88402	4.44378
27	2.59280	0.81790	4.36770
28	2.52170	0.75134	4.29206
29	2.45060	0.68434	4.21686
30	2.37950	0.61691	4.14209
31	2.30840	0.54903	4.06777
32	2.23730	0.48071	3.99389
33	2.16620	0.41194	3.92046
34	2.09510	0.34273	3.84747
35	2.02400	0.27306	3.77494
36	1.95290	0.20294	3.70286
37	1.88180	0.13237	3.63123
38	1.81070	0.06134	3.56006
39	1.73960	−0.01014	3.48934
40	1.66850	−0.08207	3.41907
41	1.59740	−0.15445	3.34925
42	1.52630	−0.22728	3.27988
43	1.45520	−0.30056	3.21096
44	1.38410	−0.37429	3.14249
45	1.31300	−0.44847	3.07447
46	1.24190	−0.52308	3.00688
47	1.17080	−0.59814	2.93974
48	1.09970	−0.67364	2.87304
49	1.02860	−0.74957	2.80677
50	0.95750	−0.82594	2.74094
51	0.88640	−0.90272	2.67552
52	0.81530	−0.97994	2.61054
53	0.74420	−1.05757	2.54597
54	0.67310	−1.13562	2.48182
55	0.60200	−1.21407	2.41807
56	0.53090	−1.29293	2.35473
57	0.45980	−1.37219	2.29179
58	0.38870	−1.45185	2.22925
59	0.31760	−1.53189	2.16709
60	0.24650	−1.61232	2.10532

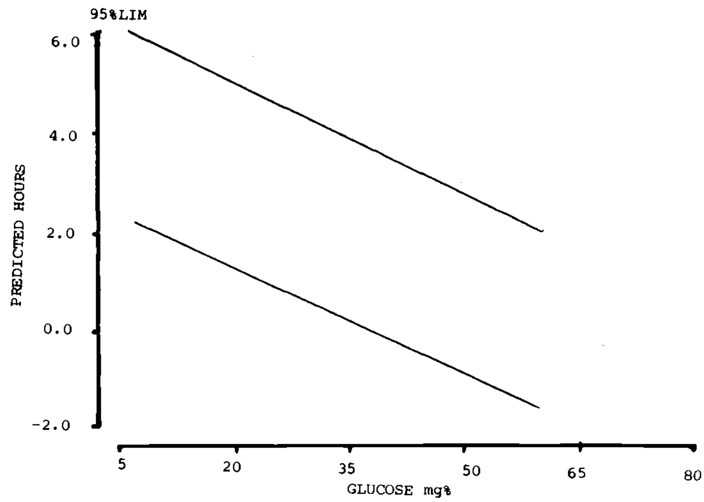

FIG. 1—*Graph of the upper and lower prediction limits for Table 1.*

Discussion

This study confirms the work of Hamilton-Patterson and Johnson [*2*]. Glucose will diminish in concentration with time until glycolysis is complete. They reported the process took 3.5 to 7 h. Our results are similar.

Coe [*1*] indicated the decline of postmortem vitreous glucose in humans was erratic and precipitous. In blacktailed deer, the decline is fairly linear and predictable (Fig. 2).

The 31 deer under 23 kg (50 lb) examined in the field study were treated as a separate group. The muscle mass in the rump provided less insulation for the deep muscle temperature, which declined at a more rapid rate, consequently, use of the combined glucose and temperature creates an extended time bias when compared to the actual time reported by the hunter. The values for the first 3 h are accurate, however the 3- to 7-h range is falsely extended. By utilizing only the glucose and omitting the temperature entirely (Table 2 and Fig. 3), all 31 deer were within the prediction interval. It must be noted that the $R^2 = 0.828$. The upper and lower prediction intervals will be slightly larger when only using glucose. Schoning and Strafus [*5*] stated that after 3 h glucose values appeared to be unrelated to time or temperature in dogs. It is apparent here that the decline in glucose in deer is independent of temperature, within the limitation of this study, and remains relative to time for a longer period.

The body temperature decline is apparently related to muscle mass. The temperature decline of the adult deer in the control study fit within the time versus temperature parameters previously established by Carlson and Kistner [*6*].

Headshot deer in the field study were also independently examined. As indicated earlier, those deer that had actual damage to the eye socket or eyes or both were not sampled. Of the 27 deer in this category, three deer were found to be outside the prediction interval, however, they had complimentary body temperature and glucose values. The time of death reported by the hunters in all three cases was excessively short and outside the frequency of times reported for other deer with similar values. Variability in glucose values because of hydrostatic shock from the bullet in these deer was not realized.

The sum of headshot deer (27), deer under 23 kg (50 lb) (31), and the 23- to 68-kg (50 to 150-lb) deer (129) equals 187 deer. Ten (5.3%) were found to be outside the parameters of the prediction interval in the first 8 h. In all ten cases, the time reported by the hunter was less than that given by the prediction interval.

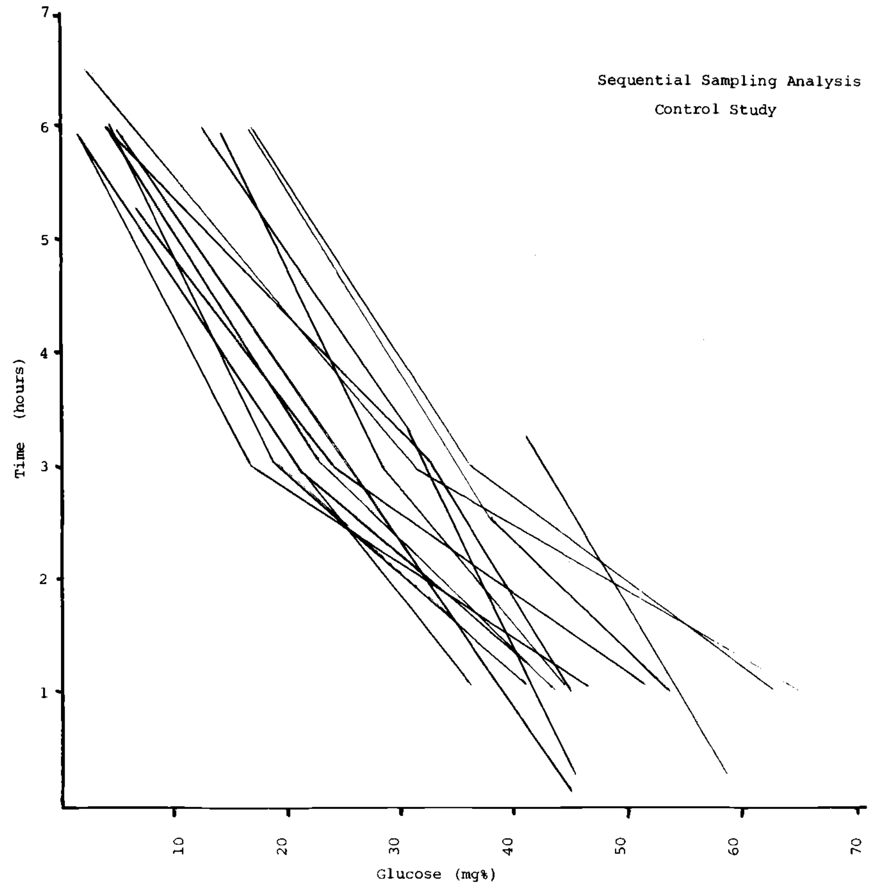

FIG. 2—*Sequential sampling analysis control study.*

The following is an actual case.

Case 1—At 9:00 a.m., a hunter transported a 54-kg (120-lb) four-point buck to the McDonald Forest Check Station. The deer was field dressed and in excellent physical condition. The three-and-one-half-year-old buck was transported in the back of a pickup approximately 16 km (10 miles). The outside temperature was 13°C.

At the check station, the hunter reported the TOD as 7:15 a.m. and the body temperature was 27°C (82°F). Subsequent eye fluid examination revealed the glucose to be 8 mg/100 mg. The mean prediction intervals was calculated to be 7.1 h, however the range is 5.1 to 9.1 h. Legal shooting time was approximately 6:30 a.m. The probable TOD was 2:00 a.m., but could have been as late as 4:00 a.m.

Some shooting was reported in the management area during the night, but no one was apprehended.

The following case demonstrates an alternative applicability.

Case 2—A game officer stopped a hunter at 2:00 a.m. Spotlight activity and shooting had been reported in the area. The hunter was transporting a 54-kg (120-lb) buck deer in a van. The animal was field dressed and had not been skinned. The 30-min sequential body temperatures are 37.8 and 36°C (100 and 98°F). Subsequent glucose determination reveals aqueous humor glucloses of 37 and 32 mg/100 mg.

The mean prediction interval was 1.71 h, however the range was from 0 to 3.49 h. The deer was probably killed at about 12:15 a.m., but could have been killed as early as 10:30 p.m. The

TABLE 2—*Prediction interval for glucose only.*

Glucose, mg/100 mg	*Y*-PRED (Mean)	L-PRED (Lower)	U-PRED (Upper)
5	5.59500	3.63082	7.55918
6	5.49400	3.53514	7.45286
7	5.39300	3.43928	7.34672
8	5.29200	3.34324	7.24076
9	5.19100	3.24702	7.13498
10	5.09000	3.15062	7.02938
11	4.98900	3.05404	6.92396
12	4.88800	2.95727	6.81873
13	4.78700	2.86031	6.71369
14	4.68600	2.76317	6.60883
15	4.58500	2.66583	6.50417
16	4.48400	2.56831	6.39969
17	4.38300	2.47060	6.29540
18	4.28200	2.37269	6.19131
19	4.18100	2.27459	6.08741
20	4.08000	2.17630	5.98370
21	3.97900	2.07782	5.88018
22	3.87800	1.97913	5.77687
23	3.77700	1.88026	5.67374
24	3.67600	1.78118	5.57082
25	3.57500	1.68191	5.46809
26	3.47400	1.58244	5.36556
27	3.37300	1.48278	5.26322
28	3.27200	1.38291	5.16109
29	3.17100	1.28284	5.05916
30	3.07000	1.18258	4.95742
31	2.96900	1.08212	4.85588
32	2.86800	0.98145	4.75455
33	2.76700	0.88059	4.65341
34	2.66600	0.77953	4.55247
35	2.56500	0.67826	4.45174
36	2.46400	0.57680	4.35120
37	2.36300	0.47514	4.25086
38	2.26200	0.37328	4.15072
39	2.16100	0.27121	4.05079
40	2.06000	0.16896	3.95104
41	1.95900	0.06650	3.85150
42	1.85800	−0.03616	3.75216
43	1.75700	−0.13901	3.65301
44	1.65600	−0.24206	3.55406
45	1.55500	−0.34531	3.45531
46	1.45400	−0.44875	3.35675
47	1.35300	−0.55239	3.25839
48	1.25200	−0.65622	3.16022
49	1.15100	−0.76024	3.06224
50	1.05000	−0.86446	2.96446
51	0.94900	−0.96887	2.85687
52	0.84800	−1.07347	2.76947
53	0.74700	−1.17825	2.67225
54	0.64600	−1.28323	2.57523
55	0.54500	−1.38839	2.47839
56	0.44400	−1.49374	2.38174
57	0.34300	−1.59927	2.28527
58	0.24200	−1.70498	2.18898
59	0.14100	−1.81088	2.09288
60	0.04000	−1.9165	1.99696

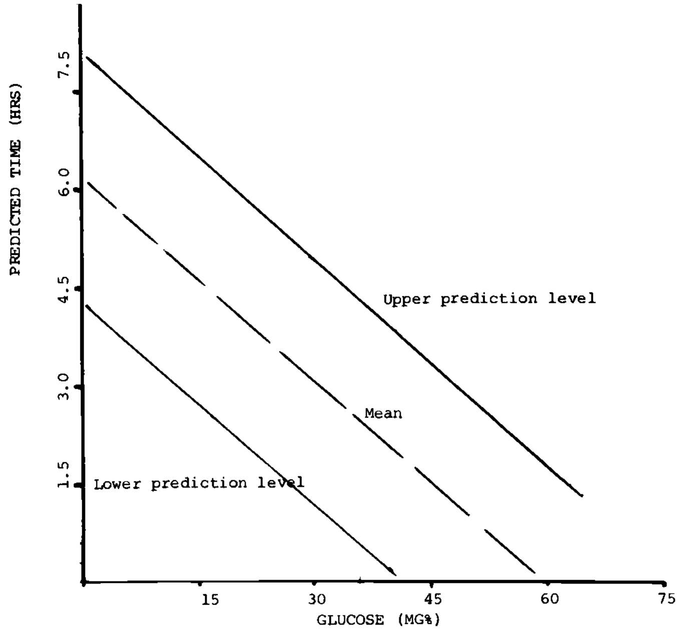

FIG. 3—*Graph of the upper and lower prediction limits for the aqueous glucose in Table 2.*

drop in body temperature and glucose for the 30-min interval is consistent with the control study.

Sunset was at 6:45 p.m.

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