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Postmortem Biochemical Changes in Canine Vitreous Humor

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ABSTRACT: Time and temperature effects on postmortem vitreous humor from 60 adult mongrel dogs were studied. After death the dogs were held at 4, 20, or 37°C for intervals of 3, 6, 12, 24, or 48 h. Antemortem and postmortem vitreous was analyzed for sodium, chloride, potassium, urea nitrogen, glucose, and creatinine. Potassium levels rose with increases in temperature and time. Sodium, chloride, and urea nitrogen values were stable at 4°C for 48 h; they were less stable at higher temperatures. Glucose dropped to less than half within 3 h at all temperatures. Creatinine values were inconsistent. In that postmortem glucose and sodium levels remained below antemortem levels, diagnosis of hyperglycemia and hypernatremia should be possible. Consequently, a diagnosis of hypoglycemia could not be supported; the diagnosis of hyponatremia could be made in the early postmortem period.

KEY WORDS: pathology and biology, vitreous humor, postmortem examinations

Chemical analysis of postmortem vitreous humor in man reportedly can be a useful diagnostic aid, especially when autopsy is impossible or incomplete [1-5]. Vitreous humor electrolytes have been discussed in sudden infant death in man [6-11]; rise in potassium [12-19] levels has been used for estimating postmortem interval in dogs and man; and, recently, vitreous has been found to be reliable for determining content of alcohol [20-23] and drugs [24-26] in a cadaver, even though embalmed [27,28].

Many postmortem problems have not been adequately studied. For example, normal values for canine and human vitreous humor have not been published; the postmortem interval has not always been known, making reported postmortem values difficult to interpret; and postmortem temperature has seldom been controlled, and so its effect remains unknown.

In the study reported here, normal antemortem canine vitreous humor values for chloride, sodium, potassium, glucose, urea nitrogen, and creatinine have been recorded. The effect of time and temperature on postmortem chemical values was determined. Change should be comparable with that of humans even though values might vary.

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Materials and Methods

Sixty adult mongrel dogs, weighing 9 to 32 kg, were divided into 15 groups of four each. For consistency, each group was handled on the same day. After fasting for 16 to 24 h, the dogs were anesthetized intravenously with sodium pentobarbital and placed on their left sides for the remainder of the experiment.

With a 15-gauge needle attached to a 12-cm³ syringe, 1.5-cm³ of vitreous humor was removed from the right eye of each anesthetized dog. The dogs were killed with sodium pentobarbital and placed in a Walk-In Environmental Room (Forma Scientific, Inc., Marietta, Ohio).

Five groups were held at 4°C, relative humidity 50%, for 3, 6, 12, 24, or 48 h; five groups at 20°C, relative humidity 50%, for the same time intervals; and five groups at 37°C, relative humidity 25%, for the same time intervals. At 3, 6, 12, 24, or 48 h later, the dogs were removed in the same sequence that they entered the chamber. The antemortem technique was used to remove vitreous humor from the left eye.

Antemortem and postmortem samples were centrifuged within 2 h after being collected. The supernatants were removed and frozen at -40°C until analyzed.

Sodium and potassium values were obtained with a Model 143 flame photometer (Instrumentation Laboratories); glucose and urea nitrogen with an ABA-100 semiautomated analyzer (Abbott Labs, N. Chicago, Ill.); chloride with a Buchler-Cotlove chloridometer (Scientific Products, N. Kansas City, Mo.); and creatinine by the bench method [29].

Analysis of variance procedures were used to determine if differences among the 15 groups were significant ($P < 0.05$). Duncan's multiple range test [30] was applied to the significant analysis of variance results to compare all group means.

Results

Antemortem means, ranges, standard deviations, and sample sizes are listed in Table 1.

Collection of postmortem samples was influenced by time and temperature. From the 37°C dogs, no samples were obtained at 48 h, one sample was obtained at 24 h, three samples were obtained at 12 h, and three samples were obtained at 6 h. Vitreous samples were aspirated from all 40 dogs held at 4 and 20°C.

Postmortem chloride values remained stable in dogs held at 4°C but were less stable in those held at higher temperatures (Table 2). Postmortem chloride values were statistically the same for all dogs held at 4°C. The antemortem-postmortem differences were uniform and ranged from a rise of 1 meq/litre to a drop of 3 meq/litre; 20°C values remained stable for 24 h, and then there was a large drop at 48 h; and 37°C values remained stable for 12 h and then dropped markedly.

Sodium fluctuated inconsistently with no apparent relation to time or temperature,

TABLE 1—Antemortem chemistries of canine vitreous humor.

Constituent	Sample Size ^a	Mean	Range	Standard Deviation
Chloride, meq/litre	57	127.14	114-138	4.56
Sodium, meq/litre	60	154.18	146-175	5.88
Potassium, meq/litre	60	6.58	5-9.7	1.12
Urea nitrogen, mg/dl	60	14.55	4-31.7	5.54
Glucose, mg/dl	59	73.46	54-102	10.04
Creatinine, mg/dl	40	0.7	0.36-1.01	0.17

^aInitial attempts to use an SMA/12-60 resulted in "plugging" of the instrument with too little vitreous left for reanalysis; therefore, some sample sizes are less than 60.

TABLE 2—Postmortem means (upper number), difference means (lower number) between postmortem and antemortem means, and sample size for chloride (meq/litre) in canine vitreous humor.

Temperature	Postmortem Hours				
	3	6	12	24	48
4°C	128.75 ^{a*} <i>n</i> = 2 1 ^{z**}	125.25 ^{ab} <i>n</i> = 4	130.5 ^a <i>n</i> = 4	127.25 ^{ab} <i>n</i> = 4	128.75 ^a <i>n</i> = 4
20°C	126.5 ^{ab} <i>n</i> = 3 - 3.6 ^{yz}	128.75 ^a <i>n</i> = 4	117.5 ^b <i>n</i> = 4	126.6 ^{ab} <i>n</i> = 3	107 ^c <i>n</i> = 4
37°C	117.25 ^b <i>n</i> = 4 - 0.25 ^z	124.75 ^{ab} <i>n</i> = 4	118 ^b <i>n</i> = 4	104 ^c <i>n</i> = 1 - 22 ^x

* Postmortem means with different superscripts are significantly different ($P < 0.05$).

** Difference means with different superscripts are significantly different ($P < 0.05$).

except for major drops at 20°C at 48 h and at 37°C at 24 h (Table 3). The postmortem mean values ranged from 125 to 164.6 meq/litre.

Potassium levels were influenced by both time and temperature (Table 4). The levels rose with time for each temperature group. Just as important, however, potassium increased with temperature when time was held constant. That change was not obvious under 6 h.

As indicated by differences in values from 52 samples (Table 5), vitreous urea nitrogen was stable for 48 h at 4°C, 24 h at 20°C, and 12 h at 37°C. Postmortem means for these stable groups ranged from 10.875 to 28.5 mg/dl, with a drop from antemortem values of 1.225 mg/dl to an increase of 6.9 mg/dl. In groups held at 20°C for 48 h and 37°C for 24 h, the urea nitrogen rose too high to be of any diagnostic value.

Glucose in all three temperature groups dropped to less than half of the antemortem value within 3 h (Table 6). The initial 3-h drop was equal in all three temperature groups. Further drops in glucose values appeared to be unrelated to either time or temperature.

For 28 postmortem levels evaluated, creatinine appeared to increase with time after an initial drop, as indicated by the antemortem-postmortem differences (Table 7). However,

TABLE 3—Postmortem means (upper number), difference means (lower number) between postmortem and antemortem means, and sample size for sodium (meq/litre) in canine vitreous humor.

Temperature	Postmortem Hours				
	3	6	12	24	48
4°C	164.6 ^{a*} <i>n</i> = 3 8.3 ^{z**}	147.25 ^{bc} <i>n</i> = 4	152 ^{ab} <i>n</i> = 4	150 ^b <i>n</i> = 4	152.25 ^{ab} <i>n</i> = 4
20°C	148.75 ^{bc} <i>n</i> = 4 - 0.75 ^{xyz}	149.75 ^{bc} <i>n</i> = 4	136.25 ^{cd} <i>n</i> = 4	146.3 ^{bc} <i>n</i> = 3	125.3 ^d <i>n</i> = 3
37°C	143.5 ^{bc} <i>n</i> = 4 - 5.75 ^{xyz}	152 ^{ab} <i>n</i> = 4	147 ^{bc} <i>n</i> = 3	125 ^d <i>n</i> = 1 - 24 ^{uv}

* Postmortem means with different superscripts are significantly different ($P < 0.05$).

** Difference means with different superscripts are significantly different ($P < 0.05$).

TABLE 4—*Postmortem means (upper number), difference means (lower number) between postmortem and antemortem means, and sample size for potassium (meq/litre) in canine vitreous humor.*

Temperature	Postmortem Hours				
	3	6	12	24	48
4°C	10.4 ^{cd*} <i>n</i> = 3	14.1 ^c <i>n</i> = 4	9.35 ^d <i>n</i> = 4	13.9 ^c <i>n</i> = 4	18.3 ^b <i>n</i> = 4
20°C	4.5 ^{uv**}	8.1 ^v	3.225 ^u	8.2 ^v	12.475 ^w
	9.2 ^d <i>n</i> = 4	10.625 ^{cd} <i>n</i> = 4	13.4 ^c <i>n</i> = 4	20.13 ^b <i>n</i> = 3	33.6 ^a <i>n</i> = 3
37°C	3.575 ^{u**}	3.725 ^u	6.875 ^{uv}	13.03 ^w	26.26 ^x
	13.225 ^c <i>n</i> = 4	12.5 ^{cd} <i>n</i> = 3	20.9 ^b <i>n</i> = 3	30 ^a <i>n</i> = 1	...
	5.675 ^{uv}	5.86 ^{uv}	14.56 ^w	23 ^x	...

*Postmortem means with different superscripts are significantly different (*P* < 0.05).
 **Difference means with different superscripts are significantly different (*P* < 0.05).

TABLE 5—*Postmortem means (upper number), difference means (lower number) between postmortem and antemortem means, and sample size for urea nitrogen (mg/dl) in canine vitreous humor.*

Temperature	Postmortem Hours				
	3	6	12	24	48
4°C	10.96 ^{e*} <i>n</i> = 3	28.15 ^c <i>n</i> = 4	15.525 ^{de} <i>n</i> = 4	10.875 ^c <i>n</i> = 4	20.2 ^{cdc} <i>n</i> = 4
20°C	2.2 ^{x**}	6.95 ^x	-1.225 ^x	5.275 ^x	3.025 ^x
	14.425 ^{de} <i>n</i> = 4	12.8 ^{de} <i>n</i> = 4	17.325 ^{de} <i>n</i> = 4	19.275 ^{cde} <i>n</i> = 4	48.35 ^b <i>n</i> = 4
37°C	0.2 ^x	1.725 ^x	2.125 ^x	4.1 ^x	27.65 ^y
	12.975 ^{de} <i>n</i> = 4	17.325 ^{de} <i>n</i> = 4	23 ^{cd} <i>n</i> = 4	86.4 ^a <i>n</i> = 1	...
	0.575 ^x	0.8 ^x	5.35 ^x	77.9 ^z	...

*Postmortem means with different superscripts are significantly different (*P* < 0.05).
 **Difference means with different superscripts are significantly different (*P* < 0.05).

TABLE 6—*Postmortem means (upper number), difference means (lower number) between postmortem and antemortem means, and sample size for glucose (mg/dl) in canine vitreous humor.*

Temperature	Postmortem Hours				
	3	6	12	24	48
4°C	32.3 ^{a*} <i>n</i> = 4	13.5 ^c <i>n</i> = 4	9.75 ^{cd} <i>n</i> = 4	5 ^{ef} <i>n</i> = 4	4.25 ^{ef} <i>n</i> = 3
20°C	-53.3 ^{z**}	-55.25 ^z	-74 ^x	-73.75 ^x	-73.66 ^{xy}
	25.5 ^b <i>n</i> = 4	11.25 ^{cd} <i>n</i> = 4	1.75 ^f <i>n</i> = 4	1.5 ^f <i>n</i> = 4	10 ^{cd} <i>n</i> = 4
37°C	-51.25 ^z	-63.75 ^{xyz}	-67.5 ^{xyz}	-72.25 ^{xy}	-60.5 ^{yz}
	11.75 ^{cd} <i>n</i> = 4	8.25 ^{de} <i>n</i> = 4	3 ^f <i>n</i> = 4	7 ^{ef} <i>n</i> = 1	...
	-51.25 ^z	-71.75 ^{xy}	-56 ^z	-69 ^{xyz}	...

* Postmortem means with different superscripts are significantly different (*P* < 0.05).
 ** Difference means with different superscripts are significantly different (*P* < 0.05).

TABLE 7—Postmortem means (upper number), difference means (lower number) between postmortem and antemortem means, and sample size for creatinine (mg/dl) in canine vitreous humor.

Temperature	Postmortem Hours				
	3	6	12	24	48
4°C	0.6525 ^b	0.74 ^b	...
	<i>n</i> = 4	<i>n</i> = 4	...

20°C	0.595 ^b	0.655 ^b	0.673 ^b	0.965 ^a	...
	<i>n</i> = 3	<i>n</i> = 4	<i>n</i> = 3	<i>n</i> = 2	...
	-0.246 ^x	0.12 ^y	0.183 ^y	0.24 ^y	...
37°C	0.7525 ^b	0.745 ^b	1.085 ^a
	<i>n</i> = 4	<i>n</i> = 2	<i>n</i> = 1
	-0.1725 ^x	-0.1 ^{xy}	0.13 ^y

* Postmortem means with different superscripts are significantly different ($P < 0.05$).

** Difference means with different superscripts are significantly different ($P < 0.05$).

the range of postmortem means was small: 0.595 to 1.085 mg/dl. When time was held constant, creatinine values were higher in dogs held at 37°C than at 4 or 20°C.

Discussion

Several authors [1,3,4] have reported postmortem chloride values as being stable in man. This work confirms this finding in dogs with the notable exception that stability was lost after 24 h at room temperature and after 12 h at 37°C. Postmortem chloride remained stable in dogs held at 4°C. Therefore, chloride values from cadavers held at 4°C should be reliable for at least 48 h. Values would be less dependable for dogs dead for undetermined lengths of time at 20°C or higher.

Postmortem sodium values are known to remain stable [1,3,4]. In this study neither time nor temperature appeared to influence changes, except for major drops at 20°C for 48 h and at 37°C for 24 h. Mean values probably fluctuated because of variations in individual dogs rather than because of postmortem change.

Antemortem hypernatremia [2,4,8,9] has resulted in elevated sodium concentrations in vitreous humor. This work supports that finding in that the highest antemortem mean was 164 meq/litre; the highest postmortem mean was 164.5 meq/litre. Therefore, sodium values that were high before death probably would be high after death.

Potassium concentrations have been considered to be indicators of the postmortem interval. Although most workers [11-19] agree that the potassium level increases after death, its accuracy for determining postmortem interval has been questioned [11-16]. In this study temperature as well as time directly and consistently affected potassium values. So, apparently, considering temperature influences would result in a more nearly accurate assessment of time of death.

Urea nitrogen was found by Coe [2] to be the most reliable of postmortem chemistries studied, and his findings were supported by Leahy and Farber [4]. Naumann [5], however, found an increase in urea nitrogen values postmortem in hospital patients. This study indicated that urea nitrogen values, as well as sodium and chloride, are stable at 4°C for 48 h. Values were not reliable after 24 h at 20°C or after 12 h at 37°C.

Although the differences were statistically the same, both antemortem and postmortem urea nitrogen ranges were wide: antemortem, 5.6 to 21.2 mg/dl; postmortem, 10.96 to 28.15 mg/dl (exclusive of unreliable values). Therefore, a vitreous urea nitrogen value exceeding 28.15 mg/dl would have diagnostic value only if it were within the time and temperature limits mentioned.

Studying postmortem vitreous is useful in determining antemortem hyperglycemia [2]. In this study, postmortem glucose values dropped rapidly at all temperatures to less than half the antemortem value within 3 h. Elevated antemortem glucose levels would probably be reflected in postmortem vitreous humor, suggesting a possibility for diagnosing hyperglycemia. Since postmortem glucose drops rapidly, diagnosing hypoglycemia would not be likely.

Creatinine levels in man have been found to be stable after death [4,5]. From 40 antemortem and 28 postmortem samples, significant interpretations could not be made for dogs. Furthermore, the normal values had such a small range (0.445 mg/dl difference) that variations would be difficult to explain.

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