POSTMORTEM POTASSIUM AND HYPOXANTHINE LEVELS IN VITREOUS HUMOR OF DOG AND CATTLE IN RELATION TO DEATH TIMING AND CAUSES, PARTICULARLY SLAUGHTERING.

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SUMMARY

Hypoxanthine [Hx] and potassium are constituents of the vitreous humor and they elevate after death. They have been used to estimate postmortem interval [PMI] due to the linear fusion of increasing. Determination of potassium was done in vitreous humor of cattle and dogs at 4 different temperature degrees. A significant increase in potassium concentration was determined with time and temperature. [Hx] in vitreous humor of dogs was estimated and found to be increased with time in more persuasive manner than potassium. Cause of death was studied as a factor affecting relation between potassium and PMI. All groups caused significant increase except strangulation. [Hx] also showed significant increase in slaughtered dogs. [Hx] might be used in differentiation between antemortem and postmortem slaughtering as it highly increases in deaths associated with antemortem cerebral hypoxia.

INTRODUCTION

Examination of time elapsed from death is important. Its determining is difficult and accuracy is impossible. (Knight, 1987).Many postmortem changes begin to take place in the body immediately or shortly after death and progress in fairly orderly fashion until the body disintegrated. Each change has its own time factor or rate. Unfortunately, these rates of development of those changes are strongly influenced by unpredictable endogenous and environmental factors. Consequently, the longer the postmortem interval the less precise is the estimate of time of death. Moreover, necropsy is not always possible in all cases even when death was quite recent. Accordingly, it was important to bring about parameters other than postmortem evidences to aid in estimating the time of death. Relationship between the rise of potassium

concentration in vitreous humor and the time since death has been studied by several workers and recently reviewed by (Madea et al, 1989). It was established that, probably the single most accurate determination of PMI is by potassium content of vitreous humor, which shows a linear rise with time (12-100 hours) postmortem, and that rate of rise is fairly independent of environmental influence, (Bocaz et al, 2002) and (Henry and Smith, 1980).Moreover, estimation of PMI by determining potassium in vitreous humor has advantages over analysis of blood and cerebrospinal fluid, (James et al, 1997).Potassium ion concentration in vitreous body has also showed by many workers to be increased in high temperature, (Schoning and Strafuss,1980) and (Mc Laughlin and Mc Laughlin,1987).External factors which influence the test are; sampling techniques, analytical instrumentation, and environmental temperature, while the internal factors are; age, duration of terminal episode and presence or absence of nitrogen retention, (Coe, 1989). Cause of death has recently proposed as an extra factor which modifies the relationship and gives even greater precision in estimating PMI, (Munoz et al 2002).Other point is that hypoxanthine (HX),which is formed by hypoxic degradation of adenosine monophosphate (AMP) and might be elevated due to antemortem hypoxia , is recommended as an indicator of prolonged (cerebral) hypoxia,and may also be used as an indicator for PMI as it increases in a linear fashion ,(Madea et al,1994 , Rognum et al,1991,and . James et al, 1997)

The purpose of the present study is to corroborate the roll of potassium with temperature variation as a postmortem estimating parameter using narrow time interval less than that used by the previous workers, and to study the cause of death as a factor affects postmortem potassium level, with special reference to slaughtering which could guide to perceptible facility to differentiate post and ante mortem slaughtering. Cattle and dogs were used to study the variables (time, concentration and temperature) while dogs only was involved in studying causes of death, using potassium and hypoxanthine (HX).

MATERIALS AND METHODS

For studying the effect of postmortem temperature on the relation between potassium concentration and PMI, a total number of 90 adult cattle of different species which were slaughtered in the slaughter house and included in this study. Also 30 dogs of mixed

strains collected randomly were used. Just before slaughtering the cattle, a vitreous humors sample was aspired from one eye by inserting a needle (16-gauge) connected to a syringe into the center of the vitreous body. After slaughtering both eyes were enucleated, kept in a plastic page and labeled (number of sample, date of slaughtering, time of slaughtering) keeping in mind that both eyes of the same animal are considered one sample. Samples were divided into three groups each of 30 samples and incubated in three different temperature conditions (6^0C, 24^0C and 37^0C).Vitreous samples were collected after (2,4,6,8,10,12,16,20,24,32,40 and 48 hours from incubation starting (12 postmortem samples).

Dogs were anesthetized using 0.5-1.0 mg/kg body weight diazepam and 0.03-0.05 mg/kg body weight atropine I.V as premedication and then general anesthesia was induced with I.V thiopental sodium 5% in a dose of 20-30 mg/kg body weight in order to inspire the antemortem vitreous sample from one eye. After euthanatization by rapid IV injection of thiopental sodium 50-100 mg/kg body weight, both eyes were enucleated, kept in a plastic page and labeled (number of sample, date of slaughtering, time of slaughtering).Samples were grouped in three groups each of 10 and inspired as just as in cattle.

For studying the effect of cause of death on the relation between potassium concentration and PMI, a total number of 18 dogs nearly of the same age and weight were used. Dogs were divided into 3 groups 6 animals in each. Dogs were anesthetized as before in order to inspire the ante mortem vitreous sample from one eye. Dogs in the first group were immersed under fresh water surface until death and left in thermostatically controlled water temperature (21^0C, $\pm$ 2^0C) along the experiment (drowning group).In the second group, dogs were slaughtered (cut throated) left bleeding until death, and kept in controlled room temperature (21^0C, $\pm$ 2^0C).Dogs of the third group were strangulated until death and kept at the same temperature .Vitreous humor sample is taken in the same interval as the first experiment for 48 hours. All vitreous samples were filtered just after draining and then frozen until analyzed. Biochemical determination of vitreous potassium was performed by flam photometry. Both eyes were used as source for sample as, no statistically difference was found between the potassium concentrations in the two eyes of the same subject (Tagliaro et al, 2001).

A small experiment was done by analyzing samples of vitreous humor from euthanatized dogs and slaughtered dogs and detecting the level of hypoxanthine by (HPLC) Rognum et al, 1988 .The involved samples were; antemortem sample, and 6,10,20,40 hours postmortem samples. All samples were incubated at 24^{0}C to exclude the expected effect of temperature rising. All vitreous samples were filtered just after draining and then frozen until analyzed.

RESULTS

Table (1)and fig.(1)shows that there is a significant increase in the level of vitreous humor potassium concentration in cattle after death in relation to both time and postmortem incubation temperature. At 6^{0}C there was significant increase at 10 hours postmortem, while there was highly significant increase at 12 hours and the following time examined samples. At 24^{0}C and 37^{0}C there was significant increase at 2 hours postmortem, while there was highly significant increase at 4 hours and the following time examined samples. Table (2) and fig. (2) shows that there is a significant increase in the level of vitreous humor potassium concentration in dogs after death in relation to both time and postmortem incubation temperature. At 6^{0}C and 24^{0}C there was significant increase at 2 and 4 hours postmortem, while there was highly significant increase at 6 hours and the following time examined samples. At 37^{0}C there was highly significant increase in time examined samples. Table (3) and fig. (3) shows that when temperature variable fixed at 24^{0}C, potassium concentration in vitreous humor of dogs in the drowning and slaughtering groups showed highly significant increase at 8 hours postmortem and the following tested time samples when postmortem potassium concentration in the euthanatized group considered control .While in strangulation group there was no significant change in potassium concentration along the postmortem tested intervals. Results also showed that, the postmortem concentration of hypoxanthine (HX) in the vitreous humor of both euthanatized and slaughtered dogs increased with time after death .Moreover, there was significant increase in the concentration of vitreous humor hypoxanthine in slaughtered dogs, when compared with those of euthanatized group at the same postmortem interval (table 4& fig.4).

DISCUSSION

Estimation of the postmortem interval by using postmortem vitreous humor potassium concentration has been indicated by many workers (Madea et al, 1989 and Bocaz et al, 2002). Temperature must be also respected as potassium level increases with temperature as well as with time (Komura andOshiro, 1977). Mc Laughlin and Mc Laughlin, 1987, found that potassium concentration in vitreous humor of cattle after 48 hours at 4^0C, 20^0C,37^0C were; 9.2,12.5and18.6 mEq/l respectively. In the present study potassium concentration increased significantly in progressive order with time and temperature recording 10.1,13.2 and17.2 mEq/l after 48 hours at 6^0C,24^0C,37^0C respectively. In the group incubated at 6^0C, potassium significantly increased after10 hours postmortem, which agrees with (Bray, 1984), who reported that the rate of rise of potassium concentration diminished in cold and chilled conditions. Potassium increased to approximately 55% more than the immediate postmortem level (Wilkie and Bellamy,1982) in the present work that figure reached 88% after 24 hours and 191%after 48 hours in the group incubated at 37^0C.That difference may be because temperature factor was not considered in the mentioned study.

Potassium concentration in vitreous humor of dogs increased definitely after death (Crowell and Duncan, 1974).In the present work, potassium concentration increased significantly with increase in time and temperature in all tested time intervals. Potassium reached 18.1,29.2 and 29.8 after 48 hours when incubated at 6,24 and 370C respectively ,while (Schoning and Strafuss, 1980) reported that it reached 33.6 after 48 hours at 370C. On the other hand, the recent results revealed that using of narrow pmi turns the curves less liner Studying of the postmortem vitreous humor hypoxanthine [Hx] concentration in dogs revealed clear and obvious relation with time. No articles were available concerning [Hx] in vitreous humor of dog but same results were reported by (Gardiner et al, 1989) in avian and procine and by (Rognum et al, 1991) in human. In spit of the increase in potassium level postmortem , cause of death showed marked modification in the relation between postmortem interval and potassium concentration on condition that temperature factor was fixed. In both slaughtering and drowning groups the level elevated after 8 hours postmortem, while in strangulation no significant change observed. (Maha and Ghanem, 1994) found that, potassium increased in the vitreous

humor of rats in all groups except in group of strangulation. This may be attributed to the increased permeability of brain cells and blood brain barrier. Postmortem vitreous humor hypoxanthine records higher concentration in deaths where violence or antemortem cerebral hypoxia is expected, than in other cases where hypoxemia has not occurred prior to death (Rognum and Saugstad, 1991 and Rognum et al, 1988). In the present study hypoxanthine was dramatically increased in the group of slaughtering (71.2 after 48 hours postmortem) when compared with the other group of euthanatized dogs. Significant increase in Hx concentration in the group of slaughtering, where animal bled to death, could be expounded by the preceding antemortem hypoxia. Accordingly, animals died due to causes other than slaughtering may not show that [Hx] elevation. Although no literature concerning the relation between [Hx] and cause of death in dog, one can consider results in human as, the amount of [Hx] in both human and canine serum are very similar (Assenza and Brown, 1980).Both potassium and [Hx] concentration in vitreous humor can be used in estimation of PMI on considering temperature factor .In cold postmortem conditions (6^{0}C), potassium may not be a good evidence specially at early PMI determination.

Conclusion

We can conclude that, using of [Hx] in postmortem interval determination could be more accurate than potassium considering temperature and causes of death. Postmortem vitreous humor [Hx] level in cases of death associated with hypoxia namely bleeding, increases clearly with obvious significance .Accordingly, it might be excellent in differentiation of ant and postmortem slaughtering. Similar studies are recommended to be done in cattle so as to be applicable.

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Table (1): Concentration of potassium "mEq/l"in vitreous humors incubated at"6°C, 24°C and 37°C"after different postmortem interval in cattle.

Incubation °C / Time interval		potassium concentration(mEq/l) after incubation at :		
		6^0C	24^0C	37^0C
Ante mortem (control)		4.21± 0.72	4.21± 0.72	4.21± 0.72
Post-mortem	2 hours	5.10±0.22	5.60±0.02*	5.9±0.23*
	4 hours	5.24±0.35	6.25±0.01**	7.02±0.42**
	6 hours	5.40±0.02	6.41±0.24**	7.18±0.05**
	8 hours	5.31±0.02	6.53±0.07**	8.06±0.14**
	10 hours	5.86±0.18*	7.61±0.32**	9.25±0.87**
	12 hours	6.20±0.12**	8.00±0.04**	9.91±1.02**
	16 hours	6.82±0.31**	8.22±0.19**	9.74±0.23**
	20hours	7.31±0.11**	8.75±0.16**	10.5±1.05**
	24 hours	7.28±0.34**	8.69±1.05**	11.1±0.02**
	32 hours	7.94±0.73**	10.02±0.09**	12.4±1.20**
	40 hours	9.24±0.64**	12.77±1.12**	15.8±1.08**
	48 hours	10.1±0.52**	13.2±1.07**	17.2±1.76**

Data are expressed as mean ± S.E

* Significant at (P≤0.05). ** Highly significant at (P≤0.01).

Table (2): Concentration of potassium "mEq/l"in vitreous humors incubated at"6°C, 24°C and 37°C"after different postmortem interval in dogs.

Incubation°C / Time interval		potassium concentration(mEq/l) after incubation at :		
		6^0C	24^0C	37^0C
Ante mortem(control)		5.98±0.82	5.98±0.82	5.98±0.82
Post-mortem	2 hours	8.6±0.76*	10.1±1.12*	12.1±1.86**
	4 hours	10.4±1.71*	11.5±1.01*	13.3±1.68**
	6 hours	11.6±0.41**	12.7±1.21**	14.2±1.7**
	8 hours	11.9±1.11**	12.5±0.09**	13.9±1.61**
	10 hours	12.6±0.23**	13.5±0.18**	15.7±1.81**
	12 hours	12.8±0.04**	15.7±1.08**	18.8±0.77**
	16 hours	14.2±0.88**	18.7±0.05**	21.3±1.14**
	20hours	14.8±1.22**	23.4±0.13**	26.1±1.22**
	24 hours	15.6±0.36**	25.6±2.01**	28.2±0.02**
	32 hours	16.8±1.11**	27.1±0.11**	28.7±2.01**
	40 hours	18.4±1.95**	27.7±1.13**	28.1±0.67**
	48 hours	18.1±1.21**	29.2±0.12**	29.8±0.57**

Data are expressed as mean ± S.E

* Significant at (P≤0.05). ** Highly significant at (P≤0.01).

Table (3): Concentration of potassium "mEq/l"in vitreous humors of dogs in cases of death due to; drowning, slaughtering, and strangulation at 24°C

Time interval		Vitreous humor potassium concentration(mEq/l)			
		Euthanatized control	drowning	slaughtering	strangulation
Ante mortem→		5.98±0.82	5.98±0.82	5.98±0.82	5.98±0.82
Post-mortem	2 hours	10.1±1.12	11.1±1.4	10.5±0.02	9.9±2.2
	4 hours	11.5±1.01	12.7±1.02	11.9±0.04	11.8±1.06
	6 hours	12.7±1.21	14.1±0.02	12.9±1.04	12.5±1.04
	8 hours	12.5±0.09	15.7±0.08**	14.1±0.07**	12.7±1.00
	10 hours	13.5±0.18	17.6±0.05**	15.9±0.03**	13.7±1.15
	12 hours	15.7±0.08	20.3±1.03**	18.2±0.20**	14.9±0.08
	16 hours	18.7±0.05	23.4±0.02**	21.2±0.01**	17.6±1.12
	20hours	23.4±0.13	26.6±1.03**	24.7±0.61*	22.8±0.05
	24 hours	25.6±0.01	29.2±0.07**	27.7±0.05**	25.9±1.06
	32 hours	27.1±0.11	31.4±1.15**	29.7±0.09**	27.7±1.05
	40 hours	27.7±1.13	33.1±0.06**	31.1±0.06**	28.2±1.10
	48 hours	29.21±0.12	34.7±1.03**	32.1±0.2**	28.7±1.07

Data are expressed as mean ± S.E

* Significant at (P≤0.05). ** Highly significant at (P≤0.01).

Table (4): Ante and postmortem hypoxanthine (HX) concentration (mumol/l) in vitreous humor of euthanatized and slaughtered dogs incubated at 24^{0}C

Hypoxanthine concentration	Antemortem control	After 6 hours	After 10 hours	After 20 hours	After 40 hours
euthanatized dogs	6.42±0.03	26.2±0.02*	38.12±0.05*	46.2±0.05*	51.11±1.01*
Slaughtered dogs	6.42±0.03	42.22±0.8* #	54.3±0.13* #	63.1±0.02* #	71.2±0.05* #

* Highly significant at (P≤0.01) in postmortem values,compared with antemortem as a control.

Highly significant at (P≤0.01) in slaughtered dogs, compared with euthanatized samples as a control.

Fig.(1)postmortem changes in potassium concenteration in vitreous humor of cattle at different temp.

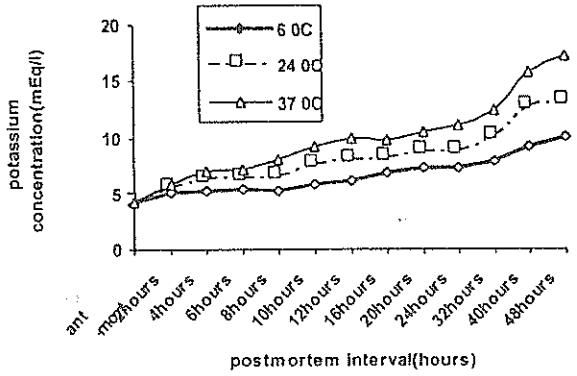
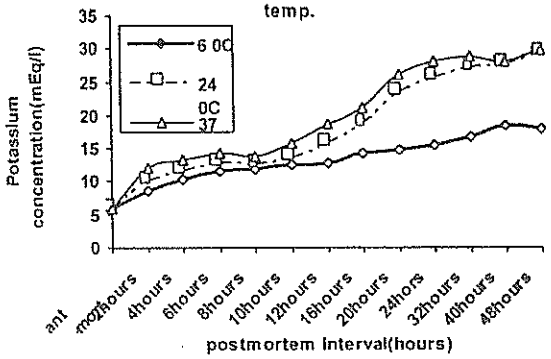


Fig.(2)Postmortem changes in potassium concentration in vitreous humor of dogs at different temp.



(Fig.3)Effect of cause of death on the relation between vitreous pot. conc. and pmi in dogs

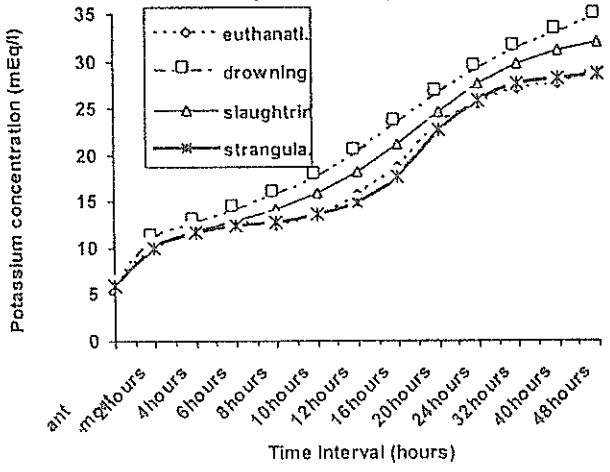
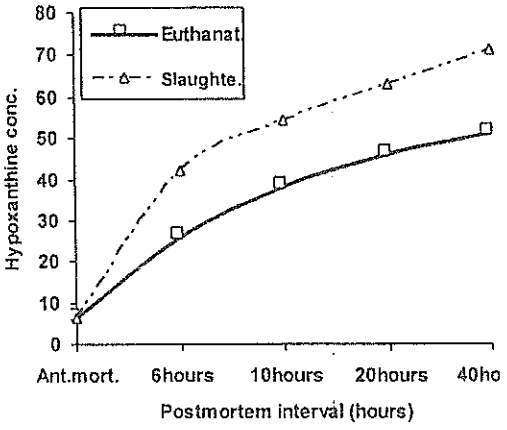


Fig.(4):Hypoxanthine conc. in vitreous humor of euthanatized and slaughtered dogs



الملخص العربي

مستوى البوتاسيوم والهيبوزانسين بعد النفوق في السائل الزجاجي للماشية والكلاب وعلاقته بتحديد وقت النفوق وأسبابه خاصة الذبح

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يعد البوتاسيوم و الهيبوزانسين من مكونات السائل الزجاجي في العين و هما من المواد التي يتزايد تركيزها بعد النفوق. و تستخدم هاتان المادتان لتحديد الزمن الذي مضى على النفوق و ذلك لأنهما تتزايد خطيا . و في هذه الدراسة تم تعيين مستوى البوتاسيوم في السائل الزجاجي للماشيه و الكلاب و ذلك في ثلاث درجات حرارة مختلفة .و قد لوحظ وجود ارتفاع معنوي في مستوى البوتاسيوم مع زيادة الوقت و ارتفاع درجة الحرارة . و قد وجد أيضا أن تركيز الهيبوزانسين في السائل الزجاجي في الكلاب يزيد بزيادة الوقت بشكل أدق مما في البوتاسيوم.و بدراسة أسباب النفوق من ناحية تأثيرها على العلاقة بين البوتاسيوم و الوقت المنقضي على النفوق في الكلاب ، أظهرت كل المجموعات زيادة معنوية ماعدا مجموعة الخنق بالحبل . و قد لوحظ أيضا زيادة معنوية في مستوى الهيبوزانسين في السائل الزجاجي في الكلاب المذبوحة. ومن هذه الدراسة تتضح أهمية الهيبوزانسين في دراسة الوقت المنقضي على النفوق .

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