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POSTMORTEM TIMING USING CHEMICAL ANALYSIS OF EYE FLUIDES IN CATTLE

(With 5 Tables and 2 Figures)

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تحديد وقت النفوق باستخدام التحليل الكيميائي لسوائل العين في الأبقار

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استخدم في هذا البحث عيون مائة من الأبقار الصحيحة ظاهرياً والتي استئصلت مباشرة بعد الذبح وقسمت إلى مجموعتين ، اشتملت كل منها على عشر من المجموعات الفرعية المتكونة من خمس حيوانات. تم وضع المجموعة الأولى في درجة حرارة ٢٣±٢°م والأخرى في ٣٧±٢°م. وأخذت العينات من سوائل العين في الفترات المحددة وهي صفر ، ٦ ، ١٢ ، ١٨ ، ٢٤ ، ٣٦ ، ٤٨ ، ٦٠ ، ٧٢ و ٨٤ ساعة . وقد تم قياس مستويات عناصر البوتاسيوم والصوديوم والماغنسيوم والكالسيوم وكذلك نشاط خميرة الفوسفاتاز الحمضي. أورت النتائج وجود علاقة موجبة خطية بين مستويات البوتاسيوم في جسمي العين المائي والزجاجي وأوقات ما بعد النفوق. وتبين من التحليل تناقص عنصر الماغنسيوم بمعدلات غير منتظمة ولم توضح النتائج وجود علاقة بين مستويات عنصري الصوديوم والكالسيوم بالنسبة إلى فترات ما بعد النفوق. ولقد دل التحليل الكيميائي عن وجود علاقة خطية إيجابية بين مستوى نشاط خميرة الفوسفاتاز الحمضي الكلي والفترات ما بعد النفوق. وتبين وجود علاقة خطية بين تركيز البوتاسيوم في الجسم المائي بالنسبة لتركيزه في الجسم الزجاجي وبالنظر في قيمة أنحدار المنحنى يتضح سرعة تزايد معدلات البوتاسيوم في العينات المتواجدة في درجة حرارة ٣٧ °م عنها في ٢٣ °م. ولقد دل التحليل على سلوك خميرة الفوسفاتاز الحمضي المشابه لمسلك عنصر البوتاسيوم. ويتضح من هذه الدراسة التأثير الواضح لإرتفاع درجة حرارة البيئة المحيطة والذي يجب اعتباره عند تحديد فترة ما بعد النفوق باستخدام تقدير عنصر البوتاسيوم وكذلك مستوى نشاط خميرة الفوسفاتاز الحمضي في جسمي العين المائي والزجاجي.

SUMMARY

Eyes of one hundred apparantely healthy cattle aged 1-3 years were randomly collected from slaughterhouses. Eyes were obtained at once from slaughtered animals and divided into two groups. Each one had ten

subgroups for different postmortem time intervals (5 animals each). One group was stored at a 23 ± 2°C environment, while the second at a 37 ± 2°C environment. Potassium, sodium and calcium, magnesium and total acid phosphatase were estimated in both aqueous and vitreous humor. There are highly significant progressive increase in the aqueous and vetreous potassium concentration after death. This change is correlated to the time since death. It was found a linear correlation between postmortem interval (PMI) and potassium levels either in aqueous or vitreous humor at different temperatures. The obtained magnesium values were irregularly decreased with the time after death in both aqueous and vitreous humor, while sodium and calcium values showed no significant relationship with PMI. There was a gradual linear increase in the total acid phosphatase activity in both aqueous and vitreous humor, which was more rapidly at higher temperatures during the period of the study. The results of the present study indicate that, during 0-84 h postmortem period in cattle, a linear relathioship of high correlation exists between the potassium and total acid phosphatase concentration in both aqueous and vitreous humor and PMI takeing in consideration that the slope of both parameters was steeper at higher temperatures.

Key words: *Postmortem Timing, Chemical Analysis, Eye Fluides, Cattle*

INTRODUCTION

Attempts to devise a reliable chemical method for estimation of postmortem interval (PMI) have been directed towards revealing some constituent (s) of a body fluid, the concentration of which ranges within narrow limits during life and varies in a time-related manner post mortem (Coe, 1977; Henry and Smith, 1980 and Querido, 1990). In addition, the concentration of the constituent at any given postmortem interval should display minimal individual variation and should be independent of environmental influences. In man, the determination of time since death is a well-studied subject in forensic science. In particular the estimation concerning the first hours and days after death are well investigated (Neis et al., 1999). The most reliable single method for chemical estimation of PMI in man is that devised by Adjutantis and Coutselinis, (1972). Their technique relies upon the progressive postmortem increase in potassium concentration in vitreous humor, first demonstrated by Nauman, (1959) and subsequently confirmed by Jaffe,

(1962). This increase occurred linearly throughout most of the 0-100-h postmortem period.

Vitreous humor has been used in human with varying amounts of success in the estimation of time of death, diagnosis of electrolyte and carbohydrate disturbances, diagnosis of drowning, and in the identification of selected toxicological substances such as ethyl alcohol. Use of PM vitreous humor as a diagnostic aid in domestic animals has been investigated only to a limited extent (Crowell and Duncan, 1974, Schoning and Strafuss, 1980 and Wilkie and Bellamy, 1982). Chemical analysis of aqueous and vitreous humor was used as a helpful tool for estimating time after death in donkeys and camels (Ibrahim et al., 1991 and Salem, 1998).

This study aimed to determine the relationship between some chemical constituents of cattle eye fluides and the time after death within different temperatures.

MATERIALS and METHODS

Eyes of one hundred apparantely healthy cattle were used in this study, which were randomly obtained from slaughterhouses. The animals aged 1-3 years. Complete eyes were carefully removed at once after slaughtering and divided into two groups. Each group had ten subgroups for different postmortem time intervals (5 animals each). Eye samples were randomly selected for sampling at each of the PM intervals (0, 6, 12, 18, 24, 36, 48, 60, 72, and 84 hours). The first group was stored in a 23 ± 2 °C environment, while the second in a 37 ± 2°C environment. Eye samples stored in a 37 ± 2°C environment could not be aspirated after 48 hours interval as putrefaction and autolysis occurred. Using a 20-gauge needle attached to a 3-ml syringe, aqueous and vitreous humor were collected by aspiration once from each eye at the predesignated PM interval. Zero time samples were collected in the slaughterhouses once animals slaughtered. The obtained samples were kept at -18°C and analysis was done within 5 days after sampling.

Potassium, sodium and calcium were determined by using Corning Flame Photometer 410 as reported by Brandstein et al. (1963). Magnesium was determined by Varian flame atomic absorption spectrometer (Spectr A A.30, Varian Techtron Pty.Ltd., Springvale, Australia) accompanied with Varian DS-15 Data Station and Epson LX-800 printer.

Total acid phosphatase enzyme was measured by using *Enzyline® Phosphatase acide optimise unitaire* test kits *(bioMérieux sa, Franc)* as resported by Roy et al. (1971). Spectronic® Genesys™ 5 Spectrophotometer was used for kinetic determination of the enzyme. The obtained results were statistically analyzed according to Kalton (1967).

RESULTS

The obtained results of potassium, magnesium, sodium and calcium levels in both aqueous and vitreous humor of cattle are illustrated in Tables (1, 2, 3 and 4) and Figure (1). Total acid phosphatase enzyme concentration is shown in Table (5) and Figure (2).

DISCUSSION

The deremination of the accurate time of death in medico-legal investigations has alwayes been important. There is a continuous need for the development of an accurate method, by which the time of death can be determined to within a few minutes. The investigation of the postmortem interval (PMI) by determining potassium levels in the vitreous humor (KV) of human has been a subject of many forensic pathology researches (Adelson et al., 1963; Sturner, 1963; Coe, 1969; Adjutantis and Coutselinis, 1972; Stephen and Richards, 1987; Madea et al., 1989; Madea et al., 1990 and Gamero et al., 1992). The numerous studies to date have yielded a variety of linear or piecewise-linear relationships between KV and PMI, i.e., different estimated intercepts and slopes of regression line(s) as well as different reliabilities of these estimates (Lange et al., 1994).

The data of this study revealed that the mean concentrations of potassium in aqueous and vitreous humor immediately following death (time zero values, figure 1 and table 1) were of the same order of magnitude. The levels of potassium were increased after death and continuing for 84 h in both aqueous and vitreous humor. There is highly significant progressive increase in the aqueous and vitreous potassium concentration after death. This change is correlated to the time since death. The results indicated a linear correlation between PMI and potassium levels either in aqueous or vitreous humor at different studied

two temperatures. Potassium levels in vitreous humor and PMI had 0.976 correlation coefficient with a slope of 0.206 mmol/l per h and an intercept of 8.645 mmol/l at 23°C stored eyes, while the correlation coefficient, the slope and the intercept were 0.975, 0.306 mmol/l per h and 7.683 mmol/l at 37 °C stored samples. Aqueous humor potassium levels had 0.984 correlation cofficient and slope of 0.205 mmol/l per h with an intercept of 7.955 mmol/l at 23°C stored eyes and 0.996, 0.291 mmol/l per h and 7.213 mmol/l at 37°C (Table 1 and Fig. 1). The values of aqueous and vitreous potassium had a correlation coefficient of 0.997 and 0.986 respectively, at both temperatures.

Madea et al. (1987) studied the vitreous potassium in 170 cases of human being. They found that there was a linear relationship between the vitreous potassium and the time after death up to 120 h. The slope was 0.203 mmol/per h and the intercept was 5.99 mmol/l. The same relationship was recorded by Choo-Kang et al. (1983) in 105 autopsy cases and Sparks et al. (1989) in 91 persons of varying ages and different ambient temperatures. Ibrahim et al. (1991) and Salem (1998) studied the relationship of potassium in aqueous and vitreous humor of donkeys and camels in relation to PMI. They found also that potassium had a linear relationship with time after death. The slope was 0.085 and 0.0429 mmol/l per h and the intercept was 5.885, 6.704 mmol/l in aqueous and vitreous humor, while the correlation coefficient was 0.995 and 0.928 for aqueous and vitreous fluid in donkeys (Ibrahim et al., 1991). The correlation coefficient of potassium levels in vitreous humor of camels and postmortem intervals was 0.985 with a slope of 0.173 mmol/l per h and an intercept of 9.537 mmol/l at 23°C stored eyes, while the correl. Coeff., slope and intercept were 0.964, 0.296 mmol/l per h and 7.754 mmol/l at 37°C. Camels aqueous humor potassium levels had 0.980 and 0.969 correlation coefficients and slopes of 0.177 and 0.304 mmol/l per h with intercepts of 8.807 and 6.786 mmol/l at 23°C and 37°C stored eyes, respectively (Salem, 1998).

A consistent rise in the level of potassium commencing shortly after death and continuing for 125 h with no significant difference between refrigerated bodies and those kept at room temperature was recorded (Jaffe, 1962). Potassium concentration in rabbit eyes also increased with the PMI in both aqueous and vitreous humor in a linear fashion (Bito and Salvador, 1970). They found also a marked difference in the slope for eyes incubated at 3°C compared with those incubated at 37°C.

This study showed that both aqueous and vetreous potassium levels were rapidly increased in the higher temperature, which indicated by a steeper slope (0.291 and 0.306 mmol/l per h at 37°C for vitreous and aqueous humor in comparison with 0.206 mmol/l per h at 23°C). Bodies present at warmer temperature had higher rates of KV increases than those kept at lower temperature (Coe, 1973). In the same situation, (Komura and Oshiro, 1977) found a linear relationship between values of potassium and PMI. The slope of the line for ambient temperatures of 26-29°C was considerably steeper than the slope of specimens obtained from bodies residing in temperatures of 13-17°C. Bray, (1984) found that potassium levels rose slowly in vitreous of refrigerated sheep eyes compared to values from animals maintained at room temperature.

Bray, (1985) studied 25 cases of deaths due to submersion in water and found that when the water was cold, the vitreous potassium concentration increased in a linear fashion for seven days, while the potassium concentration from bodies submerged in warm water rose much more rapidly for 3-4 days after death. This study showed that aqueous and vitreous potassium had a linear relationship with each other and time after death, which means that potassium, was elevated either in aqueous or vitreous humor consecutively with PMI even at the different temperatures (Table 1 and Figure 1). Schoning and Strafuss (1980) experimenting with mongrel dogs demonstrated that the rise of potassium values with an increasing PMI was temperature dependent. Their experiment established that there were different lines of potassium increase for 4°C, 20 °C and 37°C.

Coe, (1989) reviewed that there is a marked variation in the slope of the values derived from various investigators, varying from a value of 0.14 mmol/l per hour found by (Sturner, 1963 and Sturner and Gantner, 1964) through various increasing values up to 0.55 mmol/l per h determined by (Adjutantis and Coutselinis, 1972). The majority of slopes ranged closely around 0.17 mmol/l per h. The obtained values of potassium slope were 0.206 mmol/l per h in both aqueous and vitreous humor of cattle specimens stored at 23°C, where, the slopes of samples stored at 37°C were 0.306 and 0.291 mmol/l per h, respectively.

Certain external elements recognized as influencing the final result are sampling techniques, analytical instrumentation and environmental temperature during the PMI (Coe, 1989). In his final conclusion, he reported that the environmental temperature is the single most significant factor discovered to date and must be considered

carefully whenever vitreous studies are being used to determine the PMI. In this respect, Madea et al., (1989) also reported that ambient temperature may be such a factor and our first results with bodies kept at different constant ambient temperatures within 3 h after death (5°C or 20°C) up to 40 h postmortem indicate that there might be a different slope in these two ambient temperature groups. From the previous studies, it could be concluded that the main cause of the variation in potassium slope is the change in the temperature as the other factors are considerably fixed. The same behaviour was obtained in camels (Salem, 1998).

In their study to diagnose magnesium antemortem imbalances through vitreous analysis, (Lincoln and Lane, 1985) concluded that postmortem vitreous humor Mg determination in cattle could be a useful diagnostic aid for at least 48 hours after death, provided the postmortem environmental temperature did not exceed 23 °C after 24 hours. Low environmental temperature (4°C) had no effect on postmortem vitreous Mg concentration. However, high environmental temperature (30°C) significantly ($P<0.05$) reduced postmortem vitreous Mg concentrations at the 36-hour postmortem interval. The recorded magnesium values were irregularly decreased with the time after death in both aqueous and vitreous humor of cattle (Table 2). This decrease of Mg levels in relation to PMI occurred in the latest intervals especially at 23°C.

Devgun and Dunbar, (1986) and Madea et al. (1989) reported that sodium, calcium, chloride and urea are stable in the postmortem interval up to 120 hours. The detemined sodium and calcium levels in both aqueous and vitreous humor of cattle showed no relationship with PMI at different temperatures, as their concentrations had no significant variation after death. In contrast, Balasooriya et al. (1984) found that there is a significant reduction in the vitreous sodium concentration, in proportion to the time since death. This reduction appears linear during the first 85 h after death.

It is obvious from the obtained data that there are highly significant changes between the total acid phosphatase levels in both aqueous and vitreous humor and time elapsed after death of cattle. There is a gradual linear increase in the total acid phosphatase activity in both aqueous and vitreous humor which more rapidly at higher temperatures during the period of study. The linear correlation between acid phosphatase enzyme levels in aqueous and vitreous humor and postmortem intervals was indicated by 0.974 and 0.962 correlation

coefficient at 23°C stored eyes, respectively. The enzyme activity had slopes of 1.240 & 1.124 U/l per h, and intercepts of -10.691 and -8.7915 U/l in both aqueous and vitreous humor of 23°C environment. Total acid phosphatase activity in samples kept at 37°C showed correlation coefficient of 0.966 and 0.961, slopes of 1.33 and 1.38 U/l per h and intercepts of -7.697 and -8.490 in both aqueous and vitreous humor, respectively. The levels of total acid phosphatase in vitreous and in aqueous humor had a correlation coefficient of 0.992 and 0.994 in both temperatures, respectively (Table 1 and Fig. 2), that means the enzyme activity was increased in both aqueous and vitreous humor in the same interval at different temperatures. It was noticed also from the data that the rise in enzyme levels was much more rapid in samples kept at higher temperatures resulting in a steeper slope. The same findings were recorded by (Ibrahim et al., 1991 and Salem, 1998), who found a linear relationship between acid phosphatase activities in aqueous and vitreous humor of donkeys and camels and PMI.

Acid phosphatase enzyme is a lysosomal enzyme and its high level may confuse other lysosomal activity condition (Wilkinson, 1976). The release of the enzyme depends on membrane permeability that increases after death. In this respect, it should be mentioned here that some aqueous and vitreous samples were found to contain zero level of total acid phosphatase at zero time interval.

In conclusion, the results of the present study indicate that, during 0-84 h postmortem period in cattle (and under the conditions peculiar to this study), a linear relathioship of high correlation exists between the potassium and total acid phosphatase concentration in both aqueous and vitreous humor and PMI takeing in consideration that the slope of both parameters was steeper at higher temperatures. On the other hand use of potassium and acid phosphatase enzyme could be a helpful and good diagnostic aid in conjunction with other methods to increase the accuracy of determining the PMI.

REFERENCES

Adelson, L.; Sunshine, I.; Rushforth, N.B. and Mankoff, M. (1963): Vitreous po·assium concentration as an indicator of the postmortem interval. J. Forensic Sci. 8:503-514.

Adjutantis, G. and Coutselinis, A. (1972): Estimation of the time of death by potassium levels in the vitreous humor. Forensic Sci. 1:55-60.

Balasooriya, B.A.W.; Hill, C, A. St. and Williams, A.R. (1984): The biochemistry of vitreous humor. A comparative study of the potassium sodium and urate concentration in the eyes at identical vitreous humor. J. Forensic Sci. Int. 26: 85-91.

Bito, L.Z. and Salvador, E.V. (1970): Intraocular fluid dynamics II. Postmortem changes in solute concentrations. Exp. Eye Res. 10:273-287.

Brandstein, M.; Castellano, A. and Mezzacappa, C. (1963): Am. J. Clin. Pathol. 400: 583.

Bray, M. (1984): The effect of chilling, freezing and rewarming on the postmortem chemistry of vitreous humor. J. Forensic Sci. 29:404-411.

Bray, M. (1985): Chemical estimation of fresh water immersion intervals. Am. J. Forensic Med. Pathol. 6: 133-139.

Choo-Kang, E.; McKoy, C. and Escoffrey, C. (1983): Vitreous humor analytes in assessing the postmortem interval and antimortem clinical status. W. I. Med.J. 32: 23-26.

Coe, J.I. (1969): Postmortem chemistries on human vitreous humor. Am. J. Clin. Pathol. 51:741-750.

Coe, J.I. (1973): Further thoughts and observations on postmortem chemistry. Forensic Sci. Gaz. 5 (5):2-6.

Coe, J.I. (1977): Postmortem chemistry of blood, cerebrospinal fluid and vitreous humor. In Tedeschi, G. G.; Eckert, W. G. and Tedeschi, L. G. (eds.), Forensic Medicine, Vol. II, W. B. Saunders Co., Philadilphia, pp. 1033-1060.

Coe, J.I (1989): Vitreous potassium as a measure of the postmortem interval: an historical review and critical evaluation. Forensic Sci. Int. 42:201-213.

Crowell, W.A. and Duncan, J.R. (1974): Potassium concentration in vitreous humor as an indicator of the postmortem interval in dogs. Am. J. Vet. Res. 35:301-302.

Devgun, M.S. and Dunbar, J.A. (1986): Biochemical investigation of vitreous: applications in Forensic Medicine, especially in relation to alcohol. Forensic Sci. Int. 31:27-34.

Gamero, L.J.J.; Romero, J.L.; Ramos, H.M.; Arufe, M.I. and Vizcaya, M.A. (1992): Precision of estimating time of death by

vitreous potassium-comparison of various equations. Forensic Sci. Int. 56:137-145.

Henery, J.B. and Smith, F.A. (1980): Estimation of the postmortem interval by chemical means. Am. J. Forensic Med. Pathol. 1:341-347.

Ibrahim, Th.A.; Shehata, A.; Seddek, A.Sh. and Abdel-Nasser, M. (1991): Aqueous and vitreous humor analysis as a diagnostic aid for postmortem timing. Assiut Vet. Med. J. 50:140-150.

Jaffe, F.A. (1962): Chemical postmortem changes in the intraocular fluid. J. Forensic Sci. 7:231-237.

Kalton, G. (1967): Introduction to statistical ideas from scientists, 2nd ed., Addison Willey Publ. Inc. London. pp. 1-75.

Komura, S. and Oshiro, S. (1977): Potassium levels in the aqueous and vitreous humor after death. Tohoku J. Exp. Med. 122:65-68.

Lange, N., Swearer, S. and Sturner, W.Q. (1994): Human postmortem interval estimation from vitreous potassium: an analysis of original data from six different studies. Forensic Sci. Int. 66:159-174.

Lincoln, S.D. and Lane, V.M. (1985): Postmortem magnesium concentration in bovine vitreous humor: Comparison with antimortem serum magnesium concentration. Am. J. Vet. Res. 46:160-162.

Madea, B.; Henssge, C.; Hoeig, W. Gerbracht, A. (1987): References for determining the time of death by potassium in vitreous humor - presented at the 11th meeting of the International Assoc Forensic Sciences, Vancouver B.C., Canada, August 1987.

Madea, B.; Henssge, C.; Hoenig, W. Gerbracht, A. (1989): References for determining the time of death by potassium in vitreous humor. Forensic Sci. Int. 40:231-243.

Madea, B.; Herrman, N. and Henssge, C. (1990): Precision of estimating the time since death by vitreous potassium – comparison of two different equations. Forensic Sci. Int. 46:277-284.

Nauman, H.N. (1959): Postmoortem chemistry of the vitreous body in man. Arch. Opthalmol. 62:356-363.

Neis, P.; Hill, R.; Paschke, M.; Pilwat,G.; Schnabel, A.; Neiss, C. and Bratzke, H. (1999): Strontium90 for determination of time since death. Forensic Sci. Int. 99: 47-51.

Querido, D. (1990): Double logarithmic, linear relationship between plasma sodium/potassium concentration ratio and postmortem interval during the 6-96-h postmortem period in rats. Forensic Sci. Int. 44:125-134.

Roy, A.V.; Brower, M.E. and Hayden, J.E. (1971): Sodium thymolphthalein monophosphate. A new acid phosphatase substrate with greater specificity for the prostatic enzyme in serum. Clin. Chem., 17: 1093-1102.

Salem, D.A. (1998): Relationship between postmortem intervals and some chemical changes in aqueous and vitreous humor of Camelus Dromedarius. Zagazig Vet. J. 26 (1): 111-120.

Schoning, P. and Strafuss, A.C. (1980): Postmortem biochemical changes in canine vitreous humor. J. Forensic Sci. 25:53-59.

Sparks, D.L.; Oeltgen, P.R.; Kryscio, R.J. and Hunsaker III, J.C. (1989): Comparison of chemical methods for determining the postmortem interval. J. Forensic Sci. 34:197-206.

Stephen, R.J. and Richards, R.G. (1987): Vitreous humor chemistry, the use of potassium concentration for the prediction of the postmortem interval. J. Forensic Sci. 32:503-509.

Sturner, W.Q. (1963): The vitreous humor: postmortem potassium changes. The Lancet, 1:807-808.

Sturner, W.Q. and Gantner, G.E. (1964): The postmortem interval: a study of potassium in the vitreous humor. Am. J. Clin. Pathol. 42:137-144.

Wilkie, I.W. and Bellamy, J.E.C. (1982): Estimation of antemortem serum electrolytes and urea concentration from vitreous humor collected postmortem. Can. J. Comp. Med. 46:146-149.

Wilkinson, J.H. (1976): The principles and practice of diagnostic enzymology. 1st ed., Edward Arnold. Ltd. London, pp. 113-141.

Table 1. Concentrations of potassium (mmol/L) in aqueous and vitreous humor in relation to postmortem intervals (PMI) at different temperature.

Postmortem Interval (hours)	23 ± 2 °C		37 ± 2 °C	
	Aqueous	Vitreous	Aqueous	Vitreous
0	6.138 ± 0.13	6.650 ± 0.21	6.882 ± .18	6.394 ± 0.23
6	8.184 ± 0.26**	8.719 ± 0.23**	8.417 ± 0.15**	8.912 ± 0.15**
12	10.69 ± 0.31**	10.895 ± 0.28**	11.207 ± 0.25**	11.696 ± 0.19**
18	12.539 ± 0.31**	13.134 ± 0.22**	12.974 ± 0.24**	14.230 ± 0.21**
24	13.885 ± 0.29**	15.624 ± 0.39**	14.276 ± 0.18**	16.788 ± 0.36**
36	16.624 ± 0.36**	17.452 ± 0.19**	17.88 ± 0.22**	18.903 ± 0.36**
48	18.714 ± 0.39**	19.205 ± 0.39**	20.716 ± 0.21**	20.972 ± 0.37**
60	20.588 ± 0.26**	21.483 ± 0.22**	-	-
72	22.123 ± 0.37**	23.018 ± 0.18**	-	-
84	24.041 ± 0.29**	24.297 ± 0.70**	-	-
Correl. Coeff.	*0.984027423*	*0.976338542*	*0.995935482*	*0.975135776*
Slope	*0.205503968*	*0.205635714*	*0.290722056*	*0.306337325*
Intercept	*7.954857143*	*8.644814286*	*7.21257485*	*7.683203593*
Correl. Coeffi. (aqueous & vitreous)	*0.997427096*		*0.985769417*	

* Significant at p<0.05 ** Significant at p<0.01

Table 2. Concentrations of magnesium (mmol/L) in aqueous and vitreous humor in relation to postmortem intervals (PMI) at different temperature.

Postmortem Interval (hours)	23 ± 2 °C		37 ± 2 °C	
	Aqueous	Vitreous	Aqueous	Vitreous
0	0.441 ± 0.05	0.658 ± 0.068	0.482 ± 0.065	0.581 ± 0.088
6	0.411 ± 0.08	0.864 ± 0.073	0.494 ± 0.045	0.397 ± 0.093
12	0.494 ± 0.04	0.987 ± 0.02	0.494 ± 0.074	0.411 ± 0.075
18	0.411 ± 0.08	0.987 ± 0.034	0.369 ± 0.133	0.411 ± 0.068
24	0.411 ± 0.04	0.679 ± 0.063	0.411 ± 0.1	0.335 ± 0.038 *
36	0.247 ± 0.06*	0.288 ± 0.031 *	0.354 ± 0.058	0.316 ± 0.064 *
48	0.329 ± 0.08	0.267 ± 0.045 *	0.319 ± 0.021*	0.320 ± 0.031*
60	0.267 ± 0.014*	0.296 ± 0.054*	-	-
72	0.123 ± 0.005*	0.165 ± 0.058 *	-	-
84	0.123 ± 0.009*	0.206 ± 0.044*	-	-
Correl. Coeff.	*-0.925350893*	*-0.845695577*	*-0.413457959*	*-0.51373068*
Slope	*-0.004193651*	*-0.009665873*	*-0.001810878*	*-0.0*
Interc	*0.476671429*	*0.887671429*	*0.46339521*	*0.48242515*
Correl. Coeffi. (aqueous & vitreou)	*0.87700067*		*-0.087998839*	

* Significant at p<0.05 ** Significant at p<0.01

Table 3. Concentrations of sodium (mmol/L) in aqueous and vitreous humor in relation to postmortem intervals (PMI) at different temperature.

Postmortem Interval (hours)	23 ± 2 °C		37 ± 2 °C	
	Aqueous	Vitreous	Aqueous	Vitreous
0	81.3 ± 5.652	79.22 ±10.78	85.44 ± 4.348	79.09 ± 6.522
6	81.74 ± 6.522	83.04 ± 5.435	76.09 ± 4.348	78.26 ± 4.348
12	78.96 ± 9.174	98.91 ± 8.174	82.61 ± 2.174	88.26 ± 6.523
18	96.52 ± 6.500	97.83 ± 6.174	78.26 ± 3.261	76.09 ± 8.692
24	84.78 ± 9.435	86.96 ± 7.870	86.96 ± 2.174	91.30 ± 2.147
36	80.44 ± 7.348	92.17 ± 3.043	86.96 ± 4.348	86.96 ± 4.348
48	86.96 ± 4.348	97.09 ± 3.261	89.30 ± 3.478	85.65 ± 2.174
60	80.44 ± 6.523	83.48 ± 6.304	-	-
72	91.30 ± 3.174	78.26 ± 4.348	-	-
84	86.96 ± 6.523	86.96 ± 4.348	-	-
Correl. Coeff.	*0.277960654*	*-0.179979103*	*0.62963766*	*0.483179726*
Slope	*0.053730159*	*-0.047801587*	*0.18250998*	*0.165583832*
Intercept	*83.00571429*	*90.11285714*	*79.90550898*	*80.25227545*
Correl. Coeffi. (aqueous & vitreous)	*0.135204303*		*0.680518179*	

* Significant at p<0.05 ** Significant at p<0.01

Table 4. Concentrations of calcium (mmol/L) in aqueous and vitreous humor in relation to postmortem intervals (PMI) at different temperature.

Postmortem Interval (hours)	23 ± 2 °C		37 ± 2 °C	
	Aqueous	Vitreous	Aqueous	Vitreous
0	11.976 ± 1.197	12.475 ± 1.248	10.978 ± 0.749	12.475 ± 0.746
6	11.477 ± 1.622	12.226 ± 0.529	11.477 ± 1.65	11.976 ± 1.62
12	12.475 ± 0.988	13.224 ± 0.402	12.475 ± 1.37	13.474 ± 0.582
18	12.974 ± 0.749	13.473 ±0.325	13.474 ± 0.48	12.475 ± 1.134
24	11.976 ± 1.354	12.475 ± 1.22	13.723 ± 0.215	12.974 ± 0.782
36	12.475 ± 0.864	12.475 ± 0.936	13.474 ± 0.179	12.475 ± 0.258
48	12.974 ± 0.645	13.972 ± 0.37	13.972 ± 0.389	13.474 ± 1.14
60	12.974 ± 0.763	13.224 ± 0.34	-	-
72	13.473 ± 0.365	12.475 ± 0.75	-	-
84	12.974 ± 0.70	12.974 ± 0.499	-	-
Correl. Coeff.	*0.453221772*	*0.213330898*	*0.712229744*	*0.483966666*
Slope	*0.010692857*	*0.004156349*	*0.03631487*	*0.016196108*
Intercept	*12.13995714*	*12.74967143*	*12.33480838*	*12.4272515*
Correl. Coeffi. (aqueous & vitreous)	*0.333095914*		*0.478940135*	

* Significant at p<0.05 ** Significant at p<0.01

Table 5. Levels of total acid phosphatase enzyme (U/l) in aqueous and vitreous humor in relation to postmortem intervals (PMI) at different temperature.

Postmortem interval (hours)	23 ± 2 °C		37 ± 2 °C	
	Aqueous	Vitreous	Aqueous	Vitreous
0	0.567 ± 0.06	0.457 ± 0.041	0.521 ± 0.06	0.63 ± 0.056
6	1.216 ± 0.120*	1.277 ± 0.18*	1.46 ± 0.13**	1.825 ± 0.16*0
12	3.65 ± 0.420**	5.11 ± 0.221**	5.84 ± 0.37**	2.19 ± 0.14**
18	7.25 ± 0.373**	9.81 ± 0.88**	8.03 ± 0.16**	7.3 ± 0.18**
24	15.38 ± 1.17**	17.70 ± 1.42**	17.88 ± 0.15**	19.71 ± 0.15**
36	29.39 ± 1.11**	25.94 ± 1.46**	43.69 ± 1.19**	40.39 ± 0.18**
48	38.07 ± 1.53**	34.68 ± 2.4**	60.25 ± 1.18**	64.02 ± 1.32**
60	60.59 ± 2.11**	45.03 ± 2.86**	-	-
72	73.73 ± 1.46**	71.54 ± 2.79**	-	-
84	109.86± 2.78**	104.03± 1.95**	-	-
Correl. Coeff.	*0.974444643*	*0.962140247*	*0.966965865*	*0.961578124*
Slope	*1.240609524*	*1.124256349*	*1.329593812*	*1.379545409*
Intercept	*-10.69164286*	*-8.915828571*	*-7.697215569*	*-8.94093413*
Correl. Coeffi. (aqueous & vitreous)	*0.992114977*		*0.994775312*	

* Significant at p<0.05 ** Significant at p<0.01

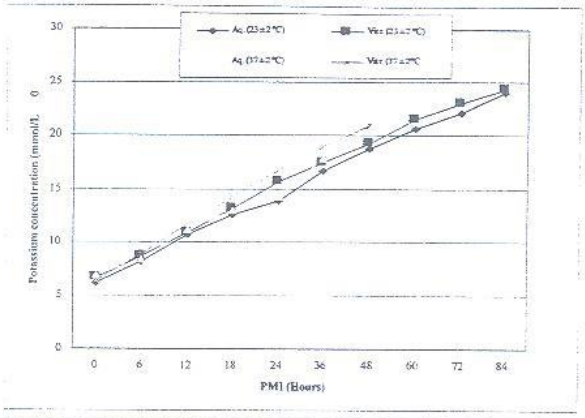

Figure 1. Concentrations of potassium in cattle aqueous and vitreous humor.

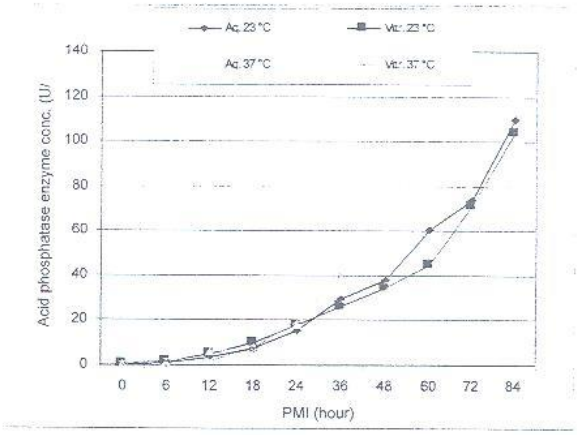

Figure 2. Levels of total acid phosphatase in cattle aqueous and vitreous humor.