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# Investigation of Electrolyte Changes in Bovine Vitreous at Different Postmortem Interval

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## Abstract

**Objective:** Investigation of the changes in electrolyte level in the Bovine vitreous at different postmortem interval under two different temperature conditions.

**Methods:** Ninety eight bovine right eyes from freshly slaughtered healthy cows were gotten from the government abattoir in Edo State, Nigeria. They were divided into two sets; forty nine right eyes were kept at 32°C while forty nine right eyes were kept at 4°C in a mobile refrigerator. The samples of vitreous were carefully aspirated from the bovine eyes within an hour of death of the animals. Measurements of the levels of cations (sodium and potassium) and anions (chloride and bicarbonate) were taken at various postmortem intervals of 2, 12, 24, 36, 48, 60 and 72 hours, using E110111 Flame Photometer.

**Results:** There was a statistically significant increase ($p<0.05$) in the potassium ion level with increased postmortem interval (PMI) at 4°C and 32°C. There were significant reductions ($p<0.05$) in sodium, chloride and bicarbonate ion levels with increased postmortem interval at 32°C, but at 4°C, the reduction in chloride ion level was not significant. The results also showed that the changes in cations and anions levels varied under the two temperature conditions after death.

**Conclusion:** The level of potassium ion increased after death, but sodium, chloride and bicarbonate ion levels in the vitreous humour of bovine eyes decreased after death. Likewise temperature affected the level of anions and cations in the bovine vitreous after death.

**Keywords:** Vitreous humour; Postmortem interval; Cations; Anions; Temperature

## Introduction

Vitreous humor has been investigated since the 1960s, with many debates occurring over the years with regard to the usefulness of its specific applications. The composition of several electrolytes in postmortem vitreous humor has been extensively studied in advanced countries but not in Nigeria. Analysis of chemical changes within intraocular fluid, postmortem, was introduced by Naumann [1] and has since generated great interest in the many applications of vitreous humor analysis. Vitreous humor is preferred for postmortem investigations because of its large volume and easy accessibility [2]. It can be obtained even in cases in which blood and urine specimens are not accessible. It is relatively inert and only slightly influenced by sudden fluctuations in the blood chemistry. The isolate nature of vitreous humor, compared to blood and cerebrospinal fluid, and its resistance to microbiological contamination with bacterial degradation makes it a very suitable medium for postmortem biochemical investigation. Moreover, its composition is more stable and less affected by postmortem changes than cerebrospinal fluid or blood [2].

Normal vitreous humor is a colorless, acellular, viscous, clear gel that fills the posterior compartment between the crystalline lens and the retina and occupies about 80% of the volume of the eyeball of humans and other vertebrates. It is present at birth and does not change much over the course of aging. It does not contain blood vessels, 99% of its volume is water with electrolytes, glucose, inorganic salt, ascorbic acid and a network of collagen fibers (type II) with the glycosaminoglycan hyaluronic acid [1-3]. Vitreous humor is the most investigated body fluid for estimation of postmortem interval (PMI) from chemical changes taking place in its constituent electrolytes after death [4]. Vitreous humor contains very measurable electrolytes and other entities such as potassium, sodium, chloride, bicarbonate, urea nitrogen and creatinine that may enable forensics determine time of death. The vitreous humor chloride values in normal individual have been found to vary from 105-135 mmol/L with an average value of 120 mmol/L, vitreous potassium values in normal individual is <15 mmol/L while normal sodium value vary from 135-150 mmol/L. In decomposition, chloride is <105 mmol/L, sodium is <130 mmol/L while potassium is >20 mmol/L [3].

Postmortem interval (PMI) is the time elapsed between death of a person and the time of autopsy [3]. Though the exact time of death can rarely be estimated on the basis of autopsy findings alone, an appropriate range of PMI can be deduced by careful interpretation of various changes that take place after death [5]. Estimation of time since death is a paramount medico-legal issue in any postmortem examination. Determination of PMI is essential in many criminal forensic investigations as well as in certain natural deaths [6]. Many chemical changes begin to take place in the body immediately or

shortly after death and progress in a fairly orderly fashion until the body disintegrates. Each change has its own time factor or rate. These changes occur in various body fluids such as blood, cerebrospinal fluid (CSF), aqueous and vitreous humour of the eye. All these fluids showed time related changes after death [6]. But the reports on changes that occur in the anions and cations level in the vitreous during different postmortem interval at varied temperature are very scanty in tropical region like Nigeria. This is why this study was carried out to determine the electrolyte changes that occur in the vitreous humor of bovine eyes at different postmortem interval under two different temperature variations.

## Methodology

Vitreous humor samples were aspirated from 98 right eyes of certified healthy cows within the ages of 10-14 years. They were reared in the same cattle ranch and slaughtered in the government abattoir in Edo State, Nigeria. The cows right eyes were collected within an hour after death, and the ninety eight right eyes were divided into two sets, forty nine right eyes were kept in sample carrier at 32°C (room temperature) and forty nine right eyes were kept at a lower temperature of 4°C in a mobile refrigerator (morgue temperature). The vitreous humor samples were aspirated, diluted and analyzed with the flame photometer by the methods proposed by Chavhan et al. [7]; Coe et al. [8]; and Rant et al. [9]; using a 20-gauge needle attached to a 10 ml syringe, a sclera puncture was made on the lateral canthus and the total extractable vitreous humor was then aspirated from each eye separately to determine the anions and cations level. Care was taken to gently aspirate the fluid and avoid tearing any loose tissue fragments surrounding the vitreous chamber; an average amount of 2.5 ml was collected from each eyeball. The samples were diluted in diluents containing 15 mmol/L of lithium aspirated into a propane-air flame, (the lithium emission signal is used as an internal standard to eliminate interferences due to variations in dilution ratios). 50 µl of each sample was added to 10 ml deionized water and was mixed thoroughly. The flame photometer was switched on and allowed to run for ten minutes to properly warm up the machine. Thereafter it was zeroed or blanked with deionized water. The machine was standardized using (standard solution of sodium=140 mEq/L and potassium=5.0 mEq/L), the sample was then introduced into the flame photometer. The flame photometer acts on the principle of emission of radiation from flames which depend on the characteristic element present in them. It also works with the principle of absorption and luminescence spectroscopy. The absorbance of light due to the electron excitation was measured using direct absorption techniques. Due to the thermal energy of the flame, the atoms get excited and there after return to ground state. In this process of return to ground state, exited atoms emit radiation of specific wavelength. This wavelength of radiation emitted is specific for every element and is visualized in the visible region of the spectrum as sharp bright lines; the intensity of radiation depends on the concentration of element. The concentrations of cations (potassium and sodium) and anions (chloride and bicarbonate) in the vitreous humour obtained at the various postmortem interval of 2, 12, 24, 36, 48, 60 and 72 hours were measured by the E110111 flame photometer and recorded. The data obtained was analyzed using descriptive statistics (mean and standard error) and the Pearson correlation test was used to show the relationship between postmortem interval and the levels of cations and anions under two temperature conditions.

## Results

Table 1a showed the mean values of bicarbonate ion level at different postmortem interval under 4°C and 32°C. In Tables 1b and 1c, Pearson correlation showed a highly significant negative correlation relationship between the bicarbonate ion level and postmortem interval at 4°C and 32°C respectively. As postmortem interval (hours) increased, there was a statistically significant reduction ($p<0.05$) in bicarbonate ion levels. At 4°C ($r=-0.941$, $p=0.002$) and at 32°C ($r=-0.977$, $p=0.000$). The results also showed that temperature had effect on the level of bicarbonate ions after death. At 4°C, the reduction in bicarbonate ion level was 94.1% while at 32°C, there was 97.7% reduction in bicarbonate ion level.

| Postmortem Interval (HRS) | $HCO_3^-$ Level (mmol/L) at 4°C ± SEM | $HCO_3^-$ Level (mmol/L) at 32°C ± SEM |
|---|---|---|
| 2 | 18.95 ± 2.49 | 20.85 ± 4.23 |
| 12 | 18.91 ± 2.37 | 20.50 ± 4.18 |
| 24 | 18.39 ± 3.15 | 20.16 ± 4.07 |
| 36 | 17.30 ± 2.18 | 16.30 ± 3.11 |
| 48 | 15.70 ± 2.05 | 15.15 ± 2.85 |
| 60 | 11.80 ± 1.90 | 12.45 ± 1.76 |
| 72 | 10.05 ± 1.70 | 10.76 ± 1.57 |
| HRS: Hours; $HCO_3^-$ : bicarbonate ion; PMI: Postmortem Interval | | |

**Table 1a:** Mean values of bicarbonate ion level in bovine vitreous at different postmortem interval under 4°c and 32°c temperature.

| | | bicarbonate ion at 4°C | Hours |
|---|---|---|---|
| bicarbonate ion at 4°C | Pearson Correlation | 1 | -.941** |

| | | | |
|---|---|---|---|
| | Sig. (2-tailed) | | 0.002 |
| | N | 7 | 7 |
| Hours | Pearson Correlation | -.941** | 1 |
| | Sig. (2-tailed) | 0.002 | |
| | N | 7 | 7 |
| **. Correlation is significant at the 0.05 level (2-tailed). | | | |

**Table 1b:** Pearson Correlation relationship between PMI and $HCO_3^-$ at 4°C.

| | | Hours | bicarbonate ion at 32°C |
|---|---|---|---|
| Hours | Pearson Correlation | 1 | -.977** |
| | Sig. (2-tailed) | | 0 |
| | N | 7 | 7 |
| bicarbonate ions at 32°C | Pearson Correlation | -.977** | 1 |
| | Sig. (2-tailed) | 0 | |
| | N | 7 | 7 |
| **. Correlation is significant at the 0.02 level (2-tailed). | | | |

**Table 1c:** Pearson Correlation relationship between PMI and $HCO_3^-$ at 32°C.

Table 2a showed the mean values of chloride ion level at different postmortem interval under 4°C and 32°C. In Tables 2b and 2c, Pearson correlation (r=−0.594, p=0.160) showed that there was a negative correlation relationship between the chloride ion level and postmortem interval at 4°C but it was not statistically significant. At 32°C, Pearson correlation (r=−0.937, p=0.002) showed a significant strong negative correlation between chloride ion level and postmortem interval which was statistically significant ($p<0.05$). As the postmortem interval (hours) increased the level of chloride ions decreased. The change in chloride ion level at different postmortem interval varied under the two temperature conditions. This showed that temperature had effect on the level of chloride ions after death. At 4°C, there was 59.4% reduction in chloride ion level, while at 32°C, there was 93.7% reduction in chloride ion level.

| Postmortem Interval (HRS) | Cl-Level (mmol/L) at 4°C ± SEM | Cl-Level (mmol/L) at 32°C ± SEM |
|---|---|---|
| 2 | 115.20 ± 1.39 | 118.70 ± 1.96 |
| 12 | 110.15 ± 1.07 | 114.35 ± 1.90 |
| 24 | 110.15 ± 1.07 | 114.20 ± 1.90 |
| 36 | 110.15 ± 1.07 | 113.45 ± 1.34 |
| 48 | 110.15 ± 1.07 | 112.25 ± 1.21 |
| 60 | 110.15 ± 1.07 | 110.10 ± 1.09 |
| 72 | 110.15 ± 1.07 | 110.10 ± 1.09 |
| HRS: Hours; Cl: Chloride Ion; PMI: Postmortem Interval | | |

**Table 2a:** Mean values of chloride ion level in bovine vitreous at different postmortem interval under 4°c and 32°c temperature.

| | | Hours | Chloride ion at 4°C |
|---|---|---|---|
| Hours | Pearson Correlation | 1 | -0.594 |
| | Sig. (2-tailed) | | 0.16 |

| | | | |
|---|---|---|---|
| | N | 7 | 7 |
| Chloride ion at 4°C | Pearson Correlation | -0.594 | 1 |
| | Sig. (2-tailed) | 0.16 | |
| | N | 7 | 7 |

Table 2b: Pearson Correlation relationship between PMI and $Cl^-$ at 4°C.

| | | Hours | Chloride ion at 32°C |
|---|---|---|---|
| Hours | Pearson Correlation | 1 | -.937** |
| | Sig. (2-tailed) | | 0.002 |
| | N | 7 | 7 |
| Chloride ion at 32°C | Pearson Correlation | -.937** | 1 |
| | Sig. (2-tailed) | 0.002 | |
| | N | 7 | 7 |
| **. Correlation is significant at the 0.05 level (2-tailed). | | | |

**Table 2c:** Pearson Correlation relationship between PMI and Cl- at 32°C

Table 3a showed the mean values of sodium ion level at different postmortem interval under 4°C and 32°C. In Tables 3b and 3c, Pearson correlation showed a highly significant negative correlation relationship between the sodium ion level and postmortem interval at 4°C and 32°C. As postmortem interval increased, there was a statistically significant reduction ($p<0.05$) in sodium ion levels. At 4°C, ($r = -0.883$, $p=0.008$) and at 32°C ($r=-0.961$, $p=0.001$). The change in sodium ion level varied under the two temperature conditions. Therefore temperature had effect on the level of sodium ions after death. At 4°C, there was 88.3% reduction in sodium ion level, while at 32°C, there was 96.1% reduction in sodium ion level.

| Postmortem Interval (hrs) | Na+ LEVEL (mmol/L) at 4°C ± SEM | Na+ Level (mmol/L) at 32°C ± SEM |
|---|---|---|
| 2 | 140.10 ± 7.31 | 148.65 ± 8.71 |
| 12 | 138.00 ± 7.05 | 141.90 ± 6.68 |
| 24 | 137.95 ± 6.31 | 138.36 ± 5.42 |
| 36 | 135.20 ± 6.08 | 137.85 ± 5.30 |
| 48 | 135.20 ± 6.08 | 137.85 ± 5.30 |
| 60 | 134.75 ± 6.05 | 135.42 ± 5.25 |
| 72 | 133.46 ± 6.02 | 135.42 ± 5.25 |
| HRS: Hours, Na+: Sodium Ion, PMI: Postmortem Interval | | |

**Table 3a:** Mean values of sodium ion level in bovine vitreous at different postmortem interval under 4°c and 32°c temperature.

| | | Hours | Chloride ion at 32oC |
|---|---|---|---|
| Hours | Pearson Correlation | 1 | -.937** |
| | Sig. (2-tailed) | | 0.002 |
| | N | 7 | 7 |
| Chloride ion at 32°C | Pearson Correlation | -.937** | 1 |

| | | | |
|---|---|---|---|
| | Sig. (2-tailed) | 0.002 | |
| | N | 7 | 7 |
| **. Correlation is significant at the 0.05 level (2-tailed). | | | |

**Table 3b:** Pearson Correlation relationship between PMI and Na+at 4°C.

| | | Hours | Sodium ion at 32°C |
|---|---|---|---|
| Hours | Pearson Correlation | 1 | -.961** |
| | Sig. (2-tailed) | | 0.001 |
| | N | 7 | 7 |
| Sodium ion at 32°C | Pearson Correlation | -.961** | 1 |
| | Sig. (2-tailed) | 0.001 | |
| | N | 7 | 7 |
| **. Correlation is significant at the 0.05 level (2-tailed). | | | |

**Table 3c:** Pearson Correlation relationship between PMI and Na+at 32°C.

Table 4a showed the mean values of potassium ion level at different postmortem interval at 4°C and 32°C while in Tables 4b and 4c, Pearson correlation showed a strong positive relationship between potassium ion level and postmortem interval. At 4°C, (r=0.878, p=0.009) and at 32°C, (r=0.920 p=0.003). As postmortem interval increased, there was a statistically significant increase ($p<0.05$) in potassium ion level. The change in potassium ion level varied under the two temperature conditions. This showed that temperature had effect on the level of potassium ions after death. At 4°C, there was 87.8% increment in potassium ion level, while at 32°C, there was 92.0% increment in potassium ion level.

| Postmortem interval (hrs) | K+Level (mmol/L) at 4°C ± SEM | K+Level (mmol/L) at 32°C ± SEM |
|---|---|---|
| 2 | 6.65 ± 0.19 | 7.18 ± 0.22 |
| 12 | 6.65 ± 0.19 | 7.25 ± 0.30 |
| 24 | 6.70 ± 0.27 | 7.32 ± 0.35 |
| 36 | 6.70 ± 0.27 | 7.32 ± 0.35 |
| 48 | 7.00 ± 0.35 | 7.50 ± 0.38 |
| 60 | 7.15 ± 0.36 | 7.84 ± 0.40 |
| 72 | 7.73 ± 0.39 | 8.16 ± 0.42 |
| HRS: hours; K+: potassium ion; PMI: postmortem interval | | |

**Table 4a:** Mean values of potassium ion level in bovine vitreous at different postmortem interval under 4°c and 32°c temperature.

| | | Hours | Potassium ion at 4°C |
|---|---|---|---|
| Hours | Pearson Correlation | 1 | .878** |
| | Sig. (2-tailed) | | 0.009 |
| | N | 7 | 7 |
| Potassium ion at 4°C | Pearson Correlation | .878** | 1 |
| | Sig. (2-tailed) | 0.009 | |

| | | | |
|---|---|---|---|
| | N | 7 | 7 |

**Table 4b:** Pearson Correlation relationship between PMI and K+ at 4°C.

| | | Hours | Potassium ion at 32°C |
|---|---|---|---|
| Hours | Pearson Correlation | 1 | .920** |
| | Sig. (2-tailed) | | 0.003 |
| | N | 7 | 7 |
| Potassium ion at 32°C | Pearson Correlation | .920** | 1 |
| | Sig. (2-tailed) | 0.003 | |
| | N | 7 | 7 |
| **. Correlation is significant at the 0.05 level (2-tailed). | | | |

Table 4c: Pearson Correlation relationship between PMI and K+ at 32°C.

## Discussions

The results of this study showed a linear rise in potassium ion level with increasing postmortem interval under the two temperature conditions, this is in agreement with the study of Prasad et al. [10]; Nilesh et al. [11] and Jashnani et al. [12]. They found a rise in potassium ion level as postmortem interval increased. The general understanding is that after death, cell membranes become permeable. Active and selective membrane transport stop, and the loss of selective membrane permeability and diffusion of ions, and other parameters according to their level gradients, start [11,13]. Although the vitreous is stable, certain vitreous elements will change with diffusion from the retinal cells. Immediately potassium begins to diffuse out of these cells into the vitreous, the level of potassium increases linearly. For this reason, potassium has been used to estimate the time since death occurred, potassium concentration in vitreous humour is a single best time honoured parameter to estimate postmortem interval [12,14]. Other studies also supported a direct positive relationship between the vitreous potassium level and the postmortem interval [15-20].

Studies have shown that the concentration of sodium and chloride fall slowly after death, these changes are reported to be in proportion to the postmortem interval [2,12]. This was seen in the results of this study where there was a gradual fall in sodium and chloride ion levels with increasing postmortem interval under the two temperature conditions.

The results of this study also showed an inverse relationship between bicarbonate ion level and postmortem interval such that as the postmortem interval increased the level of bicarbonate ion reduced under the two temperature conditions. This is in agreement with the study of Kim [3] who observed that bicarbonate values in the vitreous after death are usually low and this may be due to loss of carbon dioxide.

In conclusion, this study has shown that biochemical changes in the level of cations and anions in the vitreous of bovine eye, in relation to the time since death occurred is a useful tool that may enable forensics determine time of death. Temperature was also seen to affect the level of the cations and anions in the bovine vitreous at different postmortem interval in this study. The changes in electrolyte level were more significant at room temperature (32°C) than at morgue temperature (4°C), this may be attributed to the fact that chemical changes and disintegration occur faster at higher temperature than at cold temperature.

## References

1. Naumann HN (1959) Postmortem chemistry of the vitreous body in man. Arch Ophthalmol 62: 356-363.
2. Baniak N, Campos-Baniak G, Mulla A, Kalra J (2015) Vitreous Humor: A Short Review on Post-mortem Applications. J Clin Exp Pathol 4: 1-7.
3. Kim AC (2016) Post mortem Vitreous Analyses: Overview. Drug Dis 1-4.
4. Harper DR (1989) A comparative study of the microbiological contamination of postmortem blood and vitreous humour samples taken for ethanol determination. Forensic Sci Int 43: 37-44.
5. Chandrakanth HV, Kanchan T, Balaraj BM, Virupaksha HS, Chandrashekar TN (2013) Postmortem vitreous chemistry-an evaluation of sodium, potassium and chloride levels in estimation of time since death (during the first 36 h after death). J Forensic Leg Med 20: 211-216.
6. Henssge C, Madea B (2004) Estimation of the time since death in the early post-mortem period. Forensic Sci Int 144: 167-175.
7. Chavhan SG, Balachandran C, Nambi AP, Dhinakar Raj G, Vairamuthu S (2014) A study on vitreous humor between-eye differences and baseline values of potassium, calcium, sodium and glucose immediately after death in dogs. Ind J Vet Anim Sci Res 43: 372-375.
8. Coe JI, Apple FS (1985) Variations in vitreous humor chemical values as a result of instrumentation. J Forensic Sci 30: 828-835.
9. Rant SC, Chandel RS, Abichandani LG (2013) Study of serum electrolytes by flame photometer and autoanalyzer. J of App Sci 1: 972-974.
10. Prasad BK, Choudhary A, Sinha JN (2003) A study of correlation between vitreous potassium level and post mortem interval. Kathmandu Univ Med J (KUMJ) 1: 132-134.
11. Nilesh KT, Vipul NA, Anand PD (2014) Thanatochemistry: Study of vitreous humor potassium. Alexand J Med 50: 85-88.
12. Jashnani KD, Kale SA, Rupani AB (2010) Vitreous humor: biochemical constituents in estimation of postmortem interval. J Forensic Sci 55: 1523-1527.
13. Madea B, Musshoff F (2007) Postmortem biochemistry. Forensic Sci Int 165: 165-171.
14. Swain R, Kumar A, Sahoo J, Lakshmy R, Gupta SK, et al. (2015) Estimation of post-mortem interval: A comparison between

cerebrospinal fluid and vitreous humour chemistry. J Forensic Leg Med 36: 984-988.

15. Balasooriya BAW, Hill CAS, Williams AR (1984) The biochemistry of vitreous humour. A comparative study of the potassium, sodium and urate concentrations in the eyes at identical time intervals after death. Forensic Sci Int 26: 85-91.
16. Sturner WQ, Gantner GE (1964) The Postmortem Interval. A Study of Potassium In The Vitreous Humor. Am J Clin Pathol 42: 137-144.
17. Hughes WM (1965) Levels of potassium in the vitreous humour after death. Med Sci Law 5: 150-156.
18. Hansson L, Uotila U, Lindfors R, Laiho K (1966) Potassium content of the vitreous body as an aid in determining the time of death. J Forensic Sci 11: 390-394.
19. Lie JT (1967) Changes of potassium concentration in the vitreous humor after death. Am J Med Sci 254: 136-143.
20. Agrawal RL, Gupta PC, Bhasin S, Nagar CK (1983) Determination of the time of death by estimating potassium level in the cadaver vitreous humour. Indian J Ophthalmol 31: 528-531.