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Post-Mortem Vitreous Ammonium Concentrations in Estimating the Time of Death

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Summary. On a total of 150 specimens of vitreous humor the post-mortem ammonia value was determined by diffusion method. The results established that there existed a regular ammonia increase after a sudden death and that deviating high or low vitreous ammonia values are strongly correlated with the cause of death and with environmental temperature.
The ammonia content of the vitreous humor extracted from both eyes at the same and at different post-mortem intervals showed no differences in the concentrations between both specimens sampled at the same moment while an obvious variation in the individual rate of the vitreous ammonia increase is found, especially when hospital cases with antemortem existing disease are concerned.

Zusammenfassung. Der Ammoniakgehalt der Glaskörperflüssigkeit wurde gemessen an insgesamt 150 Entnahmen mit bekannter Todeszeit nach der Diffusionsmethode. Die Ergebnisse zeigen einen regelmässigen postmortalen Anstieg des Ammoniaks in plötzlichen Todesfällen während abweichend höhere und niedrigere Messzahlen sich eng korreliert verhalten mit der Todesursache und dem Aussentemperatur. Doppelbestimmungen des Ammoniakgehalts im Corpus Vitreum beider Augen zu gleicher und verschiedener Leichenzeit vorgenommen erweisen keine Konzentrationsunterschiede in den zu gleicher Zeit entnommenen Proben aber eine merkbare Schwankung in dem individuellen Anstieg der Ammoniakwerte des Glaskörpers, besonders wenn Hospitalfälle mit vorherigen Krankheiten betroffen sind. Wegen der Beschränkung einer notwendigen Selektion in bezug auf Ursache und Plötzlichkeit eines Sterbens ist die Methodik der Ammoniakbestimmung im Glaskörperinhalt angesichts der mit zunehmender Liegezeit wachsenden individuellen Streuung nur als zusätzliches Hilfsmittel zur Todeszeitschätzung verwendbar.

Key words: Estimating the time of Death, Vitreous Ammonium Concentration – Vitreous Ammonium Concentration, Estimating the Time of Death

The desirability of a method that would permit the forensic pathologist to determine the exact moment that death occurred is self-evident. Several workers in the field have tried to find the relation between the post-mortem biochemical changes in various

body fluids and the time since death, especially by determining the potassium content of the vitreous humor (Adelson a.o., 1963; Sturner, 1963; Hughes, 1965; Hansson a. o., 1966; Leahy and Farber; 1967; Adjutantis and Coutselinis, 1972; Coe, 1973).

The vitreous humor represents an easy obtainable isolated pool of cristal clear material suitable for various clinical and toxicological analyses in order to detect antemortem existing aberrations to determine the possible cause of death. Not only is the intraocular fluid in comparison with other body fluids for a given post-mortem interval far less subject to rapid chemical and bacterial putrifactive changes, the technical practicability of this substrate is also to be preferred.

The ammonia content of cadaver blood was in the past found to have a gradual rise after death with such wide individual variation that it could not be considered as a method in estimating the time of death (Schleyer, 1957).

Previously the ammonia levels in liquor cerebrospinalis obtained by suboccipital puncture were measured at different post-mortem intervals (Osvath, 1961; Schleyer, 1967) and the authors concluded the method to have a restricted utility during the first 30 hour period post-mortem as an additional means in estimating the moment of death. Since the ammonia concentration in the blood and the cisternal fluid by the production of proteolytic NH_3-groups in the different tissues and compartiments of the body after death shows a sharp rise in the early postmortem period, it seemed indicated to investigate the post-mortem ammonia changes in the vitreous humor.

Materials and Methods

There were 59 males and 31 females with the ages varying from 6 to 80 years in the study. Intentionally these were divided into two groups. The first group consisted of 69 cases of ‚sudden death' where no significant biochemical alteration by disease or by medical administration of pharmacals and fluids antedated death that occurred within 6 hours after the onset of the first clinical symptoms. The cause of death in most cases confirmed by autopsy was coronary occlusion, heart decompensation, pulmonary embolism, asphyxia by various intrinsic or extrinsic mechanisms and fatal accidental or incidental trauma.

The second group consisted of 21 hospital patients who died after a period of lingering diseases and where the cause of death with decreasing frequency consisted of carcinoma, cerebrovascular disease, metabolic disease and primary infections of different nature.

Within few hours after death the bodies arrived at the mortuary of the Leuven university hospital where they were stored at an environmental temperature between 5° and 18° Celsius.

All of the vitreous humor was gently extracted using a 5 ml disposable syringe and a 20 gauge needle inserted through the lateral sclera into the centre of the eyeball.

Only cristal clear samples were restrained for further examination. In 60 cases vitreous humor was drawn from both eyes at the same or at a different post-mortem interval. All specimens were taken before an autopsy was performed and were immediately filtered by means of a stalked spongy plastic device, next centrifuged and analysed at the hospital laboratory.[1]

By using the diffusion method modificated after the original micro diffusion method of Conway (Seligson, 1951 and 1957) a total of 150 ammonia values were obtained. Ammonia is separated by diffusion in penicillin bottles with a footed glass rod dipped in 1 N H_2SO_4 after saturated potassium carbonate is added to the sample. A rotator is used with a maximum diffusion occurring in 15 minutes. The liberated ammonia as ammoniumsulfate is brought into a diluted Nessler's Reagent and measured colorimetrically in a Eppendorf photometer at 420 nm. Every determination was conducted triple and only the mean value expressed in microgrammes ammonia per millilitre vitreous humor was listed for statistical analysis.

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[1] Through the kind cooperation of Prof. K. Heirwegh and the skilful assistance of miss N. Mestdagh, Laboratory of pathophysiology of the Liver, University of Leuven

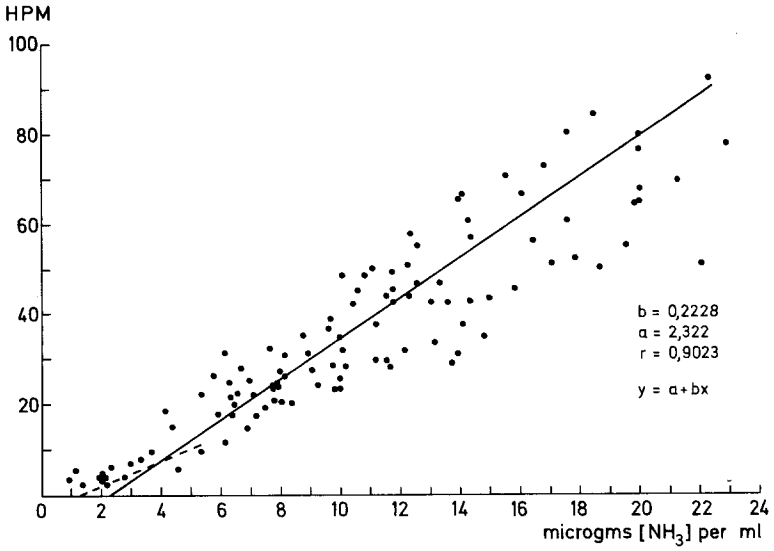

Fig. 1. Ammonia concentrations of the vitreous humor in microgrammes per millilitre during the first 100 hours postmortem of the ‚sudden death' cases

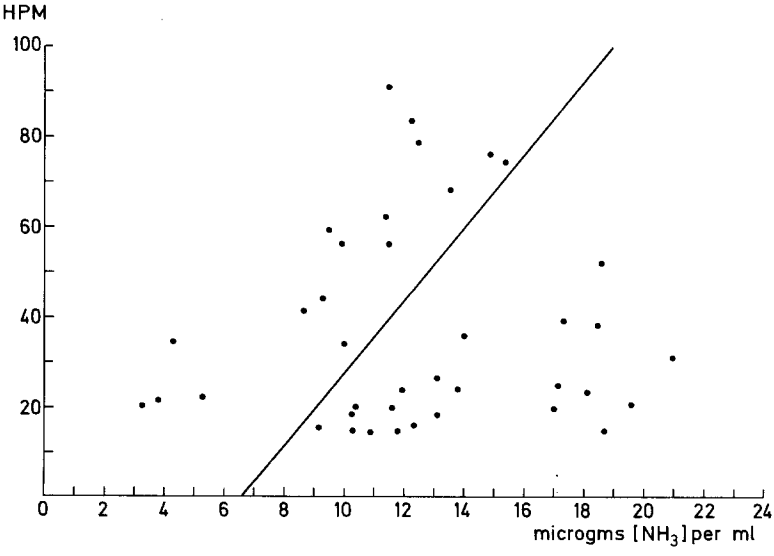

Fig. 2. Ammonia concentrations of the vitreous humor in microgrammes per millilitre during the first 100 hours postmortem of the ‚hospital' cases

Results

The diagram (Fig. 1) shows the regular increase in the ammonia concentration of the ocular fluid in the first 100 hour interval post-mortem for the sudden death group.

In 3 cases where the corpses were kept at an environmental temperature of approximately 30°C for 24 hours remarkably high NH_3-concentrations were obtained. The results of 8 samples with abnormally elevated ammonia values concerning 6 traumatic cerebral hemorrhages, an aneurisma dissecans and an electrocution had to be excluded for statistical analysis. Equally in 9 cases of sudden death by asphyxia and massive external bloodloss the extreme low NH_3-concentrations were left out. The paired determinations of 10 specimens sampled from both eyes at the same post-mortem time showed identical ammonia values (mean difference 0.09 µgper ml.)

The figures of the ammonia content of the vitreous humor extracted from the same individual at varying intervals after death expressed an obvious difference in the rate of ammonia increase.

The hourly vitreous ammonia increase averaged 0.233 µgper ml. with in the early post-mortem period up to 10 hours the mean hourly NH_3-rise amounting to 0.30 µgper ml.

The line of least squares regression gives a zero time intercept of 2.322 µgper ml. and slope 0.233 µgper hour.

In the second group of the hospital cases (Fig. 2) a much larger individual variation in the rate of ammonia increase after death was found and the overall hourly increase in this series averaged 0.124 µgper ml.

Discussion

There are relatively few publications giving post-mortem ammonia values in body fluids as a means of estimating the time since death. Investigations on the post-mortem plasma ammonia content showed that the NH_3-concentration remains stationary within a 1–3 mgms per 100 ml. range during an 8 hour period after which a sharp increase was seen so that ammonia values in the blood above 3 mg % only indicate a post-mortem interval of more than 8 hours.

In his study on the post-mortem NH_3-content of the liquor cerebrospinalis of 81 individuals, Osvath apparently included several hospital patients and considered the established increase during the first 30 hours after death as a possible aid in determining the moment that death occurred. A variation between such wide limits as 10–20 hours however and the necessary exclusion of a considerable number of causes of death (Ihm and Schleyer, 1967) reduces the practical utility of this method.

A useful correlation between the ammonia content in muscle and the time since death could not be established (Kamm and Schleyer, 1968). In the ‚sudden death' group of the present series a constant rise of the NH_3-values in the vitreous humor up to 100 hours post-mortem was observed. A notable influence, however, of the environmental temperature resulted in deviatingly elevated ammonia levels when the corps during summer time remained at atmospheric temperatures of 30°C as a visible expression of the more rapid advance of the post-mortem autolytic desamination. The obligatory eleimination of all medico-legal cases with a death by drowning or profuse external hemorrhage and the increased variation between wider limits in some cases of fatal head trauma and myocardial infarction represent a considerable

restriction of the utility of the ammonia concentration as an aid in fixing the moment of death. Nevertheless this limitation is considered to be less impairing than formerly mentioned (Ihm and Schleyer, 1967) and the correlation r = 0.9023 by means of least squares regression is good.

While the hourly increase of ammonia in the ocular fluid is 0.233 μgper ml. the rise of the NH_3-content during the first 10 hours post-mortem averages 0.3 μgper ml. and per hour with the normal in vivo ammonia concentration in the vitreous humor assumed to be 1 μgper ml. This oberservation is in accordance to the more rapid rise of the vitreous humor potassium content (Coe, 1969) during the early post-mortem interval. Control determinations on 10 frozen specimens of vitreous humor at different intervals for 5 months showed no significant alterations in the ammonia content originally measured.

In the series of the hospital deaths the observed very wide individual variation in the rate of vitreous ammonia increase was expected since the antemortem disturbances of the normal metabolic serum values are reflected in the other body fluids.

When the classic indications of temperature, lividity, rigor and decomposition are contradictory and the conditions regarding the cause and the suddenness of a death with medico-legal interest are fulfilled, the method can be of some help.

With the limitation of a necessary selection regarding the cause and the suddenness of a death the analysis of the vitreous ammonia concentration by its marked individual variation of rising rate with time after death can only be of restricted value as an additional aid in estimating the time of death.

References

Adelson, L., Sunshine, I., Rushford, N.B., Mankoff, M.: Vitreous potassium concentration as indicator of the post-mortem interval. J. Forens. Sci. 8, 503–514 (1963)

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

Coe, J. I.: Post-mortem chemistries on human vitreous humor. Amer. J. Clin. Pathol. **51**, 741–750 (1969)

Coe, J.I.: Post-mortem chemistry: practical considerations and review of the literature. J. Forens. Sci. 18, 13–32 (1973)

Hansson, L., Uotila, U., Lindfors, R., Laiho, U.: Potassium content of the vitreous body as an aid in determining the time of death. J. Forens. Sci. **11**, 390–394 (1966)

Hughes, W.M.H.: Levels of potassium in the vitreous humor after death. Med. Sci. Law **5**, 150–156 (1965)

Ihm, P., Schleyer, F.: Fehlerkritische Betrachtungen über die Todeszeitbereichnung anhand biochemischer Komponenten im Zisternenliquor und Serum. Arch. klin. Med. **214**, 20–33 (1967)

Leahy, M.S., Farber, E.R.: Post-mortem chemistry of human vitreous humor. J. Forens. Sci. **12**, 214–222 (1967)

Kamm, G., Schleyer, F.: Untersuchungen über den Ammoniak- und Milchsäuregehalt der totenstarren Muskulatur. Arch. Kriminol. **142**, 177–183 (1968)

Osvath, A.: Untersuchungen über die Beziehungen zwischen der Ammoniak-Konzentration im Zisternenliquor und dem Leichenalter. Dissertation, Bonn 1961

Schleyer, F.: Untersuchungen über den Serum-NH_3-Gehalt in Beziehung zu Leichenalter und Blutgefässgebiet. Dtsch. Z. ges. gerichtl. Med. **46**, 1–9 (1957)

Seligson, D., Seligson, H.: A microdiffusion method for the determination of nitrogen liberated as ammonia. J. Lab. Clin. Med. 38, 324–330 (1951)

Seligson, D., Hirahara, K.: The measurement of ammonia in whole blood, erythrocytes and plasma. J. Lab. Clin. Med. **49**, 962–973 (1957)

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