

From the Department of Oncology-Pathology

Karolinska Institutet, Stockholm, Sweden

**POSTMORTEM ANALYSES
OF VITREOUS FLUID**

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**Karolinska
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POSTMORTEM ANALYSES OF VITREOUS FLUID

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Postmortem Analyses of Vitreous Fluid

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ABSTRACT

The identification of various various medical conditions postmortem is often difficult. Results from analysis of postmortem blood and urine samples are not as appropriate as in living subjects, due to bacterial contamination and postmortem cell degradation. Therefore, vitreous humour – the fluid in the eyeball – has been of substantial value in forensic pathology. Vitreous fluid is easily collected, isolated, and almost bacteria- and cell free, and shows relatively stable conditions after death, making it a better matrix for postmortem biochemical analyses than e.g. blood and serum. Although postmortem analyses of vitreous fluid have been studied quite extensively, there are still many unanswered questions.

Vitreous fluid from over 3,000 deceased subjects were consecutively collected and analysed for glucose, lactate, pH, electrolytes and gas pressures.

Paper I focuses on the postmortem diagnosis of hyperglycemia. We show that vitreous glucose levels decrease the first 12-24 hours after death and are relatively stable after that. We also suggest that lactate should not be used to diagnose antemortem hyperglycemia, due to massive lactate increase from other sources – instead, glucose alone should be used. A vitreous glucose level of 10 mmol/L indicates severe antemortem hyperglycemia.

In **paper II**, we studied the postmortem increase in vitreous potassium levels, which can be used to estimate the time of death. Our results show that postmortem potassium levels are affected by the surrounding temperature and the age of the deceased. We have developed a new equation for the estimation of the time of death that includes potassium, surrounding temperature and decedent age. To facilitate the calculation for the user, we have developed a web application.

Paper III deals with the interpretation of postmortem vitreous sodium and chloride levels. We show that vitreous sodium and chloride levels slowly decrease with time of death. Postmortem vitreous sodium and chloride levels correlate well with antemortem serum sodium and chloride levels if corrected for time since death, and may be used to diagnose various antemortem sodium/chloride imbalances, such as dehydration or water intoxication. Sodium imbalances in cases of drownings are most likely due to postmortem diffusion between water and vitreous, rather than the drowning process.

LIST OF PUBLICATIONS

- I. **Zilg B**, Alkass K, Berg S, Druid H.
Postmortem identification of hyperglycemia.
Forensic Sci Int. 2009;185:89-95.
- II. **Zilg B**, Bernard S, Alkass K, Berg S, Druid H.
A new model for the estimation of time of death from vitreous potassium levels corrected for age and temperature.
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- III. **Zilg B**, Alkass K, Berg S, Druid H.
Interpretation of postmortem vitreous concentrations of sodium and chloride.
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1. BACKGROUND

1.1. General background

Postmortem analyses of different body fluids can help the forensic pathologist to determine the cause and manner of death, as well as time of death, in a wide selection of cases. Blood can be used for toxicological analyses, but because of the postmortem degradation of red and white blood cells, it is often not suitable for analyses of endogenous compounds. Other body fluids that can be used for postmortem chemistry are vitreous, cerebrospinal, pericardial and synovial fluids. Among these, vitreous fluid has become the matrix of choice in forensic pathology, not only because it is much easier to obtain, but also because of its isolated position, which makes it less affected by postmortem contamination and putrefaction. [1-3]

Vitreous fluid can be used to analyse electrolytes (sodium, potassium, chloride) and a variety of endogenous compounds like acetone, urea, hypoxanthine, glucose and lactate, and the levels of such compounds can offer the pathologist an appreciation of the antemortem medical state of the deceased [1]. For instance, high vitreous glucose levels can indicate hyperglycemia [1, 2, 4, 5], the vitreous potassium concentration is extensively used to estimate the time of death [3, 6-8]; and sodium and chloride concentrations can be used to diagnose dehydration [9, 10] or drowning [11].

This thesis deals with postmortem analyses of vitreous fluid, their applications and limitations.

1.2. Vitreous fluid

1.2.1. Anatomy & physiology

The vitreous body is a colourless, transparent gel that fills up the eyeball. It is the least studied structure of the eye and was long considered to be an amorph, lifeless water solution. It is also the hardest structure of the eye to study, since its main feature is to be transparent in order to let light travel freely through the eyeball and hit the retina.

The vitreous body measures around 16 mm in axial length and has volume of around 4.5 mL. It is almost spherical with a depression anteriorly behind the lens, the patellar fossa. It consists mainly of water (99%), collagen and hyaluronic acid. [12]

Although there are no blood vessels in the vitreous, it is still metabolically active. Its main functions are to maintain the round structure of the eye, to serve as a shock absorber, to move solvents and solutes within the eye and to act as a deposit for metabolic wastes and short term needs for the retina. [13]

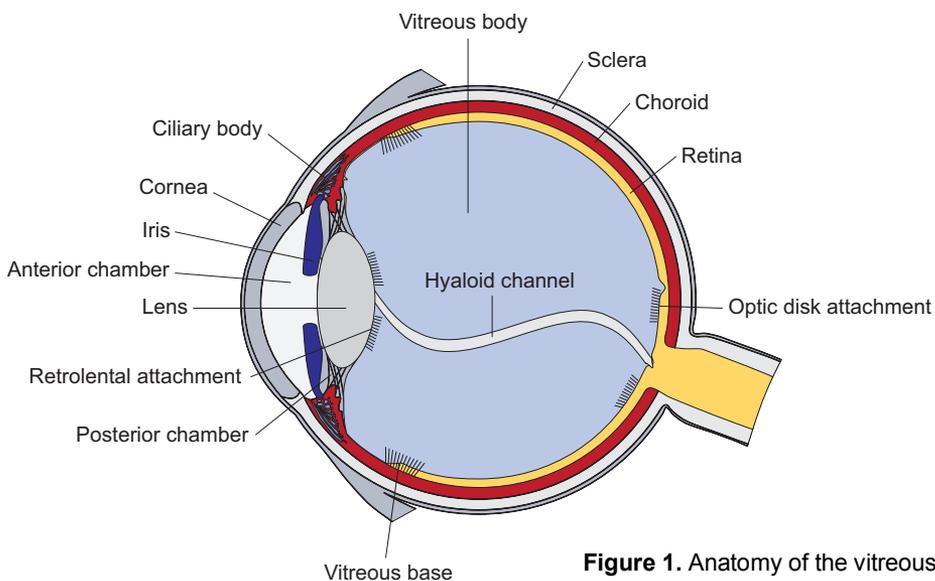


Figure 1. Anatomy of the vitreous body.

The outermost layer of the vitreous is called the vitreous cortex. It is approximately 100 µm thick and consists of tightly packed collagen fibrils, cells, proteins, and mucopolysaccharides. The fibrils run parallel and in a right angle to the retinal surface [13]. Although the cortical vitreous represents only 2% of the total vitreous volume, it is the metabolic centre of the vitreous body, because it contains hyalocytes and fibrocytes. The hyalocytes have been found to both produce hyaluronic acid and glycoproteins, as well as to function as phagocytes [14]. Inward of the cortex there is a medullary zone that contains fine, continuous fibrils that run anteroposteriorly. Most of the medullary vitreous is essentially a cell-free mixture of collagens and hyaluronic acid. In the centre of the vitreous there is an S-shaped hyaloid channel called Cloquet's canal. It runs from the lens to the optic nerve disc and is the former site of the hyaloid artery, which supplied the lens with nutrients during embryology. It has a diameter of 1-2 mm and contains no collagen. The wall of Cloquet's canal does not consist of a true membrane, it is rather formed by a vitreous condensation. [15]

There are several attachments between the vitreous body and the surrounding structures. The strongest is called the vitreous base and is located near the ora serrata, see figure 1. The vitreous base consists of thick collagen fibrils that are more densely packed and that attach to the basement membrane of the ciliary body and to the retina. Other attachments are located at the posterior lens, at the optic disc, at the macula and to retinal vessels. [13]

1.2.2. Age related changes

The vitreous of an infant is a homogeneous, gel-like substance. With age, the gel volume decreases and the liquid volume increases, a process that is called vitreous liquefaction. At the age of 40 years, the vitreous is 80% gel and 20% liquid, and by 80 years it is 50% liquid. Both HA and collagen may be affected by free radicals that cause dissolution of the HA-collagen complex: collagen fibrils move out of the collagen network, causing them to unite into

fibres and then into bands. The redistribution of collagen leaves spaces next to these bundles, allowing pockets of liquid vitreous called lacunae. [13]

1.2.3. Biochemistry

Vitreous fluid is a dilute solution of salts, soluble proteins, and hyaluronic acid contained within a meshwork of collagen. Vitreous is around 99% water and has been described as having connective tissue status and being an extracellular matrix. [13] The rest of the vitreous consist of salts (0.9%) and proteins and polysaccharides (0.1%).

a) Collagen

Collagen is the major structural protein of the vitreous. Collagen is composed of three individual polypeptides known as alpha chains, organised in a triple helix configuration. Triple helixes join together and form collagen fibrils, which in turn form collagen fibres, see Figure 2. Most of the collagen in the vitreous is type II. [14]

Vitreous collagen consists of nonbranching fibrils 7 to 28 nm in diameter. The vitreous base has the highest density of collagen fibrils, followed by the posterior and then anterior vitreous cortex. [15]

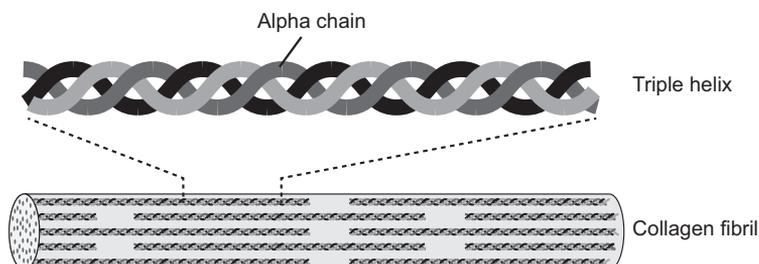


Figure 2. Structure of collagen.

b) Hyaluronic acid

Hyaluronic acid (HA) is an anionic, nonsulfated glycosaminoglycan (GAG). It is a long, unbranched molecule coiled into a twisted network. HA is connected at specific sites at collagen fibrils and maintains the space between the fibrils.

Collagen fibrils and HA form a network where the collagen fibrils provide a frame-like structure that is inflated by the hydrophilic HA, see figure 3. If collagen is removed, the remaining HA forms a viscous solution, if HA is removed, the gel shrinks. [14]

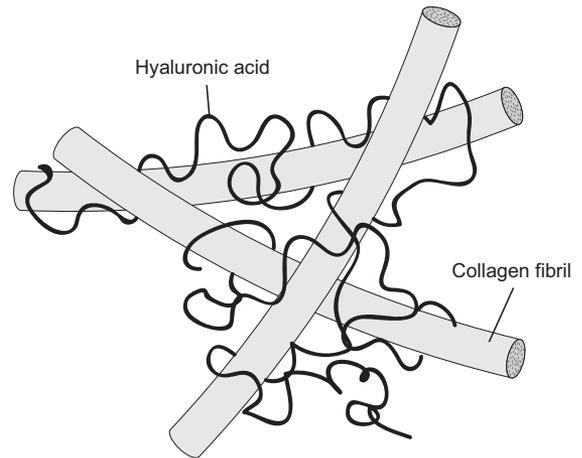


Figure 3. Collagen and hyaluronic acid interaction.

c) Cells

Cells normally found in the vitreous are hyalocytes and fibrocytes. Hyalocytes are the most abundant (90%) and are found in the vitreous cortex. They produce HA and function as phagocytes. Fibrocytes are primarily located in the vitreous base and are believed to produce collagen. [14]

d) Low molecular weight substances

Regarding low molecular weight substances like electrolytes and glucose, vitreous fluid has been studied extensively on deceased humans during postmortem investigations, where it is used as a substitute for blood, which often cannot be analysed due to postmortem degradation [1, 2]. There is however surprisingly little research on the concentrations of these substances in the vitreous of the living. Virtually all data are obtained from animals. This may be acceptable when studying for example the relationships between concentrations in vitreous fluid and blood, but absolute values of electrolyte concentrations from animals cannot be applied to humans, since studies have

shown considerable species variations. Table 1 shows published serum/plasma and vitreous levels for various substances in different species. Blood and vitreous samples were taken simultaneously during euthanasia or anaesthesia. The human data are obtained from blood and vitreous samples taken during vitrectomy.

In summary:

- Sodium levels are slightly lower in blood than in vitreous fluid.
- Potassium shows different vitreous/blood ratios, depending on species/study.
- Chloride levels are slightly higher in vitreous fluid than in blood.
- Calcium levels are lower in vitreous fluid than in blood.
- Glucose levels are lower in vitreous fluid than in blood

e) Gas pressures

Oxygen supplied by the retinal, choroidal and ciliary arteries dissolves in the vitreous body and diffuses among the surrounding tissues such as the lens, the ciliary body and the retina. The retina requires a continuous supply of oxygen to support visual processes; the lens however is damaged by high oxygen levels that can cause cataract. As a consequence, oxygen gradients exist

across the vitreous body in vivo [16]. Sakaue et al [17] measured oxygen tensions in the human eye during vitrectomy and found levels of 2.2 kPa in the anterior vitreous, 2.1 kPa in the central and 2.7 kPa in the posterior vitreous. Mathematical models have suggested larger gradients in oxygen

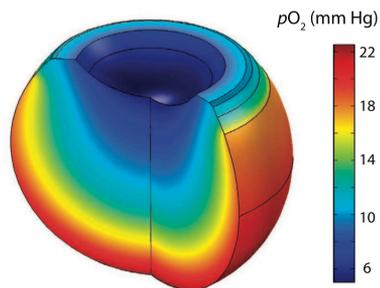


Figure 4. Mathematical model of oxygen distribution in the vitreous body. From Filas et al: "Computational model for oxygen transport and consumption in human vitreous". @ ARVO

Table 1. Comparison between plasma/serum and vitreous levels for electrolytes, glucose and lactate in different species

		Na ⁺	K ⁺	Cl ⁻	Ca ²⁺	Glu	Lac	PO ₂	PCO ₂
Reference values for human blood*		135 -145	3.5 - 5.1	95 - 110	2.1 - 2.8	3.8 - 6.1	4.5 - 19.8	4.0 - 5.3 (venous)	5.5 - 6.8 (venous)
Human [18]	Vitreous					3.5			
	Plasma					9.1			
Human [19]	Vitreous		4.2					18.6	3.3
	Plasma		3.6					6.3	6.2
Rabbit [20]	Vitreous	134	9.5	105		3.0	12		
	Plasma	143	5.6	109		5.7	10.3		
Rabbit [21]	Vitreous		5.2						
	Plasma		5.0						
Dog [21]	Vitreous		5.4						
	Plasma		3.9						
Cow [22]	Vitreous	137	4.6	120	1.4				
	Serum	145	6.3	103	2.5				
Cow [23]	Vitreous	142	5.6		1.6				
	Serum	146	6.9		2.6				
Cow [24]	Vitreous					2.4	4.4		
	Serum					3.8	4.2		
Horse [25]	Vitreous	134	4.3	113	1.9				
	Serum	140	5.2	99	3.0				
Pig [22]	Vitreous	142	5.0	118	1.4				
	Serum	152	7.5	103	2.8				
Rhesus monkey [26]	Vitreous		3.6		2.6				
	Plasma		4.2		4.9				

*reference levels used by the clinical chemistry laboratory at Karolinks Hospital, Stockholm, Sweden.

tension, see Figure 4 [16]. Sato and Tamai [19] measured the oxygen levels in the vitreous and found it to be around 18,6 kPa, and reported carbon dioxide levels around 3.3 kPa, but these values were obtained after insufflation of irrigating air.

f) Other compounds

The vitreous also contains low concentrations of various other substances, such as bicarbonate, proteins, lipids and amino acids.

1.2.4. Intraocular fluid dynamics

Metabolites enter and leave the vitreous body at two sites: via the aqueous humour chambers and via the retina.

Aqueous humour

Aqueous humour is a transparent, gelatinous fluid similar to plasma, but containing low protein concentrations. It is found in the anterior chamber (the space between the cornea and the lens, with a volume of $\sim 250 \mu\text{L}$) and the posterior chamber (the space between the posterior iris, the ciliary body and the anterior vitreous, with a volume of $\sim 60 \mu\text{L}$), see Figure 5. The aqueous humour maintains the intraocular pressure of the eye and provides nutrients for the avascular cornea and lens. [13]

Aqueous humour is produced by the ciliary body and is secreted into the posterior chamber. It flows around the lens and through the pupil into the anterior chamber. It leaves the anterior chamber through the trabecular meshwork into Schlemm's canal and to episcleral veins, see Figure 5.

Blood-ocular barriers

Much like the central nervous system, which is separated from the circulating blood by the blood-brain barrier, the ocular fluids are separated from the circulating blood by two so called blood-ocular barriers:

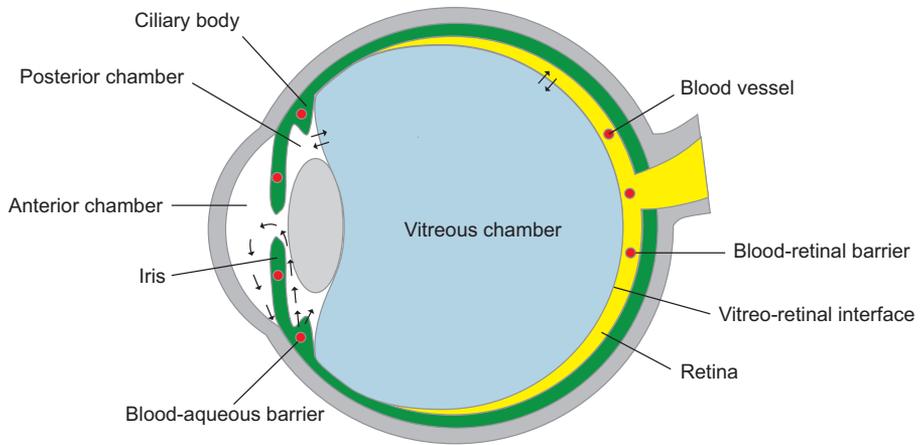


Figure 5. Blood ocular barriers.

The aqueous chambers are separated from the circulating blood by the *blood-aqueous barrier*, which consists of the ciliary epithelium and capillaries of the iris. Here, inward movements of metabolites from the blood into the eye predominate. [27]

The vitreous chamber and the retina are separated from the circulating blood by the *blood-retinal barrier*, which consists of non-fenestrated capillaries of the retinal circulation and tight-junctions between retinal epithelial cells. The blood-retinal barrier allows only a few important metabolic products to enter the retina; here outward movement from the vitreous into the blood predominates. [27]. See Figure 5.

The border between the vitreous body and the retina is called the *vitreoretinal interface*. It is not really a border, but a complex formed by the vitreous cortex and the basal laminae of the adjacent retinal cells [28]. Between the posterior chamber and the vitreous chamber there are no barriers – there is free diffusion between aqueous and vitreous humour [27].

After intravenous injection, test substances penetrate through passive or facilitated diffusion into the vitreous in minimal amounts, always reaching a higher concentration in the anterior vitreous, entering from the posterior chamber and ciliary circulation rather than through the blood-retinal barrier. [27]

The blood-ocular barriers prevent larger molecules like proteins to enter the intraocular chambers – proteins only reach 1/200 of their concentrations in plasma [27]. Low molecular weight substances, however, are not affected by the barrier system, but enter the vitreous through passive diffusion. It is therefore expected that the levels of such substances in the vitreous would be the same as in other extracellular fluids like plasma or cerebrospinal fluid. As can be seen in Table 1, this is not the case, since the metabolic activities of the vitreous cells and adjacent tissues, such as the retina, ciliary body or lens, have a significant influence on the concentration of these compounds. [29]

The blood-retinal barrier has a low permeability even for low molecular weight substances, the blood-aqueous barrier is somewhat looser. Because of these barriers, changes in the blood stream are reflected relatively slowly in the vitreous body. After an intravenous bolus injection of glucose for example, vitreous glucose levels reach 78% of the steady state concentration after 15 minutes and 93% after 30 minutes – the vitreous steady state concentration being 49% of the plasma glucose concentration. [30]

1.3. Postmortem analyses of vitreous fluid

1.3.1. Glucose and lactate

Death from diabetic coma with severely elevated blood sugar levels (hyperglycemia) can be difficult to diagnose. There are no obvious findings at

autopsy or at the microscopic examination. The analysis of blood sugar (glucose) can result in highly erratic values due to postmortem degradation of blood cells. Instead, glucose levels can be measured in the vitreous fluid and provide information on the decedent's blood sugar levels at the time of death.

There are two types of diabetic coma:

- 1.) *Diabetic coma with ketoacidosis* – these are often type I diabetics who have not taken their insulin or who not yet have been diagnosed with diabetes. Blood glucose levels are usually around 15-25 mmol/L and the hyperglycemia is accompanied with ketoacidosis.
- 2.) *Hyperosmolar syndrome* – these are often type II diabetics who have not taken their medication or who not yet have been diagnosed with diabetes. Blood glucose levels do usually exceed 30 mmol/L and there is no ketoacidosis.

Nauman [31], Sturner [4] and Coe [32] were the first to measure glucose levels in postmortem vitreous in the 1960's. Since then, analysis of vitreous glucose has been widely used in forensic medicine, but there are still some unanswered questions regarding the postmortem diagnosis of hyperglycemia.

Postmortem vitreous glucose levels cannot be simply compared to blood glucose levels in the living. Firstly, vitreous glucose levels are only about half of blood glucose levels [18, 20, 22]. Secondly, vitreous glucose levels decrease consistently, although to a limited extent, in the early phase after death. This means that in most postmortem vitreous samples collected a day or two after death, the glucose levels often are close to zero in healthy individuals.

The reason for the decrease in postmortem vitreous glucose levels is most likely that even after death, glucose keeps being consumed by surviving vitreous and retinal cells in the vitreous. When the organism is dead and no oxygen is available, surviving cells turn to anaerobic glycolysis, implying that glucose is converted to lactate. Based on this biochemical knowledge, certain authors have suggested to also analyse lactate in vitreous fluid and to evaluate the sum of glucose and lactate levels. In 1969, Traub [33] suggested that if the

sum of glucose and lactate concentrations exceeds 362 mg/dL in cerebrospinal fluid, death from hyperglycemia should be suspected. Based on the same argument, Sippel and Möttönen [5] suggested a sum value of 410 mg/dl in vitreous fluid as an indication of diabetic coma. Several other authors have adapted Traub's sum value [34-36] and the method has been used widely in forensic medicine. Since one glucose molecule yields two lactate molecules, lactate must be divided by two when using the mmol/L unit, which means that according to Sippel and Möttönen, hyperglycemia is suspected if $\text{glucose} + \text{lactate}/2 > 23 \text{ mmol/L}$.

The postmortem diagnosis of hypoglycaemia, i.e. fatally low glucose levels due to an overdose of insulin for example, is even harder to make, since, as stated earlier, postmortem vitreous glucose levels are near zero. Traub however suggested that lactate levels can be of help also in these cases, meaning that if $\text{glucose} + \text{lactate}/2 < 8.8 \text{ mmol/L}$, hypoglycaemia should be suspected.

Some of the aims of this project were to investigate the postmortem stability of vitreous glucose levels, to evaluate the applicability of Traub's formula and to suggest cut off values for postmortem vitreous glucose concentrations that indicate antemortem hyperglycemia.

1.3.2. Potassium and the estimation of the postmortem interval

An important task for a forensic pathologist is to estimate the time of death, based on calculations of the postmortem interval (PMI). Developing an easy and reliable method to estimate the PMI has become somewhat of the Holy Grail in forensic medicine. Despite all kinds of new technologies, the most common method in clinical use today is still the measurement of the rectal body temperature. This method can however only be applied during the time window when the body has still not acquired the same temperature as the ambient air, which typically means about 24 hours. Other methods include skeletal muscle responses upon mechanical or electrical stimulation, or pupil

reactions upon chemical stimulation, but these methods can also only be applied in the early postmortem period. After that, the forensic pathologist has to rely on the assessment of rigor mortis, livor mortis, state of decomposition and insect colonisation, all of which are influenced by various factors that make them less accurate.

One method that forensic scientists have been explored for decades is the evaluation of the rise in potassium concentration in the vitreous fluid. Under physiological conditions, potassium is prevalent in high and similar levels in all cells of the body, about 150 mmol/L [37]. After death, active membrane transport and selective membrane permeability are lost and gradually potassium leaks out of the cells to the surrounding extracellular fluid. The vitreous body contains very few cells, and hence in a sample from vitreous *in vivo*, the potassium level is similar to any other extracellular fluid, i.e. around 3-5 mmol/L, see Table 1. After death, a rise in vitreous potassium due to leakage from surrounding cells in the retina and choroid takes place, and since the 1960s this rise has been used to estimate the PMI [6-8, 38-52]. Still, despite extensive research there is still no agreement on the most accurate equation to describe this rise and to estimate the PMI (see examples in Figure 6 and Table 2).

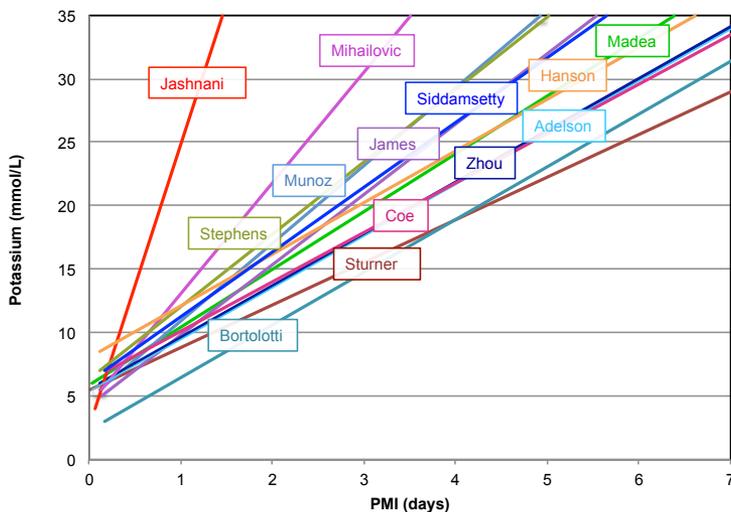


Figure 6. Different equations for estimating the PMI and their regression lines.

Table 2. Equations for the rise of potassium in vitreous.

Authors (year)	Equation (hrs)	n	Max PMI (h)	Comments
Adelson et al. (1963) [6]	$PMI = 5.88 [K^+] - 31.53$	209	21	-
Sturner & Gantner (1963) [7]	$PMI = 7.14 [K^+] - 39.1$	125	104	-
Hanson et al. (1966) [8]	$PMI = 5.88 [K^+] - 47.1$	203	310	-
Coe (1969) [32]	$PMI = 6.15 [K^+] - 38.1$	145	100	A separate equation was provided for a PMI < 6 h.
Stephens & Richards (1987) [42]	$PMI = 4.20 [K^+] - 26.65$	1427	35	Outliers, drownings, SIDS, electrolyte imbalances, and temperature extremes were excluded.
Madea et al. (1989) [43]	$PMI = 5.26 [K^+] - 30.9$	107	130	Cases involving elevated urea and prolonged agony were excluded.
James et al. (1997) [46]	$PMI = 4.32 [K^+] - 18.35$	100	80	Also included hypoxanthine.
Munoz et al. (2001) [47]	$PMI = 3.92 [K^+] - 19.04$	133	40	Only non-hospital cases were examined, there was a change in variables.
Zhou et al. (2007) [50]	$PMI = 5.88 [K^+] - 32.71$	62	27	-
Jashnani et al. (2010) [51]	$PMI = 1.076 [K^+] - 2.81$	120	50	Mostly included cases involving sepsis or tuberculosis.
Bortolotti et al. (2011) [53]	$PMI = 5.77[K^+] - 13.28$	164	110	-
Mihailovic et al. (2012) [54]	$PMI = 2.749 [K^+] - 11.98$	32	30	Repetitive sampling.
Siddamsetty et al. (2013) [55]	$PMI = 4.701 [K^+] - 29.06$	210	170	-
Present study	$PMI = \frac{\ln\left(\frac{M - C_0}{M - [K^+]}\right)}{L_0 + m_A A + m_T T}$	462	409	No cases were excluded. The proposed equation includes temperature and patient age.

Basically all previous equations are based on the approximation of the diffusion of potassium from the surrounding cells to the vitreous according to a linear model. This might be reasonable during a certain time period after death, but is not appropriate in the very early phase, and particularly not in the late postmortem interval, when vitreous potassium may be the most important means to estimate the PMI. Most likely, the diffusion curve will display an S-

shaped form, which can be right- or left-shifted, and show different slopes depending on various influencing factors. The amount of potassium in the cells in the eyeball is limited, and therefore the increase in the vitreous must form an asymptotic curve.

Several factors that might influence the rise in potassium concentration have been proposed:

- the ambient temperature [22, 40, 44, 56, 57]
- the duration of the terminal episode [6, 43]
- renal failure with increased extracellular potassium levels, reflected by elevated urea concentration [43]
- alcohol level at the time of death [43, 58]
- sampling method and instrumentation used for analysis [50, 59, 60]

One of the aims of this thesis was to investigate factors that influence the postmortem increase in vitreous potassium concentration and to improve the estimation of the postmortem interval (PMI) by means of the vitreous potassium concentration.

1.3.3. Sodium and chloride

Sodium is the major cation and chloride the major anion in the extracellular fluid. Normal serum levels for living humans are 135-145 mmol/L and 95-110 mmol/L for sodium and chloride, respectively. Sodium combined with chloride results in table salt. Excess sodium and chloride from food intake is excreted in the urine. Sodium is important for the regulation of the total amount of water in the body.

Sodium is by far the most osmotically active ion, so the serum sodium concentration normally reflects the osmolarity of the extracellular fluid. The total body sodium content also determines the volume of the extracellular

fluid compartment. Water balance and plasma osmolarity, and thereby serum sodium levels, are firmly adjusted by the kidneys, which constantly alter the urine concentration, so that constant plasma osmolarity is maintained. Extreme sodium and chloride imbalances can cause the cells in the body to swell or shrink, which can be fatal. [61]

Deranged sodium and chloride levels can arise from a variety of illnesses, such as kidney failure, liver failure, cancer or diarrhea. In a forensic setting cases may for example concern:

- *dehydration* – may be very important to diagnose properly, especially in cases of neglect of children or the elderly [1, 62];
- *salt intoxication* – mostly seen in children who have been force fed salt as a punishment or due to a Munchhausen by proxy syndrome [62, 63];
- *water intoxication* – a condition called psychogenic polydypsia, where psychiatric patients compulsively drink large amounts of water [64]. This can lead to brain swelling, seizures and death. Water intoxications can also occur during MDMA (ecstasy) use [65];
- *beer potomania* – a condition similar to water intoxication. Large ingestion of beer, together with poor food intake, can lead to severe hyponatremia [66];
- *drowning* – in theory, aspiration of water during drowning leads to hyponatremia in fresh water and to hypernatremia in salt water. Some authors have proposed that vitreous sodium/chloride levels can be used to distinguish salt water from fresh water drownings [11] or drownings from non-drownings [67].

Just like analysis of glucose and potassium, analysis of sodium or chloride levels in postmortem blood will lead to erratic results due to degradation of the many cells that are present in blood. Instead, vitreous fluid can be used to analyse sodium and chloride levels in deceased persons.

Surprisingly, there are no reliable published data on electrolyte levels in the vitreous of living humans. Studies on different animals however have shown that vitreous sodium levels are ~95% of those in blood and vitreous chloride levels ~110%. [22, 23, 25, 68]

Quite a large number of studies have been published regarding the post-mortem changes in vitreous sodium and chloride levels. However, most of these studies have focused on the usefulness of sodium and chloride levels in the estimation of the time of death and not on the diagnosis of sodium or chloride imbalances. There are no reference ranges for postmortem sodium or chloride levels and the interpretation of these is still a challenge for forensic pathologists.

Author, year	Sodium	Chloride
Coe, 1969 [32]	↓	↓
Blumenfeld, 1979 [69]	→	→
Balasoorya, 1984 [70]	↓	
Famer, 1985 [71]	↓	
Madea, 2001 [72]	→	→
Tao 2006, [73]	↓	↓
Jashnani, 2010 [51]	→	→
Tumram, 2011 [52]	→	↓
Chandrakanth, 2013 [74]	↓	↓
Mitchell, 2013 [75]	↓	↓
Siddamsetty, 2014 [55]	↓	→

Table 3. Postmortem changes of sodium and chloride according to different authors.

Table 3 summarises findings from different authors. There seems to be no real agreement about the postmortem stability of sodium and chloride, although it should be mentioned that the postmortem decrease that was found by some authors was minor.

How postmortem vitreous sodium and chloride levels should be interpreted, still remains essentially inconclusive. In a publication from 2005 on the postmortem diagnosis of hypertonic dehydration by Madea and Lachenmeier [10] it is stated that there are several conceptual problems surrounding postmortem vitreous sodium values that still have to be resolved, for example the correlation of postmortem vitreous values with antemortem serum values and the postmortem changes of vitreous sodium.

The aims of this part of the thesis were:

- To evaluate the postmortem changes of vitreous sodium and chloride.
- To investigate whether postmortem vitreous sodium levels reflect antemortem serum sodium levels and thus can be used to identify antemortem imbalances.
- To correlate sodium and chloride levels to specific causes of death.
- To establish postmortem vitreous reference concentrations for sodium and chloride.

1.3.4. Oxygen and carbon dioxide

In the alveoli of the lungs, oxygen and carbon dioxide (a metabolic waste product) are exchanged. In a living person, arterial blood can be analysed for the partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2) and give information about the person's lung function. In a forensic setting, it would be interesting to be able to analyse blood gases in order to obtain support for the diagnosis of asphyxia. Suffocation, e.g. due to external application of a pillow over the face, can cause fatal asphyxia without leaving any trace. Such a

victim may show a pallor of the skin corresponding to the object pressed against the face, and severe cyanosis on the surrounding skin, but this pattern will fairly rapidly fade after death. However, the PCO_2 would rise tremendously and PO_2 drop during an extended asphyxia, as reported experimentally [76]. Unfortunately, postmortem gas analyses in blood will give highly erratic results due to significant contact between the blood vessels and air within the gastro-intestinal system and due to a highly variable metabolism of the large amounts of cells present in the blood. The vitreous body, being isolated and with limited cell content, is expected to show a more predictable change in the gas pressures after death. Hence, it is possible that elevated PCO_2 caused by antemortem asphyxia will present as a time curve after death that parallels, but runs above the average levels in subjects with normal PCO_2 levels at the time of death.

There is only one previous study on CO_2 (CO_2 combining power) in postmortem vitreous fluid, performed by Coe [26] and briefly discussed in a later article also by Coe [1], where he found that CO_2 levels were surprisingly stable.

Since we used a blood gas instrument for analysing glucose, lactate and electrolytes in the vitreous samples, we also obtained pCO_2 and pO_2 values for each case. It is possible that these values can be used to detect cases of antemortem asphyxia, but this requires future studies.

2. AIMS

The overall aims of this study were

- to investigate the stability of vitreous postmortem concentrations of glucose, sodium and chloride and to establish reference values for these substances
- to assess the usefulness of analysing postmortem vitreous lactate values in the diagnosis of antemortem hyper- and hypoglycemia
- to investigate factors that influence the postmortem increase in vitreous potassium concentration and to improve the estimation of the postmortem interval (PMI) by means of the vitreous potassium concentration

3. MATERIAL AND METHODS

3.1. Study design

Between January 2003 and June 2006, vitreous fluid was systematically collected from consecutive deceased subjects brought to the department of forensic medicine in Stockholm as soon as possible after their arrival at the morgue. Using a 1 mL syringe equipped with an 18-gauge needle, 0.2 mL vitreous fluid was aspirated from the centre of each eye and pooled in the same syringe. Samples from severely decomposed bodies, and from infants were not consistently included, since toxicological analyses were prioritised in several of these cases. The vitreous samples were analysed with a blood gas instrument, ABL 625 (Radiometer, Copenhagen), by direct injection of the content of the syringe.

The glucose electrode contains a chamber where glucose oxidase converts glucose to gluconic acid and hydrogen peroxide. At the anode, the hydrogen peroxide will be oxidised, and the liberated electrons will cause a current in proportion to the glucose concentration in the sample. The lactate is also measured with an amperometric electrode equipped with an enzymatically active membrane, a method that has been shown to yield readings that correlate very well with those of one of the most widely used standard laboratory methods. The Radiometer instrument measures all electrolytes with ion-selective electrodes. Inter-assay imprecision for potassium, glucose and lactate was less than 0.3 mmol/L. Limit of quantification for these analytes was 0.1 mmol/L. In total, complete results for glucose, lactate, potassium, sodium and chloride were obtained for 3076 cases.

3.2. Evaluation of procedures

To investigate the robustness of the methodology the following studies were conducted. In almost all cases a second vitreous sample was collected for toxicology, during autopsy, typically performed 1–3 days after the first sample was collected. To this end, the whole vitreous from both eyes was

collected in tubes containing NaF and submitted for toxicology. In 49 cases, these samples were analysed for glucose and lactate with standard clinical chemistry technique based on enzymatic conversion yielding NADH, measured as the absorbance at 340 nm with a Hitachi 917 instrument. The comparison of the first and second sample thus included different sampling techniques, different sampling times, difference in additives and difference in analytical methods. There were only small differences in the two samples in vitreous glucose concentrations, while lactate levels had increased during the postmortem storage of the bodies.

To address the possible importance of the immediacy of analysis, portions of vitreous fluid were analysed immediately and after 4 h, respectively. To investigate the possible influence of cell debris that to a variable extent may be included in the aspirate, 21 whole vitreous samples were transferred to Eppendorff tubes and centrifuged at 8,000 g for 10 min and the pellet and supernatant were analysed separately. Ten of the samples were subjected to sonication for 30 min before centrifugation. No significant differences were seen regarding the glucose, lactate or potassium concentrations when analysing the supernatant and the pellet separately, and in fact, whole vitreous samples with macroscopic presence of cellular debris, did not differ from those with transparent and colourless fluid regarding the analytes measured.

3.3. Estimation of ambient temperature

When a body was exposed to variable ambient temperatures, such as room temperature for A hours and then a colder temperature for B hours in the morgue prior to the collection of vitreous samples, the average temperature was calculated according to the following equation:

$$\text{temp}_{\text{average}} = \frac{\text{time}_A}{\text{PMI}} \cdot \text{temp}_A + \frac{\text{time}_B}{\text{PMI}} \cdot \text{temp}_B$$

3.4. Mathematical modeling

Measurements of potassium concentration and other covariates (e.g., decedent age, average ambient temperature, blood alcohol concentration, and duration of the terminal episode) were used to develop a mathematical model for calculating a PMI distribution. Bootstrapping is a computer-based method for assigning measures of accuracy to sample estimates [77], and it was used to derive estimates of prediction errors for our non-linear model. Briefly, resampling of the data was performed by using the prediction of the model and adding a random residual to the prediction. This procedure creates a new dataset (but with similar statistics), which can be fitted with the model. Each time this was performed, the error between the prediction data and the resampled data was calculated. As a result, a distribution of errors around the prediction data could be used to estimate the real error of the prediction.

The main steps for estimating the error of such a prediction model are:

1. Selection of a dataset to fit the model.
2. Fitting of the model to the data.
3. Calculation of the residual between the PMI and the predictions.
4. Fitting of the absolute value of the PMI residuals with an exponential.
5. Normalising of the residuals with the fitted exponential so that the residuals are uniform for all of the PMI values.
6. Definition of a new dataset for which the PMI is to be estimated.
7. Application of the bootstrap method 999 times.
8. A 95% confidence interval is obtained for the prediction using the 0.025 and 0.975 quantiles.

This procedure has been implemented in the statistical programming language, R, using the non-linear, least-square fitting algorithm, *nls*, and the bootstrapping method, *boot*. To generate bootstrap samples, it was assumed that the error of the PMI was normally distributed with mean 0 and a standard deviation (SD) proportional to the exponential of the potassium concentration.

3.5. Statistics

All analytical data were compiled in a file to which data from the Swedish forensic pathology database [78] were linked. This database comprises detailed information of the decedent including – but not limited to – age, sex, cause(s) of death, manner of death, circumstantial information, medical history, additional findings and diagnoses, histopathologic and forensic toxicology results. The information is based on the autopsy results, the ancillary analyses, the police reports and, when available, medical records.

Kruskall–Wallis’ test was used for statistical comparisons of non-normally distributed groups. Statistica v7.1 (Statsoft Inc., Tulsa, OK), Statistica v10, (StatSoft Inc. Tulsa, OK) and Excel v14.5.3 (Microsoft) were used for statistical analyses.

Equations for predicting PMI based on potassium concentration and a combination of co-variates were generated and evaluated. The best-fit parameter set and confidence intervals for each model were obtained by using the R non-linear fitting routine, *nls*. The significance of decedent age and ambient temperature on increases in potassium level were estimated using t-statistics from asymptotic confidence intervals provided by *nls*.

3.6. Ethical aspects

All of the analyses were performed as a part of a forensic medicine investigation and were reported to the responsible forensic pathologist. Therefore, ethical permission was not required for the sampling and analyses conducted according to Swedish regulations. Nonetheless, ethical approval was obtained for the formation of a database for these data, and for the perusal of forensic medicine files, including police reports and medical records (Regional Ethical Review Board, Stockholm 2008/231-31/3).

4. RESULTS AND DISCUSSION

4.1. Study I: Postmortem identification of hyperglycemia

4.1.1. Vitreous glucose concentrations

A vast majority of the postmortem cases showed vitreous glucose levels falling short of 1 mmol/L. In total, 76 cases had vitreous glucose levels exceeding 10 mmol/L. Since it has been suggested that glucose levels in blood and vitreous drop with postmortem time, we explored the relationship between vitreous glucose levels with vitreous potassium levels as a proxy for the postmortem time, since the exact time of death was not be certified in most cases, see Figure 7. Known diabetic subjects are indicated in green and red. From this graph it can be appreciated that elevated vitreous glucose levels comprise both known diabetics and subjects with no such history. It is possible that some of the latter cases actually had recently contracted diabetes and developed hyperglycemia. The graph also shows that the cases with elevated vitreous glucose levels are distributed rather evenly across the potassium ranges, contradicting the notion that glucose levels continue to drop with postmortem time. For some of the cases with elevated glucose levels, the time of death was known with certainty, and several of these cases had a long postmortem interval (Figure 7, inset). However, Figure 8 shows that there is a slight drop in glucose levels in the very early phase of the postmortem interval, approximately corresponding to a concentration difference of 3 mmol/L. A closer look at the cases with a very short postmortem interval (as reflected by low potassium level), demonstrates a small, but conspicuous drop in glucose concentrations shortly after death (Figure 8).

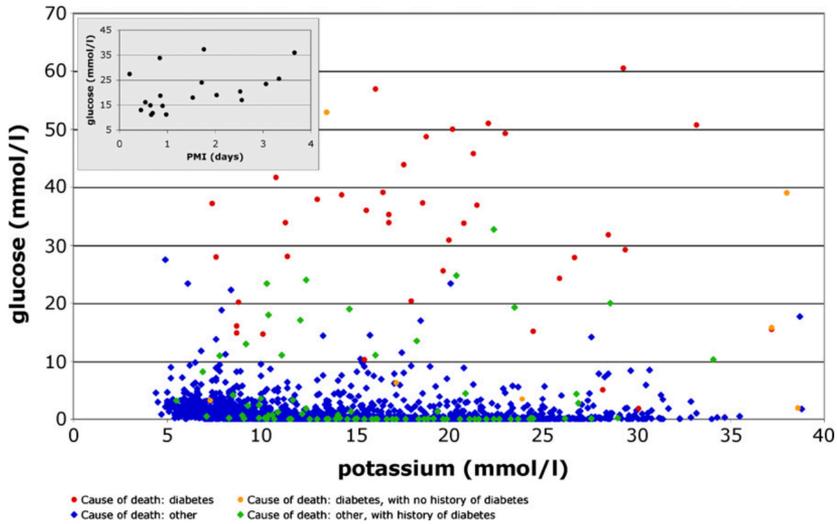


Figure 7. Vitreous glucose concentration plotted against vitreous potassium concentration. Insert shows glucose concentrations related to certified postmortem interval for select cases with elevated vitreous glucose levels.

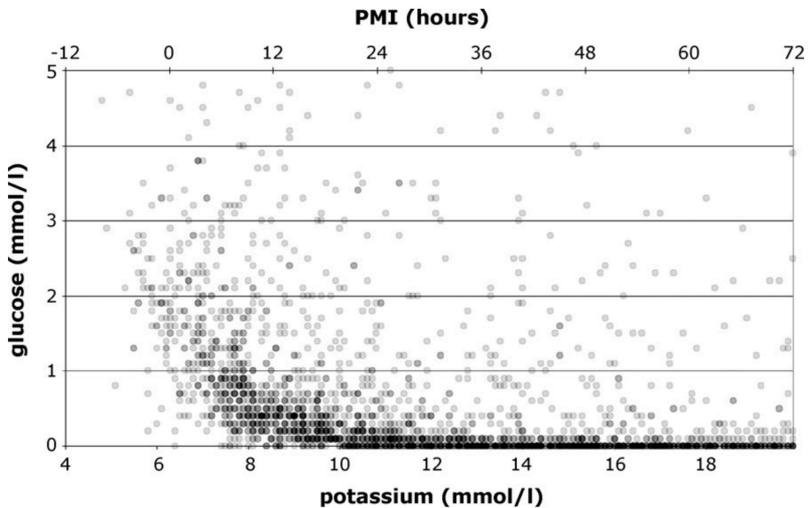


Figure 8. Vitreous glucose concentration plotted against potassium concentration in the early postmortem interval. The scale at the top shows an arbitrary scale of the postmortem interval calculated from the potassium values according to the equation suggested by Madea and Henssge [43].

The drop in glucose concentration corresponds to a steeper rise in lactate concentration with increased potassium level in the early phase (Figure 9). This pattern can be explained by anaerobic metabolism in vitreous cells shortly after death. The cases with a history of diabetes are also indicated in this figure, and as opposed to the pattern in Figure 7, these cases conform more to the general distribution of lactate levels across the range of potassium levels.

4.1.2. Evaluation of the sum value of vitreous glucose and lactate

To critically evaluate the notion that the sum value of vitreous glucose and lactate/2 reflect the original antemortem glucose level, this sum was plotted against the vitreous potassium concentration as a proxy of the postmortem interval (Figure 10). It should be observed that the initial drop in glucose

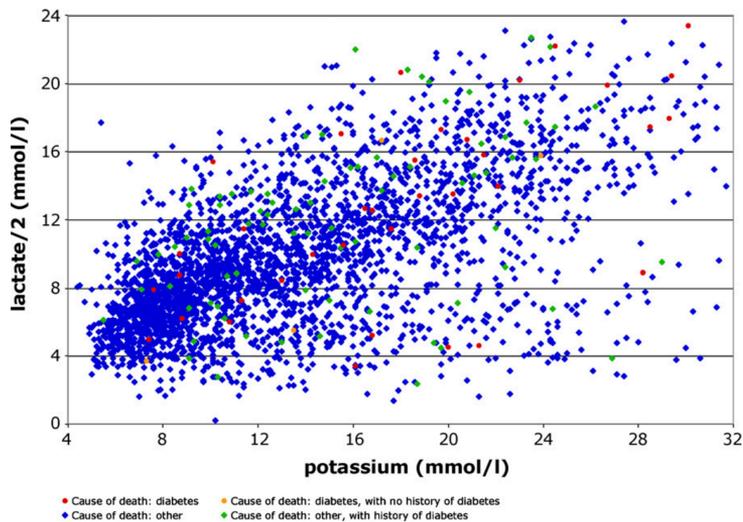


Figure 9. Vitreous lactate concentration plotted against vitreous potassium concentration.

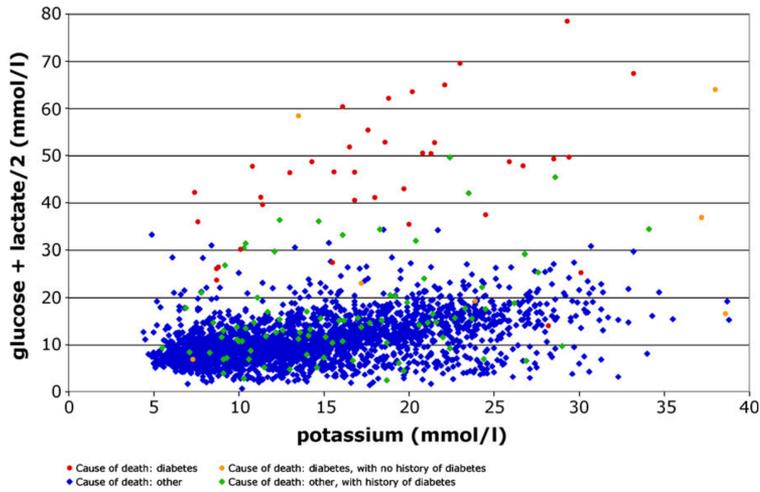


Figure 10. The sum value of vitreous glucose and lactate/2 plotted against the vitreous potassium concentration.

in the left-hand part of the graph compensates for the early, steeper rise in lactate levels, producing a straighter curve as compared to Figure 9. It should be appreciated, however, that the sum value is not constant, but increases with increasing potassium level. In fact, the sum value is significantly correlated with the potassium level. The reason is easily appreciated when the glucose, lactate and sum value curves are compared; the sum value curve is dominated by increasing lactate levels with increasing potassium level, whereas the drop in glucose levels is limited to the very early phase, and comes to a complete stop at about 24 h.

4.1.3. Hyperglycemia cases

In total, 76 subjects had a vitreous glucose level exceeding 10 mmol/L. In several cases elevated vitreous glucose levels were seen in subjects with no history of diabetes (Figure 7). In other cases with moderately elevated levels, strong physiological stress, e.g. due to brain trauma, could be assumed to

explain for this finding. In cases with certified hypothermia, a condition known to induce physiological stress, the highest vitreous glucose value was 6.0 mmol/L and in total this group only showed slightly higher average glucose level than the bulk of the study population.

4.1.4. Practical outcome

As a consequence of the introduced strategy, the frequency of deaths in diabetic coma at the department of Forensic Medicine in Stockholm doubled during the study period 2004–2006 as compared to the 10-year period 1992–2001; comprising 1.11% of all cases examined compared to 0.55% during the preceding period. At all other forensic medicine departments in Sweden, the corresponding figures were 0.50 and 0.38%, respectively. This implies that the strategy to analyse of vitreous glucose upon suspicion of diabetic come will overlook a number of such cases.

4.1.5. Discussion

We have shown that vitreous glucose levels decrease in the very early postmortem period (around 24 hours), but that the levels stay stable after 24 hours, and that glucose alone apparently is the most appropriate marker of antemortem hyperglycemia.

In order to more closely explore cases with elevated vitreous glucose levels, we decided to use an arbitrary limit of 10 mmol/L, based on our finding of a drop of vitreous glucose of about 3 mmol/L during the early postmortem phase and on clinical observations that the concentration of glucose in vitreous is about half of the concentration in blood [18]. Hence a glucose value of 10 mmol/L in a vitreous sample collected at about 1 day or more after death would theoretically correspond to an antemortem blood glucose level of about 26 mmol/L. It therefore seems likely that most subjects

displaying vitreous glucose exceeding 10 mmol/L died of diabetic coma, or that the hyperglycemic state contributed to death.

Several investigators have suggested that the sum value of vitreous glucose and lactate should be used to estimate the antemortem blood glucose level, based on the assumption that lactate is formed during anaerobic metabolism of glucose. Our observations argue against this theoretically appealing calculation, since lactate levels seem to increase after death, the major part coming from other sources than postmortem glycolysis. The sum of glucose and lactate cannot be used to detect antemortem hypoglycemia either – combined glucose and lactate/2 levels of > 8.8 mmol/L have been suggested to indicate antemortem hypoglycemia. In our material 1277 out of 3076 cases (42%) had glucose + lactate/2 levels under 8.8 mmol/L, which is an unreasonable high proportion.

We suggest that in the postmortem setting, vitreous glucose alone should be used to estimate the antemortem blood glucose concentration, and that lactate levels be left out.

4.2. Study II: A new model for the estimation of time of death from vitreous potassium levels corrected for age and temperature

4.2.1. General observations

The rise in vitreous potassium levels was not linear but followed an exponential curve. This curve became eventually asymptotic after about a week. Given that the amount of potassium present in the eye is finite, it is expected that intra- and extracellular potassium concentrations will eventually equilibrate after a certain period of time. It appears that equilibration occurs after about a week, and a concentration of ~ 35 mmol/L is achieved. (Figure 11)

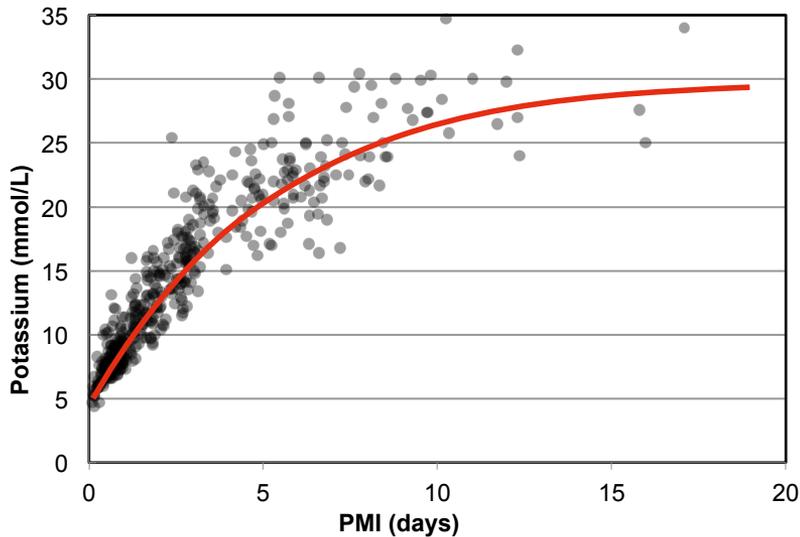


Figure 11. Potassium concentration (mmol/l) is plotted versus PMI (n = 462). The non-linear regression line is shown in red.

4.2.2. Influence of ambient temperature

Based on the basic potassium curve that was generated, various factors were assessed to evaluate their potential impact on potassium levels. First, the influence of ambient temperature was examined. Figure 12 shows that the higher the average ambient temperature, the steeper the increase in potassium concentration. In the early postmortem period (up to around four days after death), the distribution between different temperature intervals followed a clear pattern, whereas the picture was somewhat blurred at longer PMI's with fewer observations.

Adelson et al. [6], Sturner & Gantner [7], and Lie et al. [38] stated that temperature has no influence on the vitreous potassium concentration, but apart from that it has been presumed for a long time that the rise in potassium concentration is affected by the surrounding temperature. This seems

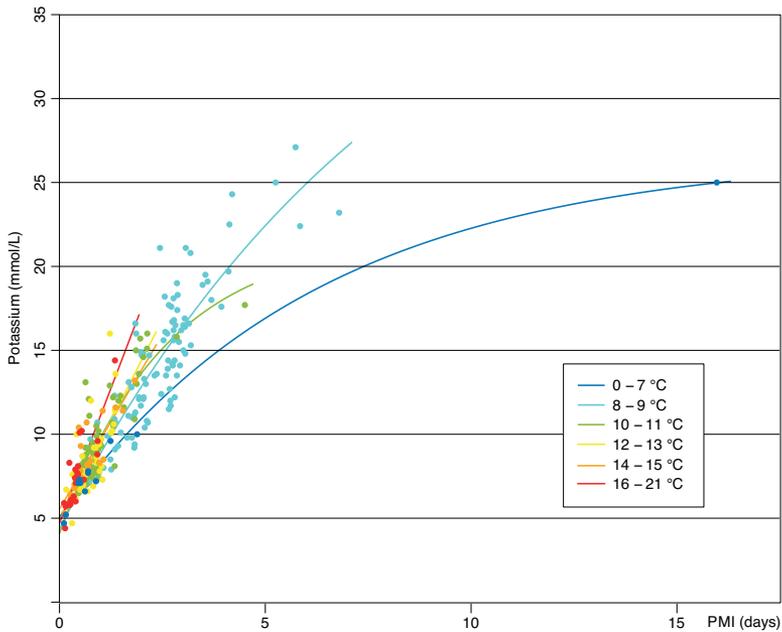


Figure 12. The influence of temperature on the postmortem rise in vitreous potassium levels. The colours represent different ambient temperatures reported in 272 cases.

reasonable, since cold temperatures slow most biological processes down. Several studies on animals have shown that the surrounding temperature affects the increase in potassium concentration [22, 40, 56, 57]. In a study of postmortem human subjects by Rognum et al. [44], different slopes were found to be associated with the rise in vitreous potassium concentrations at four different ambient temperatures. The results of this study confirm that the potassium concentration increased faster in bodies that were exposed to warm ambient temperatures.

4.2.3. Influence of decedent age

A major finding of the present study is that decedent age significantly affected the rise in potassium concentrations: the younger the subject, the

more rapid the increase (Figure 13). The differences were observed for every age group, from infancy to old age. Most previous authors have not considered decedent age at all. One exception is Coe [79], who found that vitreous potassium levels rise faster in infants than in adults. Madea et al. [80] argued that the reason for a faster potassium accumulation in the vitreous of children is due to the smaller diameter of the eye globe. A three month old child has a globe diameter of 11 mm, and therefore a shorter diffusion distance than a 14 year old child with a globe diameter of 16 mm. This seems very plausible, but it cannot explain the differences in potassium rise in the other age groups.

An explanation for the faster rise in young subjects could be, that the retina goes through a significant cell loss with age and the thickness of the retina decreases [81, 82]. A relatively larger cell mass in youth would result in a higher final potassium concentration in the vitreous (a higher value for M in the equation) and hence a steeper rise in the concentration if diffusion factors are not altered. It seems that the lines in figure 5 could be in accordance with a higher final steady state potassium concentration and hence a higher M .

4.2.4. Influence of other factors

Other factors that might influence the postmortem rise in potassium concentrations following death were examined, such as decedent gender, body weight, blood alcohol level and agonal period. None of these were found to influence potassium concentrations significantly. In the routine casework, these results are valuable since some of these factors are difficult to determine at a scene investigation.

Cause of death is sometimes suggested to influence the postmortem potassium concentrations. In Figure 14 the cases are marked in different colours for different causes of death. The cause of death does not seem to affect the rise in postmortem potassium levels.

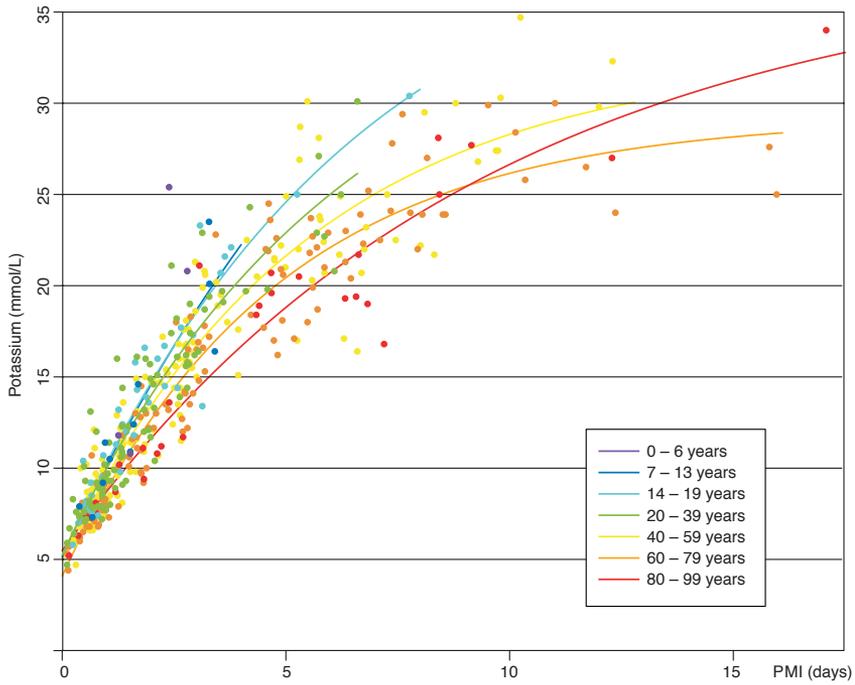


Figure 13. The influence of age on the postmortem rise in vitreous potassium levels. The colours represent the different age groups (n = 462).

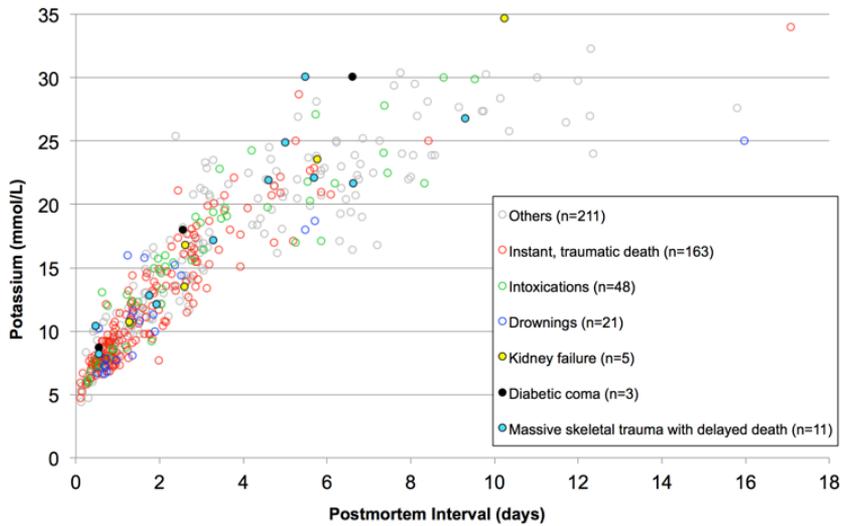


Figure 14. Potassium concentration relative to PMI for different causes of death.

4.2.5. A multivariate model for the estimation of time of death based on vitreous potassium concentration, decedent age, and ambient temperature

Based on our results, a new equation for the estimation of the PMI was created. This equation included the vitreous potassium concentration, the surrounding temperature and the decedent age.

The model for PMI prediction with $[K^+]$, age, and temperature is:

$$PMI = \frac{\ln\left(\frac{M - C_0}{M - [K^+]}\right)}{L_0 + m_A A + m_T T}$$

When the available temperature data for 272 subjects of the present cohort were used, the parameter values that gave the best fit for this equation were:

When the available temperature data for 272 subjects of the present cohort were used, the parameter values that gave the best fit for this equation were:

$$M = 34.44 \text{ mmol/L}$$

$$C_0 = 4.4 \text{ mmol/L}$$

$$L_0 = 0.135 \times 10^{-3}/\text{day}$$

$$m_A = -0.00112 \times 10^{-3}/\text{day} \times \text{year}$$

$$m_T = 0.00985 \times 10^{-3}/\text{day} \times \text{degree}$$

To simplify the calculation process for the user, we have also created a web application where the potassium concentration, decedent age, and ambient temperature (if available) can be entered into a form and the PMI with confidence intervals is calculated:

<http://dx.doi.org/10.6084/m9.figshare.1319545>

This web application can be updated with new data over time. By altering the constants of the equation accordingly, the precision of the model is expected to improve.

4.3. Study III: Interpretation of postmortem vitreous concentrations of sodium and chloride

4.3.1. Postmortem changes

This study shows that sodium and chloride levels slowly decrease with increasing PMI, here represented by potassium levels (Figure 15 and 16). One would assume that vitreous sodium and chloride levels would increase postmortem due to evaporation of vitreous fluid, but this is not the case. Vitreous sodium and chloride levels most likely decrease postmortem as a result of diffusion of these electrolytes into the surrounding cells of the retina and choroid. Intracellular fluid contains low sodium and chloride

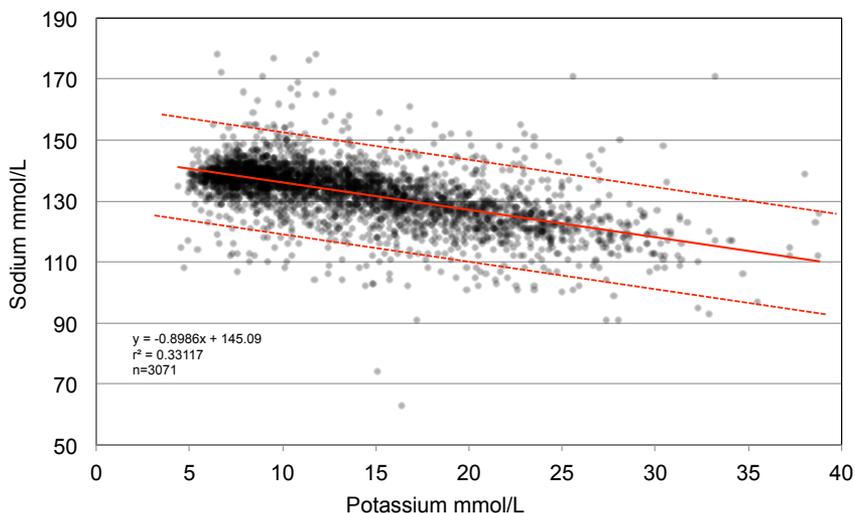


Figure 15. Sodium vs potassium. Red lines indicate mean and the 95% CI, suggesting cut off levels for hypo- and hypernatremia, considering matching potassium levels.

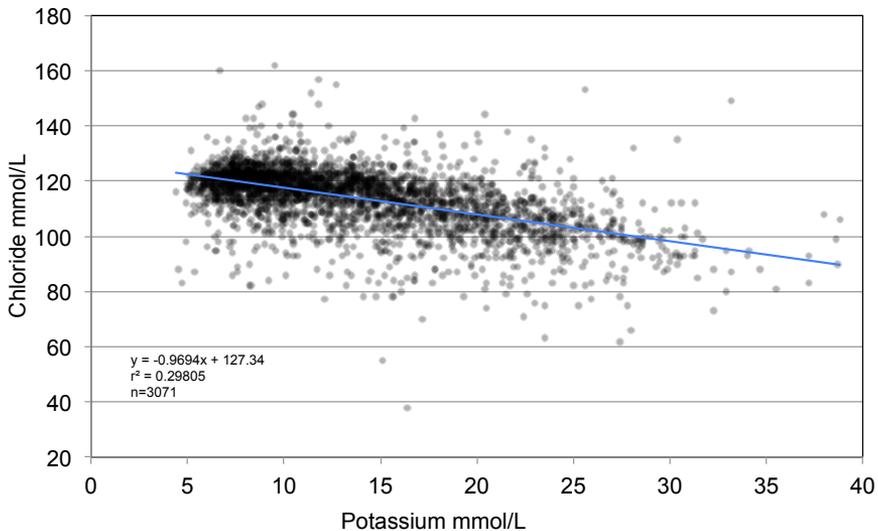


Figure 16. Chloride vs potassium.

concentrations and high potassium concentrations – interestingly, the same mechanism is responsible for the postmortem increase in vitreous potassium.

4.3.2. Correlation with antemortem serum sodium concentrations

The red lines in Figure 15 represent the mean and 95% confidence interval in relation to the potassium levels, based on linear regression analysis. All cases outside the 95% confidence interval were reviewed regarding the cause of death. In virtually all these cases, the diverging sodium and chloride levels could be reasonably explained, e.g. pneumonia, water intoxication or fresh water drowning.

In 46 cases, we had access to serum sodium values that were obtained in a hospital shortly before death. Antemortem serum sodium values correlate

very well with postmortem vitreous sodium values, especially in cases with low potassium values, i.e. short PMIs. For longer PMIs, postmortem vitreous sodium values are progressively lower, as expected. (Figure 17)

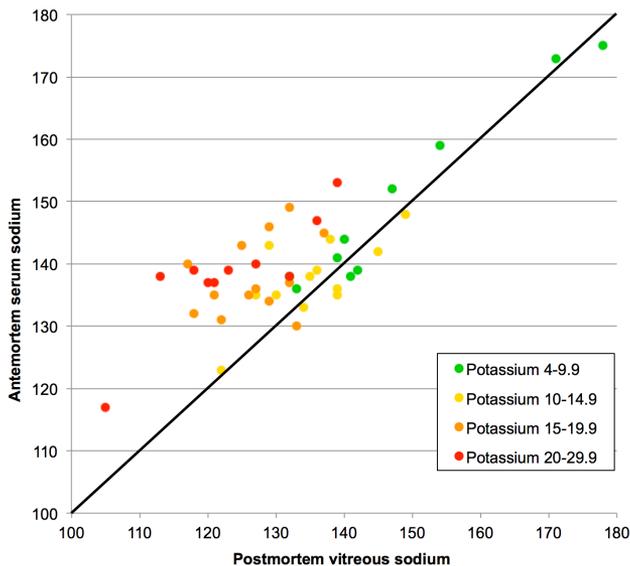


Figure 17. Comparison ante-mortem hospital serum sodium levels with postmortem vitreous. n=46. The black line indicates identity.

4.3.3. Drownings

Vitreous sodium levels in cases of drownings were examined. Figure 18 shows that cases of freshwater drownings had significantly lower sodium levels than Baltic Sea water drownings (brackish water) ($p < 0.01$) and cases of non-drownings ($p < 0.001$). Baltic Sea water drownings had virtually the same sodium and chloride levels as non-drownings ($p = 1.0$). We found that the low sodium values in freshwater drownings clearly correlate with immersion time (Figure 19).

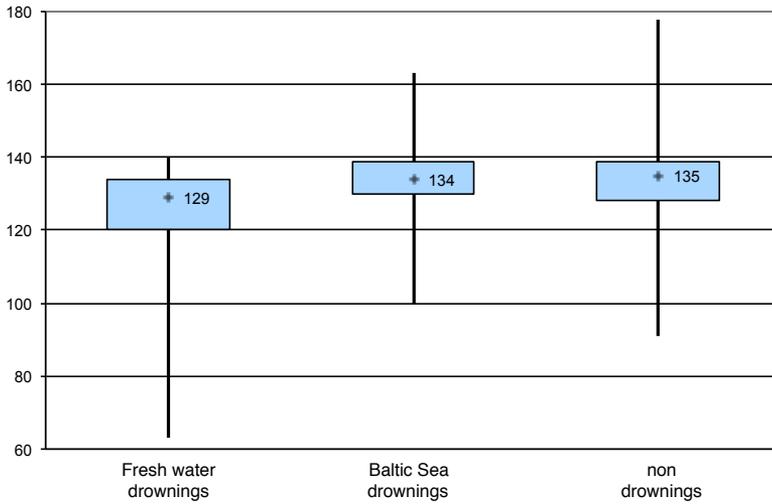


Figure 18. Sodium levels in fresh water drownings, Baltic Sea drownings and non drownings. The inside figure of each box represents the median. The lower and upper edges of the boxes represent the 25th and 75th percentiles. Upper and lower lines outside the boxes represent minimum and maximum values.

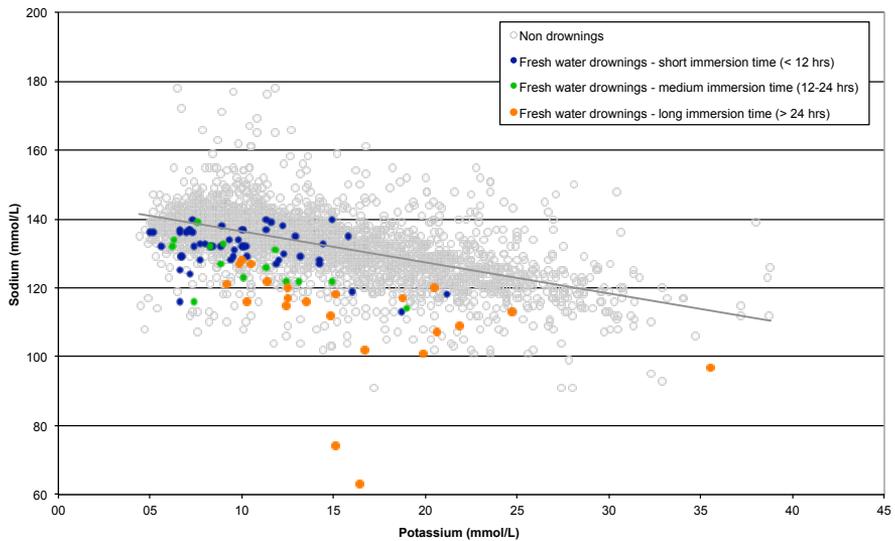


Figure 19. Sodium levels in fresh water drownings with different immersion times.

Previous studies have shown that vitreous sodium and chloride levels are increased in cases of sea-water drownings and decreased in fresh water drownings. However, there are different opinions on whether this is a result of the drowning process itself or of postmortem diffusion into the eyeball from the surrounding water.

Unfortunately, there were no sea-water drownings in this material; only drownings in the brackish water of the Baltic Sea in the Stockholm area (salinity ~0.65%, sodium ~90 mmol/L) and freshwater. Nonetheless, this study confirms that vitreous sodium and chloride levels are generally lower in fresh water drownings than in non-drownings. The sodium and chloride levels in Baltic Sea water drownings are virtually the same as in non-drownings, which seems plausible, since the salt level in the Baltic Sea is similar to human serum levels. Figure 18 shows that long immersion time was associated with lower sodium levels, suggesting that this is an effect of postmortem diffusion. It can also be seen that in cases with a very short immersion time, sodium levels are similar to sodium levels of non-drownings, suggesting that there is no effect of hemodilution due to water inhalation. Even if electrolyte imbalances do occur in blood during drowning, it is questionable if these imbalances would show in the vitreous, since it takes some time for the electrolyte blood levels to equilibrate with the vitreous levels [83].

5. Conclusions

A strategy to consistently collect and analyse postmortem vitreous fluid using a blood gas instrument was presented, providing bedside support for the forensic pathologist.

Reference ranges for postmortem vitreous glucose, sodium and chloride levels were suggested and it was concluded that vitreous glucose alone should be used to diagnose hyperglycemia.

The postmortem rise in vitreous potassium is non-linear and decedent age and the ambient temperature significantly influence the rise. A new equation for the estimation of the postmortem interval that includes decedent age and ambient temperature was created. To facilitate the calculation, a web application that rapidly provides a PMI estimate with confidence intervals based on these equations was developed. The web application also provides confidence intervals.

Postmortem vitreous sodium and chloride levels slowly decrease with postmortem interval. Postmortem vitreous sodium levels correlate very well with antemortem serum sodium levels, but should be interpreted together with postmortem vitreous potassium levels or PMI. Sodium levels can help to establish the cause of death or at least the antemortem condition of the subject prior to death in a number of cases.

6. Future directions

Since a blood gas instrument was used for all analyses, we also have values for the gas pressures in the vitreous ($p\text{CO}_2$ and $p\text{O}_2$). It would be interesting to investigate whether the gas pressures can be used to diagnose antemortem asphyxia, for example in cases of suffocation with a pillow, which often leave no other traces at autopsy, histopathology or biochemistry. Our preliminary research has shown that $p\text{CO}_2$ levels slowly decrease postmortem and $p\text{O}_2$ levels increase due to diffusion to the surrounding air. There is a large variation of the values, but it should be possible to identify cases outside the “normal” range with deviating values.

There is surprisingly little data published on the composition of vitreous fluid in living humans under physiological conditions. We have started collaboration with St Erik’s Eye Hospital in Stockholm, where we plan to obtain vitreous fluid from living patients who undergo vitrectomy. During the vitrectomy, a blood sample will be acquired from the patient, which then can be compared to the contents of the vitreous fluid regarding glucose, lactate, electrolytes and gas pressures. This will hopefully help to further understand fluid dynamics between blood and vitreous.

All analyses in this thesis were made by the use of an in house Radiometer blood gas instrument. Re-analysis of samples showed very small variations. The advantage of using a blood gas instrument is that results can be obtained within minutes. Such rapid information may be important as guidance for the continuing death investigation. We have performed a number of comparative analyses, but these need to be extended in order to evaluate how universally applicable the values obtained are, if other analytical methods are used.

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