

Postmortem chemistry update part I

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Abstract Postmortem chemistry is becoming increasingly essential in the forensic pathology routine and considerable progress has been made over the past years. Biochemical analyses of vitreous humor, cerebrospinal fluid, blood and urine may provide significant information in determining the cause of death or in elucidating forensic cases. Postmortem chemistry may essentially contribute in the determination of the cause of death when the pathophysiological changes involved in the death process cannot be detected by morphological methods (e.g. diabetes mellitus, alcoholic ketoacidosis and electrolytic disorders). It can also provide significant information and useful support in other forensic situations, including anaphylaxis, hypothermia, sepsis and hormonal disturbances. In this article, we present a review of the literature that covers this vast topic and we report the results of our observations. We have focused our attention on glucose metabolism, renal function and electrolytic disorders.

Keywords Postmortem chemistry · Biochemical markers · Glucose metabolism · Electrolytes · Renal function

Introduction

In 1993, Coe [1] published an article titled “Postmortem Chemistry Update: Emphasis on Forensic Application”, in which he reviewed postmortem chemistry and the articles pertaining to this subject over the last 15 years. In his

introduction, Coe defined forensic chemistry as “*one of the more important ancillary procedures for the forensic pathologist*”. He also emphasized that routine examinations of vitreous electrolytes, glucose and urea nitrogen alone provided significant information in determining the cause of death as well as assisting in determining the time of death in more than 5% of all cases. Moreover, forensic chemistry helped in solving forensic problems in about 10% of the routine, natural deaths.

Succeeding Coe, many other authors have approached forensic chemistry bringing new points of views, ideas, suggestions and solutions for improving sampling accuracy, the choice of the analytical technique as well as measurement precision. The number of substances that may be used for diagnostic purposes in forensic routine has increased significantly, though some studies have put back into question the usefulness of other molecules that are no longer systematically used. Postmortem determinations of a wide variety of substances are now technically possible in many biological fluids, including blood, vitreous humor, urine, cerebrospinal, pericardial and synovial fluids [2–15].

A very important array of new material on postmortem chemistry has appeared since Coe’s article; hence, forensic chemistry today seems to be even more complementary to the work of forensic pathologists.

The aim of these two articles is to propose a review of the literature covering this vast topic, for which the diagnostic potential is, in our opinion, still greatly unexplored. Unlike Coe, we have focused our attention on the molecules with the potential of offering information concerning the cause of death and did not expand upon the analyses which, historically, have been used to determine the time of death. Moreover, given that forensic chemistry analyses are currently used in our medico-legal centre, we have reported the results of our observations

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where possible. In the first part of this work, we concentrate on glucose metabolism, renal function and electrolytes.

Glucose metabolism

Glucose, lactate and hyperglycaemia

In clinical practice, glycaemia and glycated haemoglobin are the main biochemical markers for diagnosing disorders in glucose metabolism. In forensic pathology, glycaemia is of no value as a biochemical parameter due to substantial fluctuations in glucose levels after death since glycolysis continues spontaneously making blood glucose concentrations fall extremely rapidly. Furthermore, death may be preceded by agonal processes and cardiopulmonary resuscitation, which are often associated with the secretion or the administration of catecholamines. This results in further mobilisation of liver glycogen and the release of glucose into blood circulation as a counterbalancing phenomenon. Considering the difficulty in interpreting postmortem blood glucose levels, other biological fluids, such as vitreous humor and cerebrospinal fluid, have been proposed in order to estimate the antemortem blood glucose concentrations [1, 12–29].

Respecting the assumption that glycolysis continues spontaneously after death and that two molecules of lactic acid are the final product of the postmortem glycolysis of one molecule of glucose, Traub [30] proposed that postmortem glycaemia could be estimated by combining the values of glucose and lactate in cerebrospinal fluid. After Traub, other authors have proposed the establishment of the sum values of glucose and lactate in vitreous humor or cerebrospinal fluid, to estimate the antemortem blood glucose concentrations [31–36].

Karlovssek [37, 38] compared several biochemical parameters (glycated haemoglobin, glucose, lactate and combined glucose and lactate values) in vitreous and cerebrospinal fluid in 112 forensic cases divided into two diagnostic groups (41 diabetics and 71 control cases). The author proposed that vitreous glucose levels over 13 mmol/l (corresponding to 234 mg/dl) or combined glucose and lactate values in vitreous or cerebrospinal fluid over threshold values of 23.7 (427 mg/dl) and 23.4 mmol/l (422 mg/dl) respectively could indicate antemortem hyperglycaemia with a fatal outcome. The supplementary determinations of glycated haemoglobin, acetone and other ketone bodies were also recommended in order to identify diabetic ketoacidosis.

Zilg et al. [39] evaluated the usefulness of the sum value of glucose and lactate in vitreous. They applied a strategy which included the consistent sampling of vitreous as soon as possible upon arrival of the body at the morgue as well as immediate bedside analysis using a blood gas instru-

ment. Three thousand seventy-six cases were included in the study and the authors found that, after an initial drop of vitreous glucose during the very early postmortem period, the levels stayed stable for an appreciable amount of time after death. The analysis of a second sample collected at autopsy 1–3 days later showed similar results. In contrast, the vitreous lactate levels showed a steady increase, suggesting that the sum of glucose and lactate increased with postmortem time. No case with a circumstantial indication of hyperglycaemia showed only high vitreous lactate. The authors suggested that vitreous glucose alone should be employed to diagnose hyperglycaemia and indicated the limit of 10 mmol/l (180 mg/dl), theoretically corresponding to a blood glucose value of about 26 mmol/l (468 mg/dl). Regarding the methodology, they concluded that sonication, centrifugation and the addition of fluoride to the samples were unnecessary procedures when a blood gas instrument was used.

In our medico-legal centre, we observed several cases of diabetic ketoacidosis showing increased glucose levels in vitreous and cerebrospinal fluid (over 20 mmol/l in both fluids, corresponding to 360 mg/dl), glucose in urine and increased blood acetone and 3- β -hydroxybutyrate (3HB) values. There were no cases in which only increased vitreous lactate was shown. Our results confirmed the conclusions of Zilg et al. suggesting that the combined values of glucose and lactate in vitreous or cerebrospinal fluid do not add any further information in estimating antemortem blood glucose concentration. Vitreous glucose concentration is the most reliable marker for this. Its determination, together with the measurements of ketone bodies, urine glucose and glycated haemoglobin, can easily confirm the existence of a glucose metabolism disorder and a diabetic decompensation as cause of death.

Glycated haemoglobin and vitreous fructosamine

Osuna et al. and Vivero et al. [36, 40, 41] compared several biochemical parameters (fructosamine, glucose, lactate, combined values of glucose and lactate) in vitreous, in order to confirm the presence of antemortem hyperglycaemia and to diagnose diabetes mellitus.

Goullé et al. [42] studied the stability of glycated haemoglobin as a function of time in different samples with or without anticoagulants and preservatives. A total of 106 tests were performed. Samples containing ethylenediaminetetraacetic acid (EDTA) and stored at 4°C were indicated as optimal for blood conservation. The authors confirmed the usefulness of glycated haemoglobin for interpreting increased postmortem ketone body blood levels and diagnosing diabetes mellitus decompensation as the cause of death.

Uemura et al. [8] investigated 11 clinically available biochemical markers (including glycated haemoglobin, fructosamine, urea nitrogen, creatinine, C-reactive protein, total bilirubin, total protein and total cholesterol) in whole blood (for glycated haemoglobin) and postmortem serum (for the other markers) from three sampling sites (left cardiac blood, right cardiac blood and femoral vein blood) in 164 consecutive autopsy cases. Of all markers examined, glycated haemoglobin showed the smallest deviation from living subjects, negligible postmortem changes and no difference due to the cause of death. On the contrary, fructosamine showed a large deviation from living subjects. According to the authors, these differences were related to the nature of the marker: the level of glycation of haemoglobin is a process the rate of which is determined by the level of glucose concentration under the lifespan of erythrocytes, whereas the other markers, including fructosamine, are components of tissues reflecting their destruction or elimination from the blood by the organs.

Ketone bodies and diabetic ketoacidosis

Ketone bodies (acetoacetate (AcAc), 3HB and acetone), a by-product of fat metabolism, are produced in the liver as an alternative energy source when insufficient insulin is available for effective glucose use. Ketogenesis is the process by which fatty acids released from the adipocytes are converted in AcAc and 3HB in the mitochondria of hepatocytes. Ketosis is the term that refers to the excessive presence of ketone bodies in blood; ketonuria refers to the presence of ketone bodies in urine. Levels of circulating ketone bodies vary across populations of normal individuals, as a result of variations in basal metabolic rate, hepatic glycogen stores and differences in the mobilisation of amino acids from muscle proteins. Most investigators agree that normal serum levels of ketone bodies can be defined as being less than 500 $\mu\text{mol/l}$. Hyperketonemia can be defined as levels in excess of 1,000 $\mu\text{mol/l}$ and ketoacidosis as levels over 3,000 $\mu\text{mol/l}$. Physiologic ketosis occurs when mildly to moderately elevated levels of circulating ketone bodies are present in response to fasting or prolonged exercise. Diabetic ketoacidosis (DKA) is the most common pathologic cause of ketosis. In patients with type 1 diabetes, ketosis occurring in association with hyperglycaemia confirms the presence of insulin deficiency. Other possible causes of ketosis are alcoholic ketoacidosis, severe hypoxia, end-stage liver disease, hepatic ischemia, various metabolic disorders and multiple organ failure [43–45].

Postmortem investigations of ketone bodies in blood and other biological fluids have been performed by several authors in order to diagnose ketoacidosis as the cause of death [4, 33, 46–49].

In a study performed by Osuna et al. [47] on 453 forensic cases divided into two diagnostic groups (diabetics and the control group), cases showing increased vitreous glucose values over 200 mg/dl (11.1 mmol/l) also showed the highest 3HB concentrations, confirming the usefulness of determining vitreous 3HB concentrations as an alternative to blood.

Kanetake et al. [48] investigated levels of 3HB and acetone in the postmortem serum of 100 forensic cases and concluded that in cases without anatomical or toxicological causes of death, the measurement of ketone body levels could be informative. They also proposed that 3HB serum levels over 1,000 $\mu\text{mol/l}$ (10.4 mg/dl) could indicate ketoacidosis as the cause of death in cases showing physical conditions characterized by a tendency to increases in ketone body concentrations.

In our medico-legal centre, several cases of diabetic ketoacidosis showing high acetone values in blood, urine and vitreous and high 3HB values in blood, urine, vitreous, pericardial and cerebrospinal fluid were observed. Cases of diabetic ketoacidosis usually showed blood 3HB concentrations over 6,000 $\mu\text{mol/l}$ (62.5 mg/dl). Glucose levels in vitreous and cerebrospinal fluid were usually over 20 mmol/l.

Isopropyl alcohol and ketoacidosis

The presence of isopropyl alcohol (IPA) in biological fluids has been traditionally considered to indicate exposure to this alcohol via oral ingestion or inhalation, as well as postmortem production. However, IPA was observed in the biological fluids of animals suffering from acetonemia, suggesting that acetone could be metabolized to IPA by alcohol dehydrogenase in certain disease states [50]. Nevertheless, the traditional interpretation of detectable blood levels of IPA was the exposure to the substance itself.

Bailey [50] identified five diabetic patients with ketosis and detectable levels of IPA in blood. All patients had type I diabetes mellitus and were insulin-dependent. According to medical records, none of the patients had a history of IPA exposure and all were hyperglycaemic upon admission. Serum concentration of IPA ranged from 20 (332 $\mu\text{mol/l}$) to 297 mg/l (4,900 $\mu\text{mol/l}$) and serum concentration of acetone ranged from 5.8 (1,000 $\mu\text{mol/l}$) to 32 mg/dl (5,500 $\mu\text{mol/l}$). According to the author, these findings corroborated those published by Davis et al. [51], who postulated that acetone could be converted to IPA in situations in which NADH was elevated.

In a study on acetonemia and other volatile alcohols published by Teresinski et al. [52], the authors selected 75 forensic cases assigned to six groups (including hypothermia, fatal acetone and isopropyl alcohol intoxications and sudden death in alcoholics). The authors found increased

IPA concentrations not only in individuals intoxicated with this agent, but also in the groups of hypothermia cases and sudden death in alcoholics. The authors do not explain these results, though they point out the hypothesis given by Davis, Bailey and Jones and Summers [53] of an endogenous transformation of acetone to IPA in certain disease states as a possible explanation.

Jenkins et al. [54] and Molina [55] investigated the concentrations of IPA in blood and vitreous. In a retrospective study carried out by Molina on 152 autopsy cases with nine different IPA sources (including IPA exposure), the author measured blood and vitreous IPA and acetone concentrations and concluded that cases of IPA intoxications tended to have blood IPA values over 100 mg/dl and IPA/acetone ratios more than 1.0.

In our medico-legal centre, IPA is systematically measured in blood, urine and vitreous in cases of diabetes mellitus, suspected hypothermia and death in alcoholics. A blood concentration of 15 mg/l of IPA (249 $\mu\text{mol/l}$) was observed in a case of hypothermia. In a case of diabetic ketoacidosis we noted increased blood (57 mg/dl–9,800 $\mu\text{mol/l}$) and vitreous acetone (91 mg/dl–15,650 $\mu\text{mol/l}$) values and a blood IPA concentration of 50 mg/l–830 $\mu\text{mol/l}$ (49 mg/l in vitreous–813 $\mu\text{mol/l}$).

Ketone bodies, alcoholic ketoacidosis and alcoholic lactic acidosis

The syndrome of a wide-gap metabolic acidosis, malnutrition, and binge drinking superimposed on chronic alcohol abuse is most commonly referred to as alcoholic ketoacidosis [56–62]. The possible role of alcoholic ketoacidosis as cause of death has been investigated by several authors [35, 46–48, 63–66].

Thomsen et al. [64] proposed the term of “ketoalcoholic death” for cases showing blood ketone body concentrations over 531 $\mu\text{mol/l}$ in alcoholics with an otherwise unknown cause of death.

Pounder et al. [46] investigated levels of total ketone bodies in postmortem samples including vitreous, pericardial fluid and blood from different sampling sites. Vitreous ketone body levels showed good correlation with blood and pericardial fluid levels. According to the authors, increased ketone body levels (over 10,000 $\mu\text{mol/l}$ in blood and 5,000 $\mu\text{mol/l}$ in vitreous) could be indicative of severe alcoholic ketoacidosis.

Brinkmann et al. [35] investigated alcoholic ketoacidosis and alcoholic lactic acidosis (caused by thiamine deficiency) as causes of death in chronic alcoholics. In an initial series of forensic cases investigated by the authors, blood acetone levels ranged from 9.8 to 40 mg/dl (1,685 to 6,880 $\mu\text{mol/l}$) in six cases with no cause of death at autopsy. The authors suggested that blood acetone values over 9 mg/dl

(1,548 $\mu\text{mol/l}$) could indicate a fatal alcoholic ketoacidosis. However, they emphasized the importance of 3HB as a more accurate indicator of ketoacidosis. In a second series of forensic cases investigated by the authors, the cause of death was determined to be alcoholic lactic acidosis when three criteria were met: glucose and lactate sum value in cerebrospinal fluid above 300 mg/dl (16.6 mmol/l), no indication of diabetes from previous history, histology or glycated haemoglobin determination and no cause of death even after extensive toxicology.

Iten et al. [65] investigated blood concentrations of 3HB in DKA and alcoholic acidosis and proposed that blood 3HB concentrations up to 500 $\mu\text{mol/l}$ (5.2 mg/dl) can be regarded as normal, from 500 to 2,500 $\mu\text{mol/l}$ (26 mg/dl) as high and over 2,500 $\mu\text{mol/l}$ as pathological.

Elliott et al. [66] investigated the relationship between 3HB, acetone and ethanol in 350 medico-legal autopsy cases and concluded that 3HB values over 25 mg/dl (2,400 $\mu\text{mol/l}$) in blood (and to some extent in the urine) could be considered pathologically significant. The authors also concluded that 3HB in vitreous can be considered a useful alternative when blood is absent, with the same interpretative range.

In our medico-legal centre, we experienced some cases of ketoacidosis in chronic alcohol abusers with no previous diagnosis of diabetes mellitus and no cause of death on autopsy and histology. All cases showed negative toxicology, no ethanol in blood, undetectable glucose levels in vitreous and cerebrospinal fluid and normal glycated haemoglobin. Blood and vitreous 3HB concentrations ranged from 1,650 (17.2 mg/dl) to 2,400 $\mu\text{mol/l}$ (25 mg/dl). We concluded that the cause of death was not clearly identified but the increase of ketone bodies may have represented a contributing factor in death.

Ketone bodies and hypothermia

Teresinski et al. [52, 67, 68] investigated ketone body blood levels as biochemical markers of hypothermia and the usefulness of ketone body determination in blood, urine and vitreous for the diagnosis of death by hypothermia. They started from the assumption that the cases of hypothermia are usually accompanied by an increase in ketone bodies in blood and urine and that the consumption of large amounts of alcohol inhibits ketogenesis. Cases of suspected hypothermia showed an inverse statistically significant relationship between blood acetone and ethanol concentration. The authors also emphasized that ketone body concentrations in urine should be interpreted very cautiously, due to the possible lack of ketonuria in conditions of coexisting ketonemia and renal failure (shock aetiology) or in cases of decreased ketonemia and persisting ketonuria (connected to urine retention). Moreover, in case of rapidly increasing

ketonemia, severity of ketosis cannot be evaluated solely on the basis of vitreous analysis, since the equilibrium between blood and vitreous requires some delay.

In our medico-legal centre, several cases of hypothermia were observed in which the diagnosis was made on the basis of the scene investigation data and autopsy findings (frost erythema over the extensor surfaces of the large joints, Wischniewski spots in the gastric mucosa and haemorrhages in the iliopsoas muscles). The determination of 3HB in blood, urine, vitreous, pericardial and cerebrospinal fluid was performed systematically. Also performed systematically was the determination of ethanol, acetone and isopropyl alcohol in blood, urine and vitreous, the measurement of the glycated haemoglobin and the determination of glucose in vitreous and cerebrospinal fluid. Our results support the hypothesis of Teresinski et al. that suggest the existence of an inverse statistically significant relationship between ketone body blood levels and ethanol levels in cases of hypothermia.

Insulin and C peptide

Insulin is a peptide hormone secreted by the pancreatic β -cells in response to hyperglycaemia. It is basal insulin secretion that helps maintain normal fasting blood glucose, while prandial insulin is secreted in response to increases in glucose and nutrients at mealtimes. Another hormone secreted by the pancreas, glucagon, is also involved in the control of blood glucose levels. In fact, it is the opposing actions of insulin and glucagon that help fine-tune glucose homeostasis. In the presence of hyperglycemia, the pancreatic β -cells secrete more insulin, stimulating glucose transport into muscle and adipocytes, and inhibiting glucose production in the liver. These actions are counterbalanced by those of glucagon (secreted by the pancreatic α -cells), which is suppressed by hyperglycemia but stimulated during hypoglycemia. Active insulin consists of two peptides chains (A and B chains) that are linked by two disulfide bridges. Before secretion, the proinsulin molecule (inactive form) exists as a long chain with a connecting peptide (C peptide) between the A and B chains. Enzymes within the β -cell secretory granules cleave the C peptide before secretion; therefore, when the stimulus for secretion arrives, the granules release both active insulin and C peptide. Analysis of insulin and C peptide are of forensic interest in the investigation of unclear hypoglycaemia. Due to different half-lives and clearance-rate, both peptides reach their maximum concentration at different times after meals. Both insulin and C peptide are secreted in equimolar concentrations. However, since C peptide has the longer half-life, the peptides are not always present in equimolar amounts in the bloodstream, meaning that the ratio of insulin to C peptide should be near 1.0 or even slightly

higher. Degradation of insulin is mainly the result of glutathione insulin transhydrogenase in the liver, whereas C peptide is removed from the circulation by the kidneys. Less than 1% of intact human insulin is excreted into urine. The ratio of insulin to C peptide in a living person is thought to reliably distinguish hypoglycaemia due to exogenous insulin ($I/C > 1$) from hypoglycaemia due to an insulinoma or sulfonylurea overdose ($I/C < 1$). Insulin in postmortem serum is extremely difficult to measure accurately because it degrades rapidly at room temperature. Peripheral blood sample has been indicated as the best specimen for postmortem detection of insulin, because heart blood, especially the heart-side heart blood, may show concentrations higher than peripheral specimens. Peripheral blood samples should be collected in tubes containing sodium chloride or EDTA and postmortem serum should be separated from the red blood cells as soon as possible and then refrigerated or, preferably, frozen. Measurement of C peptide is essential for the accurate interpretation of insulin levels. C peptide is more stable than insulin in postmortem blood. However, collection and storage of specimens for C peptide analyses require special handling: collection in heparinised tubes, separation of postmortem serum and, without delay, storage of the sample in a freezer [13, 69–72].

Several cases of insulin overdose, in diabetic and non diabetic individuals, have been reported. Bile, vitreous and postmortem serum from right heart blood were also used to detect insulin. However, postmortem serum from peripheral blood is the sample of choice. Tissue samples of liver, brain or kidney are not recommended for insulin levels. An elevated postmortem serum insulin level, along with a suppressed C peptide level, is virtually diagnostic of an exogenous insulin administration. If an injection site is detected on the body, the excision of the surrounding dermal area should be taken into consideration: toxicological analysis and immunohistochemical demonstration of insulin in the tissues at the injection site should always be performed [73–91].

Insulin values in postmortem serum suggesting or evocating lethal dosages are difficult to propose. Patel [79] provided a table of insulin values for normal fasting (5–75 $\mu\text{UI/ml}$ —35–521 pmol/l) versus fatal insulin overdose (800–3,200 $\mu\text{IU/ml}$ —556–22,224 pmol/l). Kernbach-Wighton and Püschel [82] proposed the lower limit of 100 $\mu\text{UI/ml}$ for lethal insulin dosage. Musshoff et al. [13] reported that clearly elevated serum insulin levels could be caused by intentional insulin overdose, because levels of 1,000 $\mu\text{UI/ml}$ are rarely seen in patients with insulin-secreting tumours. Karlovsek [37, 38] proposed a summary of the biochemical indicators which would support the hypothesis of hypoglycaemia at the time of death, including low or indeterminate glucose concentration in vitreous

immediately after death; extremely low glycated haemoglobin in treated diabetics as a consequence of repeated hypoglycaemic states and biochemical and toxicological findings indicating insulin or anti-diabetic overdose.

In our medico-legal centre, two cases of insulin overdose have been recently observed. In the first case, the postmortem insulin serum level (from femoral blood) was 154 $\mu\text{IU/ml}$ (1,070 pmol/l). In the second case, the postmortem insulin serum level (from femoral blood) was 582 $\mu\text{IU/ml}$ (4,041 pmol/l). In both cases, C peptide in postmortem serum from femoral blood was under the detection limit. Moreover, insulin was demonstrated immunohistochemically in the subcutaneous tissue surrounding the needle tracks. In the second case, insulin and C peptide levels were measured in vitreous, cerebrospinal fluid, bile and pericardial fluid. The results showed detectable levels of insulin in all fluids, even in vitreous, where insulin is thought to penetrate minimally. In order to test the usefulness of postmortem insulin and C peptide determinations, we measured their concentrations in postmortem serum from femoral blood, pericardial fluid and vitreous in 20 control cases, with various causes of death. The results confirmed that insulin and C peptide penetrate the blood-vitreous barrier only minimally and to a greater extent in the pericardial fluid.

Renal function

Urea nitrogen, creatinine and uric acid

Postmortem urea nitrogen and creatinine stability in biological fluids have been tested by numerous authors. Most of them concluded that urea nitrogen is a very stable compound and that its levels in postmortem serum closely approximate the antemortem serum levels, irrespective of the methodology used, the level found and the duration of postmortem interval, even after moderate decomposition [1]. Studies performed on urea nitrogen in cerebrospinal fluid showed a slight rise after death. In general, however, postmortem values reflect the antemortem levels, as they do in vitreous and even in the synovial fluid [1–3, 92]. In addition to its importance in assessing renal function, mild urea nitrogen increases, in association with hypernatremia, can confirm antemortem dehydration, which generally causes urea nitrogen levels to rise more than creatinine levels. Similar to urea nitrogen, postmortem creatinine levels remain stable in vitreous and cerebrospinal fluid [1].

Zhu et al. [93–95] analysed urea nitrogen, creatinine and uric acid levels in pericardial fluid and postmortem serum from different sampling sites (right heart, left heart, subclavian vein and external iliac vein). In an initial study performed on 395 medico-legal autopsy cases, the authors

found that a considerable elevation of uric acid, especially in postmortem serum from right heart blood, was observed in fatal mechanical asphyxiation and drowning, suggesting that postmortem hyperuricemia in acute deaths could be associated with hypoxic skeletal muscle damage following hyperactivity or agonal convulsions. In a second study performed on 409 medico-legal autopsy cases, the authors compared urea nitrogen, creatinine and uric acid levels in postmortem serum and pericardial fluid and emphasized that, because of the nature of the pericardial fluid, in which the turnover rates are much milder than those in blood, elevations in pericardial urea nitrogen, creatinine and uric acid suggested the existence of persistent metabolic deteriorations before death. In a third study realized on 556 medico-legal autopsy cases, the authors compared the differences between pericardial fluid and postmortem serum levels of urea nitrogen, creatinine and uric acid and concluded that since significant changes in pericardial fluid levels became apparent some hours later than in the serum, elevated urea nitrogen, creatinine and uric acid levels in pericardial fluid could suggest prolonged survival of several hours.

Maeda et al. [96] analysed urea nitrogen, creatinine and C-reactive protein (CRP) postmortem serum levels in 429 medico-legal autopsy cases and found that fatal hyperthermia cases showed elevated isolated creatinine levels (over 2 mg/dl), without significant elevation of urea nitrogen and CRP.

Uemura et al. [8] investigated 11 clinically available biochemical markers including urea nitrogen and creatinine in postmortem serum from three sampling sites (left cardiac blood, right cardiac blood and femoral vein blood) in 164 consecutive autopsy cases. The differences in the measured values were classified according to sampling site and in relation to postmortem interval and aetiology of death. For urea nitrogen, no significant differences among the sampling sites were found, whereas levels of creatinine were lower in postmortem serum obtained from left cardiac blood than from femoral blood. The authors concluded that any sampling site could be used for urea nitrogen, whereas postmortem serum obtained from left cardiac blood is preferred for creatinine.

In our medico-legal centre, urea nitrogen and creatinine are systematically measured in vitreous and in postmortem serum, when available. Several cases of gastrointestinal bleeding showing increased urea nitrogen levels in both fluids with normal creatinine levels were observed. Cases of dehydration usually showed increased postmortem serum (from femoral blood) and vitreous urea nitrogen levels, increased vitreous sodium and chloride levels and normal postmortem serum and vitreous creatinine levels. In such cases, in order to confirm the presence of prerenal failure, the concentrations of urea nitrogen, creatinine and uric acid were also measured in urine and the fractional excretion of

Table 1 Summary of reports describing postmortem analysis of markers of glucose metabolism, markers of renal function and electrolytes

Marker analysed	Number of cases	Samples analysed	Time of sampling after death or postmortem interval	Analytical method	Concentration range proposed and other suggestions	Reference
Glucose	112 (41 diabetics)	VH CSF	3–72 h	Glucose peroxidase method	13 mmol/l (243 mg/dl) in VH would indicate antemortem hyperglycemic state with fatal outcome	[37, 38]
	3076	VH	After arriving at the morgue	Glucose peroxidase method	10 mmol/l (180 mg/dl) in VH would represent the value to diagnose antemortem hyperglycemia	[39]
Glycated haemoglobin	106	Whole blood	Not indicated	HPLC	Clinical range (3.5–6.25%) EDTA and storage at 4°C	[42]
	164	Whole blood from different sampling sites	0–72 h	Latex aggregation immunoassay	Clinical range (4.3–5.8%)	[8]
Ketone bodies	49	VH Urine	Not indicated	Quantitative enzymatic determination	Vitreous 3HB over 15 mg/dl (1,440 μ mol/l) in case of alcoholic ketoacidosis	[63]
	131	Blood	Not indicated	HS-GC	Ketone bodies sum concentration over 531 μ mol/l would suggest ketoacidosis as cause of death	[64]
	256 (24 chronic alcoholics, 7 diabetics) 105 (22 chronic alcoholics, 12 diabetics)	Blood Blood from different sampling sites, VH pericardial fluid	1–8 days Not indicated	GC-FID HS-GC	Blood acetone over 9 mg/dl (1,500 μ mol/l) would indicate a fatal ketoacidosis Increased ketone bodies levels (10,000 μ mol/l in femoral blood or 5,000 μ mol/l in VH) indicate alcoholic ketoacidosis. PF and VH alternative to blood	[35] [46]
Insulin	100 (25 alcoholics, 6 diabetics)	Whole blood	120 h	Available clinical laboratory methodologies (enzymatic reaction)	Blood 3HB over 2,500 μ mol/l can be considered as pathological	[65]
	100	Postmortem serum (sampling site not indicated)	0–96 h	Available clinical laboratory methodologies	3HB over 1,000 μ mol/l would indicate ketoacidosis as cause of death	[48]
Peptide C	350	Blood	Not indicated	GC-FID (ethanol and acetone) GC-MS(3HB)	Blood (and urine) 3HB over 25 mg/dl (2,400 μ mol/l) can be considered pathologically significant VH 3HB: same interpretative range	[66]
	164	Urine VH	As soon as possible, then frozen		800–3200 μ IU/ml (556–22,224 pmol/l) for insulin lethal dosage	[69, 70, 79, 82]
Urea nitrogen, creatinine, uric acid	164	Postmortem serum from peripheral blood	As soon as possible, then frozen	Urease UV method	100 μ IU/ml for insulin lethal dosage Suppressed in case of exogenous insulin administration	[69, 70]
	164	Postmortem serum from left cardiac blood for creatinine	0–72 h	Enzyme method	Clinical serum range (6–20 mg/dl)	[8]
556, 395, 409	556, 395, 409	Postmortem serum from different sampling sites and pericardial fluid	0–48 h	Urea nitrogen: urease-glutamata dehydrogenase method Creatinine: alkali-picric acid method Uric acid: uricase peroxidase method	Clinical serum range: Urea nitrogen 6–20 mg/dl Creatinine: male 0.61–1.04 mg/dl, female 0.47–0.79 mg/dl Uric acid: male 3.7–7.6 mg/dl, female 2.5–5.4 mg/dl	[93–95]

Table 1 (continued)

Marker analysed	Number of cases	Samples analysed	Time of sampling after death or postmortem interval	Analytical method	Concentration range proposed and other suggestions	Reference
Sodium, chloride		Postmortem serum, VH and CSF VH			Clinical serum range Clinical serum range (various clinical ranges were reported by several authors)	[1–3, 92] [1, 4, 6, 9, 19, 21–28, 97–99]
Calcium, magnesium	360	Postmortem serum from different sampling sites	5–48 h	Orthochoresolphthalein complexome method and xyliid blue method	Clinical serum range (Ca 8.7–10.1 mg/dl, Mg 1.8–2.6 mg/dl)	[114]
	385	Pericardial fluid	48 h	Orthochoresolphthalein complexome method and xyliid blue method	Clinical serum range for Ca, pericardial Mg levels generally higher than clinical serum reference range	[115]
Strontium	25, 133, 70, 172, 55, 150	Blood from left and right ventricles	In a study, 0–48 h. Not indicated in the other studies.	Spectrophotometry atomic absorption, graphite furnace atomic absorption spectroscopy, flameless atomic absorption spectrometry Available clinical laboratory methodologies	172 µg/l in left ventricular blood (seawater drowning) Difference in excess of 70 µg/l between left and right heart	[116–121]
	120	Postmortem serum from cardiac and femoral blood	30 h			[122]

VH vitreous humor, CSF cerebrospinal fluid, PF pericardial fluid, 3HB 3-β-hydroxybutyrate, HPLC high-performance liquid chromatography, EDTA ethylenediaminetetraacetic acid, HS-GC headspace-gas chromatography, GC-FID gas chromatography-flame ionization detector, GC-MS gas chromatography–mass spectrometry

urea was calculated. Cases of diabetic ketoacidosis showed increased postmortem serum and vitreous urea nitrogen levels, usually with normal postmortem serum and vitreous creatinine levels.

Electrolytes

Byramji et al. [97] and Ingham and Byard [98] reviewed the literature concerning electrolytes in vitreous and particularly vitreous sodium. Electrolyte levels in vitreous change after death, the degree of change depending on the conditions of body storage, postmortem interval, sample and analyte. Changes depend on the effects of cellular hypoxia, which lead to increased cell membrane and blood vessel wall permeability as well as a reduction in ATP stores, which prevent electrolyte pumps from maintaining physiological cell membrane electrical gradients. These factors result in the merging of intra- and extracellular fluid and their respective electrolytes which, coupled with autolysis and cell disintegration, lead to important and unpredictable changes in electrolyte contents in postmortem samples. Vitreous sodium levels have been determined to be relatively stable during the early postmortem period and similar to levels found in the serum in living subjects. According to several authors abnormalities in antemortem serum sodium concentrations are reflected in postmortem vitreous values, making it possible to diagnose hypo- or hypernatremia at the time of death. Like sodium, vitreous chloride concentrations show minimal falls in values during the early postmortem period and abnormalities in antemortem serum chloride are reflected in postmortem vitreous values [1, 4, 6, 9, 19, 21–28, 97–99].

Cases of hypernatremia showing vitreous sodium concentrations ranging from 155 to 210 mmol/l and chloride concentrations ranging from 139 to 147 mmol/l have been reported in the literature [1, 100–102]. Madea and Lachenmeier [103] mentioned 17 cases of hypertonic dehydration showing increased vitreous sodium and urea values. The authors also cited seven cases of increased vitreous sodium values not related to dehydration and concluded that prudence should be used in determining the cause of death on the basis of vitreous sodium and chloride values only.

Unnatural cases of hyponatremia upon autopsy [97, 104–113] include water intoxication from psychogenic polydipsia, environmental polydipsia, ingestion of dilute infant formulas, beer potomania, endurance exercise, fresh water immersion and iatrogenic causes including drug and parenteral fluid administration and surgical irrigation. If antemortem findings are not available, vitreous sodium levels lower than 120 mmol/l could support the diagnosis of fatal hyponatremia [104].

Vitreous potassium levels [1] are of no help in determining the potassium status of an individual immediately prior to death. Increased vitreous potassium levels have no diagnostic value, whereas low vitreous levels are theoretically indicative of hypokalemia.

Zhu et al. and Li et al. [114, 115] analysed postmortem calcium (Ca) and magnesium (Mg) levels in pericardial fluid and postmortem serum from different sampling sites (right heart, left heart, subclavian vein and external iliac vein). In a study performed on 360 medico-legal autopsy cases, Zhu et al. [114] found that both Ca and Mg levels in postmortem serum from cardiac and peripheral blood were significantly higher in saltwater drownings. Moreover, a significant elevation in postmortem serum from the left cardiac blood of both markers compared with other sampling sites suggested the influence of saltwater aspiration. Freshwater drowning and fire fatalities showed increased Ca postmortem serum levels, especially in postmortem serum from peripheral blood, suggesting an increase of skeletal muscle origin. In a study performed on 385 medico-legal autopsy cases, Li et al. [115] analysed postmortem Ca and Mg levels in pericardial fluid. They found that pericardial Ca levels were similar to clinical serum reference range, whereas pericardial Mg levels were generally higher. Both Ca and Mg levels were significantly higher in saltwater drowning, suggesting the influence of saltwater aspiration, whereas pericardial Ca were relatively high for hypothermia cases.

Studies on the diagnostic value of strontium (Sr) in seawater and freshwater drowning cases have been performed by Azparren et al. [116–121], who measured Sr concentrations in left and right ventricular blood, and by Pérez-Cárceles et al. [122], who measured Sr concentrations in postmortem serum from left and right ventricular blood as well as femoral vein blood. These authors confirmed the usefulness of cardiac blood (or postmortem serum from cardiac blood) Sr levels in diagnosing seawater and freshwater drownings, together with circumstantial data and morphological findings, especially in cases which have been in the water for less than 72 h [116–123].

In our medico-legal centre, vitreous sodium and chloride are systematically measured. In a series of 473 medico-legal autopsy cases, vitreous sodium averaged 136 mmol/l and vitreous chloride 125 mmol/l. No case of fatal hypernatremia was experienced in the last years in our centre, whereas cases of alcohol abusers with advanced liver cirrhosis and other signs of hepatic insufficiency (jaundice, ascites, pleural effusion, recanalisation of umbilical vein, splenomegaly and pelvic and oesophagus varices) also showing increased blood acetone and 3HB values and decreased vitreous sodium and chloride levels, were sometimes observed. Since hyponatremia is a common problem in individuals with advanced cirrhosis, directly related to the hemodynamic changes and

secondary neurohumoral adaptations, we concluded that, even if the cause of death was not clearly identified, ketoacidosis and hyponatremia may have represented a contributing factor in death (Table 1).

Conclusions

It seems more and more evident that, in the past, the aim of postmortem chemistry was almost exclusively to determine “the cause of death”, to characterize “the metabolic profile” consistent with a metabolic disorder, with or without morphological findings, which could explain the death. Cases of diabetic coma with a fatal outcome and presenting pathologically increased vitreous glucose, glycated haemoglobin and ketone body levels or insulin overdoses showing pathologically increased insulin levels and suppressed C peptide concentrations represent two examples of this approach. However, many researchers have recently focused their attention on other possible approaches, with different objectives. Today, postmortem chemistry can be employed not only to determine the cause of death, but also (and especially) to help in interpreting the cause of death and to support and enforce morphological and toxicological findings. On the one hand, it must be emphasized that postmortem chemistry should be incorporated among the routine forensic and medico-legal investigations, like radiology, histology and toxicology, to improve the quality of forensic autopsies. On the other hand, it must be stressed that the interpretation of postmortem chemistry results always requires prudence. Postmortem chemistry is of no value without history, radiological investigations, macroscopic findings, histology and toxicology. However, and with respect to this, it can undoubtedly represent one of the most important ancillary procedures for the forensic pathologist in investigating the cause and the process of death, the contributing conditions and the predisposing disorders.

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