# Postmortem Chemistry 10

VICTOR W. WEEDN, CHARLES F. SIEBERT, JR., AND JOSEPH A. PRAHLOW

## Contents

Introduction 235
Postmortem Specimens 237
Blood 237
Vitreous Humor 238
Cerebrospinal Fluid, Pericardial Fluid, Synovial Fluid, Urine 238
Solid Organ Tissues 238
Decomposition/Postmortem Interval 239
Hypoxia 240
Acid-Base Disorders 240
Hydration States 240
Diabetic States 241
Renal Function 242
Hepatic Function 242
Cardiac Function 243
Hyperlipidemia 244
Immunology/Serology and Anaphylaxis 244
Pancreatitis 245
Hormonal Disturbances 245
Thyroid 245
Steroids 245
Other Hormones 245
Neuroactive Agents 245
Metabolic Screening 246
Genetic Screening 246
References 247

## Introduction

Clinical chemistry came into widespread practice with the advent of the automated analyzer by Technicon in the late 1950s and early 1960s and has since burgeoned into a large industry and an integral part of modern medical diagnostic practice. Clinical laboratory tests are also a critical component of the postmortem investigation of death by hospital and forensic pathologists, since most such deaths are from natural causes. In fact, this postmortem testing for clinical disease has begun to broaden beyond traditional clinical chemistry into genetic, metabolic, transcriptomic, proteomic, and other more esoteric testing.

Laboratory testing in clinical practice continues to be debated as either under-utilized or over-utilized depending on skill and trust in clinical history and physical examination. Cases presented to medical examiners commonly have no known clinical history and do not permit the same physical exam; therefore, particularly in the absence of anatomic changes, they may be even more dependent on clinical chemistry. Unfortunately, the clinical chemistries are also altered postmortem. Nonetheless, it has been reported that 5–10% of cases may be resolved or substantially facilitated through postmortem chemistry testing.[1,2,3]

As death ensues, the metabolism of the individual is radically affected. Diminished tissue perfusion results in depleted oxygen reserves, anaerobic metabolism, and lactic acidosis. With loss of membrane integrity, potassium, magnesium, and other small molecules leak out of cells, while sodium, chloride, and calcium ions rush in. Fluid shifts between compartments become manifest. Most enzymes become erratic and fail, while other enzymes are unleashed from their protected environments. In sum, the chemical milieu changes substantially as the patient succumbs to the process of death.

After death, other changes continue to occur. Some enzymatic activity persists, which results in certain continued metabolism. Decomposition, consisting of cellular autolysis and putrefaction (microbial action), begins. Postmortem diffusion results in redistribution, although this is more an issue with exogenous agents. Most postmortem chemistries are of value only in the early postmortem period.

Several analytes are remarkably stable postmortem. Creatinine and urea are the most stable of analytes. They are in relatively high concentration, are robust chemicals that resist the normal environmental insults of early decomposition, and are easy to chemically analyze. However, this cannot be said of most other analytes, and their concentrations may be substantially altered by postmortem changes. Electrolytes, which are at high concentration gradients, immediately begin to diffuse out with postmortem loss of membrane integrity and ion pump activity. Antibody-based tests may not detect analytes that lose their tertiary structure from pH changes, hydration states, and denaturation, while other proteins may become exposed that cross-react. Proteins and other chemicals may be metabolized or become altered by enzymatic activity.

Accordingly, clinical reference testing tables cannot be assumed to be applicable to the interpretation of postmortem tests. Unfortunately there is a relative lack of scientific literature on the subject of postmortem interpretation for many analytes. Although there is an increasing body of literature on the subject, it pales in comparison to the literature on live patient testing. Much basic work has been done, but for only the most common analytes. It is, of course, difficult to obtain data that directly compares postmortem to live patient laboratory test values and relatively few papers are published as such. The bulk of the literature tries to establish postmortem distribution and correlation with pathologist findings. Sadly, postmortem interval is an obvious factor that is usually not well described in most of this literature. Environmental temperature and body mass as well as other environmental factors[4] are simply not issues of concern for clinical testing of live patients, but they confound postmortem testing. Embalming may be another consideration of a forensic autopsy that is not for clinical laboratory testing.[5] Furthermore, the type of sample, site of sampling, sample preparation, and storage[6] are still other important variables. Lastly, the laboratory testing methodology is significant to the results obtained. The bulk of the postmortem chemistry literature is older and has not been updated to more modern equipment and procedures. Within all these parameters measures of "normal" for

"postmortem" levels as well as disease cutoff values need to be established. Despite these problems laboratories do attempt to establish reference ranges, and pathologists do find postmortem clinical testing to be valuable.

## Postmortem Specimens

Femoral blood, vitreous fluid, and urine are generally the minimal biological fluid specimens from a forensic autopsy; these are generally sufficient for most clinical chemistry testing. Blood for toxicology is most commonly placed in "tiger top" serum separator tubes or standard "red top" tubes. A purple top tube, with EDTA anticoagulant, if the postmortem interval (PMI) is not too long, is also recommended for certain tests. Guidelines for postmortem testing have been recommended.[7,8,9] Postmortem interval (PMI) is the single most important factor affecting all postmortem specimens. Results should be interpreted in light of the PMI.

### Blood

Blood is a familiar sample source as the dominant sample source for clinical laboratory testing and has a vast literature. However, postmortem blood is not the same as a fresh pristine clinical sample from a live person. The clinical sample has been mixed within the body, but the postmortem blood has settled out and is no longer in a homogeneous state. Furthermore, the coagulation and complement cascades have been activated and may be in various states of clotting and lysis. Hemolysis is often an indicator that a clinical sample is not a useful sample for valid testing, but some degree of hemolysis is the rule postmortem.

The advantages of blood are that it is usually abundant and readily accessible. It is also a familiar source to laboratories and the medical community and has a large body of literature. Early postmortem testing was largely confined to blood samples. On the other hand, blood also has disadvantages. It is a complex matrix that undergoes great changes postmortem. Postmortem clotting and hemolysis results in variability and confounding factors. The intimate and large surface area of the intravascular spaces exposes it to the release of intracellular materials and to redistribution (e.g., hepatic glycogenolysis). The exposure to gut flora and the excellent nutrient medium renders the blood susceptible to microbial growth. Thus, while blood may be a good source for some analytes relatively unaffected by such processes, it is not a good source for others. Moreover, due to hemorrhage, postmortem clotting, or embalming, blood may not be available for testing.

To reduce the complexity of the matrix, serum or plasma may be extracted. Serum is usually collected from a "tiger top" ("marbled") serum separator tube, which contains a clotting activator and a gel to facilitate uncontaminated separation of the serum. Serum may also be obtained from a "red top" glass tube without additives or "red top" plastic tubes containing clot activators. Plasma is obtained from a "purple top" or "lavender top" tube containing EDTA to chelate potassium and prevent clotting and other metalloenzyme activity; however, since clotting is activated postmortem, pure plasma collection is not possible. "Grey top" tubes contain oxalate to prevent coagulation as well as fluoride to stop enzymatic activity and microbial growth.

Striking differences may be manifest between the right side of the heart (venous blood) and the left side of the heart (arterial blood) in concentrations of postmortem oxygen tension, pH, glucose, insulin, and levels of certain drugs (i.e., digoxin) and thus the site of blood sampling should be noted. Also, postmortem values from peripheral specimens are closest to levels found in living people and are therefore preferred for analysis.

## Vitreous Humor

Postmortem degradation and other shortcomings of blood have led the field to explore other sample sources, particularly vitreous humor. Vitreous is a simpler matrix and is protected from early decomposition. The vitreous fluid compartment is relatively small, with a more controlled exchange rate, and thus may reflect blood levels, but is also protected from agonal changes. It should be recognized that this fluid compartment is protected by a blood-brain barrier and thus some analytes are excluded (e.g., bile, most hormones, most enzymes). Moreover, vitreous is accessible and easily obtained.

Since vitreous is not homogeneous, the entire fluid volume should be collected.[3] On the other hand, overly vigorous aspiration will draw fragments of retinal tissues, which will contaminate the fluid; it should be crystal clear. Pairs of vitreous samples, if tested separately, permit some measure of replication and quality control, or conversely, when the samples are combined, an average measurement will be obtained.

A significant issue with interpretation of vitreous values is that data on clinical vitreous chemistries are unavailable.[2] Studies which have been conducted on globe enucleation from ophthalmologic diseases may not be representative due to the disease process for which the enucleation was performed. Studies conducted on animals are also of limited value due to questionable generalizability.

## Cerebrospinal Fluid, Pericardial Fluid, Synovial Fluid, Urine

Sample sources from non-vitreous biologic fluids are less commonly tested and often overlooked considerations. CSF may be used as a substitute for vitreous in cases of suspected child abuse where the eyes are saved for histological examination. As filtrates, they all have some advantage over blood: they are simpler matrices and to some extent protected from postmortem decomposition. Cerebrospinal fluid is protected by a blood-brain barrier, but the other fluids are not and may approximate serum values. However, they all suffer from nonspecific fluid accumulations which make quantitative testing difficult to interpret; this is most obvious in the case of urine. Synovial fluid permits replicate testing. Each fluid has potential special significance for organ specific disease associations: CSF for brain, pericardial fluid for heart, synovial fluid for joints, and urine for kidneys.

## Solid Organ Tissues

Solid tissues are not generally useful for clinical disease testing. They are complex matrices and are subject to autolysis. However, solid tissues may be appropriate specimens for organ specific chemistries, metabolic studies, and possibly microarray mRNA studies if sufficiently fresh.

## Decomposition/Postmortem Interval

As described above, during the process of dying, body tissues and fluids begin to become physiologically deranged due to fluid shifts, ion flows, acid-base disturbances, enzymatic dysfunction, loss of membrane integrity, and so on. Such derangements accelerate after death. Taphonomic changes, particularly autolysis from internal digestive enzymes and putrefaction from microbial enzymes, become dominant. These processes, unless inhibited, continue in some form for weeks, months, and years. It is these processes that so significantly undermine the utility of postmortem chemistry. In general, clinical chemistries are of value only in the early postmortem period. A vitreous potassium of >15 mEq/L is evidence of a prolonged postmortem interval, significant chemical decomposition, and that other clinical chemistries will not likely be of value.[10]

Researchers have investigated various aspects of decomposition as a method to determine the postmortem interval (PMI), the time period between death and the time of analysis, such as pH,[11] non-protein nitrogen,[12] free amino acids,[13] vitreous ammonia,[14] vitreous hypoxanthine,[15–17] calcium,[18] synovial fluid proteoglycans,[19] putrescine and cadaverine production, racemization of amino acid enantiomers, among others.[20] Different decompositional changes would conceivably cover different phases of the PMI. Regardless of the analyte and change measured or the phase of PMI studied, the underlying assumption is that the process follows a relatively predictable linear or curved model that is reproducible. Due to temperature, hydration, clothing, environmental exposures, nature of the death, and biologic variation, attempts to determine PMI through chemical analysis have largely failed to produce narrow time estimates, but rather merely suggest broad windows of time.

By far the most studied effect for PMI determination has been the diffusion of potassium from lining cells into the vitreous humor.[21] Upon death, potassium quickly begins to diffuse out of cells into the extracellular fluids. In the vitreous, the potassium has been shown to have a slower and more gradual rise making it more suitable for the determination of the postmortem interval. According to Sturner, vitreous potassium is a more reliable indicator of PMI than overall body decomposition, rigor mortis, or body temperature.[3]

Factors that cause variation in the vitreous potassium levels include environmental temperature,[22] age of the deceased,[23] terminal illness,[24] renal status,[10] sample collection,[3] and test method.[25,26] An incomplete sampling will cause a spuriously low result, but too vigorous extraction will cause a spuriously high result. Studies have shown differences in potassium of up to plus or minus 10% of the mean for each eye in about 20% of subjects.[27–29]

Despite its shortcomings, vitreous potassium has been shown to be the most reliable chemical method within the first 24 hours of death. Perhaps the most commonly used formulation was derived by Madea:[30]

$$\mathrm{PMI(h)} = 5.26 \times \mathrm{K\,concentration\,(mmol/L)} - 30.9$$

The confidence limits for this formula are ±20 hours in the first 100 hours postmortem. This assumes that the body has not been kept refrigerated.

By drawing vitreous samples from each eye at different times, Adjutantis and Coutselinis[31] claim to further increase the accuracy. Others (i.e., Schleyer[12]) claim increased accuracy through the use of combinations of chemical determinations.

## Hypoxia

Controlled studies in dogs have shown that asphyxial deaths (in which respiratory failure precedes cardiac failure) produce substantially lower arterial (left ventricular heart) blood $pO_2$ than cardiac deaths (in which cardiac failure precedes respiratory failure)—the former <25 mmHg and the latter >25 mmHg.[2,32] These findings seem to be borne out in humans.[33] However, this difference may become obscured by attempted resuscitation measures prior to death. The analytic method seems to be significant, as oxymetric profiles have found to be of limited value.[34]

Blood carbon dioxide content reflects antemortem levels and remains stable. Vitreous $CO_2$ levels vary from 4 to 27 mEq/L (average 15 mEq/L) using an AutoAnalyzer.[1] However, such results are method dependent, with some methods (i.e., AutoAnalyzer) showing a rapid decrease from increasing lactic acid and associated decreased $CO_2$ combining power.

Some investigators have used oxypurines (hypoxanthine, xanthine, uridine, and inosine), as ATP metabolites, to determine hypoxia.[17,35–38] Also, it has been noted that asphyxia produces an increase in terminal blood sugar.

## Acid-Base Disorders

Blood pH has been shown to decrease in proportion to the time of death. The value obtained is dependent on the site of the sample. A small study has shown that postmortem pH from a cardiac sample fell from 7.0 at the time of death to 5.5 at 20 hours after death.[11] However, the difference in pH between normal and severely acidotic individuals is sufficient to distinguish the two within the first five hours postmortem.[3]

Plasma lactic acid increases postmortem; it is significantly increased within the first postmortem hour and progressively increases to between 50 and 75 times the normal antemortem values.[2] Vitreous lactic acid also increases but only doubles (from 80–160 to 210–260 mg/dL) at 20 hours. Values are low in sudden cardiac death and are high in cases of prolonged agonal states.[39]

## Hydration States

Vitreous sodium and chloride levels remain relatively stable during the early postmortem phase. Sodium levels begin to decline immediately postmortem, but the rate of decrease is variable. Chloride also decreases, but at a relatively constant rate of up to 1 mEq/L/hr (although too variable for PMI determination[3]). Vitreous sodium levels generally range from 135 to 151 mEq/L (average of 143 mEq/L) in adults.[2] However, levels are lower in children (average of 141 mEq/L) and especially newborns (average of 139 mEq/L).[40] Vitreous chloride levels generally range from 104 to 132 mEq/L (average of 120 mEq/L), but are lower in infants (average of 113 mEq/L).[2] However, these results are method dependent, with flame photometry indicating elevated levels compared to normal levels by ion-specific electrodes (such as in an Ektachem determination).[3]

Abnormally high or low salt concentrations can be reliably diagnosed postmortem. Coe has categorized the vitreous electrolyte abnormalities into four patterns:[2,3]

1. *Dehydration or hypertonic pattern.* Increased sodium (>155 mEq/L) and chloride (>135 mEq/dL) and a moderate increase in urea nitrogen (40–100 mg/dL).
2. *Uremic pattern.* Increased urea nitrogen and creatinine without a significant increase in sodium and chloride.
3. *Low-salt or hypotonic pattern.* Decreased sodium (<130 mEq/L) and chloride (<105 mEq/L) with a relatively low potassium (<15 mEq/L).
4. *Decomposition pattern.* Decreased sodium and chloride with an increased (>20 mEq/L) potassium.

Thus, elevated sodium and chloride values may be due to either dehydration[41–43] or impaired renal function, whereas depressed sodium and chloride values may be due to hypotonicity or decomposition. Isolated hypernatremia may be due to hyperosmolar infant formula. Causes for hypotonicity include polydipsia and water intoxication[44,45] and alcoholism with fatty liver or cirrhosis.[3] Isolated hyponatremia has many specific causes including adrenal cortical disease, hypothyroidism, pituitary disorders, acrodynia, beer potomania, and Ecstasy abuse. Isolated hypochloremia may be seen with severe and prolonged vomiting.

## Diabetic States

Diabetic hyperglycemia should not normally be diagnosed from postmortem serum values alone.[3,46] It may be considered if the level is above 600 mg/dL from a peripheral blood source, without other explanation for the increased glucose level and if no resuscitative attempt has been performed.[2] Young infants (<3 mo of age) manifest higher postmortem glucose levels than normal adults (170.9 mg/dL v. 96.8 mg/dL). Blood glucose levels in the right atrium may be elevated from hepatic glycogenolysis.[2] Asphyxia and terminal stress has been associated with an increase in blood glucose. Apparently, attempted resuscitation may cause elevated blood glucose due to flushing of the hepatic circulation and terminal glycogenolysis.[2] Fatal hypothermia has also been reported to cause elevated blood glucose, presumably due to decreased glycolysis.[47,48] Thus, elevated postmortem glucose values can be found in diabetics, but also in normal individuals, those dying from asphyxia or hypothermia, and after terminal stress or attempted resuscitation.

Vitreous is the preferred source for glucose determinations. Embalming will not affect establishment of the diagnosis from vitreous chemistries.[5] Diagnosis of fatal diabetic ketoacidosis with severe hyperglycemia is generally obvious, but diagnosis of milder hyperglycemia is problematic. Zilg et al. use 10 mmol/L (~100mg/dL) postmortem vitreous glucose as indicating antemortem hyperglycemia.[49] Coe has reported on 1,000 consecutive natural deaths.[1] He found 103 cases of non-diabetics with serum glucose >500 mg/dL, of which 87 had been resuscitated—in none of these was the vitreous glucose >100 mg/dL. He found that a vitreous level of >200 mg/dL was only found in diabetic fatalities, regardless of resuscitative attempts or death by asphyxia.

Since diabetic ketoacidosis (DKA) is a metabolic state involving more than mere hyperglycemia, testing for lactate and volatile ketone bodies, specifically acetone, acetoacetate, and B-hydroxybutyrate, will permit a definitive diagnosis.[49,50] In fact, vitreous acetone alone has been used to establish DKA in a severely decomposed individual.[51] The presence of glucose and ketone bodies in the urine also suggests DKA. However, an aketotic form of sudden diabetic death has been described.[52–54] Elevated ketones in the absence of elevated glucose suggests malnutrition or potential neglect.[3]

Glycosylated hemoglobin (HbA1c) and glycated protein (fructosamine) are indicators of longer-standing uncontrolled glucose elevation.[55–59]

Although vitreous glucose is useful for diagnosis of hyperglycemia, it is not useful for hypoglycemia. Initial postmortem vitreous glucose levels are approximately 85% of antemortem levels and, due to postmortem glycolysis, may decline to zero within four to five hours.[60] Attempts to combine vitreous lactate levels with vitreous glucose to establish a diagnosis of hypoglycemia have not met with great success.[61–62] Given a clinical picture of starvation, chronic alcoholism, or islet cell tumor, a diagnosis of hypoglycemia might be entertained from a vitreous value of <20 mg/dL when drawn within two hours of death.[2] Sturner has reported a case of a motor vehicle accident caused by hypoglycemia.[63]

Postmortem serum insulin values have been reported to be higher than antemortem levels in healthy individuals, but are variable; however, Coe found generally lower values and decreasing with PMI.[3] Studies have shown values varying by a factor of 10 from different sites in the same body.[64] Nonetheless, the level will generally be below 100 microunits—except in diabetics on exogenous insulin.[2] A diagnosis of insulin poisoning can be made from postmortem serum studies.[67–70] C-peptide may also be measured in postmortem serum.[71] Insulin and C-peptide penetrate the vitreous to a minimal extent.[10] Special considerations are applicable to postmortem insulin analysis.[72,73]

## Renal Function

Both urea nitrogen and creatinine in blood, vitreous, and CSF reflect antemortem levels and remain stable for days and despite moderate decompositional changes.[1–3] Apparent postmortem increases reported by some authors, probably represents agonal changes—especially in those hospitalized prior to death.[2] Vitreous urea nitrogen closely parallels antemortem levels, but vitreous creatinine is slightly lower than serum levels (~0.2 mg/dL).[74] After embalming, vitreous urea nitrogen is minimally diluted and will permit a diagnosis of uremia.[8] Creatinine is slower to rise from terminal prerenal elevations and thus is favored by some authors.[2] Uremia is characterized by marked elevations of vitreous and serum urea and creatinine levels without a significant rise in sodium and chloride. Postmortem urea nitrogen and creatinine are sufficiently accurate to support a diagnosis of renal disease despite no antemortem history or autopsy findings.[2,75] As noted above, an associated elevated sodium and chloride indicates dehydration as a basis for the elevated urea and creatinine levels. Other nitrogenous compounds will increase after death and are not useful as postmortem renal function tests.[12]

## Hepatic Function

Significant postmortem serum bilirubin values accurately reflect antemortem jaundice, but slight postmortem elevations preclude diagnosis of minimal bilirubin elevations.[1–3,7,10,76] Small

amounts of bilirubin have been detected in vitreous, cerebral spinal fluid, and synovial fluid.[77] These results, however, have shown much variation when compared to serum. Neither bile nor urobilinogen are normally present in the urine, and positive tests suggest liver damage.[10] Urinary urobilinogen remains unchanged after death and accurately reflects premortem levels.

Serum liver enzymes (including LDH, SGOT, GPT) become erratically elevated after death.[1–3] A possible exception is gamma glutamyl transferase (GGT), which may be used for documenting chronic alcoholism.[78,79] Vitreous enzyme activities are minimal or absent and highly erratic, bearing no relation to serum levels.

Inversion of the albumin-to-globulin ratio accurately reflects the antemortem state.[1–3] Ammonia concentrations are normally less than 3 mg/dL for the first few hours after death, but rise sharply after an initial eight-hour period.[12] Hepatic coma may be diagnosed by elevated glutamine in the cerebrospinal fluid.[2,3]

Alcoholics will often have a low-salt pattern and low blood sugar, and other markers, such as GGT and CDT.[80–82,3] Sudden death in alcoholics with fatty livers may be due to an alcoholic ketoacidosis (AKA), in which ketone bodies will be present in the blood and vitreous.

## Cardiac Function

Clinical use of cardiac markers have evolved with time.[83–85] Lactate dehydrogenase (LDH) isoenzyme analysis was the cardiac biomarker used in the 1950s. LDH becomes elevated in about 10 hours and peaks in 2–3 days after the inception of a myocardial infarction. Myocardial LDH is composed primarily of LDH-1 and LDH-2 isoenzymes, but they are not entirely specific to the heart. Later, creatinine kinase (CK) isoenzyme analysis added further specificity to cardiac necrosis. It usually rises in 4 to 6 hours post-infarction but may not rise until 12 hours post-infarction. Myocardial CK is composed of CK-MB isoenzyme. In the 1990s, cardiac troponins (cTnI and cTnT) began to be used. They rise in a similar fashion to CK-MB. The troponin levels better correlate with severity and prognosis. Plasma norepinephrine, renin activity, antidiuretic hormone (ADH), and particularly beta-natriuretic peptide (BNP) rise after congestive heart failure.

Cardiac deaths constitute the overwhelming caseload of natural deaths seen by medical examiners. Most cardiac deaths do not manifest unequivocal anatomic changes of infarction or other causes of sudden cardiac death. The absence of an acute thrombus in cases of assumed myocardial infarction is the rule rather than the exception. The finding of severe atherosclerotic narrowing (>75%) is not itself a finding of an acute event. Despite the strong need for a useful cardiac marker, forensic pathologists have not found sufficient utility in these cardiac markers for routine use. Investigations of postmortem blood and pericardial fluid CK-MB, troponins, and other markers have yielded varied results—some indicating correlation with supposed ischemia and others not.[86–94] Hemolysis interferes with the analysis of these markers and may have prevented a showing of utility. Also, it appears that concentrations vary by site. Further studies are needed to understand the role of postmortem cardiac markers. Postmortem BNP has not been studied.

As mentioned above, oxygen tensions in left ventricular blood may distinguish between sudden cardiac death and other deaths, but are not totally consistent and will

be obscured if resuscitative attempts are made.[33,34] Vitreous lactate has been suggested to assist such analysis.[2]

Cardiac medications may also be determined postmortem.[95]

## Hyperlipidemia

Postmortem total serum cholesterol values have been documented to reflect antemortem values—both at high and low ranges.[1–3] Total serum cholesterol remains stable and has been used to identify familial hypercholesterolemia.[96] Other lipid profiles, including lipoproteins and triglycerides, have been found to be generally stable, at least within the first 24 hours, and reflect antemortem values by most, but not all investigators.[97–100] Methods of analysis are likely significant to differences in results between investigators. However, it seems clear that cholesterol esters are reduced by postmortem esterase activity.

Since individuals cannot be assumed to have fasted prior to death, the stomach and upper intestinal tract should be examined for food content. Accordingly, elevated levels should be interpreted with care if the stomach is not empty and a postprandial state inferred. However, one study found marked elevation in triglycerides, low-density lipoproteins, and apolipoprotein B in deaths of young individuals dying from coronary atherosclerosis compared to age matched controls, despite no difference due to stomach contents or postmortem interval.[101]

Attempts have been made to correlate lipid profiles with cardiac deaths.[101–104]

## Immunology/Serology and Anaphylaxis

Protein electrophoresis generally retains its profile in postmortem sera.[1–3,105,106] Albumin is somewhat decreased and beta globulins are modestly increased postmortem; other fractions remain generally unchanged. However, hemolysis can alter this profile. Postmortem serum electrophoresis has been used to diagnose agammaglobulinemia and multiple myeloma.[2]

Specific antibodies associated with various viruses and with autoimmune diseases will generally be able to be detected through Western blot techniques.[3]

Postmortem anaphylaxis testing generally involves serum tryptase from mast cell degranulation and radioabsorbent test (RAST) sensitivity analysis for serum IgE antibodies to the specific allergens.[107–115] Human beta tryptase rises rapidly, becoming detectable within 30 minutes and reaching peak levels in 2 to 3 hours; the half-life is 2 hours. Caution must be used in interpretation as elevations of serum tryptase occur in the absence of anaphylaxis.[116–118] Elevated IgE antibodies do not establish anaphylaxis, but rather a potential sensitivity. An insect venom-specific IgE antibody would be confirmatory evidence in a case of a suspected bee sting anaphylactic reaction. Approximately 1% of the population have IgE antibodies to hymenoptera venom. Also, anaphylaxis to an insect sting may occur in the absence of a venom-specific IgE antibody, presumably due to a cross-reactivity from a similar antibody.

Neopterin, a metabolite of GTP and a member of the pteridine chemical group, is a marker of cellular immune system activation under the control of T-helper cells, including

viral infections, bacterial infections, parasites, autoimmune disorders, malignant tumors, and allograft rejection. It has been measured postmortem in the serum and urine.[119–120]

## Pancreatitis

Amylase levels rise three to four times antemortem levels after two days postmortem.[2] Amylase can enter the vitreous.[121]

## Hormonal Disturbances

### Thyroid

Serum TSH is stable for approximately 24 hours and reflects antemortem values.[122–124] Some penetration of vitreous by TSH occurs, but levels do not reflect serum values.[124] Postmortem femoral blood thyroxin ($T_4$) and triiodothyronine ($T_3$) levels reflect antemortem clinical values.[125–127] $T_4$ levels decrease after death and may also be depressed agonally; therefore diagnosis of hypothyroidism must be made with caution.[122,128] On the other hand, serum $T_4$ may permit diagnosis of severe thyroiditis or death due to thyrotoxicosis.[129,130] $T_4$ does not penetrate the vitreous.[124] Postmortem thyroglobulin (Tg) has been measured by an immunoabsorbent assay.[131]

### Steroids

Cortisol levels remain stable postmortem, with an average of about 18.4 ug/dL.[132] 17-hydroxycorticosteroid levels are commonly elevated, but are particularly high after acute illness.[133] Steroids have not been reported to enter the vitreous.[3] Postmortem cortisol can establish a diagnosis of Addisonian crisis.

### Other Hormones

Parathormone (PTH) is stable in the early postmortem period and penetrates the vitreous humor.[126] Luteinizing hormone (LH) can be measured in the serum for up to 48 hours but does not pass into the vitreous.[124,134] Human chorionic gonadotropin (HCG) in postmortem blood and urine has been found to reflect antemortem values in the case of HCG-producing tumor.[135] Serum prolactin varies with the cause of death—becoming elevated in postoperative deaths, the chronically ill, and drug-related suicides.[136]

## Neuroactive Agents

Marked elevations in catecholamines from pheochromocytomas are manifest after death.[2] In general, catecholamines will be elevated from prolonged deaths and lower for quick deaths, such as from motor vehicle accidents.[2,137] Catecholamine levels are also altered in hyperthermia[138] and hypothermia.[139,140]

Massive increases in glutamine oxalacetic transaminase (GOT) and lactate dehydrogenase (LDH) and the appearance of alkaline phosphatase in clear cerebrospinal fluid is

good evidence of brain death.[2] Also, catecholamine excretion[141] and pituitary hormone release[142–143] are depressed after brain death.

Suicide has been associated with depressed serum testosterone levels,[144] elevated serum prolactin[136] and CSF corticotropin-releasing factor (CRF) levels,[145] and mixed results with the monoamine, serotonin, and its metabolite, 5-hydroxy indole acetic acid (5-HIAA).[3,146–150]

## Metabolic Screening

Most medical examiner offices now routinely perform metabolic screens on infants.[151–162] Tandem mass spectrometry (MS/MS) can interrogate amino acids and acylcarnitines useful for screening for 20 or more inherited metabolic conditions, some of which have been associated with sudden death, including medium chain acylCoA dehydrogenase (MCAD) deficiency and rarer disorders such as MSUD (maple syrup urine disease), citrullinemia, VLCAD (Very Long Chain Acyl CoA dehyrdogenase) deficiency, GA-I and GA-II, propionic acidemia, and methylmalonic acidemia. This testing is expensive and available from only a few sources; however, it appears that federal funding may become available for this. It should also be noted that this testing is becoming part of required panels of screening tests for all newborn infants. Testing for channelopathies appear on the horizon as a further screen of sudden unexpected infant deaths.

Creatine is not formed postmortem in the urine; its presence indicates significant breakdown of body tissue. Amino acids in urine have allowed diagnosis of methylmalonic acidemia and maple syrup urine disease.

## Genetic Screening

Genetic testing is still considered expensive and is not widely performed after death. Nonetheless, the promise of such testing looms large and will undoubtedly become increasingly commonplace. The concept of a molecular autopsy is already being discussed.

DNA, unlike RNA, is remarkably robust and survivable after extremely harsh environments. However, endogenous nucleases and bacteria will eventually enzymatically destroy the DNA. These processes are largely arrested when samples are taken and dried or cooled. Furthermore, formalin will hydrolyze and cross-link the DNA strands and thus may be problematic, yet will not necessarily preclude testing. Polymerase chain reaction (PCR)-based testing is extremely sensitive, permitting testing of only a relatively few remaining intact molecules. Thus, the most important factors for most postmortem testing are generally postmortem interval and the size of the DNA target tested.

It should not be forgotten that testing can also be performed on the relatives of the deceased in the case of hereditable genetic disease. Whenever testing of relatives is performed, the discovery of non-paternity must be considered.

Traditional genetic testing may be performed for known genetic disease, but there are many other postmortem applications that are possible.

Nucleic acid tests (NAT) for viral, bacterial, and even parasitic pathogens are widely available and may prove tremendously valuable. However, since RNA is less stable, tests for RNA viruses and RNA-based tests may be less valuable in a postmortem setting. The tests can take the form of a laboratory-based test on the tissue or fluid, or they may take the form

of fluorescent hybridization in situ on a histologic slide. There is interest in a "zebra chip" as a microarray for a panoply of pathogens.

Genetic testing may be useful for toxicologic testing, so-called pharmacogenomic testing. Certain tests can define a person as a fast or slow acetylator. For example, only half an oral dose of the antihypertensive hydralazine will reach the blood of a fast acetylator (FA) compared to that of a slow acetylator (SA). Such testing has been applied to toxicology.[163–165]

Spontaneous thromboembolism may particularly suggest testing for hereditary thrombophilias. Three sets of DNA tests have become common in this setting: antithrombin-III deficiency, Protein C and Protein S deficiency, and Factor V Leiden (activated protein C resistance from a variant form of Factor V).

Sudden death may suggest testing for a "channelopathy"—in which genetic mutations result in defects in cardiac ion channel proteins that may lead to fatal arrhythmias. These are generally characterized as a long Q-T syndrome (primarily Romano-Ward syndrome) or Brugada's syndrome in which the Q-T interval is normal.

One can envision the day that atherosclerosis, hypertension, diabetes, and other diseases, or subsets thereof, will someday be routinely tested at death, as they are currently becoming routine at birth.[166] The characterization of families may drive future health care.

## References


1. Coe JI, 1976. Postmortem chemistry of blood, cerebrospinal fluid, and vitreous humor, in *Legal Medicine Annual 1976*, ed by C.H. Wecht, New York, NY, Appleton, Century Croft, pp. 55–92.
2. Coe JI, 1977. Postmortem chemistry of blood, cerebrospinal fluid, and vitreous humor, in *Forensic Medicine*; vol 2, ed by C.G. Tedeschi, W.C. Eckert, L.G. Tedeschi, Philadelphia, WB Saunders, pp. 1030–60.
3. Sturner WQ, 2006. Chemical considerations, in *Medicolegal Investigation of Death*, 4th ed., ed by W.U. Spitz, Springfield, IL, Charles C Thomas, pp. 128–148.
4. Zhu BL, Ishida K, Taniguchi M, Quan L, Oritani S, Tsuda K, Kamikodai Y, Fujita MQ, Maeda H, 2003. Possible postmortem serum markers for differentiation between fresh-saltwater drowning and acute cardiac death: a preliminary investigation, *Leg Med* (Tokyo) 5(sup 1): 298–301.
5. Coe JI, 1976. Comparative postmortem chemistries of vitreous humor before and after embalming, *J For Sci* 21:583–6.
6. Bray M, 1984. The effect of chilling, freezing, and rewarming on the postmortem chemistry of vitreous humor, *J For Sci* 29:404–11.
7. Fekete JF, Brunsdon DFV, 1974. The use of routine laboratory tests in postmortem examinations, *Can Soc For Sci* 7:238–54.
8. Flanagan RJ, Connally G, Evans JM, 2005. Analytical toxicology: guidelines for sample collection postmortem, *Tox Rev* 24(1):63–71.
9. Uemura K, Shintani-Ishida K, Saka K, Nakajima M, Ikegaya H, Kikuchi Y, Yoshida K, 2008. Biochemical blood markers and sampling sites in forensic autopsy, *J For Leg Med* 15(5): 312–7.
10. Coe JI, 1993. Postmortem chemistry update, *Am J For Med & Path* 14(2):91–117.
11. Sawyer WR, Steup DR, Martin BS, Forney RB, 1988. Cardiac blood pH as a possible indicator of postmortem interval, *J For Sci* 33(6):1439–1444.
12. Schleyer F, Determination of time of death in the early postmortem interval, in *Methods of Forensic Science*, vol. 2, New York Interscience, John Wiley and Sons, pp. 253–293, 1963.
13. Durham DG, Dickinson JC, Hamilton PB, Ion-exchange chromatography of free amino acids in human intraocular fluids, 1971. *Clin Chem* 17(4):285–289.

14. van den Oever R, 1978. Postmortem vitreous ammonium concentrations in estimating the time of death, *Z Rechtsmed* 80(4):259–263.
15. Rognum TO, Hauge S, Oyasaeter S, Saugstad OD, 1991. A new biochemical method for estimation of postmortem time, *For Sci Int* 51(1):139–146.
16. James RA, Hoadley PA, Sampson BG, 1997. Determination of postmortem interval by sampling vitreous humor, *Am J For Med & Path* 18(2):158–162.
17. Madea B, Kaferstein H, Herman N, Sticht G, 1994. Hypoxanthine in vitreous humor and cerebrospinal fluid—A marker of postmortem interval and prolonged (vital) hypoxia? Remarks also on hypoxanthine in SIDS, *For Sci Int* 65(1):19–31.
18. Nowak R, Balanova S, 1989. Determination of calcium and magnesium in postmortem human vitreous humor as a test to ascertain the cause and time of death, *Z Rechtsmed* 102(2–3):179–183.
19. Hansen LM, Donnell M, Robinson S, Heimer R, Molinaro L, Laposata EA, 1990. Changes in synovial fluid proteoglycans as a possible marker of time of death, abstract G25, in the 42nd Annual Meeting, Amer Acad of For Sci publication no. 90–92.
20. Vass AA, Barshick SA, Sega G, Caton J, Skeen JT, Love JC, Synstelien JA, 2002. Decomposition chemistry of human remains: a new methodology for determining the postmortem interval, *J For Sci* 47(3):542–53.
21. Coe JI, 1989.Vitreous potassium as a measure of the postmortem interval: an historical review and clinical evaluation, *For Sci Int* 42:201–13.
22. Pex JO, Menneely KD, Andrews FC, 1983. Time of death estimations in blacktail deer by temperature and aqueous humor glucose, *J For Sci* 28:594–600.
23. Blumenfeld TA, Blanc WA, Devlin J, 1974. Postmortem vitreous humor concentrations of Na, K, Cl, Ca, and Mg in children, *Ped Res* 8:356.
24. Sturner WQ, Gantner GE, 1964. The postmortem interval: a study of potassium in the vitreous humor, *Am J Clin Path* 42(2):137–144.
25. Coe JI, Apple FS, 1985. Variations in vitreous humor chemical values as a result of instrumentation, *J For Sci* 30(3):828–835.
26. Tagliaro F, Manetto G, Cittadina F, Marchetti D, Bortolotti F, Marigo, M, 1999. Capillary zone electrophoresis of potassium in human vitreous humor: validation of a new method, *J Chromatography* 733(1–2):273–9.
27. Balasooriya BAW, St. Hill CA, Williams AR, 1984. The biochemistry of vitreous humor: a comparative study of potassium, sodium, and urate concentrations in the eyes at identical time intervals after death, *For Sci Int* 26(2):85–91.
28. Madea B, Henssge C, Honig W, Gerbracht A, 1989. References for determining the time of death by potassium in the vitreous humor, *For Sci Int* 40:231–43.
29. Pounder DJ, Carson DO, Johnston K, Orihara Y, 1998. Electrolyte concentration differences between left and right vitreous humor samples, *J For Sci* 43(3):604–607.
30. Madea B, Herrmann N, Henssge C, 1990. Precision of estimating the time since death by vitreous potassium: comparison of two different equations, *For Sci Int* 46:277–84.
31. Adjutantis G, Coutselinis A, 1972. Estimation of the time of death by potassium levels in the vitreous humor, *For Sci* 1:55–60.
32. Mithoefer JC, Mead G, Hughes JMB, Iliff LD, Campbell EJM, 1967. A method of distinguishing death due to cardiac arrest from asphyxia, *Lancet* 2:654–6.
33. Patrick JR, 1970. Cardiac or respiratory death, in *Sudden Infant Death Syndrome: Proceedings of the Second International Conference on Causes of the Sudden Death in Infants* (Seattle), ed by A.B. Bergman, J.B. Beckwith, C. Ray, Univ of Washington Press, p. 131.
34. Maeda B, Fukita K, Oritani S, Ishia K, Zhu BL, 1997. Evaluation of postmortem oxymetry with reference to causes of death, *For Sci Int* 87(3):201–210.
35. Harkness RA, Lund RJ. 1983. Cerebrospinal fluid concentrations of hypoxanthine, xanthine, urdine, and inosine: high concentrations of the ATP metabolite, hypoxanthine, after hypoxia, *J Clin Path* 36:1–8.

36. Saugstad OD, Rognum TO, 1988. High postmortem levels of hypoxanthine in the vitreous humor of premature babies with respiratory distress syndrome, *Pediatrics* 81:395–8.
37. Poulsen, JP, Rognum, TO, Hauge S, Oyasaeter S, Saugstad OD, 1993. Postmortem concentrations of hypoxanthine in the vitreous humor—A comparison between babies with severe respiratory failure, congenital abnormalities of the heart, and victims of sudden infant death syndrome, *J Perinatal Med* 21(2):153–163.
38. Opdal, SH, Rognum, TO, Vege, A, Saugstad, OD, 1998. Hypoxanthine levels in vitreous humor: a study of influencing factors in sudden infant death syndrome, *Ped Res 44* (2):192–196.
39. Sturner WQ, Sullivan A, Suzuki K. 1983. Lactic acid concentrations in vitreous humor: their use in asphyxial deaths in children, *J For Sci* 28:222–30.
40. Blumenfeld TA, Blanc WA, Devlin J, 1974. Postmortem vitreous humor concentrations of Na, K, Cl, Ca, and Mg in children, *Ped Res* 8:356.
41. DiMaio VJM, DiMaio SJ, 1980. Fatal water intoxication in a case of psychogenic, *J For Sci* 25:332–5.
42. Huser CJ, Smialek JE, 1986. Diagnosis of sudden death in infants due to acute dehydration, *Am J For Med & Path* 7(4):278–282.
43. Moder KG, Hurley DL, 1990. Fatal hypernatremia from exogenous salt intake: report of a case and review of the literature, *Mayo Clinic Proc* 65:1587–94.
44. Hyashi T, Ishida Y, Miyashita T, Kiyokawa H, Kimura A, Kondo T, 2005. Fatal water intoxication in a schizophrenic patient—an autopsy case, *J Clin For Med* 12(3):157–9.
45. Madea B, Lachenmeier DW, 2005. Postmortem diagnosis of hypertonic dehydration, *For Sci Int* 155(1):1–6.
46. Gormsen H, Lund A, 1985. The diagnostic value of postmortem blood glucose determinations in cases of diabetes mellitus, *For Sci Int* 28:103–7.
47. Bray M, 1984. The eye as a chemical indicator of environmental temperature at the time of death, *J For Sci* 29(2):396–403.
48. Coe JI, 1984. Hypothermia: Autopsy findings and vitreous glucose, *J For Sci* 29(2):389–395.
49. Zilg, B. Alkass, K. Berg, S Druid, H., 2009. Postmortem identification of hyperglycemia, *For Sci Int* 185:89–95.
50. Osuna E, Vivero G, Conejero J, Abenza JM, Martinez P, Luna A, Perez-Carceles MD, 2005. Postmortem vitreous humor beta-hydroxybutyrate: its utility for the postmortem interpretation of diabetes mellitus, *For Sci Int* 153:189–95.
51. Smialek JE, Levine B, 1998. Diabetes and decomposition: a case of diabetic ketoacidosis with advanced postmortem change, *Am J For Med Path*, 19(1):98–101.
52. DiMaio VJM, Sturner WQ, Coe JI, 1977. Sudden and unexpected deaths after the acute onset of diabetes mellitus, *J For Sci* 22(1):147–151.
53. Irwin J, Cohle SD. 1988. Sudden death due to diabetic ketoacidosis, A*m J For Med Path* 9:119–21.
54. Cohle SD, 1989. The authors reply, *Am J For Med Path* 10:270.
55. Chen C, Glagov S, Mako M, Rochman H, Rubenstein AH, 1983. Postmortem glycosylated hemoglobin (HbA1c): evidence for a history of diabetes mellitus, *Annals Clin Lab Sci* 13(5):407–410.
56. John WG, Scott KW, Hawcroft DM, 1988. Glycated haemoglobin and glycated protein and glucose concentrations in necropsy blood samples, *J Clin Path* 41:415–8.
57. Valenzuela A. 1988. Postmortem diagnosis of diabetes mellitus: quantitation of fructosamine and glycated hemoglobin, *For Sci Int* 38:203–8.
58. Ritz S, Mehlan G, Martz W, 1996. Postmortem diagnosis of diabetic metabolic derangement: elevated alpha 1-antitrypsin and haptoglobin glycosylation levels as an index of antemortem hyperglycemia, *J For Sci* 41(1):94–100.
59. Winecker RE, Hammett-Stabler CA, Chapman JF, Ropero-Miller JD, 2002. HbA1c as a postmortem tool to identify glycemic control, *J For Sci* 47(6):1373–9.

60. Coe JI, 1973. Further thoughts and observations on postmortem chemistry, *For Sci Gaz* 5(5):2.
61. De Letter EA, Pierre MH, 1998. Can routinely combined analysis of glucose and lactate in vitreous humour be useful in current forensic practice? *Am J For Med Path* 19(4) 335–342.
62. Peclet, C, Picotte P, Jobin, F, 1994. The use of vitreous humor levels of glucose, lactic acid, and blood levels of acetone to establish antemortem hyperglycemia in diabetic, *For Sci Int* 65(1):1–6.
63. Sturner, WQ, Sullivan A, 1983. Hypoglycemia as the responsible factor in a truck driver accident fatality, *J For Sci* 28(4):1016–1020.
64. Lindquist O, Rammer L, 1975. Insulin in postmortem blood, *Z Rechtsmed* 75(4):275–277.
65. Oksanen A, 1976. Insulin in postmortem blood: a comment, *Z Rechtsmed* 77:311–2.
66. Dickson SJ, Cairns ER, 1977. The isolation and quantitation of insulin in postmortem specimens: a case report, *For Sci* 9:37–42.
67. Sturner WQ, Putnam RS, 1972. Suicidal insulin poisoning with nine day survival: recovery in bile at autopsy by radioimmunoassay, *J For Sci* 17:514–21.
68. Hood I, Mirchandani H, Monforte J, Stacer W, 1986. Immunohistochemical demonstration of homicidal insulin injection site, *Arch Path Lab Med* 110:973–4.
69. Patel F, 1992. Fatal self-induced hyperinsulinaemia: a delayed post-mortem analytical detection, *Med Sci Law* 32:151–9.
70. Winston DC, 2000. Suicide via insulin overdose in nondiabetics: the New Mexico experience, *Am J For Med Path* 21(3): 237–240.
71. Iwase H, Kobayashi M, Nakajima M, Takatori, T, 2001.The ratio of insulin to C-peptide can be used to make a forensic diagnosis of exogenous insulin overdosage, *For Sci Int* 115 (1,2) 123–127.
72. Fletcher SM, 1983. Insulin: a forensic primer, *J For Sci Soc* 23:5–17.
73. Kernbach-Wighton G, Puschel K, 1998. On the phenomenology of lethal applications of insulin, *For Sci Int* 93(1): 61–73.
74. Piette M, 1989. The effect of postmortem interval on the level of creatinine in vitreous humor, *Med Sci Law* 29:47–54.
75. Zhu BL, Ishida K, Quan L, Taniguchi M, Oritani S, Li DR, Fujita MQ, Maeda H, 2002. Postmortem serum uric acid and creatinine levels in relation to the causes of death, *For Sci Int* 24;125(1):59–66.
76. Coe JI, 1974. Postmortem chemistries on blood: particular reference to urea nitrogen, electrolytes, and bilirubin, *J For Sci* 19:33–42.
77. Naumann HN, Young JM, 1960. Comparative bilirubin levels in vitreous body, synovial fluid, cerebrospinal fluid, and serum after death, *Proc Soc Exp Biol Med* 105:70–2.
78. Devgun MS, Dunbar JA, 1985. Postmortem estimation of gamma-glutamyl transferase in vitreous humor and its association with chronic abuse of alcohol and road-traffic deaths, *For Sci Int* 28:179–80.
79. Piette M, DeSchrijver G, 1987. Gamma-glutamyl transferase: applications in forensic pathology: I: study of blood serum recovered from human bodies, *Med Sci Law* 27(3):152–160.
80. Sturner WQ, Coe JI, 1973. Electrolyte imbalance in alcoholic liver disease, *J Forensic Sci* 18:344–50.
81. Sadler, D.W., Girela, E, Pounder, DJ, 1996. Post mortem markers of chronic alcoholism, *For Sci Int* 82 (2):153–163.
82. Osuna E, Perez-Carceles MD, Moreno M, Bedate A, Conejero J, Abenza J, Matinez P, Luna A, 2000. Vitreous humor carbohydrate-deficient transferrin concentrations in the postmortem diagnosis of alcoholism, *For Sci Int* 108(3):205–213.
83. Perez-Carceles MD, Osuna E, Vieira DN, Martinez A, Luna A, 1995. Biochemical assessment of acute myocardial ischaemia, *J Clin Path* 48(2):124–8.
84. Harrison A, Kirchick H, Mader S, Goldsmith BM, 2004. An analysis of cardiovascular biomarkers, *Advance/Laboratory*.

85. Jaffe AS, Babuin L, Apple FS, 2006. Biomarkers in acute cardiac disease: the present and the future, *J Am Coll Cardiol* 48(1):1–11.
86. Luna A, Carmona A, Villanueva E, 1983. The postmortem determination of CK isoenzymes in the pericardial fluid in various causes of death, *For Sci Int* 22:23–30.
87. Stewart RV, Zumwalt RE, Hirsch CS, Kaplan L, 1984. Postmortem diagnosis of myocardial disease by enzyme analysis of pericardial fluid, *Am J Clin Pathol* 82:411–7.
88. Arroyo A, Valero J, Marron T, Vidal C, Hontecillas B, Bernal J, 1998. Pericardial fluid postmortem: comparative study of natural and violent deaths, *Am J For Med Path* 19 (3):266–268.
89. Cina SJ, Thompson WC, Fischer JR Jr., Brown DK, Titus JM, Smialek JE, 1999. A study of various morphologic variables and troponin I in pericardial fluid as possible discriminators of sudden cardiac death, *Am J For Med Path* 20 (4):333–337.
90. Ooi DS, Isotalo PA, Veinot JP, 2000. Correlation of antemortem serum creatine kinase, creatine kinase–MB, troponin I, and troponin T with cardiac pathology, *Clin Chem* 46(3):338–344.
91. Ellingsen CL, Hetland O, 2004. Serum concentrations of cardiac troponin T in sudden death, *Am J For Med & Path* 25(3):213–215.
92. Davies SJ, Gaze DC, Collinson PO, 2005. Investigation of cardiac troponins in postmortem subjects: comparing antemortem and postmortem levels, *Am J For Med & Path* 26(3):213–215.
93. Khalifa AB, Najjar M, Addad F, Turki E, Mghirbi T, 2006. Cardiac troponin T (cTnT) and the postmortem diagnosis of sudden death, *Am J For Med & Path* 27(2):175–177.
94. Takeichi S, Nakajima Y, Osawa M, Yukawa N, Saito T, Seto Y, Nakano T, Adachi M, Jitsukata K, Horiuchi K, Wang T, Nakajima K, 1997. The possible role of remnant-like particles as a risk factor for sudden cardiac death, *Int J Legal Med* 114(4):213–9.
95. Coe JI, 1981. Forensic aspects of cardiac medications, *Am J For Med & Pathol* 2:329:32.
96. Leadbeatter S, Stansbie D, 1984. Postmortem diagnosis of familial hypercholesterolaemia, *BMJ* 289:1656.
97. Tilvis R, Miettinen TA, 1980. Squalene, methyl sterol, and cholesterol levels in human organs, *Arch Pathol Lab Med* 104:305–40.
98. Sarkoja T, Yla-Herttuala S, Solakjui T, Nikkari T, Hirvonen J, 1988. Stability of plasma total cholesterol, triglycerides, and apolipoproteins B and A-1 during the early postmortem period, *J For Sci* 33:1432–8.
99. Sarikioja T, Yla-Hettuala S, Solakivi T, Nikkari T, Hirvonen J, 1988. Stability of plasma total cholesterol, triglycerides, and apolipoproteins B and A-1 during the early postmortem period, *J For Sci* 33(6):1432–1438.
100. Lehtimaki T, 1991. Determination of apolipoprotein E phenotypes from stored or postmortem serum samples, *Clin Chim Acta* 203(2–3):177–182.
101. Hiserodt JC, Perper JA, Koehler SA, Orchard TJ, 1995. A comparison of blood lipid and lipoprotein values in young adults who die suddenly and unexpectedly from atherosclerotic coronary artery disease with other noncardiac deaths, *Am J For Med & Path* 16(2):101–106.
102. Enticknap JB, 1962. Lipids in cadaver sera after fatal heart attack, *J Clin Path* 14:496–499.
103. Sturner WQ, 1971. Postmortem lipid studies: attempts to correlate with death from arteriosclerotic heart disease in the young age group, *For Sci Gaz* 2(1):5–7.
104. Hart AP, Zumwalt RE, Dasgupta A, 1997. Postmortem lipid levels for the analysis of risk factors of sudden death: usefulness of the Ektachem and Monarch analyzers, *Am J For Med & Path* 18(4):354–359.
105. Brazinsky JH, Kellenberger RE, 1970. Comparison of immunoglobulin analyses of antemortem and postmortem sera, *Am J Clin Pathol* 54:622–4.
106. McCormick GM, 1972. Nonanatomic postmortem techniques: postmortem serology, *J For Sci* 17:57–62.
107. Schwartz HJ, Squillace DL, Sher TH, Tiegland JD, Yunginger JW, 1986. Studies in stinging insect hypersensitivity: postmortem demonstration of antivenom IgE antibody in possible sting related sudden death, *Am J Clin Pathol* 85:607–610.

108. Yunginger JW, Sweeney KG, Sturner WQ, Giannandra LE, Teigland JD, Bray M, Benson PA, York JA, Biedrzycki L, Squillace DL, Helm RM, 1988. Fatal food induced anaphylaxis, *JAMA* 260(10):1450–2.
109. Yunginger JW, Nelson DR, Squillace DL, Jones RT, Holley KE, Hyma BA, Biedrzycki L, Sweeney KG, Sturner WQ, Schwartz LB, 1991. Laboratory investigation of deaths due to anaphylaxis, *J For Sci* 36(3):857–865.
110. Prahlow JA, Barnard JJ, 1998. Fatal anaphylaxis caused by fire ant stings, *Am J For Med & Path* 19(2):137–142.
111. Pumphrey RSH, Roberts IS, 2000. Postmortem findings after fatal anaphylactic reactions, *J Clin Path* 53(4):273–276.
112. Horn KD, Halsey JF, Zumwalt RE, 2004. Utilization of serum tryptase and immunoglobulin e assay in the postmortem diagnosis of anaphylaxis, *Am J For Med Pathol* 25(1):37–43.
113. Nishio H, Takai S, Miyazaki M, Horiuchi H, Osawa M, Uemura K, Yoshida K, Mukaida M, Ueno Y, Suzuki K, 2005. Usefulness of serum mast cell-specific chymase levels for postmortem diagnosis of anaphylaxis, *Int J Legal Med* 119(6):331–4.
114. Perskvist N, Edston E, 2007. Differential accumulation of pulmonary and cardiac mast cell-subsets and eosinophils between fatal anaphylaxis and asthma death: a postmortem comparative study, *For Sci Int* 169(1):43–9.
115. Greenberger PA, Rostkoff BD, Lifschulz B, 2007. Fatal anaphylaxis: postmortem findings and associated comorbid diseases, *Ann Allergy Asthma Immunol* 98(3):252–7.
116. Randall B, Butts J, Halsey JF, 1995. Elevated postmortem tryptase in the absence of anaphylaxis, *J For Sci* 40(2):208–211.
117. Nishio H, Suzuki K, 2005. Three cases of suspected hyperthermia with remarkable elevation of serum mast cell tryptase, *For Sci Int* 149(1):51–5.
118. Edston E, Eriksson O, Van Hage M, 2007. Mast cell tryptase in postmortem serum-reference values and confounders, *Int J Legal Med* 2007 121(4):275–80.
119. Ambach E, Tributsch W, Fuchs D, Reibnegger G, Henn R, Wachter H, 1991. Postmortem evaluation of serum and urine neopterin concentrations, *J For Sci* 36:1089–93.
120. Ambach E, Tributsch W, Rabl W, Fuchs D, Reibnegger G, Henn R, Wachter H, 1991. Postmortem neopterin concentrations: comparison of diagnoses with and without cellular immunological background, *Int J Leg Med* 104:259–62.
121. Devos C, Piette M, 1989. Hypothermia and combined postmortem determination of amylase and isoamylase in the serum and vitreous humor, *Med Sci Law* 29(3):218–228.
122. Coe JI, 1973. Postmortem values of thyroxine and thyroid stimulating hormone, *J For Sci* 18:20–4.
123. Ross IS, Moffat MA, Reid IW, 1983. Thyroid hormones in the sudden infant death syndrome (SIDS), *Clin Chim Acta* 129:151–5.
124. Chong APY, Aw SE, 1986. Postmortem endocrine levels in vitreous humor, *Ann Acad Med Singapore* 15(4):606–609.
125. Edston, E., Druid, H., Holmgren, P., and Ostrom, M., 2001. Postmortem measurements of thyroid hormones in blood and vitreous humor combined with histology, *Am J For Med Path* 22(1): 78–83.
126. Rachut E, Rynbrandt DJ, Doutt TW, 1980. Postmortem behavior of serum thyroxine, triiodothyronine, and parathormone, *J For Sci* 25:67–71.
127. Bader M, 1982. Normal thyroxine levels in sudden death syndrome, *JAMA* 248:3095.
128. Bonnell HJ, 1983. Antemortem chemical hypothyroxinemia, *J For Sci* 28:242–8.
129. Simson LR, 1976. Thyrotoxicosis: postmortem diagnosis in an unexpected death, *J For Sci* 21:831–2.
130. Herman GE, Kanluen S, Monforte J, Husain M, Spitz WU, 1986. Fatal thyrotoxic crisis, *Am J For Med Path* 7:174–6.
131. Tamaki K, Sato K, Katsumato Y, 1987. Enzyme-linked immunosorbent assay for determination of plasma thyroglobulin and its application to postmortem diagnosis of mechanical asphyxia, *For Sci Int* 33:259–65.

132. Finlayson NB, 1965. Blood cortisol in infants and adults: A postmortem study, *J Pediatr* 67:284–92.
133. Done AK, Ely RS, Kelly VC, 1958. Studies of 17-hydroxycorticosteroid concentrations at death in human subjects, *Am J Dis Child* 96:655–65.
134. Mendelson JH, Dietz PE, Ellingboe J, 1982. Postmortem plasma luteinizing hormone levels and antemortem violence, *Pharmacol Biochem Behav* 17(1):171–173.
135. Ludwig J, in *Current Methods of Autopsy Practice*, Philadelphia, W.B. Saunders, p. 220, 1972.
136. Jones TJ, Hallworth MJ, 1999. Postmortem prolactin as a marker of antemortem stress, *J Clin Pathol* 52(10):749–51.
137. Hirvonen J, Huttunen P, 1996. Postmortem changes in serum noradrenaline and adrenaline concentrations in rabbit and human cadavers, *Int J Legal Med,* 109(3):143–146.
138. Kortelainen M, Huttunen P, Lapinlamppi TD, 1990. Urinary catecholamines in hyperthermia-related deaths, *For Sci Int* 48:103–10.
139. Hirvonen J, Huttunen P, 1982. Increased urinary concentrations of catecholamines in hypothermia deaths, *J For Sci* 27:264–71.
140. Lapinlampi TD, Hirvonen JI, 1986. Catecholamines in the vitreous fluid and urine of guinea pigs dying of cold and the effect of postmortem freezing and autolysis, *J For Sci* 31:1357–67.
141. Feibel H, 1981. Reduced catecholamine excretion at onset of brain death, *Lancet* 1:890.
142. Powner DJ, Hendrich A, Lagler RG, Ng RH, Madden Rl, 1990. Hormonal changes in brain dead patients, *Crit Care Med* 18:702–8.
143. Zaloga GP, 1990. Endocrine function after brain death, *Crit Care Med* 29:785–6.
144. Roland BC, Morris JL, Zelhart PF, 1986. Proposed relation of testosterone levels to make suicide and sudden death, *Psychol Rep* 59:100–2.
145. Arato M, Banki CM, Bissette G, Nemeroff CB, 1989. Elevated CSF CRF in suicide victims, *Biol Psych* 25(3):355–359.
146. Ricci LC, Wellman MM, 1990. Monoamines: biochemical markers of suicide? *J Clin Psychol* 46:106–16.
147. Stanley M, Stanley B, 1990. Postmortem evidence for serotonin's role in suicide, *J Clin Psychol* 51(suppl): 22–8.
148. Endo T, Hara S, Kuriiwa F, Kano S, 1990. Postmortem changes in the levels of monoamine metabolites in human cerebrospinal fluid, *For Sci Int* 44:61–8.
149. Karkela J, Scheinin M, 1992. Tryptophan and biogenic amine metabolites in postmortem human cisternal fluid: effects of postmortem interval and agonal time, *J Neurol Sci* 107:239–45.
150. Molcho A, Stanley B, Stanley M, 1991. Biological studies and markers in suicide and attempted suicide, *Int Clin Psychopharm* 6(2):77–92.
151. Morrow G, Barness LA, Auerbach VH, DiGeorge AM, Ando T, Nyhan WL, 1969. Observations on the coexistence of methylmalonic academia and glycinemia, *J Ped* 74:680–90.
152. Hallock J, Morrow G, Karp LA, Barness LA, 1969. Postmortem diagnosis of metabolic disorders, *Am J Dis Child* 118:649–51.
153. Bennet MJ, Marlow N, Pollit RJ, Wales JKH, 1986. Glutaric aciduria type I: biochemical investigations and postmortem findings, *Eur J Ped* 145:403–5.
154. Emery JL, Variend S, Howat AJ, Vawter GF, 1988. Investigation of inborn errors of metabolism in unexpected infant deaths, *Lancet* 1988(2):29–31.
155. Coude M, Bonnefont JP, Charpentier C, Chadefaux B, Saudubray JM, Kamoun P, 1989. Aqueous humor, a possible material for postmortem methylmalonic acidaemia, *J Inherited Metab Dis* 12:95–6.
156. Bennett MJ, Hale DE, Coates PM, Stanley CA, 1991. Postmortem recognition of fatty acid oxidation disorders, *Ped Pathol* 11:365–70.
157. Touma EH, Charpentier C, 1992. Medium chain acyl-CoA dehydrogenase deficiency, *Arch Dis Child* 67:142–5.
158. Bennett MJ, Rinaldo P, 2001. The metabolic autopsy comes of age, *Clin Chem* 47(7):1145–6.
159. Green A, Preece MA, Hardy D, 2002. More on the metabolic autopsy, *Clin Chem* 48(6):964–5.

160. Christodoulou J, Wilcken B, 2004. Perimortem laboratory investigation of genetic metabolic disorders. *Semin Neonatol* 9(4):275–80.
161. Olpin SE, 2004. The metabolic investigation of sudden infant death, *Ann Clin Bichem* 41(4): 282–93.
162. Ernst LM, Sondheimer N, Deardorff MA, Bennett MJ, Pawel BR, 2006. The value of the metabolic autopsy in the pediatric hospital setting, *J Ped* 148(6):779–83.
163. Jannetto PJ, Wong, SH, Glock SB, Laleili-Sahin E, Schur BC, Jentzen JM, 2002. Pharmacogenomics as molecular autopsy for postmortem forensic toxicology: genotyping cytochrome P450 2D6 for oxycodone cases, *J Analytical Toxicology* 26(7):438–447.
164. Wong SH, Wagner MA, Jentzen JM, Schur C, Bjerke J, Glock SB, Chang CC, 2003. Pharmacogenomics as an aspect of molecular autopsy for forensic pathology/toxicology: does genotyping CYP 2D6 serve as an adjunct for certifying methadone toxicity? *J For Sci* 48(6):1406–1415.
165. Jin M, Glock SB, Jannetto PJ, Jentzen JM, Wong SH, 2005. Pharmacogenomics as molecular autopsy for forensic toxicology: genotyping cytochrome P450 3A4*1B and 3A5*3 for 25 fentanyl cases, *J Analytical Toxicology* 29(7):590–8.
166. NIH newborn screening website: http://www.nlm.nih.gov/medlineplus/newbornscreening.html (accessed 5/21/09).