Analysis of causes that led to Charles Fleming's illness and sudden death

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Abstract

Charles (Chuck) Fleming's acute illness developed on June 12, 2000 induced by the ingestion of toxic doses of creatine monohydrate and high levels of propylene glycol (PEG). Chuck was taking several medications contained PEG that increased creatine bioavability and caused acute renal failure, severe hypophosphatemia, and ketoacidosis. Chuck's serum phosphorous level was 0.1 mg/dL (normal range: 2.8-4.9 mg/dL) and his hypophosphatemia caused hemolytic crisis. Chuck's red blood cell count and hemoglobin levels on June 13th were reduced by 27% of those measured on June 12th.

The bleeding, edema, and necrosis observed in Chuck's brain were caused by the high doses of heparin and sodium bicarbonate given in the Hospital. Chuck developed acute cardiac dysfunction due to hypophosphatemia, hypokalemia, hypomagnisemia, metabolic acidosis, and metabolic alkalosis. Chuck suffered from cardiomegaly and pulmonary atrophy as a result of the chronic use of corticosteroid medications. Chuck's heart and right lung weights were 183% and 84% of normal average weight for age, respectively.

The treating physicians and the medical examiner did not measure formic acid in Chuck's blood, urine, stomach contents, or tissues. The blood methanol measurements reported on June 12th and 13th represent a false positive. It is likely that the four bottles of Gatorade containing methanol presented in court are not the same bottles of Gatorade that Diane and Chuck spiked with creatine monohydrate on June 11th. The commonwealth's allegation against Diane that she poisoned her husband with methanol is not supported by medical and scientific facts, which support Diane's innocence.

Key words: acute heart failure, brain necrosis, brain edema, bleeding in the brain, cardiomegaly, Chuck Fleming, Diane Fleming, formic acid, edema, hemolytic crisis, hemoglobinurea, heparin, hemodialysis, hemoperfusion, hypophosphatemia, hypokalemia, hypomagnesemia, methanol poisoning, methanol false positive, pulmonary atrophy, renal failure, sodium bicarbonate, splenomegaly, thrombocytopenia

1. Summary of the case and findings

Charles Linwood Fleming, Jr. (Chuck) is a 37-year-old white male. He suffered from acute illness on June 12, 2000 following the consumption of a toxic amount of creatine monohydrate mixed with Gatorade. His wife called 911 and he was admitted to Chippenham Hospital in Richmond, Virginia.

He was diagnosed as suffering from acute intoxication with methanol and treated for methanol poisoning. Chuck died on June 14th and Dr. Marcella Fierro, Chief Medical Examiner of Richmond Virginia, performed the autopsy in Chuck's case on June 15th (Case # 368-00).

Chuck also suffered from chronic intermittent symptoms of fatigue and shortness of breath over a few months prior to his death. Physicians who treated Chuck on June 12-14, 2000, Dr. Fierro, and Detective (s) from the Chesterfield County Police Department alleged that Chuck died as a result of a chronic and acute intoxication with methanol. Diane Fleming, Chuck's wife was accused of poisoning and killing her husband with methanol.

Diane was indicted by Grand jury for poisoning and killing Chuck and then arrested. She was put on trial on February 19, 2002 in the circuit court of the county of Chesterfield, Virginia and her trial lasted for two days (Cr01F01484-01,2). She was convicted of poisoning and killing Chuck by adding methanol to his drinks over a long period of time. She was sentenced to 50 years in prison (30 years for the first degree murder and 20 years for the adulteration of food) without the possibility of parole.

Diane Fleming contacted me and requested that I evaluate the medical evidence in Chuck's case to find the likely causes that led to Chuck's chronic and acute illnesses and sudden death. I am a toxicologist and pathologist with over 20 years experience in these fields. I have published over 40 articles in medical and scientific journals.

In addition, I have evaluated many cases of children who died suddenly from unexplained causes and I was able to explain the causes of death using differential diagnosis. I have also evaluated cases of children and adults who suffered from acute and/or chronic illnesses and I was able to identify the causes of their illnesses using differential diagnosis. I have served as an expert witness in many medical-legal cases involving children and adults.

In Chuck's case, I reviewed the following documents and the pertinent medical articles cited in this report. 1) Chuck's medical record obtained from Chippenham Hospital; 2) autopsy report; 3) toxicology report; 4) case history obtained from Diane covering Chuck's health and medications used during the eight years prior to his death; 5) Diane's trial transcripts. I also examined the H & E stained tissue sections obtained from Chuck's brain, lung, heart, liver, and kidney microscopically.

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These slides were purchased from the medical examiner's office in Richmond, Virginia.

I performed differential diagnosis to evaluate the medical evidence, relevant documents and articles cited in this report. Approximately 400 hours were required to evaluate the medical evidence, perform analysis, and write this report. My findings in this case include:

1) Chuck's acute illness developed on June 12, 2000 was induced by the ingestion of toxic doses of creatine monohydrate (Crm) on June 11th and 12th and significant levels of propylene glycol (PEG). PEG present in Chuck's medications increases creatine solubility in the gastrointestinal tract, absorption from the intestine, tissue uptake, and creatine bioavalablity (Section 6).

Creatine caused acute renal failure, severe hypophosphatemia, and ketoacidosis. Chuck's serum phosphorous levels on June 13th and 14th were 0.1 mg/dL and 0.0 mg/dL (normal range: 2.8-4.9 mg/dL), respectively. On June 12th, his blood pH was 7.07 and he had high blood level of beta-hydroxybutric acid (BHA) of 296 µg/mL, which is 14 times more than the average level detected in a fasting individual. His urine also had high level of ketone bodies. In addition, Chuck had high lactic acid level of 9.6 mmol/L. Lactic acid is a major metabolite of PEG.

The Commonwealth of Virginia Forensic Lab (VFL) found PEG in Chuck's blood at the level of about 200 mg/L. The blood sample was taken at 0810 on June 13th. The biological half-life of PEG in adult is about 5 hours. The expected levels of PEG in Chuck's blood at the time of his hospitalization (1830 on June 12th) and the time of his illness (0600 on June 12th) were 800 mg/L and more than 1600 mg/L, respectively. In this case, Chuck ingested a significant amount of PEG, capable of solubilizing all Crm ingested (Section 6).

2) Chuck suffered from hemolytic crisis due to the destruction of red blood cells resulting from the severe deficiency of phosphorous. Chuck's red blood cell count and hemoglobin levels on June 13th were reduced by 27% of those measured on June 12th. Urine analysis performed following Chuck admission to the hospital indicates that he had hemoglobinurea.

3) Bleeding observed in Chuck's brain on the CT scan of June 14th and during autopsy was caused by the high doses of heparin given to Chuck in the hospital as indicated by the following clinical data: a) Chuck's CT scan of the brain taken following admission on June 12th did not show bleeding or any abnormal lesion. b) Chuck's prothrombin time (PT) and the partial thromboplastin time (PPT) were significantly increased as a result of heparin use. On June 13th, Chuck's PT and PTT values were about three times and five times the normal value, respectively. c) Chuck's platelet count on June 14th was reduced by 58% of that measured on June 12th as a result of the use of heparin (Section 4).

4) The edema and necrosis observed in Chuck's brain were caused by his treatment with high doses of sodium bicarbonate in the hospital, hypophosphatemia, and bleeding. Chuck's blood pH at admission on June 12th was 7.07 and it was raised to 7.63 on June 14th as a result of the treatment with high doses of sodium bicarbonate. The treatment with high doses of sodium bicarbonate caused edema in the brain and other tissues due to anoxia. Chuck's brain was about 111% of the average normal weight for age and the increase in weight was not limited to the brain. Chuck's liver, kidneys, and spleen were 116-128% of the average normal weight for age (Section 7).

5) Chuck developed necrosis in the cardiac muscles and cardiac dysfunction due to hypophosphatemia, hypokalemia, hypomagnisemia, metabolic acidosis, and metabolic alkalosis. Chuck's ECG exam taken at 0151 on June 13th indicates that significant changes had occurred when compared with ECG of June 12th. Chuck's ECG performed at 1144 on June 13th indicates that his cardiac problems became worse.

Blood analysis revealed that Chuck had high levels of creatine kinase-MB (CKMB) and troponin, which indicate cardiac muscle damage (Section 8). The examination of the H & E stained sections of the cardiac muscles microscopically revealed the presence of necrosis in cardiac muscles.

6) Dr. Christopher Acker treated Chuck in the hospital for methanol poisoning on June 12-14, 2000. However, he did not measure formic acid in Chuck's blood and urine to confirm that Chuck's acidosis was caused by the accumulation of formic acid. He also did not do differential diagnosis to rule out other causes of acidosis in this case. Chuck's acidosis was caused by the accumulation of ketone bodies and lactic acid (Section 6).

7) The following clinical data indicate that the blood methanol measurements reported on June 12th and 13th in Chuck's case represent a false positive and Chuck did not suffer from methanol intoxication. More medical studies and observations related to this issue are described in Section 10 of this report.

a) The Medical college of Virginia (MCV) reported that Chuck had a blood methanol level of 750 mg/L. However, MCV did not measure formic acid in the blood or urine. Formic acid is the major metabolite of methanol that causes acidosis in humans and should be measured whenever methanol poisoning is suspected. Chuck's blood sample taken on June 12th was also sent to the Commonwealth of Virginia Forensic Lab (VFL) for methanol analysis. VFL reported the level of methanol in Chuck's blood to be 600 mg/L, which is 20% less than that reported by MCV. VFL also did not measure the level of formic acid in the blood.

b) MCV reported the level of methanol in Chuck's blood sample taken at 0810 on June 13th to be 200 mg/L. This sample was also tested for methanol by VFL and reported a methanol level of 100 mg/L, which is 50% of that reported by MVC. Both labs did not check for the presence of formic acid in the blood or urine samples.

c) Chuck had high levels of ketone bodies (acetone, acetoacetate and, beta-hydroxybutyrate) and PEG in his blood. The presence of these chemicals in the blood can interfere with alcohol measurements as described in Section 10 of this report.

d) Formic acid inhibits mitochondrial cytochrome oxidase activity. Individuals suffering from methanol intoxication usually develop retinal and optic nerve bleeding, necrosis,

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and atrophy. Dr. Acker examined Chuck's eyes following admission on June 12th and stated he did not observe any abnormal changes. The funduscopic exam of both eyes was normal and Chuck did not have any retinal bleeding or any other lesion associated with acute methanol toxicity (Sections 4 & 10).

8) The likely cause of Chuck's cardiomegaly and pulmonary atrophy is the chronic use of corticosteroid medications. Chuck's heart weighted 680 g, which is 183% of the normal average for age. The weight of his right lung is 83.6% of the average normal weight for age and 98.6% of his left lung. The right lung is usually bigger than the left lung and it weighs about 15% more than the left lung. The chronic use of high doses of corticosterod has known to cause cardiomegaly and myopathy as explained in Section 9 of this report.

9) Medical data that show Chuck did not die as a result of methanol poisoning was overlooked or ignored, leading me to conclude Fierro's investigation in this case is incomplete as I described in Section 5 of this report. Briefly, she did not consider in her investigation the following critical factors: a) The toxicity of creatine and the interaction of creatine with PEG present in Chuck's medications; b) the causes and the clinical significance of Chuck's severe hypophosphatemia; c) the adverse reactions of the high doses of heparin and sodium bicarbonate given to Chuck in the hospital in causing bleeding, edema, and necrosis in the brain; d) causes of Chuck's cardiomegaly and pulmonary atrophy. Furthermore, she did not measure the levels of formic acid in Chuck's blood, urine, stomach contents, and tissues to rule out false diagnosis of methanol poisoning.

10) The Commonwealth's expert witnesses did not present clinical data and medical evidence in court that show Chuck ingested and/or was exposed to methanol by any route and his acute and chronic symptoms were caused by methanol (Section 11).

11) It is likely that the methanol detected in the Gatorade bottles presented in court did not come from the windshield washer fluid as the police alleged. In addition these bottles of Gatorade, containing methanol, are not the same bottles of Gatorade that Diane and Chuck spiked with creatine monohydrate on June 11, 2000. I described studies and observations that support my conclusions in Section 11. Briefly these observations include:

a) The windshield washer fluid (WWF) bottle found in Diane's house contains 32.8% methanol and the amount of fluid needed to spike four bottles of Gatorade to yield 3.3-4.7% methanol is 274 mL. The WWF bottle found in Diane's garage contained 3820.4 mL and the tolerance limits are 3709-3860 mL. I purchased a full bottle of WWF (Peak + 32°F) from Wal-Mart and the volume of fluid in this bottle is 3700 mL.

b) I added 2 ounces of WWF (blue) to 18 ounces of each of the three types of Gatorade used by Diane and Chuck. The colors of the Gatorade changed from yellow (lemon-lime G.) to green; orange (orange G.) to brown yellow; and pink (fruit punch G.) to magenta (purplish red). The police and the State Toxicology Lab reported no change in the colors of three types of Gatorade.

c) Diane and Chuck added 22.5 g of creatine monohydrate (Crm) to each bottle of Gatorade and it is expected that more than 50% of Crm to settle at the bottom of the bottle. The solubility of Crm in water at 25°C (77.0°F) and 4°C (39.2°F) are 17 mg/mL and 6 mg/mL, respectively. The State Toxicology Lab and Det. Baker did not report that they saw sedimentation had settled at the bottoms of the Gatorade bottles.

I added 22.5 g of Crm (white powder) to 591 mL of Gatorade (lemon-lime, orange, or fruit punch) and I mixed them by vigorous shaking for an hour at room temperature (77.0°F). Then, I left these bottles on a table for 15 minutes. I observed the formation of thick ring of powder at the bottom of each bottle.

Furthermore, I added 22.5 g of Crm to 20 ounces of each of the three flavors of Gatorade containing 10% of pure methanol or 10% of WWF and mixed them vigorously for about one hour. Then, I left these bottles on a table for 15 minutes. I observed the formation of thick ring of sediment at the bottom of each bottle. It indicates that Crm is not soluble in methanol.

12) Diane was denied effective assistance of counsel in that counsel failed to present expert testimony to show a) Chuck's acute and chronic symptoms were indicative of illnesses other than methanol poisoning; b) the commonwealth's allegations that Chuck died as a result of chronic and acute methanol poisoning are not supported by medical and scientific data; c) the high doses of heparin and sodium bicarbonate given in the hospital caused bleeding, edema, and necrosis in the brain; d) the four Gatorade bottles that contained methanol are not the same bottles of Gatorade containing the creatine that Diane and Chuck added to Gatorade on June 11, 2000.

The clinical data and medical studies described in this report clearly show that Chuck's acute and chronic illnesses and death were not caused by methanol; the commonwealth's allegations against Diane Fleming that she poisoned her husband with methanol are not supported by medical and scientific facts which confirm Diane is innocent.

2. Review of Chuck's medical history and treatments prior to his hospitalization on June 12, 2000

Charles Linwood Fleming, Jr (Chuck) is a 37-year-old white male from the state of Virginia. He worked as maintenance supervisor in Philip Morris. He suffered from an acute health problem on June 12, 2000 and was admitted to Chippenham Medical Center in Richmond Virginia. He died on June 14, 2000. He did not smoke. He was a daily drinker of two to four shots of bourbon.

Chuck was an athletic man and his past medical history is significant for intermittent complaints of heartburn and loss of endurance over 3-4 months prior to his death. He was treated symptomatically. Furthermore, the review of his medical history revealed the medical problems described below. Table 1 contains a list of ten medications and supplements used by

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Chuck during the three years prior to his hospitalization on June 12, 2000. Nine of these agents contained propylene glycol (PEG) as a solubilizer [1].

1) Chuck was extremely myopic and had worn glasses since elementary school. He wore contact lenses from his early teens. There was no significant change in Chuck's vision during the ten years prior to his death and his prescription for contact lenses did not change significantly. Chuck played basketball several times a week and he depended upon clear vision. He never indicated having any visual disturbances, even on June 11 and 12, 2000 when he became ill. In the morning of June 12th, he drove to and from work, about 20 miles each way.

2) Chuck suffered from rosacea for several years before it was treated. His wife stated that he had it almost from the time they got married in 1990. It was on his face only, mostly on the bridge of his nose and along the outer nasal folds. He had reddish large flakes mostly in the nasal folds and between his eyebrows. There were never bumps, only the redness and flaky skin. He never mentioned any itching.

Chuck used betamethasone cream locally and he also tried Lotrimin and other antifungal creams. In 1999, he started to use tetracycline at about one year prior to his death on June 14, 2000.The tetracycline seemed to help a bit but the lesions did not go away. Heat or sweating made the lesion become redder, similar to when he came in from playing basketball. During the last year or so prior to his death, he also applied hydrocortisone 1% cream after his shower. He was using it on a continuing basis. His wife would purchase it for him two tubes at a time.

3) Chuck had a fungal infection on his toenails for about two years before he died. The infection lasted for a few months. He was treated with oral antifungal (Diflucan) and his toenails cleared up fairly well. However, the affected toenails remained thick and a little yellowish.

4) Chuck suffered from allergies and used beclomethasone dipropionate nasal spray (Vencenase AQ 84μg, Beconase AQ) twice daily from the beginning of 1998 until his hospitalization on June 12, 2000.

5) Chuck consumed creatine monhydrate supplement mixed in Gatorade on June 11 and 12, 2000. He and his wife mixed 1 and ½ tablespoon of creatine monohydrate (22.5 g) in 20 ounces (591 mL) of Gatorade. They prepared five bottles. He drank one bottle of the prepared mixture on June 11th and they put four in the refrigerator. Only one bottle was found in the refrigerator of the Fleming's home on June 14, 2000. Chuck took three bottles of creatine-Gatorade mixture with him to work on the morning of June 12th [1, 2].

6) Chuck was exposed to significant levels of propylene glycol (PEG) via ingestion, inhalation, and dermal absorption routes during the three years prior to his death. Nine of his medications and supplements listed in Table 1 contain PEG as a solubilizer. Creatine monohydrate (Crm) is highly soluble in PEG.

Table 1. List of medications and supplements used by Chuck during the 3 years prior to his death on June 14, 2000

Medications	Time of use	Treatment for	Solvent[1]
Betamethasone cream	Prior to 1999	Rosacea	Propylene glycol (PEG) [3]
Antifungal cream	Prior to 1999	Rosacea	PEG [4]
Tetracycline	1999-June 12, 2000	Rosacea	PEG [5]
Hydrocortisone 1% cream	1999-June 12, 2000	Rosacea	PEG [6]
Beclomethasone dipropionate nasal spray (Vancenase AQ 84 μg), twice daily	Beginning of 1998-June 12, 2000	Allergy/ Asthma	PEG [7]
Diflucan oral	1998	Fungal infection	PEG [8]
Naproxen 500 mg per day	1997- June 12, 2000	Joint pain	PEG [9]
Prevacid 30 mg per day	1998- June 12, 2000	Inhibits secretion of gastric acid	PEG [10]
Multivitamins with iron	April-June 12, 2000	Supplement	PEG [11]
Creatine monohydrate	June 11-12, 2000	Supplement	Gatorade [1,2]

[1] PEG is commonly used as a drug solubilizer [3-12].

3. Chuck's sudden illness developed on June 12, 2000 following the ingestion of creatine powder mixed in Gatorade

Diane and Chuck went to Costco in the afternoon of Sunday, June 11, 2000 and purchased a case of 24 bottles of Gatorade (20-ounce/bottle). Then, Diane and her daughter went to the General Nutrition Center (GNC) and purchased creatine monohydrate (Crm) powder for Chuck. Chuck read information about Crm stating that the use of supplement increases muscle size and Chuck wanted to use Crm to enhance his muscle performance. Chuck's recreation activities included lifting weights several times a week and playing basketball. Chuck had never used Crm before [2].

In the afternoon of Sunday, June 11th, Diane and Chuck mixed one and a half tablespoons of Crm powder (22.5 g) in one bottle of Gatorade (20-ounce). They noticed that the Crm powder did not dissolve well in Gatorade and they shook the bottle well. Then, they put it in the refrigerator to become cold. On that Sunday, Chuck drank the creatine-mixed Gatorade after a trip to the pool and after playing basketball [2].

In the evening of June 11th, Chuck brought four more bottles of Gatorade and put them on the Kitchen counter. He asked Diane to help him to mix the creatine monohydrate powder with the Gatorade. They prepared the creatine-Gatorade mixture as described above (22.5 g of Crm powder/20 ounce of Gatorade). Diane stated that the powder did not dissolve completely in the Gatorade and stayed in the bottom of the bottle like a white

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layer. They shook the bottle well to make the powder dissolve and then they put the bottles in the refrigerator [2].

Chuck ate a light dinner with some ice cream and went to bed between 9:30-10:00 pm. He woke up at about 0545 on June 12th. Diane saw him sitting on the chair to get dressed to go to work and she heard him making groaning noises. She said to him "what's wrong" and Chuck said "I really do not feel well". Diane suggested to Chuck that he stay home. Chuck did not want to stay home and he went to work at his job at Philip Morris [2].

Chuck retuned from work on the morning of June 12th after spending 1-2 hours at work because he felt very sick. He stated that he had thrown up several times at work. He also suffered from nausea. Chuck laid down on the couch. His wife asked him if he wanted to go to see a doctor. He stated that he couldn't stand ride in the car.

Diane called Chuck's doctor and the nurse in the office suggested that Chuck drink flattened Coke. He drank the Coke and he vomited. Diane called the doctor again and the nurse said that doctor would call in prescription for suppositories. He ordered Phenergan 50 mg suppositories [2].

Diane went to the Winn-Dixie pharmacy at about 1700 on June 12th and picked up the suppositories and returned home. She found Chuck upstairs laying on the bed trying to be comfortable. He was complaining about being short of breath. He said that when he laid down on the bed, he could not breathe. At this point Diane called 911 to get an ambulance to take Chuck to the hospital. The Emergency teams (EMT) received the call at 1753 and arrived at the scene at 1805. They examined Chuck and he stated that he had back pain, nausea, vomiting, and problems breathing [2].

The EMT left the scene at 1814 and took Chuck to the Chippenham Medical Center, Richmond, VA and arrived at 1835. The EMT gave Chuck Albutrol at 1815 to help him to breathe and 1000 mL of N-saline IV. They also checked his blood glucose level, pulse, blood pressure, respiratory rate, and blood-oxygen saturation levels [13]**.** He had blood glucose level of 166 mg/dL at 1807. Chuck's vital measurements are presented in Table 2.

Table 2. Chuck's vital measurements reported by EMT on June 12, 2000

Measurements	At 1807	1818	1830	Average normal
Pulse rate per min.	90	96	88	56
Blood pressure mmHg	190/100	190/110	-	120/80
Respiratory rate/min.	30	34	30	16
O_2 blood Saturation%	99	88	100	98

4. Chuck's hospitalization event on June 12-14, 2000, symptoms, diagnoses, and treatments given

The paramedics brought Chuck to Chippenham Medical Center at 1835 on June 12, 2000. He had profound metabolic acidosis. Medical college of Virginia (MCV) analyzed Chuck's blood sample taken at 2150 on June 12th and reported a blood methanol level of 750 mg/L [14].

However, MCV did not measure the formic acid and propylene glycol (PEG) levels in Chuck's blood. In addition, MVC did not measure the levels of methanol, formic acid, or PEG in Chuck's urine. I presented medical evidence in this report that shows the methanol measurements in Chuck blood represent a false positive.

Chuck was treated for methanol intoxication. He developed bleeding, edema, and necrosis in the brain as a result of his treatment with high doses of heparin and sodium bicarbonate. He also developed edema and necrosis in other organs. He died at 1630 on June 14th [14].

My review of the clinical data described below show that Chuck suffered from acute renal failure and severe hypophosphatemia due to the ingestion of toxic doses of creatine monohydrate and a high level of PEG. Chuck had taken several medications and vitamins that contain PEG as solubilizer (Table 1). PEG increased creatine solubility in the gastrointestinal tract and increased it's absorption from the intestine and tissue uptake.

Chuck's had serum phosphorous level of 0.1 mg/dL (normal range: 2.8-4.9 mg/dL).

Severe phosphorous deficiency was resulted from the involvement of several factors. These include: a) the phosphorylation of creatine in muscle by phosphocreatine kinase using ATP; b) shifting of PO_3- from the extracelluar fluid to intracellular fluid due to the reduction of PCO_2 in tissues and the activation of phosphofrucktose kinase; c) reducing the reabsoption of PO_3 from the damaged renal tubules.

Chuck developed hemolytic anemia and hemogolbinurea as a result of hypophosphatemia. His metabolic acidosis was caused by renal failure, hypophosphatemia, and lactic acidosis. He also developed hypokalemia and hypomagnesiumemia as a result of his treatment with high doses of sodium bicarbonate in the hospital.

4.1 Chuck's symptoms, clinical tests, and treatments given on June 12th

4.1.1 Physical exam

The paramedics brought Chuck to the emergency room at the Chippenham Medical Center at 1835 on June 12, 2000. The admitting physician was Dr. Christopher Acker, a nephrologist. Chuck complained of increasing shortness of breath.

Physical exam showed Chuck was agitated. Chuck's head was normocephalic and atraumatic. His eyes had intact extraocular movements. His sclerae were anicteric (without jaundice). His funduscopic exam of both eyes was normal and he did not have any bleeding.

Chuck's lungs were clear to auscultation. Cardiovascular exam showed regular rhythm and there was no pericardial friction rub. His abdomen appeared non-tender and he had no active bowel sound. His extremities were normal and his skin showed no obvious rash.

He had respiratory rate of 36/min, pulse rate of 76/min, blood pressure of 159/80 mmHg, and a body temperature of 98.7 °F. Chuck's vital measurements taken during the first four hours following admission are listed in Table 3. His respiratory rate was twice the normal rate. He also suffered from hypertension. His heart rate was increased gradually and reached a twice the normal rate at 2330.

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Table 3. Chuck's vital measurements taken in the hospital on June 12th

Time	Heart rate/min	Blood pressure mm Hg	Respiratory rate/min.
1850	56	188/80	32
1925	68	186/98	36
1945	76	159/80	32
2000	80	181/81	36
2100	78	146/77	36
2200	102	166/84	38
2330	110	173/92	-
Average normal	56	120/80	16

4.1.2 Chuck's ECG exam taken at 15 minutes following admission

Chuck's electrocardiogram (ECG) exam performed at 1850 on June 12th revealed that he had sinus rhythm with premature atrial complexes.

4.1.3 Chuck's CT scan of the head taken at 80 minutes following admission

Chuck's CT scan of the head performed at 1955 on June 12th was normal. Chuck had no abnormal brain attenuation. The ventricular system was normal and there was no abnormal extra-axial fluid collection.

4.1.4 Metabolic acidosis and low blood levels of PCO_2 and bicarbonate

Chuck's blood analysis performed at 1907 showed that he suffered from profound metabolic acidosis. His blood pH was 7.07 and his blood bicarbonate and PCO_2 levels were 2.3 mEq/L and 8.0 mmHg respectively. Chuck was treated with sodium bicarbonate but his blood pH and bicarbonate and PCO_2 levels stayed low during the four hours following admission (Table 4). The likely causes of his low levels of bicarbonate and PCO_2 are hyperventilation, renal failure, and the utilization of bicarbonate to neutralize organic acids.

Chuck's blood analysis performed at 1856 showed that he had large plasma anion gap and osmolar gap and high level of beta-hydroxybutric acid (BHA). His anion gap was 32 mmol/L and the normal range is 10-12 mmol/L. His calculated and measured osmolar gaps were 284 and 346 mOSM/kg, respectively with a gap of approximately 62 mOsM/kg. Chuck's blood level of BHA (296 (μg/mL) was more than 14 times the average level detected in the fasting individual (Table 5).

Furthermore, blood analysis performed at 2150 revealed a high lactic acid level of 9.6 mmol/L, which is about 7 times higher than the average normal level (Table 5). Lactic acid is a major metabolite of propylene glycol (PEG). Prior to admission, Chuck was taking several medications and multivitamins that contained PEG as a solubilizer (Table 1).

Table 4. Chuck's blood pH and gases measured on June 12th

Measurements	At 1907	1950	2130	Normal range
pH	7.07	7.15	7.12	7.35-7.45
HCO3 (mEq/L)	2.3	2.6	2.6	22-26
BE (mEq/L)	-26.0	-23.7	-24.4	-2-2
PCO_2 (mmHg)	8.0	7.4	8.2	35-45
PO_2 (mmHg)	144	145	149	80-100
O_2 Sat. **%**	98	98	98.3	95-100

Table 5. Chuck's blood ketone and lactic acid levels and osmolality value measured on June 12th

Measurements	Chuck's values	Reference range
Osmolality (mOSM/kg)	346	275-293 (mean=284)
Beta-hydroxybutric acid (μg/mL)	296	0.0-43.9 (mean=20.5) in fasting adult
Lactic acid (mol/L)	9.6	0.3-2.4 (mean=1.35)

4.1.5 Serum enzymes, protein, creatinine, BUN, billirubin, glucose, Na+, CL-, and Ca

The results of Chuck serum analysis performed at 1856 on June 12th are presented in Tables 6 and 7. He had a high glucose level of 181 mg/dL. He also had a high creatinine level of 1.8 mg/dL. His serum albumin and protein levels were slightly elevated. His serum billirubin, liver enzymes, lipase, and amylase levels were within the normal range (Table 6).

Table 6. Chuck's serum analysis result on June 12th

Measurements	Values	Reference range
Na+	142	136-145 mmol/L
CL-	107	98-108 mmol/L
K+	5.1	3.6-5.2 mmol/L
Ca	9.8	8.8-10.5 mg/dL
Glucose	181	70-110 mg/dL
Creatinine	1.8	0.8-1.3 mg/dL
BUN	18	7-18 mg/dL
Albumin	5.3	3.4-5.0 g/dL
T. Protein	9.7	6.4-8.2 g/dL

Table 7. Chuck's serum enzymes and billirubin levels measured on June 12th

Measurements	At 1856	Reference range
T. Bilirubin	0.3	0.0-1.0 mg/dL
Bil1. dir	0.1	0.0-0.3 mg/dL
SGOT/AST	38	15-37 U/L
SGPT/ALT	88	30-65 U/L
Alk Phos	120	50-136 U/L
CPK	85	35-232 U/L
Amylase	41	25-115 U/L
Lipase	218	105-290 U/L

4.1.6 Chuck's hematology values at 20 minutes following admission

Chuck's blood sample analyzed at 1856 on June 12th showed that his red blood cell count, hemoglobin level, and platelet

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count were within the normal range (Table 8). His white blood cell count was 12.0 x10^3/μL, which is above the normal range of 4.5-11.0 x10^3/μL. It indicates that he suffered from a bacterial infection. He was treated with antibiotics.

Table 8. Chuck's hematology values on June 12th

Measurements	Values	Reference range
RBC	5.67	4.09-5.57 x 10^6/μL
HGB	17.2	12.9-16.9 g/dL
HCT	52.8	38.7-50.7%
MCV	93	80.7-95.2 FL
MCH	30.3	27.4-32.6 PEG
MCHC	35.5	32.8-34.5 g/dL
RDW	13.4	11.2-14.7%
PLT	355	147-339 x 10^3/μL

4.1.7 Chuck's urine analysis performed on June 12th

Chuck's urine analysis performed following admission on June 12th showed high levels of ketone bodies and protein. It indicates that he suffered from ketosis and renal damage. His urine also showed a high level of blood but the examination of urine under the microscope did not reveal the presence of significant number of red blood. These data indicate that he had hemoglobinurea and suffered from hemolytic crisis (Table 9).

Table 9. Chuck's urine analysis performed on June 12th

Measurements	Values	Reference
Color	Yellow	Yellow
Appearance	Clear	Clear
Sp. gravity	1.03	1.010-1.025 (g/mL)
PH	6.0	5.0-6.0
Glucose	Normal	Normal
Bilirubin	Negative	Negative
Ketones	50	0.0 (mg/dL)
Protein	30	0-15 (mg/dL)
Nitrite	Negative	Negative
Blood	High level	Negative
RBC	0-2	0-2/HPF
WBC	0	0-5/HPF
Leuk Esterase	Negative	Negative
Hyal cast	0-1	0
Mucus	Slight	Negative

4.1.8 Chuck's blood and urine drug tests performed June 12th

Chuck's blood was tested negative for acetaminophen and salicylate. His urine was tested and found negative for drugs listed in Table 10 and his blood tested negative for ethylene glycol. However, his blood was not tested for propylene glycol (PEG). Chuck was taking several medications that contain PEG as shown in Table 1. Chuck's blood analysis performed at 2150 revealed a high lactic acid level of 9.6 mmol/L (Table 5). Lactic acid is a major metabolite of PEG.

Table 10. Result of Chuck urine drug test performed at 1856 on June 12, 2000

Measurements	Values	Reference
Barbiturate	Negative	Negative
Benzo	Negative	Negative
THC	Negative	Negative
Cocaine	Negative	Negative
Opiates	Negative	Negative
PCP	Negative	Negative
Tricyclic Antidepressant	Negative	Negative
Ethylene glycol	Negative	Negative

4.1.9 Blood methanol test and problems with the measurements

A blood sample was taken from Chuck at 2150 on June 12th and it was sent to the laboratory at the Medical college of Virginia (MCV) for chemical analysis. MCV reported that they detected methanol in Chuck's blood at the level of 750 mg/L. However, MCV did not measure formic acid in the blood or urine.

Formic acid is the major metabolite of methanol that causes acidosis in human. Methanol is oxidized in the liver by alcohol dehydrogenase to formaldehyde and the oxidation of formaldehyde to formic acid is facilitated by formaldehyde dehydrogenase [15-18].

In addition, MCV did not test the blood for the presence of PEG. Chuck was taking several medications that contain PEG as shown in Table 1. Chuck's blood analysis performed at 2150 revealed a high lactic acid level of 9.6 mmol/L (Table 5). Lactic acid is a major metabolite of PEG (Table 5).

Furthermore, Chuck's blood sample taken on June 12th was also sent to the Commonwealth of Virginia Forensic lab (VFL) for methanol analysis. VFL reported the level of methanol in Chuck's blood to be 600 mg/L, which is 20% less than those reported by MCV. VFL also did not measure the levels of formic acid and PEG in blood and urine.

In addition, MCV reported that the level of methanol in Chuck's blood sample taken at 0810 on June 13th to be 200 mg/L. This sample was also tested for methanol by VFL and reported a methanol level of 100 mg/L, which is 50% of that reported by MVC. VFL found the level of PEG in this sample to be about 200 mg/L. MCV did not check the blood for the presence of PEG and both laboratories did not check for the presence of formic acid in the blood or urine samples.

4.1.10 Treatments given

The treating physicians assumed that Chuck was poisoned with methanol based on a single measurement of methanol in the blood and without checking for the presence of formic acid and PEG in the blood and urine. Chuck was treated with sodium biocarbonate, ethanol, antibiotics, tranqulizer, and IV fluid as shown in Table 11.

Chuck was hemodialyzed and hemoperfused and large doses of heparin were used in these procedures. The use of heparin caused bleeding in the brain as shown in the CT scan of the brain taken on June 14th. Chuck's CT scan of the brain taken following admission on June 12th was normal.

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Table 11. Treatments given to Chuck in the hospital on June 12th

Treatments	Action
Ativan 2 mg (Lorazepam) IV q2-3	Tranqulizer
D5W 500 cc	IV fluid
Famotidine (Pepcid 10 mg/mL), 20 mg	Treatment for gastric ulcer
Floxin 400 mg/100 ml D5W, 100 ml Q12h	Antibiotic
Flagyl (500 mg/100 mL), IV, q6h	Antibiotic
10% Ethanol 530 ml IV, over 12 hours	Antidote for methanol
Hemodialysis and hemoperfusion (Heparin was used and doses were not given)	Remove toxins from blood
Normal saline, 5 L	IV fluid
$NaHCO_3$ 150 mEq IV in Sterile H_2O at 150 mL/hr	To neutralize acid in the blood

4.2 Chuck's symptoms, clinical tests, and treatments given on June 13th

4.2.1 Physical exam:

Dr. Peter Torrisi examined Chuck in the morning of June 13th. Chuck was poorly responsive and he had gotten increasingly worse since his admission on June 12th. He had a lot of inspiratory noises coming from his trachea. His tongue was falling back as well due to a large amount of secretions. Examination of the lungs revealed diffuse rhonchi. He was tachypneic and labored.

Chuck was afebrile and had tachycardia. His blood pressure was 116/51 mm Hg. Chuck's heart rate and blood pressure measurements obtained on June 13th are listed in Table 12. These data show that his blood pressure was gradually decreasing from 178/97 mm Hg at 0015 to 97/50 mm Hg at 2030. Examination of his abdomen revealed no masses or organomegally. His extremities showed no edema or cyanosis.

Table 12. Chuck's heart rate and blood pressure measured on June 13th

Time	Heart rate/min.	Blood pressure mmHg
0015	103	178/97
0045	88	175/92
0130	70	119/45
0215	107	82/25
0330	72	125/37
0415	97	177/79
0536	133	134/47
1745	75	86/39
1945	77	96/46
2030	75	97/50

4.2.2 Electroencephalogram (EEG):

Chuck's EEG performed on June 13th revealed abnormalities. No CT scan of the brain was performed on June 13th to observe the abnormal changes in the brain that developed within the 24 hours following admission. Chuck's CT scan of the brain taken at 80 minutes following admission was normal.

4.2.3 Metabolic acidosis and alkalosis:

Chuck's blood analysis performed at 0135 and 0300 on June 13th showed that he was still suffering from metabolic acidosis in spite of the treatment with high doses of sodium bicarbonate. Chuck's treatment with sodium bicarbonate IV continued and his blood pH was raised from 7.01 (metabolic acidosis) at 0135 to 7.53 (metabolic alkalosis) at 1400 (Table 13).

Alkalinization increases the avidity of hemoglobin to bind oxygen, impairing the release of oxygen in peripheral tissues and causing edema in the brain and other organs [19-21].

The hemoglobin-oxygen dissociation curve is normal in ketoacidosis because of opposing effects of acidosis and the deficiency of red blood cell to 2,3-bisphosphoglycerate (2,3-BPEG). Chuck suffered from ketoacidosis as shown in Table 5. If acidosis is rapidly reversed, the deficiency of 2,3-BPEG becomes manifest, increasing the avidity with which hemoglobin binds oxygen and causes reduction in the amount of oxygen delivered to the tissues [19].

Table 13. Chuck's blood gases measured on June 13th

Time	pH	HCO_3 (MEQ/L)	BE (MEQ/L)	PCO_2 (mmHg)	PO_2 (mmHg)	O_2 Sat. %
0135	7.01	6.0	-24.4	24	157	98
0300	7.01	3.5	-26.6	14	118	96
0550	7.42	9.5	-11.0	15	102	96
0900	7.42	18.1	-4.1	27.9	148.4	99
1400	7.53	19.1	-0.5	23	176	99
Normal range	7.35-7.45	22-26	-2-2	35-45	80-100	95-100

4.2.4 Hypophosphatemia, hypokalemia, hypomagnesemia, and lactic acidosis:

Chuck's blood test performed on June 13th showed that he had a serum phosphorous level of 0.1 mg/dL (normal range: 2.8-4.9 mg/dL) and he suffered from severe hypophosphatemia (Table 14). His serum phosphorus level was not measured on June 12th. The likely causes of Chuck's hypophosphatemia are the ingestion of toxic doses of creatine monohydrate and a high level of propylene glycol (PEG) present in Chuck's medications listed in Table 1.

PEG increased creatine solubility in Chuck's gastrointestinal tract and increased it's absorption from the intestine and tissue uptake. It made the creatine more bioavalable [22]. Chuck's blood analysis performed at 2150 revealed a high lactic acid level of 9.6 mmol/L (Table 5). Lactic acid is a major metabolite of PEG.

Creatine caused renal tubular necrosis and hypophosphatemia. Phosphorous deficiency was resulted from shifting of PO_3- from the extracelluar fluid to intracellular fluid due to the reduction of PCO_2 in tissues and the activation of phosphofrucktose kinase. Kidney damage also leads to the reduction in the reabsorption of PO_3 from the damaged renal tubules.

Chuck also suffered from hypokalemia, hypomagnesemia, and lactic acidosis (Table 14). Chuck's serum potassium level was dropped from 5.1 mmol/L to 3.0 mmol/L because of the use of sodium bicarbonate. In metabolic acidosis, potassium

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usually leaves the intracellular environment because the intracellular proteins bind with hydrogen, which leads to cardiac problem and paralysis of the respiratory muscles [19].

Table 14. Chuck's blood levels of phosphorous, K+, Mg++, and lactic acid observed on June 13th

Measurements	0810	1414	Normal range
Phosphorous (mg/dL)	0.1	0.1	2.5-4.9
K+ (mmol/L)	3.0	2.3	3.6-5.2
Mg++ (mg/dL)	1.0	1.6	1.8-2.4
Lactic acid (mmol/L)	3.6	NM[1]	0.3-2.4

[1]NM: not measured.

4.2.5 Electrocardiogram (ECG) exams and other cardiac function tests:

Chuck's ECG exam taken at 0151 on June 13th indicates that significant changes had occurred when compared with ECG of June 12th. These changes are listed in Table 15. Chuck's ECG performed at 1144 indicates that his cardiac problems became worse (Table 15). Blood analysis revealed that Chuck had high levels of creatine kinase-MB (CK-MB) and troponin, which indicate cardiac muscle damage (Table 16). Hypophosphotemia, hypkalemia, hypomagnesemia, metabolic acidosis and acidosis cause cardiac problems.

Table 15. Chuck's ECG findings on June 13th

Time	Findings
0151	• Sinus bradycadia • Left ventricular hypertrophy • Nonspecific ST-T Wave Changes • When compared with ECG of June 12 at 1850 significant changes had occurred.
1144	• Sinus tachycardia • Right Axis deviation • Nonspecific ST-T Wave Changes • When compared with ECG of June 13 at 0151 significant changes had occurred.

Table 16. Indicators of cardiac muscle damage observed on June 13th

Time	CKMB (ng/mL)	CK (U/L)	Troponin (ng/mL)
0140	NM[1]	55	0.04
1200	9.6	291	0.75
2200	10.8	67	0.49
Normal range	0.0-3.6	35-232	0.0-0.4

[1]NM: not measured

4.2.6 Evidence of bleeding and hemolytic anemia:

Chuck's prothrombin time (PT) and the partial thromboplastin time (PPT) were significantly increased as a result of the heparin use. At 0810 on June 13th, his PT and PTT values were three times and more than five times the average normal value, respectively (Table 17). Chuck's platelet count was also reduced by 54% as a result of the use of heparin (Tables 8, 18).

The use of heparin caused bleeding as indicated by the reduction in Chuck's red blood cell count and the hemoglobin level on June 13th as compared with the values obtained on June 12th. At 2200 on June 13th, Chuck's red blood cell count and hemoglobin level were 27% less than those measured on June 12th (Tables 8, 18).

Table 17. Blood clotting parameters measured on June 13th

Time	PT (Sec.)	INR	PTT (Sec.)
0810	28.3	2.7	155
1200	12.7	1.2	55.3
Normal range	10.0-11.9	0.9-1.1	22.6-31.6

Table 18. Chuck's hematology values on June 13th

Measurements	0810	1355	2200	Reference range
RBC	4.48	3.49	3.57	4.09-5.57x10^6/µL
HGB	14.0	10.8	10.9	12.9-16.9 g/dL
HCT	41.3	31.5	32.3	38.7-50.7%
MCV	92.0	90.3	90.5	80.7-95.2 FL
MCH	31.2	30.9	30.5	27.4-32.6 PEG
MCHC	33.9	34.2	33.7	32.8-34.5 g/dL
RDW	13.1	13.0	13.2	11.2-14.7%
PLT	275	179	162	147-339x10^3/µL

4.2.7 Significant changes in the levels of serum amylase, albumin, and protein

Chuck's blood analysis performed on June 13th showed the following significant changes as compared with the result obtained following admission on June 12th. 1) The amylase level increased from 41U/L on June 12th to 240 U/L on June 13th (Tables 7, 19). It may indicate that Chuck developed pancreatic injury resulting from the use of ethanol. 2) The albumin and total protein levels on June 13th were reduced by 57% and 53% of the levels detected on June 12th (Tables 6, 20). These data indicate that a significant amount of protein was lost in the urine due to kidney damage.

Table 19. Chuck's serum enzymes and bilirubin levels on June 13th

Measurements	6/13 (0810)	6/13 (1414)	6/13 (2200)	Reference range
T. Bilirubin	0.5	0.4	1.8	0.0-1.0 mg/dL
SGOT/AST	52	29	40	15-37 U/L
SGPT/ALT	69	49	48	30-65 U/L
GGT	38	NM[1]	NM[1]	15-85 U/L
Alk Phos	63	53	52	50-136 U/L
Amylase	240	NM[1]	NM[1]	25-115 U/L
Lipase	213	NM[1]	NM[1]	105-290 U/L

[1]NM: not measured

Table 20. Chuck's serum measurement observed June 13th

Measurements	0810	1414	2200	Reference range
Na+	141	140	139	136-145 mmol/L
CL-	108	110	109	98-108 mmol/L
Ca	8.2	7.2	8.0	8.8-10.5 mg/dL
Glucose	161	133	122	70-110 mg/dL
Creatinine	1.3	1.7	1.6	0.8-1.3 mg/dL
BUN	12	13	9	7-18 mg/dL
Albumin	3.4	2.3	2.4	3.4-5.0 g/dL
T. Protein	NM	4.5	4.6	6.4-8.2 g/dL

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4.2.8 Evidence of infection:

Blood analysis showed Chuck's white blood cell count was elevated following admission on June 12th and at 0810 on June 13th. Chuck was treated with antibiotics and his white blood cell count was reduced by 66% (Table 21). These data indicate that Chuck was suffering from bacterial infection. Hypophosphatemia usually increases the risk for infection.

Table 21. Chuck's white blood cell and differential counts measured on June 13th

Measurements	0810	1355	2200	Reference range
WBC	13.0	4.4	5.5	4.5-11.0 $x10^3/\mu L$
Neutrophil	11.0	NM	4.3	1.6-6.9 $x10^3/\mu L$
Lymphocytes	0.9	NM	0.9	0.3-3.8 $x10^3/\mu L$
Monocyte	1.1	NM	0.3	0.20-0.95$x10^3/\mu L$
Basophil	0.0	NM	0.0	0.0-0.4 $x10^3/\mu L$
Esonophil	0.0	NM	0.0	0.0-0.14 $x10^3/\mu L$

4.2.9 Treatment given on June 13th

Tables 22 and 23 list treatments given to Chuck on June 13th. He was treated for hypophosphatemia, hypomagnesemia, hypokalemia, metabolic acidosis, methanol intoxication, and bacterial infection. The hemodialysis and hemoperfusion were continued from June 12th. Chuck's health condition became worse as compared with his condition following admission on June 12th.

Table 22. Treatments given to Chuck at 0145-1145, June13th

Time	Treatments
0145	$NaHCO_3$ solution, IV Hemodialysis and hemoperfusion (Heparin was used)
0300	Normal saline, 1000 mL, IV bolus $NaHCO_3$ solution, 100 mEq IV Ethanol 50 mL/hr
0540	Ethanol dose was doubled
0800	Diprivan 10 mg/ml, 100 mL
1000	Sodium chloride solution (0.9%), 100 mL, q12 h
1100	Pyridoxine HCl (Pyridoxine 100 mg/mL vial), 500 mg
1045	Ethanol 0.6 g/kg per hour $NaPO_4$ 12 mmol in 100 cc NS, IV over 10 hrs $MgSO_4$ 2g in 100 cc NS, IV over 10 hrs
1125	Antizol 15 mg/kg IV loading dose then Antizol 10 mg/kg IV, q12 x 4 doses.
1145	Cardizem 15 mg IV bolus

Table 23. Treatments given to Chuck at 1220-2300. June 13th

Time	Treatments
1220	Hespan IV, one dose
1300	Levaquin (levofloxacin) 500 mg/100 mL D5W, q24h
1315	Hespan IV, one dose
1530	75 mEq NaHco3 in ½ NS 5000 cc IV at 130 cc/hr 40 mEq KCl in 100 D5W, IV over 3 hours
1600	Levoquin (levofloxacin) 500 mg IV
1605	Pavulon 2 mg IV
1615	Pyridoxine HCl (Pyridoxine 100 mg/mL vial), 500 mg, IM QID x 3 doses
1645	1 mg folate, IV
1730	Antizol 15 mg/kg IV loading dose
1800	Flagyl (500 mg/100 mL), IV, q8H Mannitol 25% IV
1815	KCl 40 mEq in 1000 cc D5W over 3 hours, IV infusion Ethanol 100 cc/hr while on dialysis
1845	Dopamine 400 mg
1900	Multivitamins, folic acid 1 mg
2100	Antizol 10 mg/kg IV, q12 Sodium bicarbonate (75 mEq in ½ NS)
2300	$MgSO_4$ 2g in 100 cc NS, IV, 250 cc
0530	MgSO4 1 gm in 250 cc over 2 hours KCl 20 mEq in 500 cc x 3 doses Heparin (dose was not given)
1000	Sodium phosphate (9 mmol in 50 cc NS IV over 6 hours) Thiamine HCl(Thiamine 100 mg/mL vial), 100 mg
1400	Sodium phosphate (9 mmol in 50 cc NS IV over 6 hours)
1500	½ NS 150 cc/hour
1815	KCL 40 mEq in 100 cc D5W, IV over 3 hours
2300	Hespan 500 cc IV
0140-2300	Albumin Dextrose 5% in water IV solution, 100 mL

4.3 Chuck's health condition on June 14th

Chuck was in a coma on June 14th. His CT scan of the brain performed on June 14th showed the following abnormal changes: (1) Bilateral large basal gangliar hemorrhage measuring 5 x 3 cm on the right and 5.2 cm on the left; (2) blood within the temporal horns and left occipital horn; (3) edema and compression of the cisterns around the brain stem consistent with transtentorial herniation and uncal herniation; (4) compression of the ventricles; (5) cerebelar edema.

Chuck's electroencephalogram (EEG) was abnormal. A decision was made to discontinue life support. The life support was discontinued at 1630 and Chuck subsequently expired and was pronounced dead by the house officer. Below are the clinical data collected on June 14th and their significance.

4.3.1 Metabolic alkalosis:

Chuck's blood analysis performed at 0400 on June 14th showed that his blood pH was 7.63 and he suffered from metabolic alkalosis (Table 24). His alkalosis is resulted from the excessive treatment with sodium bicarbonate. The treatment

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with high doses of sodium bicarbonate also interferes with the release of oxygen from hemoglobin and cause brain edema and edema in other organs.

Table 24. Chuck's blood gases measured on June 14th

Measurements	0400	0954	Normal range
PH	7.63	7.46	7.35-7.45
HCO_3 (MEQ/L)	25	25.2	22-26
BE (MEQ/L)	6.8	2.6	-2-2
PCO_2 (mmHg)	23	35.4	35-45
PO_2 (mmHg)	197	227	80-100
O_2 Sat. %	99	99.5	95-100

4.3.2 Hypophosphatemia and hypokalemia:

Chuck continued to suffer from severe hypophosphatomia in spite of his treatment with sodium phosphate. His serum phosphorous (Pho) level was 0.0 mg/dL at 0355 and the average normal Pho level is 3.9 mg/dL (Table 25). These data indicate that there was shifting of Pho from the extracellular fluid to intracellular fluid due to the increase in tissue pH resulting from the use of sodium bicarbonate. Chuck also suffered from hypokalemia as a result of the use of high doses of sodium bicarbonate (Table 25).

Table 25. Evidence of hypophosphatemia and hypokalemia June 14th

Measurements	0355	1315	Normal range
Phosphate (mg/dL)	0.0	0.3	2.5-4.9
K+ (mmol/L)	2.4	2.7	3.6-5.2
Mg++ (mg/dL)	1.4	1.8	1.8-2.4
Lactic acid (mmol/L)	2.3	NM[1]	0.3-2.4

[1] NM: Not measured.

4.3.3 Elevation of serum amylase level

Blood analysis performed at 0335 on June 14th showed Chuck's serum amylase level increased from 240 U/L on June 13th to 646 U/L. These data may indicate pancreatic injury resulting from the use of ethanol, hypophosphatemia, and metabolic problems. The levels of other enzymes in serum were within the normal range (Table 26).

Table 26. Chuck's serum enzymes and bilirubin levels measured on June 14th

Measurements	0355	Reference range
T. Bilirubin	1.7	0.0-1.0 mg/dL
SGOT/AST	46	15-37 U/L
SGPT/ALT	46	30-65 U/L
GGT	54	15-85 U/L
Alk Phos	54	50-136 U/L
Amylase	646	25-115 U/L
Lipase	121	105-290 U/L

4.3.4 Hypoproteinemia

Chuck's serum albumin and protein levels measured at 1315 on June 14th were very low. His serum creatinine level was elevated (Table 27). These data indicate that significant amount of protein was lost in the urine due to kidney damage.

Table 27. Chuck's serum analysis performed on June 14th

Measurements	0355	1315	Reference range
Na^+	144	154	136-145 mmol/L
CL^-	113	125	98-108 mmol/L
Ca	8.4	8.5	8.8-10.5 mg/dL
Mg	1.4	1.8	1.8-2.4 mg/dL
Glucose	106	120	70-110 mg/dL
Creatinine	1.8	1.8	0.8-1.3 mg/dL
BUN	11	10	7-18 mg/dL
Albumin	NM[1]	2.1	3.4-5.0 g/dL
T. Protein	NM[1]	4.3	6.4-8.2 g/dL
Ammonia	12	NM[1]	11-32 µmol/L

[1] NM: Not measured.

4.3.5 Indicators of cardiac damage

Chuck's blood analysis performed at 0355 on June 14th revealed very high levels of creatine kinase-MB (CKMB) and troponin (Table 28). His troponin blood level on June 14th was increased by 8 folds as compared to his level detected on June 13th (Table 16). These data indicate that Chuck's cardiac damage became worse. Hypophosphotemia, hypokalemia, hypomagnisemia, and metabolic problems cause cardiac dysfunction.

Table 28: Indicators of cardiac damage observed June 14th

Measurements	0355	1315	Normal range
CKMB (ng/mL)	11.2	NM[1]	0.0-3.6
CK (U/L)	67	NM[1]	35-232
Troponin (ng/mL)	6.1	0.47	0.0-0.4

[1] NM: Not measured.

4.3.6 Chuck's PT and PTT values on June 14th

Chuck's prothrombin time (PT) and the partial thromboplastin time (PPT) measured on June 14th (Table 29) were significantly reduced from the levels observed on June 13th (Table 17). This reduction resulted from the reduction in the heparin dose received.

Table 29. Blood clotting parameters measured on June 14th

Time	PT (Sec.)	INR	PTT (Sec.)
0355	14.7	1.4	33.6
1315	NM[1]	NM[1]	35.0
Normal range	10.0-11.9	0.9-1.1	22.6-31.6

[1] NM: Not measured.

4.3.7 Chuck urine analysis performed on June 14th

Chuck's urine osmolarity and sodium levels were 85 mOsm/L (normal rang: 250-900) and 20 mmol/L (normal range: >20), respectively. These data indicate that he had diluted urine as a result of kidney failure.

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4.3.8 Chuck's hematology values obtained on June 14th

On June 14th, Chuck's red blood cell count and hemoglobin levels were 65% of their levels obtained on June 12th (Tables 8, 30). This reduction was caused by the destruction of red blood cells due to hypophosphatemia and bleeding due to the use of heparin. Chuck's platelet count on June 14th was reduced by 58% of that measured on June 12th as a result of the use of heparin (Tables 8, 30). Chuck's white blood cell count was within the normal range on June 14th (Table 31).

Table 30. Chuck's hematology values on June 14th

Measurements	0355	Reference range
RBC	3.68	4.09-5.57 x 10^6/μL
HGB	11.3	12.9-16.9 g/dL
HCT	33.2	38.7-50.7%
MCV	90.0	80.7-95.2 FL
MCH	30.8	27.4-32.6 PEG
MCHC	34.2	32.8-34.5 g/dL
RDW	12.9	11.2-14.7%
PLT	150	147-339 x 10^3/μL

Table 31. Chuck's white blood cell count and differential count measured on June 14th

Measurements	0355	Reference range
WBC	5.8	4.5-11.0 x10^3/μL
Neutrophil	4.4	1.6-6.9 x10^3/μL
Lymphocytes	1.0	0.3-3.8 x10^3/μL
Monocyte	0.3	0.20-0.95x10^3/μL
Basophil	0.1	0.0-0.4 x10^3/μL
Esonophil	0.0	0.0-0.14 x10^3/μL

5. Autopsy findings in Chuck's case, allegation of methanol poisoning, and overlooked medical data

Chuck died at Chippenham Hospital at 1630 on June 14, 2000. Dr. Marcella F. Fierro, Chief Medical Examiner of Richmond Virginia, performed the autopsy at 1400 on June 15th (Case # 368-00). Prior to autopsy, Life Net harvested soft tissues and bones from his body for donations, at 1900 on June 14th [23, 24].

These tissues include: corneas; mandible; valves from heart; pericardium; skin; bones & associated tissues of upper extremities; bones & associated tissues of lower extremities; ribs; and saphenous veins. In this procedure, heparin (5000 units/L) was used to flush the saphenous veins prior to removing them from the body [24].

Fierro examined Chuck's body externally and she did not see any evidence of trauma and abnormal lesion. His weight and length on June 15th were 170 lb (77.3 kg) and 71 inches (177.5 cm), respectively. Fierro examined certain organs grossly and microscopically and observed edema and congestion in the lungs; edema, necrosis, and bleeding in the brain; ventricular hyperatrophy and ischemia in the heart; necrosis in both kidneys; mild fatty change in the liver; and evidence of gastric ulcer (Table 32).

Fierro alleged that Chuck died as a result of acute and likely chronic methanol poisoning. She issued her report on November 8, 2000 [23]. My review of the clinical evidence and supporting data in this case and Fierro's autopsy findings indicates that that Fierro's allegations given in this case are not supported by the medical and scientific data as described in this report.

Chuck died as a result of acute renal failure and severe hypophosphatemia due to the ingestion of toxic doses of creatine monohydrate and high levels of propylene glycol present in his medications. He also developed bleeding, edema, and necrosis in the brain due to his treatment with high doses of heparin and sodium bicarbonate in the hospital**.**

I also find Fierro's investigation of this case to be incomplete. She overlooked many medical data that show Chuck did not die as a result of methanol poisoning. Below is a list of specific medical data that support my conclusions:

1) Chuck suffered from acute renal failure and severe hypophosphatemia and these conditions were induced by the ingestion of high doses of creatine monohydrate (Crm) and propylene glycol (PEG). Chuck ingested 22.5 g of Crm on June 11th. He also ingested more creatine on June 12th. He took three bottles of creatine mixed in Gatorade with him to work and each bottle contains about 22.5 g Crm.

PEG increases creatine solubility in the gastrointestinal tract and its absorption from the intestine and tissue uptake. It makes creatine more bioavalable [22]. I added 14 g of Crm to a test tube containing 20 mL of PEG and I mixed them for 10 minutes. The Crm and PEG formed white suspension. I left the tube standing in a holder for 40 minutes and I did not see any separation or settling of Crm in the bottom of the tube. Chuck was taking several medications that contained PEG as a solubilizer as shown in Table 1.

Chuck's serum phosphorous (Pho) levels on June 13th and 14th were 0.1 mg/dL and 0.0 mg/dL, respectively and the normal range is 2.8-4.9 mg/dL (Tables 14, 25). Severe Pho deficiency resulted from the involvement of several factors. These include: a) the phosphorylation of creatine in muscle by phosphocreatine kinase using ATP; b) shifting of PO_3- from the extracellular to intracellular fluid due to the reduction of PCO_2 in tissues and the activation of phosphofructose kinase; c) reducing the reabsorption of PO_3 from the damaged renal tubules.

2) Chuck had profound metabolic acidosis at the time of admission in the hospital on June 12th. His blood pH was 7.07 and his blood bicarbonate and PCO_2 levels were 2.3 mEq/L and 8.0 mmHg, respectively. The clinical data show that Chuck's acidosis resulted from renal damage, ketosis, and lactic acidosis. These data include:

a) Chuck had high lactic acid level of 9.6 mmol/L at 3 hours following admission. Lactic acid is a major metabolite of propylene glycol (PEG). Chuck had PEG level of about 200 mg/L on June 13th. Furthermore, individuals poisoned with PEG also suffer from metabolic acidosis and have large anion gap and osmolar gap as found in Chuck's case (Table 5). Barnes *et al.* evaluated 13 individuals who received toxic levels of PEG with medications and found

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osmol gap alone can predict serum PEG concentrations in the blood [25].

b) On June 12th, Chuck had a high level of beta-hydroxybutric acid (BHA) of 296 µg/mL, which was 14 times more than the average level detected in a fasting individual (Table 5). Chuck's urine analysis performed on June 12th also showed high levels of ketone bodies (Table 9). Individuals suffering from hypophosphatemia usually have high blood levels of ketone bodies due to the use of fatty acid as a source of energy. The tissues of these individuals lack the ability to phosphorylate and utilize glucose.

c) Formic acid, a major metabolite of methanol that is responsible for metabolic acidosis was not measured in the blood, urine, or any other tissue in Chuck's case. In addition, there are no other clinical data and biomarkers presented that show formic acid was responsible for the metabolic acidosis in Chuck's case.

3) Chuck suffered from hemolytic crisis due to the destruction of red blood cells resulting from the severe deficiency of phosphorous. The red blood cells were not able to phosphorylate and utilize glucose to produce ATP that is required for keeping cell membranes intact. Chuck's red blood cell count and hemoglobin levels on June 13th were reduced by 27% of those measured on June 12th (Tables 8, 18).

In addition, urine analysis performed following Chuck's admission to the hospital indicates that he had severe hemoglobinurea (Table 9). Furthermore, Chuck had an enlarged spleen (splenomegaly) and people suffering from hemolytic anemia also have splenomegaly.

4) Chuck developed necrosis in the cardiac muscles and cardiac dysfunction due to the hypophosphatemia, hypokalemia, hypomagnesiumemia, metabolic acidosis and alkalosis. Chuck's ECG exam taken at 0151 on June 13th indicates that significant changes had occurred when compared with ECG of June 12th. These changes are listed in Table 15. Chuck's ECG performed at 1144 on June 13th indicates that his cardiac problems became worse. Blood analysis revealed Chuck had high levels of creatine kinase-MB (CKMB) and troponin, which indicate cardiac muscle damage (Table 16).

5) Chuck developed kidney failure as result of the ingestion of toxic doses of creatine and the effects of high level of PEG and hypophosphatemia.

6) The clinical data described below indicate that the bleeding, edema, and necrosis observed in Chuck's brain on the CT scan of June 14th and in autopsy were caused by the use of high doses of heparin and sodium bicarbonate in the hospital and from the resulting hypophosphatemia.

a) Chuck's CT scan of the brain taken following admission on June 12th did not show bleeding or any abnormal lesion.

b) Chuck's prothrombin time (PT) and the partial thromboplastin time (PPT) were significantly increased as a result of heparin use. On June 13th, Chuck's PT and PTT values were about three times and five times the normal values, respectively (Table 17). Furthermore, Chuck's platelet count on June 14th was reduced by 58% of that measured on June 12th as a result of the use of heparin (Tables 8, 30).

c) The medical examiner indicates that the bleeding in the brain was fresh. I examined the H & E stained sections of the brain and observed fresh bleeding (Figures 1 & 2). The bleeding probably occurred within the 24 hours prior to death.

d) Chuck's blood pH on June 12th was 7.07 and it was increased to 7.63 on June 14th as a result of the excessive treatment with sodium bicarbonate (Table 24). Alkalinization increases the avidity of hemoglobin to bind oxygen and impairs the release of oxygen in peripheral tissues. It causes edema and necrosis of the brain [19].

7) The following clinical data indicate that the blood methanol measurements reported on June 12th and 13th in Chuck's case represent a false positive and Chuck did not suffer from methanol intoxication.

a) The Medical College of Virginia (MCV) reported that Chuck had a blood methanol level of 750 mg/L. However, MCV did not measure formic acid in the blood or urine. Formic acid is the major metabolite of methanol that causes acidosis in humans and should be measured. Methanol is oxidized in the liver by alcohol dehydrogenase to formaldehyde and the oxidation of formaldehyde to formic acid is facilitated by formaldehyde dehydrogenase.

b) On June 12th, Chuck had high levels of lactic acid and ketone bodies as a result of the ingestions of high levels of PEG and suffered from severe hypophosphatemia. The presence of these chemicals in the blood can interfere with alcohol measurements. Chuck's blood sample taken on June 12th was also sent to the Commonwealth of Virgina Fornsic Lab (VFL) for methanol analysis. VFL reported the level of methanol in Chuck's blood to be 600 mg/L, which is 20% less than that reported by MCV. VFL also did not measure the level of formic acid in the blood.

In addition, MCV reported that the level of methanol in Chuck's blood sample taken at 0810 on June 13th to be 200 mg/L. This sample was also tested for methanol by VFL and reported a methanol level of 100 mg/L, which is 50% of that reported by MVC. VFL also found the level of PEG in this sample to be about 200 mg/L. MCV and VFL did not check for the presence of formic acid in the blood or urine samples.

c) Formic acid inhibits mitochondrial cytochrome oxidase activity. Individuals suffering from methanol intoxication usually develop retinal and optic nerve bleeding, necrosis, and atrophy [26-28]. Dr. Christopher Acker examined Chuck's eyes following admission on June 12th and he did not observe any abnormal changes. The funduscopic exam

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of both eyes was normal and Chuck did not have any retinal bleeding or any other lesion.

8) The likely cause of Chuck's biventricular hyperatrophy and cardiomegaly is the chronic use of corticosteroid medications listed in Table 1. In addition, the weight of Chuck's right lung is 83.6% of the average normal weight for age and 98.6% of his left lung. The right lung is usually bigger than the left lung and it weighs about 15% more than the left lung. The right lung has three lobes and left lung has two lobes. These data indicate that Chuck suffered from atrophy of the right lung. The chronic use of high doses of corticosteroid has known to cause cardiomegaly and myopathy as explained in Section 9 of this report.

9) The following medical data show that Fierro's investigation in this case is incomplete. She overlooked many medical data that show Chuck did not die as a result of methanol poisoning.

a) She did not analyze the blood samples collected on June 12th, 13th, 14th, and 15th for formic acid.

b) She did not analyze the urine samples collected on June 12th for the presence of methanol and formic acid.

c) She did not analyze samples of organs and stomach content taken at autopsy for the presence of methanol and formic acid as required in medical-legal investigations. For example, Ferrari *et al.* investigated fifteen cases of fatal massive methanol intoxication. Body distribution of methanol and formic acid, as the main metabolite, was analyzed in blood and in different organs (brain, kidney, lung and liver). Formic acid concentrations were found to be between 30 and 1100 mg/L in the samples understudy. A good correlation was found between the levels of formic acid in the blood and the brain [29].

In addition, Tanaka *et al.* determined the levels of formic acid in the blood, stomach contents, urine, and organs of two men who were fatally intoxicated with methanol. Formic acid was measured by headspace gas chromatography**.** The average postmortem concentrations of formic acid in blood and tissues were 0.28 mg/mL in the blood, 1.37 mg/mL in urine, 0.64 mg/g in the brain, 0.53 mg/g in the liver, and 0.66 mg/g in the kidneys. The average total amount of formic acid in the gastric contents for these men was 65.6 mg [15, 30]**.**

In another case of methanol poisoning, a man died at 40 hours following hospitalization. The postmortem methanol concentrations in the body fluids of this man were: bile 175 mg/dL, vitreous humor 173 mg/dL, and blood 142 mg/dL. Postmortem methanol concentrations in his tissues are given in decreasing order: brain 159 mg/100 g, kidney 130 mg/100 g, lung 127 mg/100 g, spleen 125 mg/100 g, skeletal muscle 112 mg/100 g, pancreas 109 mg/100 g, liver 107 mg/100 g, and heart 93 mg/100 g. The total amount of methanol in his gastric contents was 73 mg [31].

d) She did not examine Chuck's retina and optic nerve grossly or microscopically. These tissues are usually damaged by formic acid as described above and they should be examined in cases of individuals who suffer from acute and chronic methanol poisoning. For example, Brent *et al.* evaluated 11 individuals who presented with methanol poisoning at a hospital. Seven of them initially had visual abnormalities [32]. In addition, Sharpe *et al.* conducted histopathologic evaluation of optic nerve of four individuals who died as a result of intoxication with methanol**.** They observed myelin damage behind the lamina cribrosa in each nerve [33].

Furthermore, Naeser evaluated the eyes and optic nerves microscopically in a 37-year-old man who died as a result of methanol poisoning. He observed bilateral central necrosis of the optic nerves from behind the lamina cribrosa to the orbital apex [34].

Also, Fujihara *et al*. evaluated the retina of a 37-year-old man who suffered from methanol intoxication. The retinal profiles were evaluated by optical coherence tomography (OCT) and fluorescein angiography during the course of treatment. OCT demonstrated peripapillary nerve fiber swelling and accumulation of intraretinal fluid in the acute phase. In the chronic phase, the retinal thickness was diffusely decreased [26].

e) She did not investigate the causes of hypophosphatemia, hemolytic anemia, and cardiomegaly observed in Chuck's case.

f) She did not consider the toxicities of PEG and creatine in her differential diagnoses in this case. High doses of these agents are known to cause renal damage. In addition, exposure to PEG at high levels is known to cause metabolic acidosis.

g) She did not consider the adverse reactions of heparin and sodium bicarbonate given to Chuck in the hospital in her evaluation in this case. Heparin caused the bleeding in Chuck's brain as indicated by the significant increases in PT and PTT and the reduction in the platelet count during the course of the treatment (Tables 8, 30). Treatment with high doses of sodium bicarbonate causes tissue anoxia and brain edema. During treatment, Chuck's blood pH was increased from 7.07 to 7.63 (Table 24).

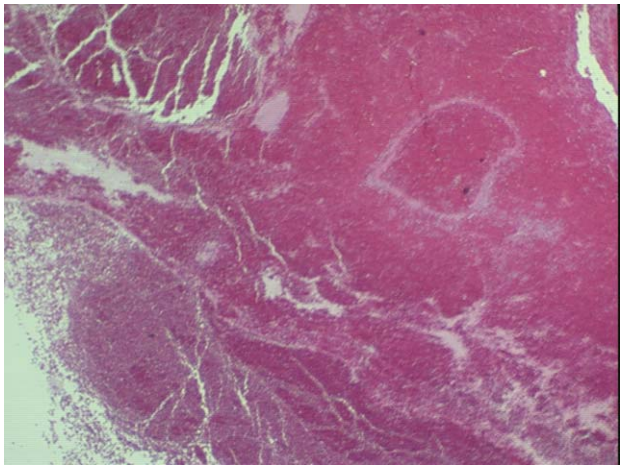

Figure 1. H & E stained section of Chuck's brain showing fresh bleeding covering large area of the brain and blood clot in the ventricle (x=3)

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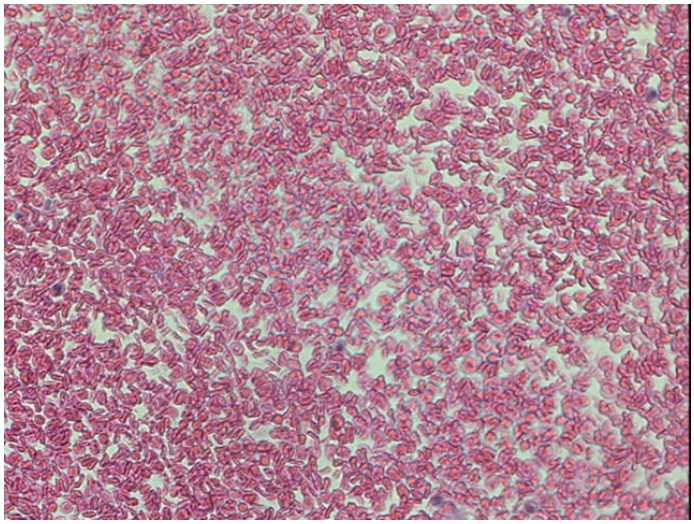

Figure 2. H & E stained section of Chuck's brain showing fresh bleeding and intact red blood cells that indicate the bleeding occurred within 24 hour prior to death (x=31)

6. Causes of renal failure, hypophosphatemia, and metabolic acidosis observed in Chuck's case

Chuck ingested high doses of creatine monohydrate (Crm) on June 11th and June 12th of 2000. He was also taking five medications and a multivitamin and these agents contain a significant amount of propylene glycol (PEG) as a solubilizer (Table 1). The solubility of Crm in water is very low but it is very soluble in PEG.

The solubility of Crm in water at 25°C (77.0°F) and 4 °C (39.2°F) are 17 mg/mL and 6 mg/mL, respectively [35]. I added 7 g and 14 g of Crm separately into two test tubes containing 20 mL of PEG per tube, respectively. I mixed these tubes for 10 minutes. The Crm and PEG formed a white suspension. I left these tubes standing in a holder for 40 minutes and during this time I did not see any separation or settling of Crm in the bottom of the tubes.

PEG increases the bioavalibilty of creatine (Cr) in the gastrointestinal tract, enhancing absorption from the intestine, and increasing tissue uptakes. The elimination of high level of Cr through the kidneys in Chuck's case caused glomerular and tubular damage and renal failure.

Chuck suffered from severe hypophosphatemia on June 12th. His serum phosphorous level on June 13th was 0.1 mg/dL (normal range: 2.8-4.9 mg/dL). His hypophosphatemia was induced by renal damage and reduction of PCO_2 levels in tissues due to hyperventilation. At admission, Chuck's respiratory rate was 32/min, which is twice the average normal rate of 16/min. Chuck's blood levels of bicarbonate and PCO_2 were 2.3 mEq/L (normal range: 22-26) and 8.0 mmHg (normal range: 35-45), respectively.

Table 32. List of lesions observed in Chuck's organs by the medical examiner

Organs	Gross and microscopic findings
Brain	•Gross examination of the brain revealed very soft brain with diffuse swelling. The brain's weight was 1,479 g. A preliminary cut showed bilateral intercerebral hemorrhages. The fixed brain showed fresh hemorrhage and necrosis involving both basal ganglia and adjacent thalamus. The ventricles contained blood clot.
	•Examination of the H & E stained sections from multiple areas of the brain revealed the presence of fresh hemorrhage and necrosis in basal ganglia in the absence of vascular changes or malformation.
Lungs	•Lungs were congested and petechial hemorrhages were noted in the posterior region. A small amount of exudates was present in major bronchi. The weights of the right and left lungs were 554 g and 562 g, respectively. Sectioning of the lungs showed the lungs were watery.
	•Microscopic examination of the H & E stained sections of the lungs showed the lungs are within normal limits.
Heart	•Gross examination of the heart revealed globoid biventricular hypertrophy, predominantly left-sided**.** The right ventricle showed moderate to severe hypertrophy. The coronary arteries showed no significant atherosclerosis. The heart weighed 680 g.
	•Microscopic examination of the H & E stained section of the heart revealed the myofibrils were wide and show nuclear enlargement. There were occasional contraction bands and patchy ischemic change. Also noted was focal cell necrosis.
Liver	•Liver was soft tan and weighed 1,942 g.
	•Microscopic examination of the H & E stained section of the liver showed mild fatty change and congestion.
Kidneys	•The kidneys were soft with normal cortical surfaces and thickness. The right and left kidneys weights were 190 g and 204 g, respectively.
	•Microscopic examination of the H & E stained section of the kidney showed necrosis of convoluted tubule lining cells.
Spleen	•Splenomegaly. Spleen weight was 200 g.
Esophagus	•The esophagus showed edema of the mucosa.
Stomach	•The stomach contained 10 ml of red liquid and showed some tube ulcers.

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Reduction of intracellular PCO2 and elevation of pH increase the activity of phosphofructose kinase. The increases in the phosphorylation of glucose intermediates leads to an increase in the cellular uptake of phosphours and hypophosphatemia. Hypophosphatemia causes hemolytic crisis, ketoacidosis, and tissue necrosis [19].

Chuck's blood analysis performed at 1907 on June 12th showed that he suffered from profound metabolic acidosis. His blood pH was 7.07. His metabolic acidosis was caused by renal failure, hypophosphatemia, lactic acidosis, and ketoacidosis. In addition, Chuck received high doses of sodium bicarbonate that caused metabolic alkalosis, hypokalemia, and hypomagnesemia.

6.1 Creatine doses and interaction with propylene glycol (PEG)

The normal daily intake of creatine ($C_4H_{10}N_3O_5P$) from diet (meat and vegetable) in adult is 1-2 g. If the dietary supply of creatine (Cr) is limited, the body can synthesize it from the amino acids glycine, arginine, and methionine. The kidneys use glycine and arginine to make guanidinoacetate and the liver uses methylates guanidinoacetate to form Cr, which is transported to the muscle cells for storage. Cr is also stored in the kidneys and brain. The maximum amount of Cr the body can store is about 0.3 g per kg of body weight.

Creatine monohydrate ($C_4H_{11}N_3O_3$) supplement is taken by people as a source for creatine ($C_4H_9N_3O2$), which is marketed as a supplement that increases muscle size and power. It has been stated that creatine (Cr) is converted to phosphocreatine ($C_4H_{10}N_3O_5P$) by phosphocreatine kinase using ATP [36]. Phosphocreatine kinase is found in the muscle, heart, and brain.

On June 11, 2000, Chuck took creatine monohydrate (Crm) for the first time. He and his wife mixed 1 and ½ tablespoon of Crm (22.5 g) in 20 ounces (591 mL) of Gatorade and put it in the refrigerator. Chuck drank the prepared bottle of Gatorade-Crm in the afternoon of June 11th.

Furthermore, Chuck and his wife prepared four more bottles of Crm mixed with Gatorade (22.5 g Crm/20 ounces) on June 11th and put them in the refrigerator. One bottle was found in the refrigerator by Diane and the police on June 14th. Diane stated that Chuck took three bottles of Crm/Gatorade mixture with him to work in the morning of June 12th and he consumed about ½ bottle of Gatorade on the morning of June 12th [2].

The total amount of Crm mixed in the five bottles of Gatorade was 112 g and each gram of Crm contains 0.879 g of Cr. The estimated total amount of Cr in the five bottles is 95.5 g. The maximum amount of Cr that can be stored in the body is about 0.3 g per kg body weight. Chuck's weight was 77 kg and the maximum amount of Cr his body can store is 23.1 g.

Crm has poor solubility in water. The solubility of Crm in water at 25 °C (77.0 °F) and 4 °C (39.2 °F) are 17 mg/mL and 6 mg/mL, respectively [35]. However**,** Crm has high solubility in propylene glycol (PEG) [22**].** I added 14 g of Crm to 20 mL of PEG and it was dissolved with 10 minutes of mixing. Chuck's blood analysis performed at 2150 revealed that he had a high lactic acid level of 9.6 mmol/L (Table 5). Lactic acid is a major metabolite of PEG.

Furthermore, the Commonwealth of Virginia Forensic lab (VFL) found PEG in Chuck's blood at the level of about 200 mg/L. The blood sample was taken at 0810 on June 13th. The biological half-life of PEG in adult is about 5 hours [12]. The expected levels of PEG in Chuck's blood at the time of his hospitalization (1830 on June 12th) and the time of his illness (0600 on June 12th) were 800 mg/L and more than 1600 mg/L, respectively. In this case, Chuck ingested a significant amount of PEG that is capable of solubilizing all Cr ingested.

The estimated maximum amount of Cr that can be stored in Chuck's body is 23.1 g. The estimated total amount of Cr in the five Gatorade bottles prepared by Chuck and his wife on June 11th is 95.5 g, which is more than 4 times the storage capacity of the body for Cr. In this case, a large portion of the ingested Cr was eliminated through Chuck's kidneys and caused severe tubular necrosis and kidney failure.

The medical examiner examined H & E stained section of Chuck's kidney microscopically and observed necrosis of the convoluted tubule lining cells. I also examined the H & E stained tissue section of Chuck's kidney microscopically and observed severe tubular necrosis (Figure 3).

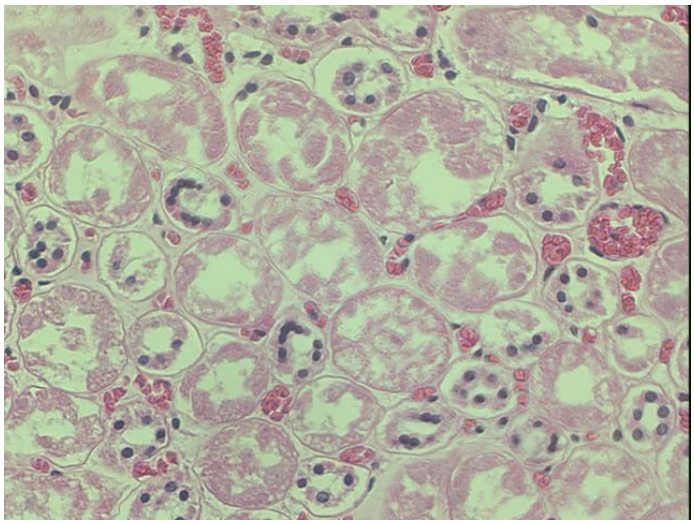

Figure 3. H & E stained section of Chuck's kidney showing widespread necrosis of the convoluted tubule lining cells (x=31)

6.2 Acute renal failure and biomarkers

The clinical data described in this report indicate that Chuck developed kidney failure following the ingestion of toxic doses of creatine monohydrate (Crm). Crm damaged the glomeruli and the convoluted tubules and caused renal failure. Below are the biomarkers of renal failure observed in Chuck's case.

6.2.1 Elimination of protein in urine:

Large quantities of plasma proteins normally flow through the glomerular capillaries but do not enter the urinary space. Both charge and size selectivity prevents virtually all of albu-

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min, globulin, and other large-molecular-weight protein from crossing the glomerular wall. The glomerular basement membranes trap most of large protein except in cases of damage to the membranes, which allow the passage of protein into the urine [19].

Chuck's serum albumin and protein levels measured on June 13th were 55% and 53% less than those measured on June 12th, respectively (Table 33). Urine analysis performed following Chuck's admission in the hospital on June 12th revealed that he had a high level of protein (30 mg/dL) in his urine. The specific gravity of his urine (1.030 mg/mL) is also higher than the normal range of 1.010-1.025 g/mL (Table 9). These data indicate that a large amount of protein was passing into the urine as a result of severe kidney damage.

Table 33. Chuck's serum levels of albumin and total protein

Date	Time	Albumin	Total Protein
06/12	1856	5.3	9.7
06/13	1414	2.3	4.5
06/13	2200	2.4	4.6
06/14	1315	2.1	4.3
Normal range		3.4-5.0 g/dL	6.4-8.2 g/dL

6.2.2 Elevation of serum creatinine

Creatinine (Crn) is a small and freely filtered solute by the glomeruli of the kidney. Crn is produced from the breakdown of creatine in muscle. A reduced glomerular filteration rate (GFR) leads to retention of Crn in the blood. If we assume that Crn is produced at a constant rate in an individual, then a 50 percent reduction in GFR results in proximate doubling of the plasma Crn concentration [19].

Chuck's serum Crn level following admission on June 12 was 1.8 mg/dL and the normal range in healthy individuals is 0.8-1.3 mg/dL (Table 6). Chuck's serum level of Crn is about 171% of the average Crn level in healthy individuals. These data indicate that the glomerular filteration in Chuck's case was reduced by 25-50% of the normal rate due to kidney damage.

6.2.3 Metabolic acidosis, large anion gap and osmolar gap, and hypertension

Chuck's blood analysis performed following admission on June 12th revealed that he suffered from profound metabolic acidosis. His blood pH was 7.07. His blood bicarbonate and PCO_2 levels were 2.3 mEq/L and 8.0 mmHg, respectively. He also had large plasma anion gap (AG) of 32 mmol/L and large osmolar gap of 62 mOsM/kg.

It was alleged that Chuck's metabolic acidosis and large AG and osmolar gap were caused by methanol toxicity due to the accumulation of formic acid. However, measurements of formic acid in blood, urine, and/or tissues were not made in Chuck's case. The clinical data described below show that the metabolic acidosis and the increase in AG and osmolar gap were caused by renal failure, hypophosphatemia, ketone bodies, and lactic acidosis.

Blood analysis performed following admission showed Chuck had high blood level of BHA (296 µg/mL), which is more than 14 times the average level detected in the fasting individual. Urine analysis revealed high level of ketone bodies in Chuck's urine. Chuck had a serum phosphorous level of 0.1 mg/dL (normal range: 2.8-4.9 mg/dL).

In severe hypophosphatemia, tissues use fatty acid and produce ketone bodies (KB). KB are three water soluble compounds that are produced as by-products when fatty acids are broken down for energy. These compounds are acetoacetate, beta-hydroxybutyrate, and acetone, although beta-hydroxybutyrate is not technically a ketone but a carboxylic acid. These compounds are used as a source of energy in the heart and brain [19].

Chuck also had a high lactic acid level of 9.6 mmol/L, which is about 7 times higher than the average normal level (Table 5). Lactic acid is a major metabolite of PEG and lactic acidosis has been observed in individuals exposed to high levels of PEG present in medications [12].

Furthermore, individuals poisoned with PEG also suffer from metabolic acidosis and have large anion gap and osmolar gap. Barnes *et al.* evaluated 13 individuals who received toxic levels of PEG present in medications as a solubilizer and that these individuals had high osmol gap. They found osmol gap alone can predicted serum PEG concentrations ($r(2)=0.532$, $p<0.05$). A PEG dose that produces an osmol gaps above 10 is usually associated with toxicity [25].

Chuck had large plasma anion gap (AG) of 32 mmol/L (normal range: 10-12 mmol/L). AG represents all those unmeasured anions in the plasma, which includes anionic proteins, phosphate, sulfate, and organic anions. AG can be calculated as follows: Anion gap = ($[Na^+]+[K^+]$) - ($[Cl^-]+[HCO_3^-]$). When acid anions, such as acetoacetate and lactate, accumulate in extracelluar fluid, the AG increases, causing a high AG-acidosis [19, 37]. AG also increases due to the accumulation of other organic anions in the extracellular fluid due to renal failure.

Chuck's blood analysis performed at 1856 showed that he had large plasma osmolar gap. His calculated and measured osmolar gaps were 284 and 346 mOSM/kg, respectively with a gap of approximately 62 mOsM/kg (Table 5). Osmolality is the solute or particle concentration of fluid and it is expressed as mosmol/kg of water.

The major extracellular fluid particles are Na^+ and its accompaying ions Cl^- and $HCO3^-$. The normal plasma osmolality is 275 to 290 mosmol/kg and is kept within a narrow range by a bio-mechanism capable of sensing a 1 to 2 percent in tonicity. To maintain a steady state, water intake must equal water excretion [19].

In adults, kidneys usually filter 180 L/a day of plasma water and 99% of the filtered water is usually reabsorbed by the renal tubules. The average volume of urine excreted per day is about 1.5 L. Kidney damage prevents the reabsorption of water from the renal tubules and it leads to the excretion of diluted urine. Chuck's urine osmolarity and sodium levels on June 14th were 85 mOsm/L (normal rang: 250-900) and 20 mmol/L (normal range: >20), respectively. These data indicate that he had diluted urine as a result of kidney failure.

Reduction in the elimination of solute due to renal failure usually causes hypertension [19]. Chuck's blood pressure was measured by the paramedics and it was highly elevated (190/110 mm Hg). The elevation of Chuck's blood pressure

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was caused by high heart rate and retention of solutes due to kidney failure.

6.2.4 Necrosis of convoluted tubule lining cells

The medical examiner examined H & E stained section of Chuck's kidney microscopically and reported necrosis of convoluted tubule lining cells. I also examined the H & E stained section of the Chuck's kidney microscopically and observed necrosis of the convoluted tubule lining cells (Figure 3).

Furthermore, Chuck's kidney weights were significantly increased due to edema. The weights of Chuck's right and left kidneys were 190 g and 204 g, respectively. The expected average weights for the right and left kidneys are 162 g and 160 g [38**]**. Chuck's right and left kidneys were 117% and 128% of the average normal weight.

6.3 Causes and biomarkers of hypophoshatemia

Chuck's serum phosphorous (Pho) level on June 13th was 0.1 mg/dL (normal range: 2.8-4.9 mg/dL) and he suffered from severe hypophosphatemia (Table 14). Severe hypophosphatemia is defined as Pho levels in serum below 1.0 mg/dL (0.3 mmol/L) [19, 39]. Hypophosphatememia resulted due to renal damage induced by creatine and hyperventilation.

Chuck had a respiratory rate of 32/min (average normal = 16/min) at the time of admission on June 12th (Table 3). Hyperventilation resulted in the significant reduction of PCO_2 levels in tissues and the activation of phosphofrucktose kinase, which led to shifting of PO_3- from the extracellular fluid to intracellular fluid. Chuck's blood bicarbonate and PCO_2 levels at the time of admission were 2.3 mEq/L and 8.0 mmHg respectively.

Phosphorous (Pho) is the most abundant intracellular anion and is critical for membrane structure, energy storage, and intracellular and extra cellular transport function. The Pho in extracellular fluid is in a freely diffusible form that (1) permits excretion of hydrogen ions as phosphate buffer into the urine and (2) is in diffusion equilibrium with cytosolic inorganic phosphate in cells. The role of phosphate ions in tissues explains the systemic nature of cellular injury consequent to Pho deficiency.

The symptoms of the severe hypophosphatemia include: metabolic acidosis; glucose intolerance; muscle weakness and respiratory insufficiency; rhabdomylosis; cardiomyopathy; erythrocyte dysfunction and hemolysis; leukocyte dysfunction and infection; and seizures, encephalopathy, and peripheral neuropathy [19, 40-43].

Sheldon and Grzyb stated that hypophosphatemia may be associated with confusion, hyperventilation, and neuromuscular irritability. If inorganic phosphate levels fall below 1.0 mg/dL, diminished red cell glycolysis occurs with low erythrocyte levels of 2,3 diphosphoglycerate and adenosine triphosphate. Lowered red cell organic phosphates are associated with increased hemoglobin oxygen affinity. If severe hypophosphatemia occurs, hemolytic anemia, which is correctible by phosphate infusion, may result. In addition, leucocyte function is impaired by low levels of serum inorganic phosphate [42].

Furthermore, Subramanian and Khardori stated that severe hypophosphatemia has significant morbidity and potential mortality. Depletion of adenosine triphosphate (ATP) would explain most of the derangement noted in cellular functions. Phosphate plays a key role in the delivery of oxygen to the tissue. Lack of phosphate, therefore, leads to tissue hypoxia and hence disruption of cellular function. Chronic hypophosphatemia results in hematologic, neuromuscular, and cardiovascular dysfunction, and unless corrected, the consequences can be life threatening [44].

In addition, Berkelhammer and Bear stated that symptoms of severe hypophosphatemia include reversible depression of myocardial function, acute respiratory failure, coma, rhabdomyolysis, osteomalacia, renal tubular acidosis and hemolysis [45]. Lee *et al.* reported three cases of individuals who developed coma as a result of severe hypophosphatemia [46].

The clinical data described in Sections 3 and 4 of this report show that Chuck developed the symptoms of hypophosphatemia described above. Below are clinical data and studies that describe the pathological and clinical changes caused by hypophosphatemia in Chuck's case.

6.3.1 High levels of glucose and ketone bodies in serum

The tissues of individuals suffering from severe hypophosphatemia usually lack the ability to phosphorylate and utilize glucose as a source of energy. These individuals usually have high blood levels of glucose and ketone bodies. Chuck's glucose level on June 12th was 181 mg/dL and it was reduced to a normal level of 106 mg/dL as a result of the treatment with sodium phosphate (Table 27).

On June 12th, Chuck's blood level of BHA (296 (µg/mL) was more than 14 times the average level detected in the fasting individual (Table 5). He also had high level of ketone bodies in his urine (Table 9). The high levels of ketone bodies in the blood and urine resulted from the use of fatty acid by the tissues as a source of energy as described above.

6.3.2 Destruction of red blood cells and hemoglobinurea:

Severe deficiency of phosphorous (Pho) usually affects the red blood cells ability to phosphorylate and utilize glucose to produce ATP which is needed to keep the cell membrane intact. Furthermore, the lack of ATP leads to hemolysis. Additionally, the 2,3-biphosphoglycerate (2,3-BPEG) content of the red blood cell is also reduced in individuals suffering from hypophosphotemia. The red cell is the only tissue in the body capable of producing this substance.

Both 2,3-BPEG and ATP facilitate dissociation of oxyhemoglobin and promote oxygen delivery to tissue. Reduced 2,3-BPEG and ATP both enhance affinity of oxygen for hemoglobin and reduce tissue oxygenation [19, 41, 47].

Van Dissel *et al.* reported a case of refeeding-associated hypophosphatemia in a 24-year-old malnourished man with anorexia nervosa. He had a reduction in red-cell ATP and 2,3-diphosphoglycerate and suffered from hemolytic anemia [48]. In addition, Shilo *et al.* described the cases of two individuals in whom acute hemolytic anemia developed secondary to severe (0.19-0.35 mmol/l) hypophosphatemia [49].

Furthermore Sarg and Pitchumoni, described three case of male alcoholics who developed acute hemolytic anemia secon-

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dary to hypophosphatemia [50]. In addition, Altuntas *et al.* reported case of an individual who suffered from hypophosphataemia due to complications of gastric Billroth II anastomosis surgery and developed severe haemolytic anaemia. He also developed rhabdomyolysis, hepatic dysfunction and intestinal osteopathy [40]. Also, Melvin and Watts reported a case of a 3-year-old child who developed severe intravascular hemolysis and RBC morphologic defects due to hypophosphatemia. His/her blood phosphate level was 1.7 mg/dL [51].

The clinical data indicate that Chuck suffered from severe hemolytic crisis on June 12-14, 2000. His urine showed a high level of blood but the examination of the urine under the microscope did not reveal the presence of a significant number of red blood cells. The data indicate that he had hemoglobinurea due to the destruction of red blood cells (Table 9).

Furthermore, Chuck's red blood cell count, hemoglobin level, and the hematocrit values on June 13th were reduced by 37-38% from those measured on June 12th (Table 34).

Table 34. Chuck's hematology values measured in hospital

Measurements	Date	Values
RBC x 10^6/µL	06/12	5.67
RBC x 10^6/µL	06/13	3.57
HGB g/dL	06/12	17.2
HGB g/dL	06/13	10.9
HCT %	06/12	52.8
HCT %	06/13	32.3

6.3.3 High white blood cell count:

Phosphorous deficiency results in impaired phagocytosis and opsonization. It increases susceptibility to bacterial and fungal infections. Chuck's white blood cell count was elevated following admission on June 12th and at 0810 on June 13th. Chuck was treated with antibiotics and his white blood cell count was reduced by 66% (Table 21). These data indicate that Chuck had a bacterial infection.

7. Causes of the bleeding, necrosis, and edema observed in Chuck's brain

Dr. Fierro examined Chuck's brain grossly and microscopically. She observed edema, bilateral intercerebral hemorrhage (fresh), and necrosis involving both basal ganglia and adjacent thalamus (Table 32). She concluded that these changes in the brain were caused by the ingestion of a lethal dose of methanol.

I also examined the H & E stained sections of Chuck's brain microscopically and observed fresh bleeding and necrosis of the brain (Figures 1 and 2). My review of the medical data and the medical literature pertinent to Chuck's case indicates that the lesions in Chuck's brain were not caused by methanol. Formic acid was not measured in the blood, urine, or tissues in Chuck's case and there is no clinical evidence that shows the acidosis was caused by formic acid.

The CT scan of Chuck's brain performed at about 80 minutes following admission to the hospital does not show bleeding, fluid, necrosis or any other abnormal lesion in the brain. The lesions in Chuck's brain developed after Chuck was admitted to the hospital. Abnormalities of the brain were induced by metabolic changes (hypophosphatemia and metabolic acidosis) which resulted from high doses of heparin and sodium bicarbonate given in the hospital as described below.

7.1 High doses of heparin causes bleeding

Bleeding in Chuck's brain was caused by the administration of high doses of heparin in the hospital. Chuck was hemodialyzed and hemoperfused following his admission to the hospital on June 12, 2000. Heparin was used in association with these procedures to prevent the clotting of blood. However, systemic heparinization, causes impairment of normal coagulation properties and significantly increases the risks of major bleeding complications [52-58].

Heparin is a heterogeneous group of straight-chain anionic mucopolysaccharides, called glycosaminoglycans, having anticoagulant properties. It inhibits reactions that lead to the clotting of blood and the formation of fibrin clots both in vitro and in vivo, acting on multiple sites in the normal coagulation cascade. Clotting time is prolonged by full therapeutic doses of heparin in most cases [52].

Hemorrhage can occur at virtually any site in individuals receiving heparin. Individuals suffering from anemia, any unexplained symptoms, and/or having low blood pressure are at the greatest risk of having serious hemorrhagic events after receiving a therapeutic dose of heparin. Heparin has been found to induce the formation of white clot due to the aggregation of platelets and to reduce the platelet count due to consumption. Reduction in platelet counts are also observed in individuals treated with heparin as a result of immune reactions.

For example, Davenport *et al.* studied the effectiveness of heparin as anticoagulants in a variety of extracorporeal circuits in 17 patients with combined acute hepatic and renal failure. They reported 8 major hemorrhages and 2 deaths from intracerebral hemorrhage during 600 h of anticoagulation with heparin [58].

Many clinical biomarkers indicate that the high doses of heparin Chuck received in the hospital on June 12th and 13th caused numerous related injuries which were overlooked or not noted during Chuck's substandard autopsy. Chuck's prothrombin time (PT), international normalized ratio (INR) and the partial thromboplastin time (PPT) were significantly increased as a result of heparin use. At 0810 on June 13th, his PT and PTT values were three times and more than five times the average normal values, respectively. Furthermore, Chuck's platelet count on June 13th was 54% less than his platelet count measured on June 12th prior to receiving heparin (Table 35).

PT and INR are measures of the *extrinsic pathway* of coagulation. PT measures factors II, V, VII, X and fibrinogen. PTT is a performance indicator measuring the efficacy of both the *intrinsic and the common coagulation* pathways. It is also used to monitor the treatment effects with heparin [19, 52].

The occurrence of heparin-induced thrombocytopenia (HIT), a serious allergic drug reaction, following exposure to heparin has been widely observed in children and adults. Thrombocytopenia was defined as a 50% decline in baseline platelet counts

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or an absolute platelet count < 100,000/μL [59-69]. Chuck's platelet count was reduced by 54% of baseline as a result of his treatment in the hospital.

Wallis *et al.* studied the complications of heparin-induced thrombocytopenia including thrombosis and death. They performed a retrospective analysis of 113 individuals with heparin-induced thrombocytopenia diagnosed by platelet aggregometry. Thirty-eight percent of these individuals developed thrombosis and 27% died [61].

Table 35. Chuck's blood clotting parameters measured in the hospital

Date	Time	PT (Sec.)	INR	PTT (Sec.)	Platelet ($x10^3/\mu L$)
06/12	1856	-[1]	-	-	355
06/13	0810	28.3	2.7	155	275
06/13	1200	12.7	1.2	55.3	-
06/13	1355	-	-	-	179
06/13	2200	-	-	-	162
06/14	0355	14.7	1.4	33.6	150
06/14	1315	NM	NM	35.0	-
Ref. range		10.0-11.9	0.9-1.1	22.6-31.6	147-339

[1] (-): Not measured

7.2 The likely causes of edema and necrosis observed in Chuck's brain

The gross examination of Chuck's brain revealed a very soft brain with diffuse swelling. His brain's weight was 1,479 g. Examination of the H & E stained sections from multiple areas of the brain revealed the presences of necrosis in basal ganglia. Chuck's CT scan of the brain taken following hospital admission was normal. These lesions were developed in the hospital as shown below.

1) Chuck's blood pH at admission on June 12th was 7.07 and it was raised to 7.63 on June 14th as a result of treatment with high doses of sodium bicarbonate. The treatment with high doses of sodium bicarbonate caused edema in the brain and other tissue due to tissue anoxia. Alkalinization of the blood with sodium bicarbonate increases the avidity of hemoglobin to bind oxygen, thus impairing the release of oxygen in peripheral tissues [19]. The treatment with sodium bicarbonate has caused brain edema in children and adults [20, 21].

Chuck's brain was about 111% of the average normal weight for age [70]. The increase in the brain weight was caused by the accumulation of fluid and bleeding. The increase in weight was not limited only to the brain. The weights of his liver, kidneys, and spleen were also significantly increased as shown in Table 36. These data indicate that the changes in the brain were not specific and other organs were also affected in a similar manner and these changes were caused by the use of sodium bicarbonate.

Table 36. Chuck's organ weights and as percentage of average normal weight for age

Organs	Chuck (1) Weight (g)	Reference (2) Weight (g)[1]	(1) as % of (2)
Brain	1479	1336	111
Liver	1942	1677	116
Right kidney	190	162	117
Left kidney	204	160	128
Spleen	200	156	128

[1] [38,70].

2) Bleeding causes edema, necrosis, and inflammation in the brain [71-73]. For example, Mayer *et al.* performed paired consecutive CT and ^{99}mTc-hexamethylpropylenamine oxime single-photon emission computed tomography (SPECT) scans during the acute (mean, 18 hours) and subacute (mean, 72 hours) phase of intracerebral hemorrhage (ICH) in 23 individuals. Hematoma and edema volumes were traced and calculated from CT images. They found that the ICH volume (18 mL) did not change but the mean edema volume was increased by 36% (from 19 to 25 mL, $P<0.0001$). Perilesional edema on CT always corresponded topographically with perfusion deficits on SPECT [72].

3) Bleeding causes inflammation and necrosis of the brain. For example, Gong *et al.* conducted a study in rats to evaluate the development of brain edema following the induction of intracerebral hemorrhage (ICH). Immunocytochemistry for polymorphonuclear leukocyte marker (myeloperoxidase, MPO), microglia marker (OX42) and intracellular adhesion molecule-1 (ICAM-1) was performed in control, and 1, 3, 7 and 10 days after the injection of 100 μL autologous blood in the right basal ganglia. They observed an inflammatory response in the brain after ICH. Infiltrating leukocytes and activated microglia may release cytotoxic mediators contributing to secondary brain injury and edema formation [73].

Furthermore, Koeppen *et al.* injected 100 μL of autologous whole blood intracerebrally in adult rabbits. They found that the extravasation of blood elicits a cellular reaction in the adjacent surviving tissue where the lesion activates resident microglia and attracts many more phagocytes from the blood stream. The cellular responses to the injections were studied by iron histochemistry and immunocytochemistry for ferritin, the ferritin repressor protein (FRP), the glial fibrillary acidic protein (GFAP), and the complement receptor CR3. The lesions caused initial destruction of astrocytes in the perifocal zone as judged by GFAP- and FRP-immunoreactivity [74].

4) Hypophosphatemia induces tissue necrosis. The tissues of individuals suffering from severe hypophosphatemia usually lack the ability to phosphorylate and utilize glucose as a source of energy. Furthermore, ATP and the 2,3-biphosphoglycerate (2,3-BPEG) content of the red blood cell are reduced in individuals suffering from hypophosphotemia. Reduced 2,3-BPEG and ATP enhance the affinity of oxygen for hemoglobin and reduce tissue oxygenation [19, 41, 47]. In Chuck's case, the necrosis was not limited to the brain. Necrosis was also ob-

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served in the cardiac muscles. In addition, the liver showed fatty change.

8. Causes and biomarkers of Chuck's acute heart failure

Chuck suffered from acute heart failure on June 12-14, 2000. His initial heart problem on June 12th was caused by hypophosphatemia. Chuck's serum phosphorous level on June 13th was 0.1 mg/dL (normal range: 2.8-4.9 mg/dL). His heart condition became worse during his hospitalization due to the treatment with high doses of sodium bicarbonate as shown by the data presented in Tables 37 and 38. It caused hypokalemia and hypomagnesiumemia and anoxia.

Chuck's blood pH at admission on June 12th was 7.07 and it was raised to 7.63 on June 14th as a result of the treatment sodium bicarbonate. His serum potassium level dropped from 5.1 mmol/L to 3.0 mmol/L. In metabolic acidosis, potassium usually leaves the intracellular environment because the intracellular proteins bind with hydrogen, which leads to cardiac problem and paralysis of the respiratory muscles [19]. His serum magnesium level on June 13th was 1.0 mg dL whereas the normal range is 1.8-2.4 mg/dL.

Chuck's electrocardiogram (ECG) exams performed in the hospital show that his heart condition worsened between June 12th and 14th (Table 37). Chuck's blood analysis performed at 0355 on June 14th revealed very high levels of creatine kinase-MB (CK-MB) and troponin. His troponin blood level on June 14th was increased 8 fold relative to the level detected on June 13th (Table 38). Troponin and CK-MB are released into the blood from the damaged cardiac muscles [75-82].

The microscopic examination of the H&E stained section of the heart performed by the medical examiner revealed the evidence of patchy ischemic change and focal cell necrosis. I also examined the H & stained sections of Chuck's heart microscopically and observed necrosis of the cardiac muscle. Gross examination of the coronary arteries showed no significant atherosclerosis.

The necrosis of the heart developed during Chuck's hospitalization as indicated by the ECG exams and the high levels of CK-MB) and troponin levels in serum (Tables 37, 38). However, the medical examiner did not investigate the causes of Chuck's acute cardiac failure.

Table 37. Chuck's ECG results show progressive derioration of his heart

Date	Time	Findings
June 12	1850	• Sinus rhythm with premature atrial complexes other normal ECG.
June 13	0151	• Sinus Bradycadia • Left ventricular hypertrophy • Nonspecific ST-T Wave Changes • Significant changes had occurred, when compared with ECG of June 12 at 1850.
June 13	1144	• Sinus Tachycardia • Right Axis deviation • Nonspecific ST-T Wave Changes • Significant changes had occurred, when compared with ECG of June 13 at 0151.

Table 38: Serum level of biomarkers for cardiac damage

Date	Time	CK-MB (ng/mL)	CK (U/L)	Troponin (ng/mL)
6/13	0140	NM[1]	55	0.04
	1200	9.6	291	0.75
	2200	10.8	67	0.49
6/14	0355	11.2	67	6.1
Normal Range		0.0-3.6	35-232	0.0-0.4

[1]NM: not measured

9. Causes and biomarkers of Chuck's cardiomegaly and pulmonary atrophy

The autopsy findings show that Chuck suffered from cardiomegaly and pulmonary atrophy, especially of the right lung (Table 39). Chuck was treated with corticosteroid compounds via inhalation and dermal routes during the two years prior to his death (Table 40). The treatment with high doses of corticosteroids for a significant period of time caused myopathy of the respiratory and the skeletal muscles and cardiomegaly.

9.1 Evidence of cardiomegaly and pulmonary atrophy observed in Chuck's case

Chuck's heart was examined grossly by Dr. Fierro and her examination revealed globoid biventricular hypertrophy, predominantly left-sided**.** The right ventricle showed moderate to severe hypertrophy. The microscopic examination of the H & E stained section of the heart revealed the myofibrils were wide and showed nuclear enlargement.

Chuck's heart weighted 680 g, which is 183% of the normal average for age (Table 39).

Hanzlick and Rydzewski reviewed the autopsy data for 218 white men (20-39 years of age) and found the average weight of the heart for this group is 371 g. A coefficient for heart weight expressed as a percentage of body weight was lower in heavy individuals than lightweight individuals; it ranged between 0.38% and 0.55%, with a mean of 0.48% [83]. Chuck died at the age of 37 years and his weight was 77 kg. His heart weight was 0.88% of his body weight.

The weight of Chuck's right lung was 83.6% of the average normal weight for age and 98.6% of his left lung. The right lung is usually larger than the left lung and it weighs about 15% more than the left lung (Table 39). In humans, the right lung has three lobes and left lung has two lobes. These data indicate that Chuck suffered from atrophy of the right lung.

Fierro stated that Chuck's lungs were congested and she observed petechial hemorrhages in the posterior region. A small amount of exudates was present in major bronchi. One section the lungs were boggy. Ferro's observations indicate that Chuck's lungs contained blood and other fluid. Chuck's combined lung weight was 1116 g and it is likely that his actual lung weight is significantly less than 1116 g. In this case, he suffered from atrophy of the right and the left lungs.

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Table 39. Chuck's lung and heart weight as compared with the reference weight.

Organs	Chuck (1) Weight (g)	Reference (2) Weight (g)	(1) as % of (2)
Heart	680	371	183.3
Right lung	554	663	83.6
Left lung	562	583	96.4
Lungs	1116	1246	89.6

9.2 Chuck's treatment with corticosteroids and adverse reactions

Chuck used corticosteroids via inhalation and dermal routes for his allergy and skin problem during the two years prior to his death (Table 40). His chronic treatment with high doses of corticosteroids caused problems in his heart and lung and probably affected other organs. Corticosteroids reduce protein synthesis in tissues and cause myopathy of both skeletal and respiratory muscles and cardiomegaly [85-88].

Furthermore, Lipworth conducted a computerized database search from January 1, 1966, through July 31, 1998, using MEDLINE, EMBASE, and BIDS and using appropriate indexed terms. He evaluated reports dealing with the systemic effects of inhaled corticosteroids on adrenal gland, growth, bone, skin, and eye. He found that all inhaled corticosteroids exhibit dose-related systemic adverse effects [89].

Table 40. Chuck's treatment with corticosteriods and durations

Medications	Time of use	Treatment for
Betamethasone cream	Prior to 1999	Rosacea
Hydrocortisone 1% cream	1999-June 12, 2000	Rosacea
Beclomethasone dipropionate nasal spray (Vancenase AQ 84 µg) twice daily	Beginning of 1998-June 12, 2000	Allergy/Asthma

9.3 Corticosteroid induced respiratory and skeletal muscles myopathy

Corticosteroids cause myopathy of the skeletal and respiratory muscles. The short-term treatment with high doses of steroids has caused in some individuals acute myopathy of the skeletal and respiratory muscles that it is characterized by generalized muscle atrophy and rhabdomyolysis [87]. Acute myopathy in patients treated for severe asthma has been recognized with increasing frequency since first being described in 1977 [90].

Chronic steroid myopathy has occurred after prolonged treatment with moderate doses. It is characterized by the gradual onset of proximal limb muscle weakness and may be accompanied by reduced respiratory muscle force [87]. Below are clinical studies that show the development of myopathy in individuals treated with corticosteroids:

1) Weiner *et al.* evaluated functional alterations in the inspiratory muscles strength in eight individuals receiving corticosteroids for diseases other than respiratory. They found a gradual decrease in both inspiratory muscle strength and endurance following corticosteroid administration. After 8 weeks of corticosteroid treatment, the inspiratory muscle endurance decreased from 84.4 +/- 2.4 to 67.9 +/- 3.1 percent ($p < 0.001$), while inspiratory muscle strength dropped from 126.9 +/- 9.6 to 86.5 +/- 7.4 cm H2O ($p < 0.005$). Gradual steroid dosage tapering resulted in marked improvement in both strength and endurance [91].

2) Bowyer *et al.* assessed sixty steroid-treated patients with asthma for the presence of muscle weakness by use of both manual muscle testing and the Cybex II isokinetic dynamometer. Hip flexor weakness developed in sixty-four percent of the individuals (16/25) taking greater than or equal to 40 mg per day of prednisone as indicated by the manual muscle testing. Forty-eight percent of the individuals (12/25) taking prednisone (greater than or equal to 40 mg per day) had hip flexor strength greater than or equal to 2 SD below the mean of age and sex-matched control subjects by Cybex testing [92].

3) Decramer and Stas reported the cases of two individuals with asthma and one with chronic obstructive pulmonary disease (COPD) who developed steroid-induced myopathy during prolonged treatment with high doses of corticosteroids. They showed significant reduction in their quadriceps force and respiratory muscle force. Tapering of treatment with corticosteroids resulted in important recovery of quadriceps force and respiratory muscle force. In all three individuals, a correlation between muscle forces and steroid dose was present during reduction of the dose [93].

4) Janssens and Decramer reported two cases in woman who developed a characteristic steroid-induced myopathy following treatment with corticosteroid for connective tissue disease. Gradual steroid dosage tapering resulted in prompt clinical improvement and marked increases in respiratory muscle strength. The maximal inspiratory pressure increased by 33 percent in one woman and by 70 percent in the other [94].

5) Vallet *et al.* evaluated a case of a 37-years-old woman who developed acute myasthenic respiratory failure following two years corticosteroid treatment for myasthenia gravis. She exhibited a progressive 12 kg weight loss with muscular weakness and atrophy. Peripheral and diaphragmatic electromyography as well as histological study were consistent with a steroid-induced myopathy. Discontinuation of corticosteroid treatment was followed by a rapid weight gain with general improvement and allowed weaning from mechanical ventilation with a complete recovery [95].

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9.4 Corticosteroids induced cardiomyopathy and cardiomegaly

Corticosteroids-induced cardiomyopathy and cardiomegaly have been very well documented in premature infants who received high doses of corticosteroids for a few weeks. Hyperatrophic cardiomyopathy was also reported in children treated for infantile spasms with high-dose of adenocorticotropin. In addition, a cardiac hypertrophy was observed in an eight-year-old child treated with high doses of prednisone. Below are the descriptions of these studies:

1) Werner *et al.* evaluated the potential induction of cardiac effects by high-dose dexamethasone therapy in 13 respirator-dependent infants with bronchopulmonary dysplasia by means of two-dimensional and M-mode echocardiography. The initial divided dose of dexamethasone was 500 micrograms/kg per day, tapered progressively for as long as 6 weeks. Evaluations were made before treatment and at 3, 7, 14, 21, 28, 35, and 42 days after the start of dexamethasone therapy. They found a significant (p less than 0.01) increase in thickness of the interventricular septum, diastolic left ventricular free wall, and diastolic right ventricular free wall [88].

2) Evans 1994 evaluated twenty preterm infants using Doppler echocardiography to document changes in myocardial thickness associated with dexamethasone treatment for chronic lung disease. Ventricular septa and left ventricular posterior wall thickness was increased in all 11 infants in whom it was measured. The median increase was 0.9 and 0.8 mm, respectively [96].

In most infants this increase was small (less than 1 mm). However two infants developed marked septal hypertrophy with Doppler evidence of left ventricular outflow tract obstruction. In addition, myocardial hypertrophy occurs in most infants and in some of them it was severe [96].

3) Korsch *et al.* examined seven preterm infants with eight treatments of dexamethasone retrospectively. The therapy was associated with a significant increase of the mean thickness of the interventricular septum and of the left ventricular posterior wall. After the termination of dexamethasone therapy the abnormale chocardiographic findings disappeared [97].

4) Israel *et al.* conducted a retrospective review of one preterm infant who received a 26-day course, and 13 preterm infants who received at least one 42-day course of dexamethasone, and who had serial echocardiographic data available. Left ventricular hypertrophy was noted in 8 of 14 (57%) infants; hypertrophy usually was noted near the end of the treatment course. Five of these eight affected infants died; the hypertrophic cardiomyopathy was considered to have contributed to mortality in three of these five infants [98].

5) Boeuf *et al.* reported four cases of premature infants who developed hypertrophic cardiomyopathy during glucocorticoid (dexamethasone and/or betamethasone) treatment for bronchopulmonary dysplasia. In one of them, septal hypertrophy led to left ventricular outflow tract obstruction and congestive heart failure. The first echocardiographic changes appeared between the 4^{th} and 15^{th} day of the glucocorticoid course. The cumulated dose of the glucocorticoid was 1.82-3.86 mg/kg. Hypertrophic cardiomyopathy resolved completely between 2 and 4 weeks after cessation of the treatment [99].

6) Brand *et al.* reported three infants who developed hypertrophic obstructive cardiomyopathy during dexamethasone treatment for bronchopulmonary dysplasia. In all three infants, echocardiography had ruled out cardiac abnormalities prior to the dexamethasone course. The hypertrophic obstructive cardiomyopathy appeared and progressed during dexamethasone therapy and resolved completely after its cessation [100].

7) Yunis *et al.* reported three cases of newborns whose mothers were treated with betamethasone prenatally at different doses and duration of time. They developed various degrees of hypertrophic cardiomyopathy (HCM), which was diagnosed by echocardiography. These changes appear to be dose-and duration-related [101].

8) Haney *et al.* reported two cases of premature babies who developed symptomatic myocardial hypertrophy and left ventricular outflow tract obstruction following treatment with dexamethasone. Changes in the heart were documented by M-mode echocardiography and Doppler studies. A normal heart was recorded on echocardiography before and after dexamethasone treatment [102].

9) Fritz and Bhat reported three cases of premature infants who developed clinically significant septal hypertrophy and left ventricular outflow tract obstruction following treatement with dexamethasone for bronchopulmonary dysplasia [103].

10) Miranda-Mallea *et al.* reported two cases of hypertrophic cardiomyopathy in two preterm newborns secondary to dexamethasone treatment. Full recovery occurred after discontinuing steroids [104].

11) Bobele *et al.* evaluated eighteen children treated for infantile spasms with high-dose of adenocorticotropin for adverse effects of corticosteroid on the heart. Abnormal ventricular hypertrophy occurs in the majority of these patients. Many of these patients developed hypertrophic cardiomyopathy with dramatic asymmetric septal hypertrophy. Abnormal cardiac hypertrophy was seen in 13 (72%) of 18 patients. Five of 18 patients developed hypertrophic cardiomyopathy with asymmetric septal hypertrophy and concentric left ventricular hypertrophy was seen in eight patients [105].

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12) Some Nina *et al.* reported a case of a two month-old girl who developed hypertrophic myocardiopathy following 45 days treatment with betamethasone (0.3 mg/kg/day) for a hemangioma of the eyelid. The discovery of a systolic cardiac murmur motivated a cardiac sonography that showed signs of an obstructive hypertrophic myocardiopathy. The progressive reduction of the corticosteroids led to the regression of this disease [106].

13) Lhasbellaoui *et al.* reported a case of a girl who developed a cardiac hypertrophy following treatment with prednisolone for ulcerative colitis. This girl had ulcerative colitis developed at the age of 8 years despite prednisolone and total parenteral nutrition. She was then given intravenous methylprednisolone (2 mg/kg/d). Polypnea appeared 25 days later while blood pressure remained normal. Echocardiography revealed a cardiac hypertrophy associated with a systolic anterior motion of the mitral valve. Cardiac symptoms and echography findings returned to normal after reduction of corticosteroid therapy [107].

10. Clinical data that argue against the allegation of methanol poisoning given in Chuck's case

It was alleged that Chuck died as a result of acute and chronic poisoning with methanol. The clinical data described in the previous sections of this report show that Chuck developed acute renal failure and severe hypophosphatemia as a result of the ingestion of toxic doses of creatine monohydrate and high levels of propylene glycol. He also developed bleeding, edema, and necrosis in the brain due to his treatment with high doses of heparin and sodium bicarbonate in the hospital. The following are specific clinical and medical data that show the allegation of methanol poisoning given in Chuck's case is false.

1) Blood, tissues, and urine samples were not analyzed for formic acid in Chuck's case:

Chuck's blood pH at the time of admission on June 12th was 7.07 and he suffered from metabolic acidosis (MA). MA is a clinical disturbance characterized by a relative increase in total body acid and it can be caused by infectious and toxic agents and metabolic problems (diabetes, hypophospphotemia, etc). Proper clinical evaluation and differential diagnosis should be performed to identify the cause(s) of acidosis.

Measurements of formic acid in the blood should be taken in all suspected methanol-poisoning cases to confirm that metabolic acidosis is caused by the accumulation of formic acid. Measurements of methanol and formic acid in urine are also helpful. Furthermore, in case of death, methanol and formic acid levels should be determined in the stomach content, brain, liver, and kidney of the victim or the deceased.

Methanol is oxidized in the liver by alcohol dehydrogenase to formaldehyde and the oxidation of formaldehyde to formic acid is facilitated by formaldehyde dehydrogenase. There is a direct correlation between the formic acid concentrations in blood and tissues and increased morbidity and mortality. Formic acid has been shown to inhibit cytochrome oxidase in the mictochondria [16-18].

Formic acid was not measured in Chuck's blood, urine, stomach content, brain, and other tissues to show that metabolic acidosis in Chuck's case was resulted from the accumulation of formic acid in the blood and tissues. Dr. Fierro performed the autopsy on Chuck's body on June 15, 2000 and took samples from several tissues (liver, brain, and, kidney), blood, small bowel contents, gastric contents, and bile for toxicology. However, none of these samples was analyzed for the presence of formic acid and methanol as it has been done in many other medical and legal investigations.

For example, Tanaka *et al.* determined the levels of formic acid in the blood, stomach contents, urine, and organs of two men who were fatally intoxicated with methanol. Formic acid was measured by headspace gas chromatography**.** The average postmortem concentrations of formic acid in blood, urine, and tissues were 0.28 mg/mL in the blood, 1.37 mg/mL in urine, 0.64 mg/g in the brain, 0.53 mg/g in the liver, and 0.66 mg/g in the kidneys. The average total amount of formic acid in the gastric contents for these men was 65.6 mg [15, 30]**.**

In addition, Ferrari *et al.* investigated fifteen cases of fatal massive methanol intoxication. Body distribution of methanol and formic acid, as the main metabolite, was analyzed in blood and in different organs (brain, kidney, lung and liver). Formic acid concentrations were found to be between 30 and 1100 mg/L in the samples understudy. A good correlation was found between the blood and the brain [29].

Also, methanol was measured in the body fluids and tissues of a man poisoned with methanol and died at 40 hours following hospitalization. The postmortem methanol concentrations in the body fluids of this man were: bile 175 mg/dL, vitreous humor 173 mg/dL, and blood 142 mg/dL. Postmortem methanol concentrations in his tissues are given in decreasing order: brain 159 mg/100 g, kidney 130 mg/100 g, lung 127 mg/100 g, spleen 125 mg/100 g, skeletal muscle 112 mg/100 g, pancreas 109 mg/100 g, liver 107 mg/100 g, and heart 93 mg/100 g. The total amount of methanol in his gastric contents was 73 mg [31].

Clinical data show that Chuck's acidosis was resulted from renal damage, lactic acidosis, and ketosis. On June 12th, Chuck had high level of beta-hydroxybutric acid (BHA) of 296 µg/mL, which was 14 times more than the average level detected in a fasting individual (Table 5). Chuck's urine analysis performed on June 12th also showed high levels of ketone bodies (Table 9).

Chuck's serum phosphorous (Pho) levels on June 13th and 14th were 0.1 mg/dL and 0.0 mg/dL, respectively and the normal range is 2.8-4.9 mg/dL (Tables 14, 25). Individuals suffering from hypophosphatemia usually have high blood levels of ketone bodies due to the use of fatty acid as a source of energy. The tissues of these individuals lack the ability to phosphorylate and utilize glucose.

In addition, Chuck had high lactic acid level of 9.6 mmol/L at 3 hours following admission. Lactic acid is a major metabolite of propylene glycol (PEG). Chuck had PEG level of about 200 mg/L on June 13th. Individuals poisoned with PEG also suffered from metabolic acidosis and have large anion gap and osmolar gap as found in Chuck's case (Table 5). Barnes *et al.* evaluated 13 individuals who received toxic levels of PEG with medications and found osmol gap alone can predicted serum PEG concentrations [25].

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2) Measurements of methanol in the blood taken in Chuck's case represent a false positive:

There are clinical data that indicate methanol measurements in the blood reported on June 12th and 13th in Chuck's case represent a false positive and Chuck did not suffer from methanol intoxication. These data include:

a) The Medical college of Virginia (MCV) reported that Chuck had a blood methanol level of 750 mg/L on June 12th. This blood sample was also sent to the Commonwealth of Virginia Forensic Lab (VFL) for methanol analysis. VFL reported the level of methanol in the blood to be 600 mg/L, which is 20% less than that reported by MCV. Both labs did not measure the levels of formic acid and propylene glycol (PEG) in the blood sample.

b) MCV reported the level of methanol in Chuck's blood sample taken at 0810 on June 13th to be 200 mg/L. This sample was also tested for methanol by VFL and reported a methanol level of 100 mg/L, which is 50% of that reported by MVC. Both labs did not measure the levels of formic acid in the blood samples.

c) On June 12th, Chuck had high levels of lactic acid and ketone bodies (acetone, acetoacetate and, beta-hydroxybutyrate) in his blood as a result of the ingestion of high levels of PEG and severe hypophosphatemia. VFL reported the level of PEG in Chuck's blood sample taken at 0810 on June 13th to be about 200 mg/L. The presence of these chemicals in the blood can interfere with alcohol measurements.

For example, Jones and Rössner reported a case of a 59-year-old man who underwent weight loss with very low calorie diets (VLCD), who attempted to drive a car that was fitted with an alcohol ignition interlock, but the vehicle failed to start. VLCD treatment leads to ketonemia with high concentrations of acetone, acetoacetate, and beta-hydroxybutyrate in the blood.

The ignition interlock device determining alcohol (ethanol) in breath also responds to other alcohols (e.g., methanol, n-propanol and isopropanol). Acetone is reduced in the body to isopropanol by hepatic alcohol dehydrogenase (ADH), which explains the false-positive result [108].

Furthermore, Jones and Andersson reported a case of a man who was tested positive for ethanol by a breath screening test with an electrochemical instrument (Alcolmeter S-L2) and he was suspected of driving under the influence of alcohol. The analysis of his blood revealed the presence of acetone (0.45 mg/mL) and isopropanol (0.17 mg/mL) but without the presence of ethanol. This man was treated for hyperglycemia by special dietary control and developed severe metabolic ketoacidosis. Biotransformation of the abnormally high concentration of blood-acetone to isopropanol occurs through the alcohol dehydrogenase pathway. It led to the false positive in this case [109].

3) Methanol targets the retina and optic nerve and Chuck had no vision problem:

Dr. Acker examined Chuck's eyes following admission on June 12th and he did not observe any abnormal changes. The funduscopic exam of both eyes was normal and Chuck did not have any retinal bleeding or other lesion. Furthermore, Dr. Fierro performed the autopsy on Chuck's body on June 15th and she did not report any abnormal changes in the retina and optic nerve grossly.

Formic acid inhibits mitochondrial cytochrome oxidase activity leading to ocular toxicity. Retinal damage and bilateral optic nerve necrosis and atrophy have been observed in people who suffered from methanol intoxication [26-28, 34]. Chuck died after two days following the development of his acute illness. Individuals who have ingested toxic levels of methanol usually develop ocular lesions within 24 hours of developing acute symptoms of methanol poisoning.

For example, Brent *et al.* evaluated 11 consecutive individuals who presented with methanol poisoning at a hospital. Seven of them initially had visual abnormalities [32]. In addition, Sharpe *et al.* conducted histopathologic evaluation of the brain and optic nerve of four individuals who died as a result of intoxication with methanol**.** They observed myelin damage behind the lamina cribrosa in each nerve [33].

Furthermore, Naeser evaluated the eyes and optic nerves microscopically in a 37-year-old man who died as a result of methanol poisoning. He observed bilateral central necrosis of the optic nerves from behind the lamina cribrosa to the orbital apex [34]. Also, Fujihara *et al.* evaluated the retina of a 37-year-old man who suffered from methanol intoxication. The retinal profiles were evaluated by optical coherence tomography (OCT) and fluorescein angiography during the course of treatment. OCT demonstrated peripapillary nerve fiber swelling and accumulation of intraretinal fluid in the acute phase. In the chronic phase, the retinal thickness was diffusely decreased [26].

4. Heparin, sodium bicarbonate, and metabolic problems caused the bleeding and other lesions observed in Chuck's brain:

The CT scan of Chuck's brain performed at about 80 minutes following admission did not show bleeding, fluid, necrosis or any other abnormal lesion in the brain. These lesions in the brain were developed in the hospital. They were induced by metabolic changes (hypophosphatemia and metabolic acidosis) and the high doses of heparin and sodium bicarbonate given in the hospital as described in Section 5 of this report.

5) Formation of edema was not specific for the brain:

Chuck's brain was about 111% of the average normal weight for age and the increase in weight was not limited to the brain. The weights of his liver, kidneys, and spleen were 116%, 122%, and 128% of average normal weight, respectively (Table 36). These data indicate that the changes in the brain were not specific and other organs were also affected in a similar manner and these changes were caused by the use of sodium bicarbonate.

6) Chuck's chronic general symptoms can be explained by the development of cardiomegaly and pulmonary atrophy. His heart and lung problems were induced by the treatment with high

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doses of corticosteroids as described in Section 9 of this report. These clinical data clearly show that the allegation of chronic poisoning with methanol given in Chuck's cases is false.

11. Review and analysis of the Commenwealth's theory and evidence presented at Diane's trial

Chuck Fleming suffered from acute illness on June 12, 2000 following the consumption of a toxic amount of creatine mixed with Gatorade. His wife, Diane Fleming called 911rescue and he was taken to Chippenham Hospital in Richmond, Virginia. Chuck died on June 14, 200 and Diane was accused of poisoning him with methanol. It was alleged that Diane added methanol to Chuck's drinks over a long period of time. Diane was indicted by grand jury for poisoning and killing Chuck and then arrested.

Diane was put on trial on February 19, 2002 in the circuit court of the county of Chesterfield, Virginia and her trial lasted for two days (Cr01F01484-01,2). She was convicted of poisoning and killing her husband by adding methanol to his drinks over a long period of time. She was sentenced to 50 years in prison (30 years for the first degree murder and 20 years for the adulteration of food) without the possibility of parole [2].

My review of the medical evidence in this case, the Commonwealth's theory, and the testimonies of the police and the Commonwealth's expert witnesses reveals that Diane was convicted based on a false theory. The Commonwealth's expert witnesses did not present clinical data nor did they present medical evidence that show Chuck ingested and/or was exposed to methanol by any route nor was evidence presented showing that his acute and chronic symptoms were caused by methanol.

In addition, below are medical data and observations that show 1) the methanol detected in the Gatorade bottles did not come from the windshield washer fluid as it was alleged by the police; 2) these bottles did not contain the creatine that Diane and Chuck added to the Gatorade on June 11, 2000.

Furthermore, Diane was denied effective assistance of counsel in that counsel failed to present expert testimony to show 1) Chuck's acute and chronic symptoms were indicative of illnesses other than methanol poisoning; 2) the commonwealth's allegations that Chuck died as a result of chronic or acute methanol poisoning are not supported by Chuck's medical history, records, and the medical and scientific data; 3) the high doses of heparin and sodium bicarbonate given in the hospital caused bleeding, edema, and necrosis in the brain; 4) the four Gatorade bottles tested and used as evidence and reported to contain methanol are not the same bottles of Gatorade containing the creatine that Diane and Chuck added to Gatorade on June 11, 2000.

11.1 Acker did not present medical data to support the allegation of methanol poisoning

Dr. Christopher G. Acker, a nephrologist testified as an expert witness for the Commenwealth [2: page 133-141]. He treated Chuck for acidosis and methanol ingestion on June 12-14, 2000 at the Chippenham Hospital. He believed that Chuck's acidosis and death was caused by the ingestion of a lethal dose of methanol. In addition, he also claimed that Chuck suffered from chronic methanol poisoning. The following clinical and medical data show that Acker's diagnoses of chronic and acute methanol poisoning given in this case are not valid.

1) He did not provide information to the court on the levels of formic acid in Chuck's blood, urine, tissues or stomach contents to show that the acidosis in Chuck's case was caused by formic acid. Formic acid measurements in the blood and other biological samples should be performed in all suspected methanol-poisoning cases to confirm that metabolic acidosis is caused by the accumulation of formic acid as described in Section 10 of this report. Formic acid was not measured in the blood, urine or tissues in Chuck's case. The acidosis was caused by the accumulation of ketone bodies and lactic acid in Chuck's blood and tissues.

2) He did not present any information in court on the levels of methanol in Chuck's blood to show that Chuck was ingesting and/or exposed to toxic levels of methanol acutely and/or chronically.

3) He did not reveal to the court that Chuck had hypophosphatemia, ketoacidosis, and hemolytic anemia. These illnesses were caused by the ingestions of toxic levels of creatine and high levels of propylene glycol present in Chuck's prescribed medications.

4) He did not disclose or present information on the adverse reactions resulting from the high doses heparin and sodium bicarbonate given to Chuck in the hospital. Heparin caused bleeding in Chuck's brain and sodium bicarbonate caused brain edema and edema in other organs.

5) He did not discuss the causes of Chuck's cardiomegaly and the impact of this condition on Chuck's health. Chuck developed cardiomegaly and pulmonary atrophy as a result of the chronic use of significant doses of corticosteroids. These chronic illnesses explain Chuck's symptoms observed during the few months prior to his death on June 14, 2000.

11.2 Saady's testimony did not show Chuck was intoxicated with methanol

Dr. Joseph Saady, toxicologist from the State of Virginia toxicology Lab testified as an expert witness in the field of toxicology for the Commenwealth [2: page 175-186]. He stated that approximately 75 ml to 120 ml of methanol is needed to cause the death of an individual. However, he did not present data on the levels of methanol and formic acid in Chuck's blood, urine, stomach contents, and/ or tissues to show that Chuck ingested methanol or had been exposed to methanol by other route(s).

He analyzed samples from the four Gatorade bottles and windshield wiper fluid (WWF) for the presence of methanol. The concentrations of methanol detected in the four Gatorade bottles and WWF were 3.3-4.7% and 32.8%, respectively (Table 41). Saady described the Gatorade in his report as lemonline and fruit punch flavored Gatorade [110]. He did not report changes in the colors of the Gatorade or the presence of precipitation of powder in the bottom of these bottles.

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Saady's observations disapprove the police's allegation that the methanol found in the four bottles of Gatorade came from the WWF. I added 2 ounces of Peak WWF (blue color) purchased from Wal-Mart to 18 ounces of three flavors of Gatorade (G). The WWF changed the color of the lemon-lime G. from yellow to green; the orange G. to yellow brown; the pink color fruit punch G to magenta (purplish red).

In addition, Diane and Chuck added 22.5 g of creatine monohydrate (Crm) to each bottle of Gatorade and it is expected that more than 50% of Crm will settle at the bottom of each bottle. The solubility of Crm in water at 25°C (77.0°F) and 4°C (39.2°F) are 17 mg/mL and 6 mg/mL, respectively [35].

Table 41. Quantities of methanol detected in the Gatorade and WWF submitted to the State Toxicology Lab

Sample[1]	Descriptions	% Methanol	Methanol (mL)
Item 6A	Windshield washer fluid bottle (3820 mL), blue color	32.8	1253
Item 7	Full 20-ounce (591mL) Gatorade bottle	3.3	19.5
Item 11A	Full 20-ounce Lemon Lime Gatorade bottle	3.6	21.3
Item 11B	Full 20-ounce (591mL) Gatorade bottle of Fruit Punch	3.6	21.3
Item 13	70% full 20-ounce (432 mL) Gatorade bottle of Fruit unch	4.7	20.3

[1]Detective E Ruth Baker stated that she recovered these items on June 14, 2000 from the following locations: items 6A and 7 from the garage and the kitchen's refrigerators at Diane's house; Items 11 A, II B, and 13 from Chuck's office at Phillips Morris. No changes in color or formation of precipitations were reported in Items 7, 11A, IIB, and 13.

11.3 Scientific observations that disagree with Detective Baker's claims presented in court

Detective E. Ruth Baker from the Chesterfield County Police Department testified in court on February 19, 2000 [2; page 145-167]. She alleged that 1) the methanol detected in the four bottles of Gatorade described in Tables 41, 42 came from the windshield wiper fluid container found in Diane's house. 2) These four bottles of Gatorade are the same bottles of Gatorade containing the creatine that Diane and Chuck added to the Gatorade on June 11, 2000. Below are scientific studies and observations that disagree with Baker's allegations.

1) The Windshield washer fluid (WWF) found in Diane's house contains 32.8% methanol and the amounts of fluid needed to spike the bottles of Gatorade to yield 3.3-4.7% methanol are 59.5-84.8 mL of WWF per bottle as shown in Table 42. I added 2 ounces of WWF (blue) to 18 ounces of the three types of Gatorade used by Diane and Chuck. The colors of the Gatorade changed from yellow (lemon-lime G) to green; orange (orange G) to brown yellow; and pink (fruit punch G) to magenta (purplish red). Det. Baker and the State Toxicology Lab reported no change in the colors of three types of Gatorade.

It seems that the methanol detected in the four bottles of Gatorade that were analyzed by the State Toxicology Lab came from spiking Gatorade with pure methanol. Pure methanol is colorless. I add 2 ounces of pure methanol to 18 ounces of Gatorade (lemon-lime, orange, and fruit punch) to yield 10% methanol and did not result in color change of any of the Gatorade.

2) Baker found a full bottle of windshield washer fluid (WWF) in Diane's garage that contains 3820.4 mL and alleged that WWF is the source of methanol detected in the four bottles of Gatorade. The WWF bottle found in the garage appeared full but could hold up to an additional 40 mL according the tolerance limits of 3709-3860 mL reported by the factory. However, 274 mL of WWF was needed to yield the mounts of methanol detected in the four bottles of Gatorade as shown in Table 42.

In addition, I purchased a full bottle of WWF (Peak + 32 °F) from Wal-Mart in Dixon California. The total volume of fluid in this bottle is 3700 mL, which is 120 mL less than the volume of fluid found in Diane's bottle. These data show Baker's claim is scientifically invalid.

3) Diane and Chuck added 22.5 g of creatine monohydrate (Crm) to each bottle of Gatorade and it is expected that more than 50% of Crm to settle at the bottom of the bottle. The solubility of Crm in water at 25 °C (77.0 °F) and 4 °C (39.2 °F) are 17 mg/mL and 6 mg/mL, respectively [35].

I added 22.5 g of Crm (white powder) to 591 mL of Gatorade (lemon-lime, orange, and fruit punch) and I mixed them by vigorous shaking for an hour at room temperature (77.0 °F). Then, I left these bottles on a table for 15 minutes and I observed the formation of thick ring of powder that settled at the bottom of each bottle. The State Toxicology Lab and Det. Baker did not report that they saw precipitation of powder in the bottoms of the Gatorade bottles.

Furthermore, I added 22.5 g of Crm to 20 ounces of each of the three flavors of Gatorade containing 10% of pure methanol and 20 ounces of each of the three flavors of Gatorade containing 10% of WWF and mixed them vigorously for about one hour. Then, I left these bottles on a table for 15 minutes. I observed the formation of thick ring of powder that settled at the bottom of each bottle I tested.

Crm is not soluble in methanol. I add 7 g of Crm to a test tube containing 20 ml of pure methanol (99.9%) and mixed them for 20 minutes. Then, I left the test tube standing in a holder for 20 minutes. The Crm precipitated almost totally at bottom of the test tube leaving the colorless methanol at the top.

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Table 42. Expected quantities of pure methanol or windshield washer fluid and creatine precipitation present in the Gatorade bottles analyzed by the State Toxicology Lab

Gatorade Bottle Location[1]	% Methanol	Methanol (mL) colorless	Windshield washer fluid (mL) Blue color[2]	Expected creatine (g) setteled at the bottom of the bottle of Gatorade
Diane's refreg.	3.3	19.5	59.5	18
Chuck's office	3.6	21.3	64.9	12.5-18
Chuck's office	3.6	21.3	64.9	12.5-18
Chuck's office	4.7	27.8	84.8	12.5-18
Total		89.9	274	52-76

[1] Volume of fluid in bottle =591 mL

[2] Windshield washer fluid contains 32.8% methanol

12. Conclusions

Chuck Fleming suffered from acute illness on June 12, 2000 following the consumption of a toxic amount of creatine mixed with Gatorade and he was admitted to Chippenham Hospital in Richmond, Virginia. He died on June 14, 2000. His wife was accused, arrested, and convicted of poisoning and killing him with methanol. The clinical data and the medical studies described in this report show the followings:

1) The most likely cause of Chuck's chronic symptoms, biventricular hyperatrophy and cardiomegaly, and pulmonary atrophy is the use of significant amount of corticosteroid medications during the last three years prior to his death.

2) Chuck's acute symptoms that developed on June 12, 2000 were induced by the ingestion of toxic doses of creatine monohydrate (Crm) on June 11th and 12th and the ingestion and the inhalation of significant amount of propylene glycol (PEG) present in his medications. PEG increased the solubility of Crm in Chuck's gastrointestinal tract and bioavability. Crm caused severe hypophosphatemia, hemolytic crisis, acidosis, and damaged many organs (kidney, heart, brain, and liver).

3) The blood methanol measurements reported on June 12th and 13th in Chuck's case represent a false positive and Chuck did not suffer from methanol intoxication. His acidosis observed on June 12th resulted from renal damage, ketosis, and lactic acidosis and it was not caused by the accumulation of formic acid in the blood and tissues. There is no evidence to support claims of injury from formic acid as no tests for formic acid were done.

4) The bleeding, edema, and necrosis observed in Chuck's brain on the CT scan of June 14th and in autopsy were caused by the use of high doses of heparin and sodium bicarbonate in the hospital and hypophosphatemia.

5) Chuck developed necrosis in the cardiac muscles and cardiac dysfunction due to the hypophosphatemia, hypokalemia, hypomagnisemia, metabolic acidosis and alkalosis.

6) Dr. Christopher Acker treated Chuck in the hospital on June 12-14, 2000. He did not measure formic acid in Chuck's blood and urine to confirm that Chuck's acidosis was caused by the accumulation of formic acid. He also did not do differential diagnosis to rule out other causes of acidosis in this case. Chuck's acidosis was caused by the accumulation of ketone bodies and lactic acid.

7) The medical and the scientific data indicate that Dr. Fierro's investigation in this case is incomplete and she overlooked many medical data that show Chuck did not die as a result of methanol poisoning.

8) The Commonwealth's expert witnesses did not present clinical data and medical evidence in court that show Chuck ingested and/or exposed to methanol by any route and his acute and chronic symptoms were caused by methanol.

9) It is likely that the methanol detected in the Gatorade bottles presented in court did not come from the windshield washer fluid. In addition these bottles of Gatorade containing methanol are not the same bottles of Gatorade that Chuck and Diane intentionally spiked with creatine monohydrate on June 11, 2000.

10) Diane was denied effective assistance of counsel in that counsel failed to present expert testimony to show the commonwealth's allegations against Diane of poisoning Chuck with methanol are not supported by medical and scientific data.

11) The commonwealth's allegations against Diane Fleming, that she poisoned Chuck chronically and acutely with methanol are not supported by medical and scientific facts. Evidence and facts reviewed by me in this case indicate that Chuck was not poisoned by Diane and that Diane is innocent.

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