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CASE REPORT

Forensic application of ESEM and XRF-EDS techniques to a fatal case of sodium phosphate enema intoxication

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Abstract Sodium phosphate enemas and laxatives are widely used for the treatment of constipation, even if a number of cases of significant toxicity due to alterations of the fluid and electrolyte equilibria (hypernatremia, hyperphosphatemia, and hypocalcemia) have been reported. We present the case of an 83-year-old man who died of fecal and chemical peritonitis secondary to an iatrogenic colon perforation (produced performing a Fleet® enema through the patient's iliac colostomy) with peritoneal absorption of sodium phosphate. Environmental scanning electron microscopy coupled with an X-ray fluorescence energy dispersive spectrometry discovered multiple bright crystals formed of calcium, phosphorus, and oxygen in the brain, heart, lung, and kidney sections of the victim. The absence of these kinds of precipitates in two control samples chronically treated with Fleet enemas led us to assume that the deceased had adsorbed a great quantity of phosphorus ions from the peritoneal cavity with subsequent systemic dissemination and precipitation of calcium phosphate bindings.

Keywords ESEM · XRF-EDS · Sodium phosphate · Enema intoxication · Electron microscopy

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Introduction

Sodium phosphate (SP) enemas and laxatives are widely used for the treatment of constipation and for preoperative bowel preparation [1, 2]. The hyperosmolarity of these solutions draws fluid into the intestinal lumen to stimulate peristalsis. A number of cases of significant toxicity secondary to oral or rectal administration of hypertonic SP have been reported [3–5].

Toxic effects due to the absorption of phosphate ions from the intestinal lumen arise from alterations in the fluid and electrolyte equilibria, most commonly hypernatremia, hyperphosphatemia, and hypocalcemia [6–8].

Animal toxicological studies have found that peritoneal absorption of SP solutions is faster than oral or rectal absorption and gives higher plasma concentrations of phosphorus. Lochbühler et al. noticed that after intraperitoneal infusion of 3–4 g/kg body weight of SP, more than 80% of the rats died of severe acidosis with hypernatremia (>160 mmol/l), hypocalcemia, and hyperphosphatemia [9]. We are unaware of other reports on human intoxication due to the peritoneal absorption of SP solutions, except for the paper of Lochbühler et al. [9].

Herein, we present the case of an 83-year-old man who died of fecal and chemical peritonitis secondary to an iatrogenic colon perforation (produced performing a Fleet® enema through the iliac colostomy) with peritoneal absorption of sodium phosphate.

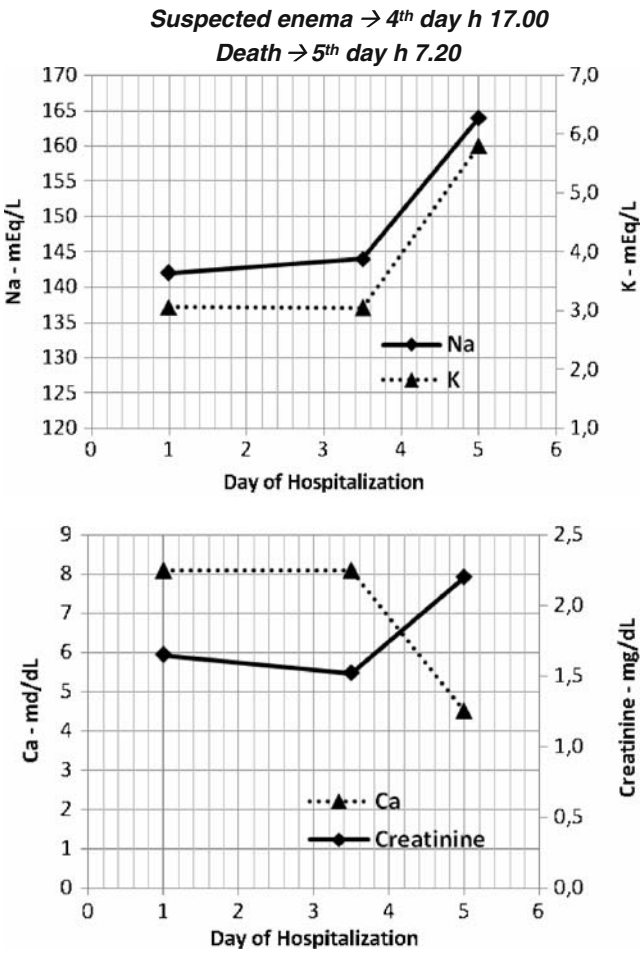
The colon perforation and the consequent electrolyte disturbances were clinically misdiagnosed and the physician who performed the enema has always denied the Fleet® administration asserting that he had only done a manual exploration of the colostomy.

In order to get forensic evidence on this point, tissue sections of brain, heart, lung, and kidney were analyzed with an environmental scanning electron microscope (ESEM) equipped with an X-ray fluorescence energy dispersive spectrometer (XRF-EDS).

Case report

An 83-year-old man (height 175 cm, weight 68 kg) was admitted to hospital with vomiting and abdominal pain. His past medical history was characterized by chronic constipation, recurrent fecal impaction, ischemic heart disease, and arterial hypertension. Two years before presentation, he had undergone a permanent colostomy for intestinal necrosis due to a volvolus. At admission, a plain abdominal radiograph showed dilatation of the entire colon with many fecal stones. Laboratory tests revealed leukocytosis (16,000/mm^3), increased serum creatinine (1.65 mg/dl), and urea (96 mg/dl) levels. During the first day of hospitalization, two 250-ml enemas (each containing 40 g of monobasic SP and 15 g of dibasic SP) were administered through the colostomy with laxative effects after 1 h and gradual improvement of the general condition. Nevertheless, on the fourth day of hospitalization, abdominal pain and vomiting presented again. At 5.00 p.m., a manual exploration of the colostomy was registered in the medical records; 2 h later (7.00 p.m.), the patient's conditions gradually worsened. He became restless, drowsy, confused, and finally profoundly comatose (11.30 p.m.). Plasma electrolyte analysis showed a fall of calcium to 4.5 mg/dl, an increase of sodium to 164 mmol/l, and an increase of potassium to 5.8 mmol/l (Fig. 1). Serum levels of creatinine raised to 2.2 mg/dl, while phosphorus plasma levels were not determined. The following day, at 7.00 a.m., the patient became pale and unconscious and a pulseless electrical activity was registered. After 20 min of cardiopulmonary resuscitation procedures, he was pronounced dead. Clinicians could not identify the etiology of the rapid general worsening or the cause of death.

The patient's daughter testified in court that she had seen a doctor administering an enema through her father's iliac colostomy the day before he died (at about 5 p.m.). This procedure was not registered in the medical records and the physician has always denied it.



	Interval of normality	Day of Hospitalization 1 h 07.00	1 h 11.00	3 h 07.00	4 h 17.00	5 h 01.00	5 h 07.20
Na^+ *mEq/L*	135 – 150	142	2 Enemas	144	Suspected Enema	164	Death
K^+ *mEq/L*	3.5 – 5.3	3.06		3.05		5.80	
Ca^{++} *mg/dL*	8.6 – 10.3	8.08		8.09		4.50	
Creatinine *mg/dL*	0.6 – 1.3	1.65		1.52		2.20	

Fig. 1 Sketch showing the time courses of Ca^{2+}, Na^+, K^+ plasma levels, and creatinine serum levels. After the suspected enema administration (fourth day of hospitalization, 5.00 p.m.), a fall of Ca^{2+} and a raise of Na^+, K^+, and creatinine concentrations are evident

Materials and methods

Autopsy

Forensic autopsy was performed 2 days after the death.

Histopathology

Tissue samples of brain, heart, lungs, spleen, liver, kidneys, colon, and peritoneum were fixed in 4% buffered formalin for 24 h, embedded in paraffin, cut by microtome in 7-μm sections, and stained with hematoxylin and eosin and Von Kossa histochemical reaction. All the sections were examined with a Leica DM4000B optical microscope (Leica, Cambridge, UK).

ESEM–XRF-EDS

ESEM instrumentation is an upgrade from the standard SEM. Instead of working only in high vacuum mode inside the sample chamber, as the conventional SEM does (10^{-4}

Torr or lower), ESEM can work at low vacuum pressure (0–1 Torr), filling the chamber in controlled mode with gas (H_2O).

Under these conditions, three operating modes are possible: back-scattered electron mode (BSE), gaseous secondary electron mode (GSE), and energy dispersive X-ray spectrometer mode (EDX). BSE and GSE are different visualization modalities (useful to detect inorganic material inside tissues), whereas EDX mode provides the elemental composition of the analyzed area.

In our experiment, tissue sections (thickness=30 μm) of brain, heart, lung, and kidney of the reported case and of two control samples were analyzed with an XL 30 ESEM TMP microscope (Philips Electron Optics, Eindhoven, The Netherlands) equipped with an integrated energy dispersive X-ray spectrometer XRF-EDS (EDAX system, Mahwah, NJ, USA). For each tissue section of brain, heart, and lung, 50 noncontinuous and not overlapped zones at 200× were analyzed in both GSE and BSE modes. Kidney sections were divided into medullary and cortical regions; for each region, 25 noncontinuous and not overlapped zones at 200× were analyzed in both GSE and BSE modes. The foci of different transparency (spots) were visualized at higher magnification (×1,600–3,200) and analyzed with the EDX mode to identify their chemical composition.

Results

Clinical data

The time courses of electrolyte plasma levels and creatinine serum levels are depicted in Fig. 1.

The known enemas were administered on the first day of hospitalization (11 a.m.), whereas the suspected enema was probably administered on the fourth day of hospitalization (5 p.m.).

Autopsy

Forensic autopsy identified a colon perforation localized 12 cm above the iliac colostomy with ragged hemorrhagic edges. No fecaloma was found.

The peritoneal surface appeared dull and lusterless with a creamy exudate. Fecal material was present in the cavity. The other organs were swollen and congestive.

Histopathology

Colon	The margins of the perforation were sharply demarcated with submucosal edema and granulocytic infiltration. In the vicinity of the perforation, neither acute nor chronic inflammatory changes were present.
Peritoneum	Acute fibrinous peritonitis characterized by loss of mesothelium, leukocytes and fibrin exudates, and submucosal edema with granulocytic infiltration
Lungs	Diffuse alveolitis with filling of the alveolar spaces by granulocytes and necrotic pneumocytes
Myocardium	Wavy myocardial fibers with lipofuscin pigment and interstitial edema
Liver	Confluent ischemic necrosis
Kidneys	Acute tubular necrosis with mesangial and interstitial deposits of calcium salts revealed by Von Kossa staining (Fig. 2a, b)

ESEM–XRF-EDS

In all organs, multiple bright crystals of different diameters (ranging from 0.5 to 100 μm) were identified (Fig. 3a–f). In the kidneys, especially in the external part of the cortical

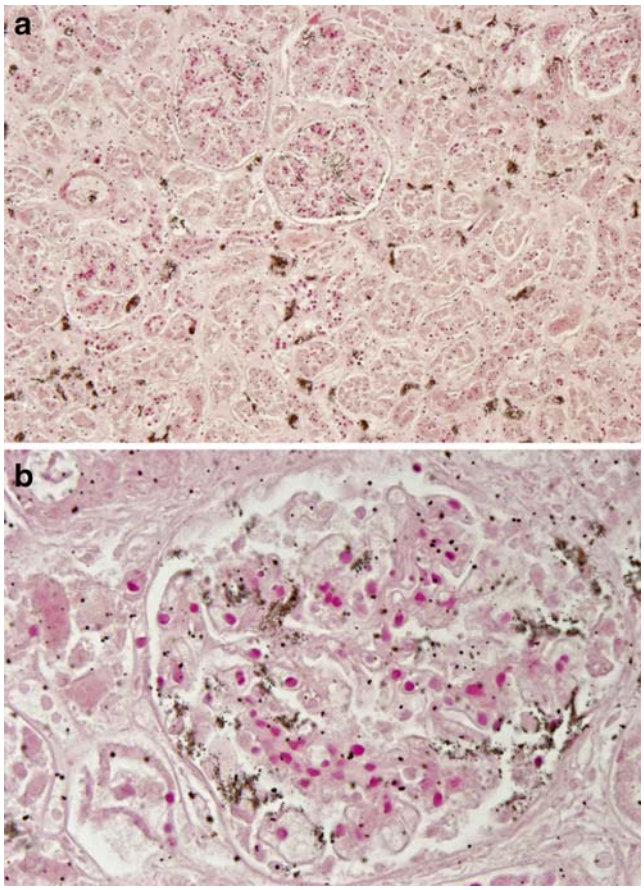


Fig. 2 **a** Acute tubular necrosis with mesangial and interstitial deposits of calcium salts (Von Kossa staining, ×80); **b** high power view of a glomerulus with mesangial and interstitial deposits of calcium salts (Von Kossa staining, ×320)

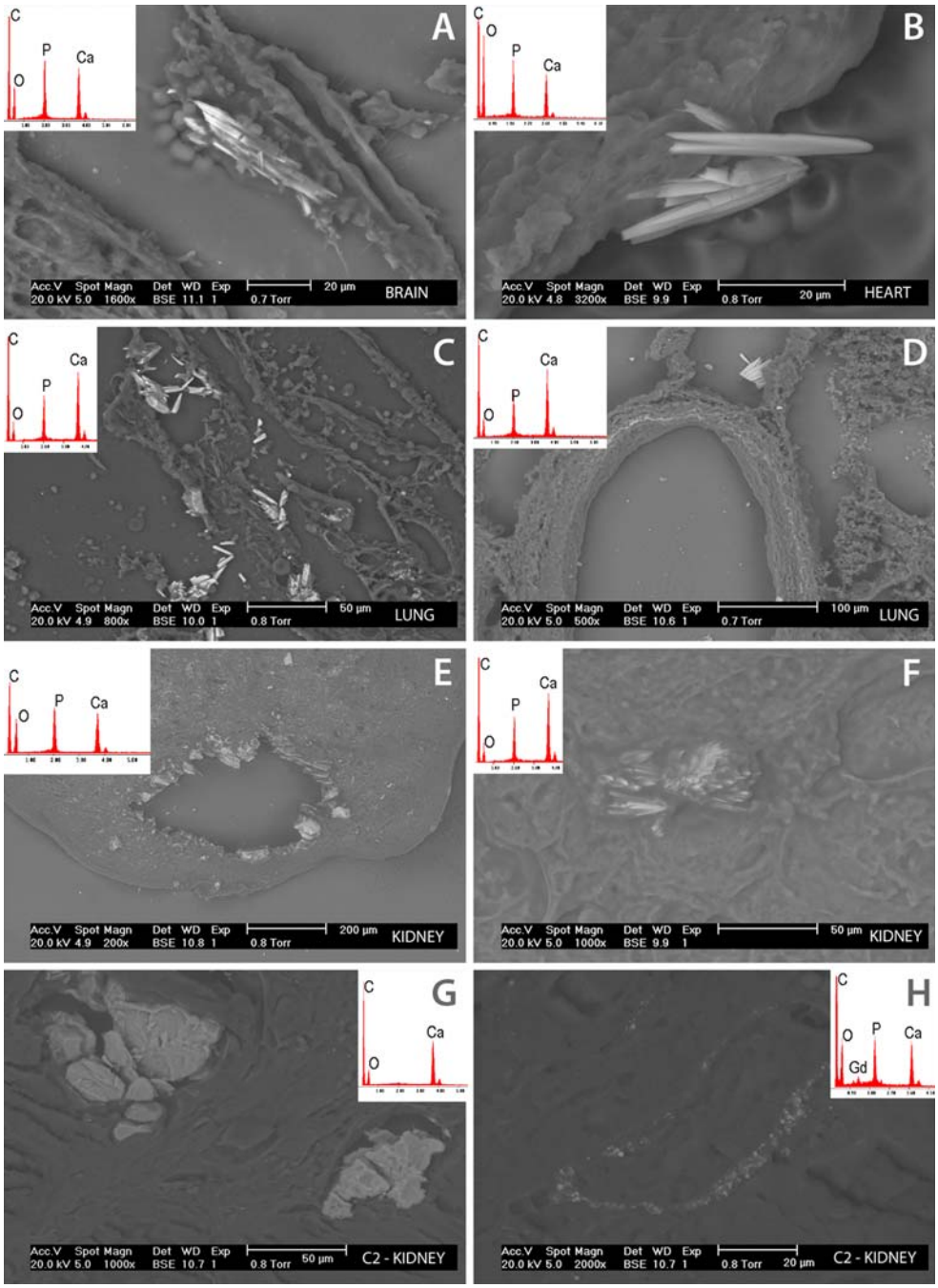


Fig. 3 Electron microscopic analysis of brain, heart, lung, and kidney tissues using an ESEM microscope equipped with an X-ray fluorescence energy dispersive spectrometer. *White boxes* show EDS spectra of the crystals. *C* carbon, *Gd* gadolinium, *O* oxygen, *Ca* calcium, *P* phosphorus. **a** Rectangular bright crystal (length≈40 µm) covered by needle-like spines in the cerebral parenchyma; **b** two rectangular crystals (length≈20 µm) in the myocardium; **c**, **d** several ovoid crystals (diameter≈10–20 µm) in the lung; **e** numerous bright ovoid crystals (diameter≈50–100 µm) in the external part of the cortical region of the kidney; **f** magnification of one of them (×1,000) showing its irregular surface; **g** huge bright crystal (diameter≈50 µm) in the cortical region of the kidney of control 2. EDS does not show the presence of phosphorus indicating that the crystal is probably formed of calcium carbonate; **h** small sodium phosphate precipitates (diameter<1 µm) in the cortical region of the kidney of control 2. EDS shows traces of gadolinium, probably administered as a contrast medium and englobed in the crystal

region, the crystals were rectangular or ovoid and had a huge diameter (50–100 µm; Fig. 3e, f). At higher magnification, the surface appeared to be covered by irregular needle-like spines (Fig. 3f). In the other organs (brain, heart, and lung), the crystals were roundish, ovoid, or rectangular with a diameter of 0.5–50 µm and an irregular surface (multiple needle-like spines at high magnification, ×1,600 or ×3,200; Fig. 3a–d).

Elemental composition of the crystals, analyzed with XRF-EDS system, differed significantly from that of the surrounding tissues revealing the presence of calcium, phosphorus, and oxygen. In contrast, the surrounding tissues appeared to be formed of carbon and oxygen. Although XRF-EDS spectrometer cannot identify the chemical structure of a compound, the great quantity of calcium, phosphorus, and oxygen measured in the crystals besides their morphological characteristics allowed us to conclude that they were calcium phosphate precipitates.

As control samples, brain, heart, lung, and kidney sections of a 48-year-old man who died of myocardial infarction (control 1) and of a 40-year-old man affected by severe chronic renal insufficiency recurrently treated with

SP enemas for constipation (control 2) were analyzed with the same XRF-EDS technique. In control 1, no calcium phosphate crystals were observed neither in the kidney nor in the brain, heart, or lung. In control 2, some huge ovoid crystals (diameter 50–80 μm) were present in the cortical region of the kidney, but XRF-EDS analysis revealed that they were formed of calcium carbonate (Fig. 3g). In the external part of the cortical region, several small calcium phosphate precipitates (diameter<1 μm) were detected, but they were roundish with a regular and smooth surface (Fig. 3h). No crystalline formations were detected in brain, heart, and lung.

Discussion

The major forensic challenge in this case was to establish if a SP enema had been administered through the colostomy and if this particular administration had played a role in causing the death.

The location and morphology of the intestinal tear (roundish with ragged hemorrhagic margins), in the absence of any vascular alterations, ischemic necrosis, or specific inflammatory changes of the wall in the vicinity of the perforation left no doubt about its etiology, excluding a spontaneous rupture and connecting it clearly to an iatrogenic origin.

More difficult was to get forensic evidence of a possible administration of a hypertonic SP enema through the colostomy with consequent intraperitoneal absorption. Plasma electrolyte analyses, showing a fall of calcium to 4.5 mg/dl, an increase of sodium to 164 mmol/l, and an increase of potassium to 5.8 mmol/l (Fig. 1), were indicative of an acute alteration of the fluid and electrolyte equilibria. Although setting the suspicion of a massive absorption of a hypertonic solution containing sodium phosphate with hyperphosphatemia, intracellular and extracellular calcium phosphate binding, and consequent hypocalcemia, the hypernatremia and the hypocalcemia alone could not prove the administration of a hypertonic enema [10, 11].

It is well known that high phosphate plasma levels can result in the precipitation of calcium phosphate in both intracellular and extracellular environments resulting in severe hypocalcemia [12, 13]. Additionally, intracellular calcium phosphate binding may lead to the influx of calcium into cells further reducing the extracellular calcium concentration [14, 15].

In our case, if a great amount of phosphorus ions had actually been absorbed intraperitoneally, calcium phosphate bindings would have precipitated in both intravascular and extravascular compartments. Considering that the hematic turbulence raises blood's kinetic energy, thus hindering the crystallization process, we can reasonably deduce that the largest and most regular-shaped precipitates would have formed in the extravascular environment.

Hence, in order to detect possible calcium phosphate crystals, brain, heart, lung, and kidney sections were analyzed using an environmental scanning electron microscope coupled with an X-ray fluorescence energy dispersive spectrometer [16].

The size, the morphology (surface covered by needle-like spines; Fig. 3a–f), the ubiquity (they were present in brain, heart, lung, and kidney), and the number of crystals detected led us to assume that the patient had adsorbed a great quantity of phosphorus ions from the peritoneal cavity with systemic dissemination and precipitation of calcium phosphate bindings.

The subsequent hypocalcemia due to the calcium phosphate precipitation caused a diffuse mitochondrial damage with cellular swelling and cerebral edema that brought the patient to coma about 6 h after the SP administration. Besides these electrolyte disturbances, the colon perforation, producing a massive vascular dissemination of bacteria and toxins, induced a sepsis with consequent multi-organ failure (MOF).

This physiopathological reconstruction, derived from autopsy, histology, and ESEM–XRF-EDS findings, allowed us to conclude that the death was caused by a MOF due to fecal and chemical peritonitis.

In the absence of a colon perforation and thus without an intraperitoneal mechanism of absorption of SP (also considering that the probable administrated dose was 55–110 g), it seems unlikely that a systemic precipitation of calcium phosphate crystals could have happened.

Indeed, ESEM–XRF-EDS investigation on tissue sections collected from two control patients (controls 1 and 2), the first affected by coronary sclerosis and died of myocardial infarction at 48 years old and the second affected by severe chronic renal insufficiency and recurrently treated with SP enemas for constipation (control 2), failed to identify any sodium phosphate crystals in brain, heart, and lung tissues.

In control 2, some small sodium phosphate precipitates were detected in the cortical region of the kidney (Fig. 3h). However, their size (diameter<1 μm) and their morphology (roundish and smooth) were completely different from those observed in our case (diameter of 50–100 μm, needle-like spines covering the surface; Fig. 3e, f).

Moreover, only in the reported case (not in the control samples) the aggregates were evident also with a Von Kossa staining (magnification ×80 and ×320; Fig. 2a, b), although histology could not tell us the composition and origin of the precipitates.

The location, shape, and dimensions of the huge crystals formed in the cortical region of the kidney could probably be explained by the high concentration of substrates (calcium and phosphorus) reached in the interstitial space after renal filtration and tubular reabsorption.

In conclusion, although future investigations are required to ascertain if the proposed microscopic analysis could be successfully applied to oral or rectal intoxications with hypertonic SP solutions (and thus without an intestinal perforation), this case report suggests a novel forensic application of the ESEM–XRF-EDS technique.

ESEM and confocal electron microscopy methods have already been used in soil and gunshot residues analysis [17–19], in questioned document examination [20], in dental comparison [21], in the diagnosis of work-related pulmonary diseases [22], and in cutting and shooting crime investigation [23, 24] demonstrating them to be powerful diagnostic tools that can sometimes provide strong forensic evidence.

References

1. Curran MP, Plosker GL (2004) Oral sodium phosphate solution: a review of its use as a colorectal cleanser. Drugs 64:1697–1714
2. Davies C (2004) The use of phosphate enemas in the treatment of constipation. Nurs Times 100:32–35
3. Biberstein M, Parker BA (1985) Enema-induced hyperphosphatemia. Am J Med 79:645–646
4. Fass R, Do S, Hixson LJ (1993) Fatal hyperphosphatemia following Fleet Phospo-Soda in a patient with colonic ileus. Am J Gastroenterol 88:929–932
5. Martin RR, Lisehora GR, Braxton M Jr, Barcia PJ (1987) Fatal poisoning from sodium phosphate enema. Case report and experimental study. JAMA 257:2190–2192
6. Davis RF, Eichner JM, Bleyer WA, Okamoto G (1977) Hypocalcemia, hyperphosphatemia, and dehydration following a single hypertonic phosphate enema. J Pediatr 90:484–485
7. Ehrenpreis ED, Wieland JM, Cabral J, Estevez V, Zaitman D, Secrest K (1997) Symptomatic hypocalcemia, hypomagnesemia, and hyperphosphatemia secondary to Fleet's Phospho-Soda colonoscopy preparation in a patient with a jejunoileal bypass. Dig Dis Sci 42:858–860
8. Rohack JJ, Mehta BR, Subramanyam K (1985) Hyperphosphatemia and hypocalcemic coma associated with phosphate enema. South Med J 78:1241–1242
9. Lochbühler H, Sachs J, Raute-Kreinsen U (1995) The pharmacological effect of sodium phosphate after absorption from the peritoneal cavity. Eur J Pediatr Surg 5:84–87
10. Marraffa JM, Hui A, Stork CM (2004) Severe hyperphosphatemia and hypocalcemia following the rectal administration of a phosphate-containing Fleet pediatric enema. Pediatr Emerg Care 20:453–456
11. Thatte L, Oster JR, Singer I, Bourgoignie JJ, Fishman LM, Roos BA (1995) Review of the literature: severe hyperphosphatemia. Am J Med Sci 310:167–174
12. Everman DB, Nitu ME, Jacobs BR (2003) Respiratory failure requiring extracorporeal membrane oxygenation after sodium phosphate enema intoxication. Eur J Pediatr 162:517–519
13. Kirschbaum B (1998) The acidosis of exogenous phosphate intoxication. Arch Intern Med 158:405–408
14. Kaye M (1995) Hypocalcemia after an acute phosphate load is secondary to reduced calcium efflux from bone: studies in patients with minimal renal function and varying parathyroid activity. J Am Soc Nephrol 6:273–280
15. Yu GC, Lee DB (1987) Clinical disorders of phosphorus metabolism. West J Med 147:569–576
16. Stokes DJ (2003) Recent advances in electron imaging, image interpretation and applications: environmental scanning electron microscopy. Philos Transact A Math Phys Eng Sci 361:2771–2787
17. Molina DK, Martinez M, Garcia J, DiMaio VJ (2007) Gunshot residue testing in suicides: Part I: analysis by scanning electron microscopy with energy-dispersive X-ray. Am J Forensic Med Pathol 28:187–190
18. Pye K, Croft D (2007) Forensic analysis of soil and sediment traces by scanning electron microscopy and energy-dispersive X-ray analysis: an experimental investigation. Forensic Sci Int 165:52–63
19. Neri M, Turillazzi E, Riezzo I, Fineschi V (2007) The determination of firing distance applying a microscopic quantitative method and confocal laser scanning microscopy for detection of gunshot residue particles. Int J Legal Med 121:287–292
20. Egan WJ, Galipo RC, Kochanowski BK, Morgan SL, Bartick EG, Miller ML, Ward DC, Mothershead RF (2003) Forensic discrimination of photocopy and printer toners. III. Multivariate statistics applied to scanning electron microscopy and pyrolysis gas chromatography/mass spectrometry. Anal Bioanal Chem 376:1286–1297
21. Fairgrieve SI (1994) SEM analysis of incinerated teeth as an aid to positive identification. J Forensic Sci 39:557–565
22. Fassina A, Corradin M, Murer B, Furlan C, Guolo A, Ventura L, Montisci M (2009) Detection of silica particles in lung tissue by environmental scanning electron microscopy. Inhal Toxicol 21:133–140
23. Saville PA, Hainsworth SV, Rutty GN (2007) Cutting crime: the analysis of the "uniqueness" of saw marks on bone. Int J Legal Med 121:349–357
24. Turillazzi E, Karch SB, Neri M, Pomara C, Riezzo I, Fineschi V (2008) Confocal laser scanning microscopy. Using new technology to answer old questions in forensic investigations. Int J Legal Med 122:173–177