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TECHNICAL NOTE

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Stability of Cholesterol Gall Stones After 165 Years of Burial

ABSTRACT: A woman who died in 1837 was exhumed for the purposes of moving the grave to another location. During the excavation, small white deposits of stone were uncovered in the right abdominal region, inferior to the rib cage and superior to the ilium blade. These stones were analyzed for cholesterol, bilirubin, and calcium following solubilization using methyl *tert*-butyl ether as a solvent. The results of these clinical chemistry analyses showed that these stones consisted primarily of cholesterol. Under these particular soil conditions encountered in this case, cholesterol gall stones are stable for at least 165 years.

KEYWORDS: forensic sciences, cholesterol, gall stones, exhumation

Gallstones are solid concretions that commonly form in the gall bladder but occasionally within the bile ducts as the result of supersaturation by cholesterol and unconjugated bilirbuin. These stones consist largely of cholesterol, and in a mixture with bilirubin, bile acids, and phospholipids (1). As there appears to be a genetic predisposition towards forming gallstones, descendants of individuals with stones may benefit from this history.

In the spring, 2002, the Connecticut Office of State Archaeology was asked to remove individuals from a family, whose history in New England dates to the 1630s, from Northeast Cemetery, Wolcott, CT. The cemetery is an ancient burying ground no longer in use, and is situated in a wooded area, overgrown with vegetation and extensively vandalized. Due to the desecrated condition of smashed and defaced tombstones in the cemetery, the descendants had requested that the remains of three individuals, a man (died 1800, age 84), his son (died 1826, age 81), and his grandson (died 1834, age 60), be removed and reburied in an historic cemetery in town that has been maintained.

In conducting the archaeological removal of the son, a shallow (3.5 ft. depth) burial of a woman was unexpectedly encountered where the son's broken tombstone lay. This individual turned out to be his wife, who died in 1837, age 86, 11 years after her husband. The woman was buried in the same grave shaft as her husband, who reposed underneath her at a depth of 5.5 ft. While excavating the skeleton remains of wife, small white deposits of stone were uncovered in the right abdominal region, inferior to the rib cage and superior to the ilium blade (Fig. 1*A* and *B*).

Soils in Northeast Cemetery consist of well-drained Charlton fine sandy loams. Soil pH readings (4.9 to 5.1) suggest moderately acidic conditions, which are not ideal for the preservation of organic remains. Nonetheless, preservation permitted the removal of individual skeletal elements, including low bone density phalanges, and the recovery of the small white stones. These stones were submitted for clinical chemistry analysis at Hartford Hospital.

One 30 mg white stone was rinsed with distilled water to remove the dirt and debris and put into 3.0 mL of methyl *tert*-butyl ether (Sigma-Aldrich, Milwaukee, WI). This solvent has been shown to be effective in dissolving cholesterol-based gall stones (2). The mixture was incubated for 3 h at 37°C. The majority of the stone was solubilized under these conditions. One hundred microliters of the dissolved stone and solvent was spiked into 0.9 mL of a plasma pool and tested in triplicate for cholesterol, bilirubin and calcium using the Cobas Integra 700 clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Results were compared against a blank containing the solvent alone. The total cholesterol content significantly increased to an average of 66.4 mg/dL with the addition of the dissolved stone relative to the background cholesterol reading. The calcium and total bilirubin concentration did not significantly increase. This produced a recovery for the cholesterol content of 66.4%. This concentration is lower than previous analysis of gallstones containing cholesterol ranging from 83–96% (3). The result in this case was artifactually low due to the presence of dirt and insoluble particulates that could not be washed from the stone.

This case was unusual because of the duration in time that the stones had been buried (165 years). Based on these analyses, we conclude that cholesterol gallstones are stable within the soil conditions encountered, and can be retrospectively tested for historic and genetic purposes. The findings described in this paper may not be applicable to other soil conditions, thus the absence of stones does not preclude their existence.

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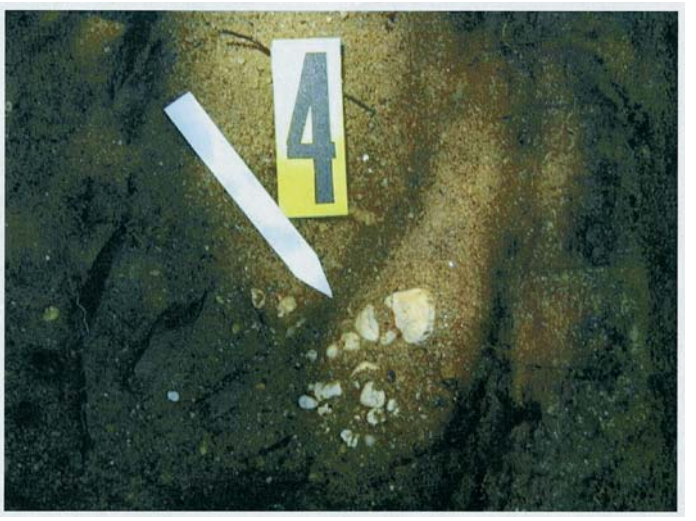

FIG. 1—A. *Remains of the subject in question.* B. *Enlarged view of the stones found in the abdominal area.*

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