TECHNICAL NOTE

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Histologic Detection of Fat Emboli

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ABSTRACT: We describe a procedure for detection of fat emboli in formalin fixed tissue using osmium tetroxide postfixation. Intravascular fat in tissue postfixed in osmium tetroxide, embedded in epoxy or paraffin, and stained with toluidine blue, hematoxylin, or Oil Red O is more easily visualized than in frozen tissue that is stained with Oil Red O. With these methods, fat emboli may be detected years after the initial autopsy.

KEYWORDS: pathology and biology, embolisms, injuries, tissues (biology)

Fat embolism syndrome, occurring most frequently in fractures of long bones and with from 10 to 90% mortality [*1*], has long been a poorly understood complication of trauma. Recent studies have documented that fat emboli can occur in the absence of a fracture in disorders such as acute pancreatitis and diabetic coma [*2*].

The typical signs and symptoms occur between 24 to 72 h after injury. Patients present with tachycardia, fever, tachypnea, and central nervous system (CNS) changes. Petechiae are a classic finding, developing somewhat later than the other signs and are most prominent in the axillary creases, the chest, and the conjunctivae. Patients at highest risk are younger individuals that have single or multiple fractures of long bones.

Respiratory insufficiency is the most clinically significant complication of fat embolism. The mechanism of lung injury results from both mechanical blockage of alveolar capillaries by fat emboli and inflammation resulting from release of free fatty acids (FFA) brought about by the breakdown of the emboli [*1*].

History

A 22-year-old white male working in the body shop of a car dealership became pinned against a cement wall by a car. At the scene he was conscious, alert, and oriented. He complained of severe pain in both lower extremities. Upon arrival at the emergency room he was lethargic but was aware of his surroundings. X-ray examination revealed a transverse fracture of the left femur, nondisplaced left tibia and fibula fractures, and an open right tibial fracture. He was taken to the operating room where he underwent open reduction and internal fixation of his left femur fracture and splinting of his tibial fractures. He failed to regain

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consciousness postoperatively and could not be aroused. Blood gases were pH 7.38, PCO_2 34, PO_2 61. Neutral fat globules were noted in the urine and blood. He remained hypoxemic and was determined to be brain dead. He was pronounced dead two days after the accident occurred.

At autopsy there was extensive undermining of the subcutaneous tissue of the left thigh. Petechial hemorrhages were seen in the conjunctiva and the visceral pericardium. The lung parenchyma was edematous. No natural disease was found.

Microscopic examination of Oil Red O (ORO) stained frozen sections of lungs, heart, brain, and liver was performed. Fat emboli were present within capillaries of all of these organs, but their identification was much more difficult because of considerable background staining.

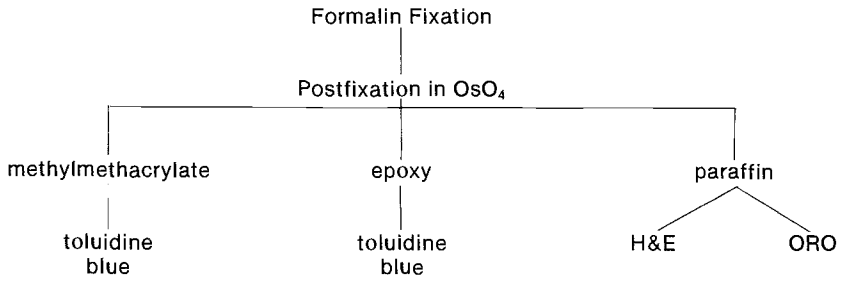
Because fat embolism is not always considered at the time of initial autopsy, frozen sections and ORO stains are not generally performed at that time. In a search for a better method of fat emboli detection, we postfixed formalin fixed lung tissue from the case mentioned above and five other trauma related deaths in 1% osmium tetroxide (OsO_4). Osmium tetroxide (OsO_4), used routinely in the processing of tissue for electron microscopy, preserves lipids such as those in cell membranes. We cut the lung samples into 1- by 1- by 0.3-cm pieces, and embedded some sections in paraffin, others in epoxy resin, and a third group in methyl methacrylate (Table 1). The epoxy and methyl methacrylate blocks were cut at 1 to 2 μm thick and stained with toluidine blue. Two 4-μm sections were cut from each paraffin block. One section was stained with hematoxylin and eosin (H&E), and the other was exposed to a 3% hydrogen peroxide solution (to bleach out the OsO_4), stained with Oil Red O (ORO), and counterstained with hematoxylin.

In the lungs of the patient described herein, and in three out of the five other cases intravascular fat globules were seen. In lung sections embedded in epoxy resin, the fat had the appearance of brown translucent material within the vessels (Fig. 1). Sections from tissue embedded in methyl methacrylate fragmented and were not interpretable. In the paraffin sections that were treated with hydrogen peroxide, stained with ORO, and counterstained with hematoxylin, the fat appeared as red globules in the vessels (Fig. 2). In H&E stained sections the fat globules were dark blue (Fig. 3).

Discussion

Agrawal and Patil postfixed lung and kidney sections in OsO_4 from 115 accident victims. Fat emboli were found in 111 (95%), although they were the principal cause of death in only 15 (13%). The only stain used was H&E [3]. In our study, the fat globules were easily discernible from background material in epoxy embedded sections and in the paraffin embed-

TABLE 1—*Fat emboli detection.*



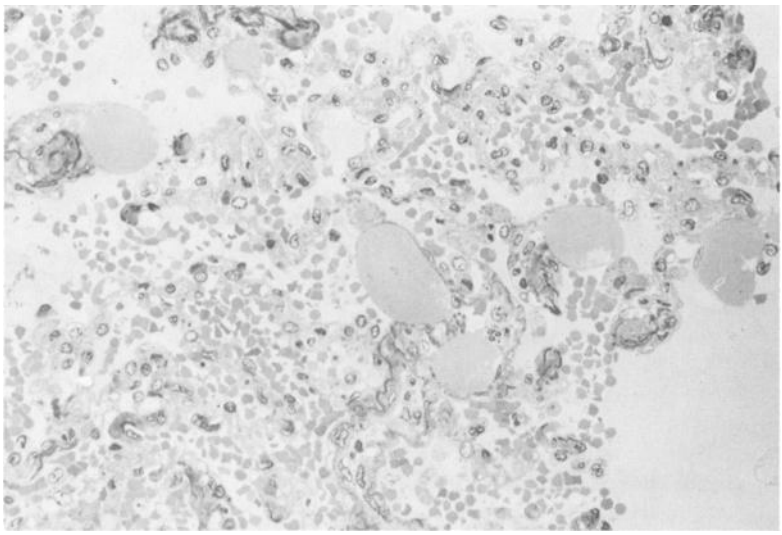


FIG. 1—*Lung section with numerous vessels containing brown translucent material indicating fat. Note the numerous intraalveolar erythrocytes (epoxy-toluidine blue ×100).*

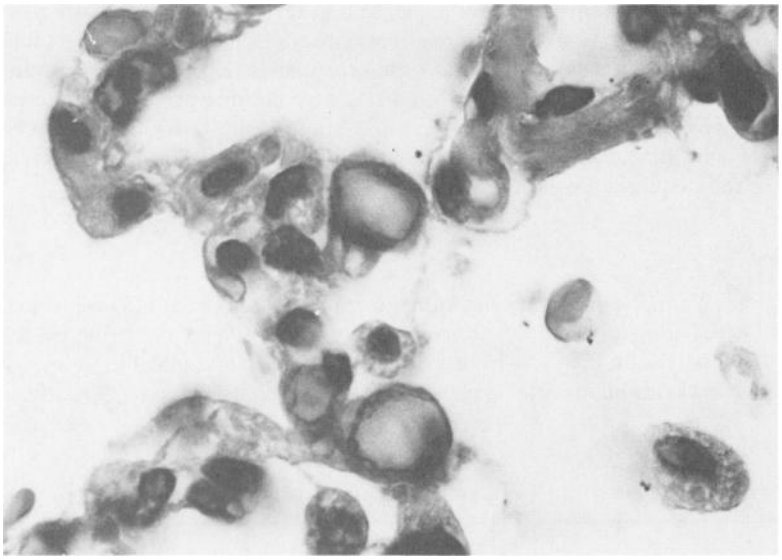


FIG. 2—*Capillaries engorged with fat as demonstrated by Oil Red O stain (paraffin Oil Red O ×250).*

ded sections stained with ORO. In the paraffin embedded sections stained with H&E, the black staining intravascular fat may be confused with anthracotic pigment at scanning power. We feel that the methyl methracrylate embedded sections fragmented because of improper penetration of the N-N^1-dimethyl aniline used as a hardener during the final steps of embedding. To avoid fragmentation, tissue blocks would have to be less than 1 mm thick.

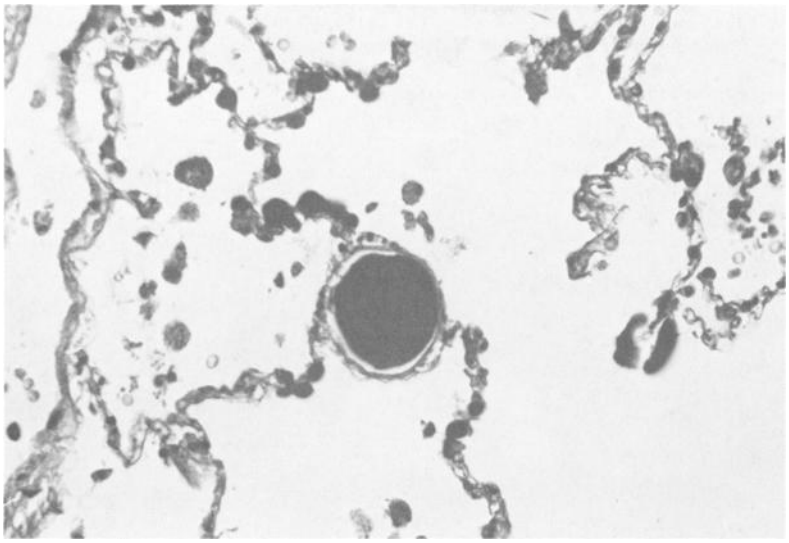


FIG. 3—*Pulmonary capillary with fat globule (paraffin-H&E ×250).*

We believe that the best method for demonstrating intravascular fat is formalin fixation, postfixation with OsO_4, embedding in epoxy resin, and staining with toluidine blue. The 1- to 2-μm sections produced by epoxy embedded sections permit the most detailed microscopic examination. If epoxy plastics are not available good alternative methods include formalin fixation, osmium postfixation, paraffin embedding, and staining with ORO or H&E. These methods, characterized by less background staining and using formalin fixed tissues, are superior to the "standard" for fat stains, namely, ORO staining of frozen tissue.

Conclusion

In conclusion, it is not necessary to do frozen sections on lung and other organs when fat embolization is suspected. Osmium fixed tissue not only provides for superior detection of fat in tissue, but enables the prosector to search for fat embolization years after the gross autopsy.

Acknowledgment

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