CASE REPORT

Howard C. Adelman,¹ M.D.; Paul C. Peterson,² Ph.D.; and Loreé J. Sorger,² B.S.

Identification of Wooden Instrument by Scanning Electron Microscopy from Splinters Left in Victim

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ABSTRACT: Case presentation concerns a twelve-year-old boy who was sexually assaulted, beaten, and murdered. A broken wooden broomstick was used in the attack to impale the victim through the rectum. Following the autopsy, splinters from the body were found by light microscopy. Macrodissected splinters were removed from the anus, rectum, and urinary bladder of the victim and examined by scanning electron microscopy. The splinters were identified as the same type of wood as the broomstick.

KEYWORDS: pathology and biology, criminalistics, criminal sex offenses, microscopy, foreign bodies, scanning electron microscopy

Background

Vlad II, Dracul, Prince of Wallachia (d. 1476) was known as Vlad the Impaler by the citizenry of his kingdom because of his use of impaling as a favorite method of sport and executions. Today, Vlad is better known as Dracula [1].

King Edward II was murdered on 21 Sept. 1327 in Berkeley Castle near Gloucester, England. He was assassinated by a rival political faction which supported the queen. Because his murderers did not wish to display any outward manifestations of physical violence, the horn of a cow was inserted into his anus. He was then impaled with a red hot iron which was thrust through the hollow horn. Ironically, King Edward was a homosexual, and it is thought, that this behavior led to the political disorder that brought about his assassination [2].

In 1983, Torre and Varetto [3] reported a case of murder by impalement. The murder took place two years before their report on the grounds of a mental hospital where the victim, a 27-year-old oligophrenic man, was staying. He was killed by a psychopath who impaled his victim with a sharp stick which was thrust into his perineal region penetrating the entire

¹Director of laboratories, St. Joseph Riverside Hospital, Warren, OH.

²Professor of biology and graduate student, respectively, Youngstown State University, Youngstown, OH.

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torso through the liver and into the pleura. Several penetration wound tracks were found and the victim was known to have taken part in "passive homosexual practices with other inpatients" on many occasions. On the night of the murder, the "active partner" probably inserted a broom handle (blunt end) into the victim "after or instead of normal anal intercourse." When the victim fled, his partner caught him and "repeatedly stuck him in the anus and perineum with another stick" which had a sharpened end. The authors reported this case because "Death from impalement is rarely, though not exceptionally, observed in ordinary medicolegal practice, and it is mainly due to accidental causes. Murder by impalement, however, must be regarded as absolutely exceptional" [3].

The anus often becomes a home to many foreign objects, from bedknobs to broomsticks and fireworks [4-8]. Many clinical articles regarding their removal have appeared in the medical literature [9-19]. Anal intercourse and insertion of objects into the rectum for sexual gratification have been well documented and can be a part of autoerotic as well as homosexual and heterosexual activity [20-25].

In forensic medicine, a necessary task in evaluating death by violence is the identification of the weapon or an instrument which may have been employed.

Case History

Returning home one evening, a 12-year-old boy pushed his bicycle through a vacant lot often used as a shortcut. He was accosted by two males, 17 and 18 years of age. At first, the older boys indicated that they would steal the younger boy's bicycle. Soon, however, the situation degenerated to the point where the 2 older boys dragged the younger boy into the bushes and beat him. The violence increased to rape and the boy was sodomized rectally by one and possibly by both older boys.

Tiring of the activities, the two older boys left their victim moaning on the ground. As they walked away, the younger boy staggered out of the bushes crying and asking them to get his mother to help him. With that, the two older boys dragged the twelve-year-old into another area and brutally beat him. They then strangled him with his underpants which they set on fire. When they thought he was dead, the older boys left. The victim was discovered by his father who searched for his son when he did not return home that evening. He was rushed to the hospital where he died after two days in a coma.

At autopsy, the victim's anus was markedly dilated and blood gushed from the orifice as though under a great pressure. Linear lacerations and contusions were prominently displayed in the anus and in the anorectal area. A circular imprinted contusion measuring 2.5 cm was found on the anterior wall of the rectum. Adjacent to this area was a perforating injury (Fig. 1) which extended into the urinary bladder. The object which could have caused this injury soon became the focus of inquiry and the possibility of a broomstick was considered. About four days after his death (six days after his hospital admission), a broken wooden broomstick was found. Exposed to severe weather conditions which included heavy rains, no chemical evidence of blood or tissue was found on the stick.

Following the autopsy, routine light microscopic examination of tissue sections revealed the presence of numerous plant-like cells within the tissues of the rectum, anus, and urinary bladder. These foreign bodies were surrounded by exudate and hemorrhage (Fig. 2). Splinters from the stick (instrument) were submitted for routine histologic preparation and paraffin embedding. Sections were cut and compared with the foreign material found in the anus, rectum, and urinary bladder. There was striking morphologic similarity between them (Fig. 3). Scanning electron microscopy was then performed.

Identification or comparisons or both of woods by scanning electron microscopy involves a detailed understanding of the minute anatomic structures which characterize hard and soft woods. Accurate determinations can be obtained only through the evaluation of a broad spectrum of characters which are found in combinations on any of the transverse, radial, or



FIG. 1—Fresh autopsy specimen of rectum showing perforation (right) and imprint mark of instrument (left).



FIG. 2—Light microscopic view of splinters in the rectum with adjacent tissue exudate ($\times 400$).

tangential surfaces (Fig. 4). Characters which have proven to be of significant diagnostic value include the vessels and vessel elements, rays (tangential and radial), longitudinal tracheids, and fibers (Fig. 5). Care must be taken to detect those characters best observed on one of the three surfaces.

Porous woods (hard woods) are characterized by the presence of vessels which have developed through the fusion of longitudinal cells to form a composite tube-like structure. Perforations are often found at or near the end of the vessel elements to permit communication between contiguous vessel elements. Soft woods lack vessels or vessel elements and are considered as nonporous woods. Distribution, pattern, and shape of the pores; the diameter and numbers of the pores/mm²; and the thickness of the walls of the vessels have been used for microscopic identification of woods.

Rays are ribbon-like aggregates of cells extending radially into the trunk of trees. Most hard wood possesses rays ranging from 2 to 30 cells in width with the maximum width



FIG. 3—Comparison photo of histologic appearances of wooden splinters found in the rectal tissues (right) and splinters from the instrument following routine histologic preparation (\times 400).



FIG. 4—A cube of Eastern white pine (Pinus strobus) (× 75) (photo by Arnold Day from Ref. 26, p. 112).

(300 μ m) development found in oaks. Some hard woods contain 2 sizes of rays such as those found in the sugar maple (Acer saccharum) and beech (Fagus sp.). As viewed tangentially, rays are also variable in height and spacing. These ray characteristics can be used extensively in the identification of both hard and soft woods.

Tracheids are long tubular lignified cells which function for support and the conduction of water. They are distinguished from vessels by having tapered closed ends and communicate with other tracheids through numerous bordered pits. Tracheids exhibit a number of modifications some of which are of diagnostic value. Particularly significant are the type of pitting, the pattern of distribution, and the presence of spiral thickenings in certain species.

In soft woods (pines), more than 90% of the volume consists of longitudinal tracheids with lengths varying from 3 to 4 mm. Conversely, the hard woods have a much more complex structure because of the addition of different cell types in their composition, for example, vessels and vessel elements.



FIG. 5—SEM photomicrograph showing morphologic similarities between the splinters of the instrument (left) and the splinters found in the rectal tissues (right).

Fiber cells include all of the remaining long, narrow cells with closed ends other than tracheids. In some species, the fibers are septate in that thin transverse walls (septa) form within the cell. These cells often compose 50% or more of a wood volume. Identification of woods based on fiber variability is based on the thickness of the walls and the volume (number) of these cells entering into the structure of wood.

In this study, we used the scanning electron microscope to maximize the number of characters observable in small wood samples (1 mm). Under such conditions, not all surfaces are present and care must be taken to orient the sample to optimize the number of characters needed for purposes of identification.

Method

Using an American Optical stereomicroscope, tiny splinters were carefully dissected from the anus, the rectum, and the bladder. The maximum length of these splinters measured approximately 1 mm.

The minute splinters were washed in 80% ethyl alcohol and transferred to a sonicator where they were washed a second time to remove cells and tissue fluids. Upon removal from the sonicator, the specimens were air-dried, mounted, and sputter coated with 2-nm gold before observation. The same procedure was employed using samples of the stick (instrument) as well as other randomly selected woods for comparison.

Observations were made with a Hitachi Scanning Electron Microscope Model 450 S and photographed with Type 55 Polaroid film.

Results

Examination of both the splinters from the autopsy and the splinters removed from the instrument reveal a strikingly similar development and positioning of the tracheids and rays (Fig. 6). The qualitative comparisons of these splinters are: the position of the tracheids and rays, the barrel shaped vessel elements, and the rays are one cell layer thick and homocellular without resin canals. The quantitative comparisons of these splinters are: average length of vessel elements—250 μ m, average ray width—8 to 10 μ m, and mean diameter of rays cells—6 to 7 μ m.

Morphologic comparisons with known samples of various woods were made. These woods



FIG. 6—SEM longitudinal section taken from the instrument. Spindle shaped cells resembling opened tubes are the tracheids. The rays are laterally directed linear structures. The trachid/ray ratio indicates a primitive wood.

included: Idaho pine (Pinus monticola) (Fig. 7), American beech (Fagus grandifilia) (Fig. 8), paper birch (Betula papyrifera) (Fig. 9), English oak (Fagacene quercus) (Fig. 10), sugar maple (Acer saccharum), gray elm (Ulmus americana), red elm (Ulmus rubra), ash (Fraxinus americana), white oak (Quercus alba), and an unknown (that is, unidentified) wooden match sample (Fig. 11). The morphologic characteristics and measurements of the anatomic structures (trachids, vessel elements, rays, and ray volumes) of these woods were markedly different from the wood of the instrument.

Discussion

When confronted with a specimen of unknown wood, it should be noted that both gross and microscopic examinations are most dependable for a positive identification. In most cases, gross features of color of the wood, odor, and the appearance of the grain provide all that is needed for identification. In other cases it is necessary to examine those structural characteristics observable only with the microscope [26].

The scanning electron microscope has proven to be an invaluable aid in the identification and comparison of small wooden fragments extracted from tissues and from the instrument. Ideally, it is requisite for positive determinations to extract those combinations of characters which are found on the three surfaces of the wood. Optimally, these features include (in descending order of usefulness) morphological aspects of the vessels; the number, occurrence, and dimensions of the rays; and the type, pattern, and distribution of the tracheids. In this study, we have been able to obtain from the tangential and radial surfaces only, sufficient numbers of characters to match positively the splinters extracted from the rectal tissues with those obtained from the instrument and even tentatively identify the type of wood involved.



FIG. 7-SEM tangential section of Idaho pine (Pinus monticola).



FIG. 8-SEM tangential section of American beech (Fagus grandifilia).



FIG. 9-SEM tangential section of paper birch (Betula papyrifera).



FIG. 10-SEM tangential section of English oak (Fagacene quercus).



FIG. 11-SEM tangential section of an unidentified wooden match stick.

Conclusions

With respect to this case, we are positively able to identify the splinters as being the same type of wood as the instrument. Criteria we used included the position of the tracheids and rays; the shape and length of the vessel elements; and the position, size, and morphologic characteristics of the ray cells.

Identification of the *species* of wood is tentative and ongoing. What is known is that both the splinters from the boy's body and from the instrument are hard woods identified tentatively as species assigned to either the genus *Fraxinus* (ash) or *Quercus* (oak).

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Address requests for reprints or additional information to

Howard C. Adelman, M.D.

Director of Laboratories

St. Joseph Riverside Hospital

1400 Tod Ave., N.W.

Warren, OH 44485.