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The Diagnostic Value of the Diatom Test for Drowning, II. Validity: Analysis of Diatoms in Bone Marrow and Drowning Medium

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ABSTRACT: In part two of this series, we investigated the characteristics of diatom frustules recovered from bone marrow and samples of putative drowning medium in case of freshwater drownings. A total of 52 cases of freshwater drowning with diatoms in the femoral bone marrow in which a sample of the putative drowning medium was also collected were available for analysis. The same types of diatoms were found in the bone marrow and putative drowning medium in 47 cases (90%) indicating that the water samples were representative of the site of drowning in at least 90% of freshwater drownings. In the remaining 5 cases (10%), the diatoms in the water samples did not match those in the bone marrow indicating that the site of body and water sample recovery were likely geographically remote from the site of drowning. In cases with matching diatoms in the bone marrow and drowning medium, the diatoms were consistently the smallest and most abundant types represented in the water samples. In addition, there were highly stereotyped physical characteristics of typical "drowning-associated" diatoms with small pennate diatoms representing the most common type of frustule found in the bone marrow. In an additional 34 cases of putative drowning in water that lacked detectable diatoms, 29 cases (85%) lacked diatoms in the bone marrow. Analysis of the diatom content of samples of putative drowning medium by month revealed that winter months had the highest frequency of samples devoid of diatoms. These data indicate that the true positive rate of the diatom test for drowning is at least 90% and that small pennate frustules are most commonly associated with drowning, particular in non winter months.

KEYWORDS: forensic science, diatoms, drowning, forensic pathology

In part one of this series, we have assessed the utility of the diatom test for drowning by analyzing the outcome of the test in 738 cases of freshwater drowning and 33 cases of domestic water drowning (1). We found that the presence of diatom frustules in bone marrow can be used to diagnose drowning in ~30% of freshwater drowning case and that a monthly variation in positive test outcomes correlated with diatom population dynamics. The correspondence of the high incidence of positive test outcomes with peak months of diatom blooms indicates that the water concentration of diatoms is an important factor in determining test outcome. In addition, the size of diatoms were implicated as important variables for a positive test outcome.

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Many investigators have suggested that the presence of diatom in bone marrow can be used as a definitive proof of drowning only if the source of diatoms can be determined to be the drowning medium (2–6). This criterion avoids false positive tests due to laboratory contamination with exogenous diatoms. In addition, we have previously argued that the demonstration of diatoms in tissue extracts must not be interpreted as a true positive test result unless the size, species distribution, and number of frustules conform to certain characteristics (1).

In this study, we compared the features of diatom frustules recovered from the bone marrow and drowning medium in cases of freshwater drowning in which diatoms were recovered from the bone marrow and a sample of the drowning medium was available for analysis. The results indicate that diatoms extracted from the bone marrow very often match those in the putative drowning medium. These "drowning-associated" diatoms form a distinctive subset of diatoms in freshwater and are less likely to be found in Ontario freshwater in the winter.

Materials and Methods

Retrospective Review of Cases

All cases of freshwater drowning, in which a sample of putative drowning medium was available for analysis, processed for the diatom test for drowning at the Office of the Chief Coroner for Ontario between 1977–1993 were used for this retrospective study (n = 254). In all instances, femoral bone marrow and samples of putative drowning medium were used for extraction of diatoms using the nitric acid digestion method as previously described (1). The putative drowning medium was defined as the water the body was immersed in at the time of body recovery and may not, necessarily, have represented the site of drowning. The cases used for the current analyses defined two subsets of the total case population: (i) cases with diatoms in the sample of putative drowning medium (n = 220); and (ii) cases without diatoms in the sample of putative drowning medium (n = 34). In the 220 cases with diatoms in the putative drowning medium, cases with frustules recovered in both the femoral bone marrow (positive test) and putative drowning medium (n = 52) were used to assess the frequency of concordance of diatom type recovered in the marrow and putative drowning medium. The monthly frequency of concordant tests was determined. In the 34 cases without diatoms in the sample of putative drowning medium (n = 34), the frequency of a positive test (presence of frustules in the bone marrow) was determined and correlated with season of drowning.

Comparison of Diatoms in Bone Marrow and Putative Drowning Medium

Cases with diatom frustules recovered in the femoral bone marrow (positive test) and putative drowning medium (n = 52) were also reanalyzed for morphologic characteristics, size, and proportional abundance of each represented type of diatom. Specific reference was made to identifying physical features of the diatom frustules that were commonly observed in the bone marrow extracts.

Analysis of Samples of Putative Drowning Medium

To determine the relationship between the outcome of the diatom test and the presence of diatoms in the putative drowning medium, the presence or absence of diatoms in the samples of putative drowning medium (n = 254) was studied by month of drowning. In addition, the presence of three genera of freshwater diatoms (*Cocconeis*, *Cymbella*, and *Navicula*), often associated with drowning in Ontario, were tabulated in a randomly selected subset of samples of putative drowning media.

Results

Concordance of Diatoms Type Extracted from Bone Marrow and Drowning Medium

In 52 cases of freshwater drowning with bone marrow diatoms, a sample of the drowning medium was available for comparison with the bone marrow extract. Forty-seven (90%) of these cases had diatom types in the bone marrow that were also represented in putative drowning medium (Table 1). In five cases (10%), the type(s) of frustules found in the bone marrow differed from those in the putative drowning medium. In addition, in 34 cases of drowning in water that was devoid of diatoms, 29 cases (85%) lacked demonstrable diatoms in the bone marrow (concordant with putative drowning medium) (Table 1).

The monthly incidence of positive diatom tests with concordance of diatoms in the bone marrow and putative drowning medium (Fig. 1) indicated that concordant test were most frequent in April, July, and August and lowest in February and December (no cases were available for analysis in January). Similarly, there was great seasonal variation in the frequency of positive diatom tests with concordance of diatoms in the bone marrow and putative drowning medium (Fig. 2). Most concordant tests occurred in the summer and the winter had the least proportion of concordant tests.

TABLE 1—*Relationship between presence of diatoms in bone marrow and the presence of diatoms in putative drowning medium.*

		Diatoms in Bone Marrow*	
		Positive	Negative
Diatoms in Putative Drowning Medium†	Positive	47/52 (90%)‡	134/202 (66%)
	Negative	5/34 (15%)	29/34 (85%)

*Numerator.
†Denominator.
‡Concordant diatom types; True positives.

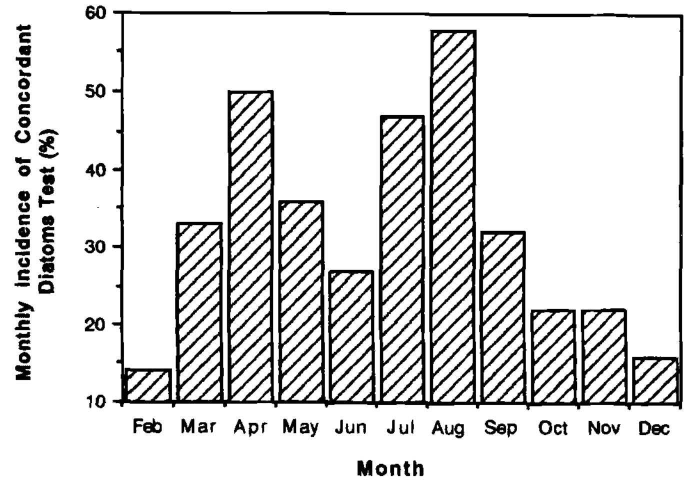

FIG. 1—*Monthly proportions of freshwater drownings cases with bone marrow diatoms that match those in sample of putative drowning medium.*

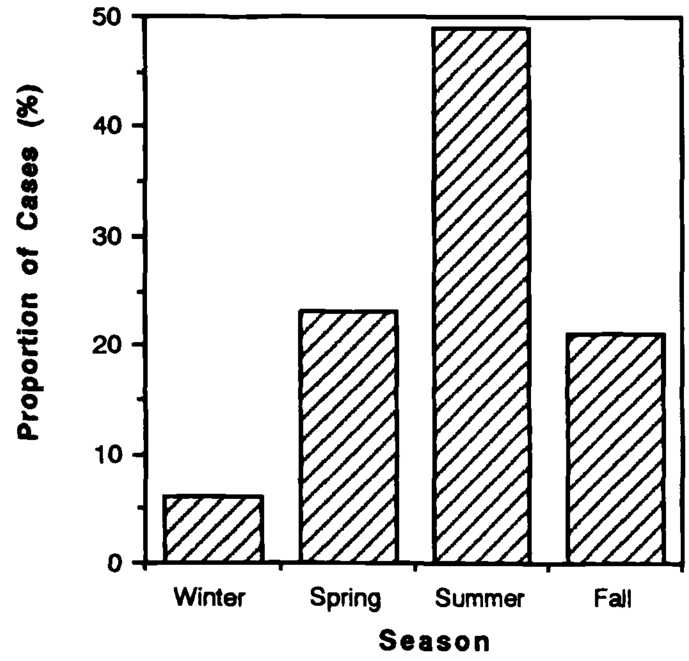

FIG. 2—*Seasonal proportions of freshwater drownings cases with bone marrow diatoms that match those in sample of putative drowning medium.*

Comparison of Frustules in Bone Marrow and Putative Drowning Medium

In most cases, no more than three distinctive species of diatom were found in any given bone marrow extract, frustules were <30 μm in size, and less than ten frustules per femoral extract were observed. Both pennate and radial diatom frustules were routinely encountered in bone marrow extracts, but, pennate-type diatoms predominated in most cases. Pennate diatoms were defined as frustules with their longest and shortest axes of symmetry intersected at 90° in the center of the frustule. These pennate diatoms were usually oval or elliptical in outline and could be characterized using simple geometrical ratio. The ratio of the longest and shortest dimension of these pennate diatoms (aspect ratio) ranged from 1:~2 to 1:~5 and was seldom less than 1:5. In addition to the size of the diatom, this characteristic was the most important common feature of the frustules extracted from bone marrow. These diatoms were typically represented by of the genus, *Cocconeis*, *Navicula*, and *Cymbella* (7).

In most cases, the number of distinctive types of diatoms in the water samples vastly exceeded the number of species in the bone marrow. On the basis of qualitative analysis, the diatom species found in the bone marrow, usually, represented the smallest diatoms in the putative drowning medium. When more than one distinctive type of diatom was present in the bone marrow, the relative abundance of the different types of diatoms in the bone marrow was similar to that in the putative drowning medium. In addition, most of the water samples examined contained diatom types that were greatly in excess of 30 μm in size (e.g., many *Pinnularia* spp.) and these diatoms were never found in the bone marrow extracted. However, on occasion, small (<30 μm) fragments of typically large diatoms were recovered in bone marrow extracts and could be found in whole and fragmented forms in the putative drowning medium. In these circumstances, the frustule fragments were usually near elliptical in shape with the axial ratio of 1:~5. In some instances, small numbers of *Synedra* frustules in excess of 30 μm in maximal dimension could be observed in some bone marrow extracts and could be found in putative drowning medium. Such frustules were always fragmented and had an axial ratio of 1:4 to 1:8.

Diatom Content of Putative Drowning Medium

The diatom content of samples of putative drowning media was determined on a monthly basis, and revealed that December freshwater was largely devoid of diatoms (Fig. 3). In contrast, freshwater samples collected in the summer and autumn months usually contained diatoms. Most samples of freshwater contained diatom genera that were typically associated with drowning (Fig. 4). The monthly rate of concordant positive diatom tests was determined after correcting for negative tests due to drowning in water devoid of diatoms (Fig. 5). This analysis revealed a periodic cycle of positive tests that may be related to monthly differences in the type of diatoms that predominant in freshwater at the time of drowning.

Discussion

This study was designed to determine if the same types of the diatoms are recovered from the bone marrow and putative drowning medium in a series of freshwater drownings. The main findings

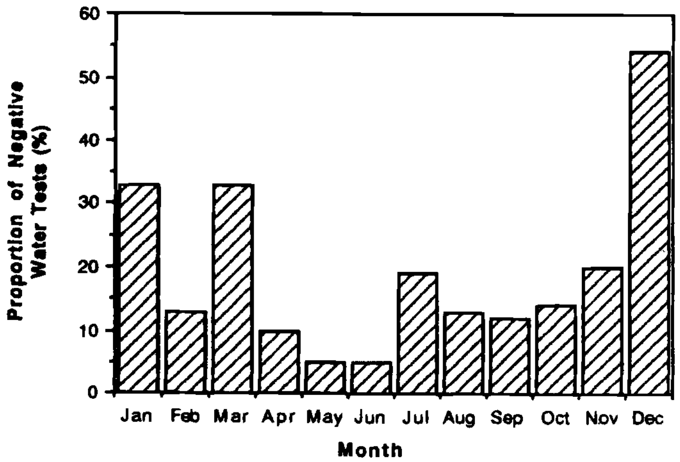

FIG. 3—*Monthly frequency of freshwater samples of putative drowning medium devoid of diatoms.*

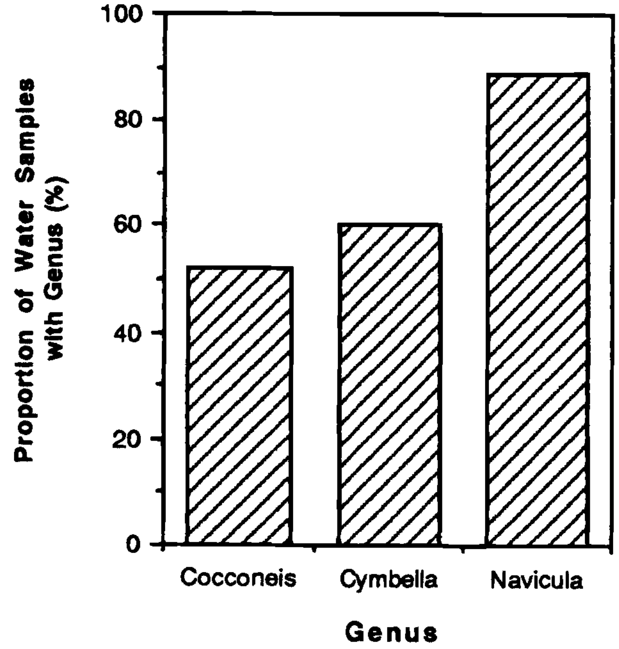

FIG. 4—*Proportion of freshwater samples of putative drowning medium with representative diatom genera often associated with freshwater diatoms.*

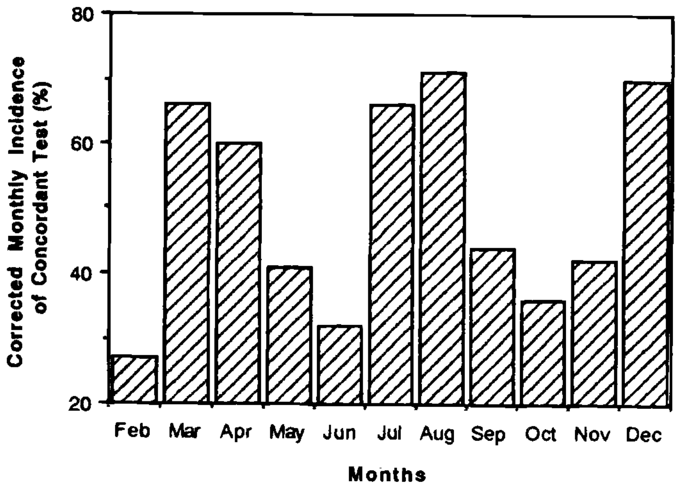

FIG. 5—*The monthly rate of concordant positive diatom tests corrected for negative tests due to drowning in water devoid of diatoms.*

of the study are that: (i) the types of diatom frustules in the femoral bone marrow often match those in the putative drowning medium; (ii) a highly stereotyped subset of freshwater diatoms are found in bone marrow; and (iii) monthly variation in the frequency of a positive diatom test is influenced by the monthly differences in the diatom content of freshwater.

The frequent concordance of diatom types in the putative drowning medium and bone marrow is important corroborating evidence for the validity of the diatoms test. However, there was a minor proportion of cases studied with a discordance in the type of diatoms recovered in the bone marrow and putative drowning medium. This may be explained by the regional and temporal variation in diatom distribution in freshwater because the site of body recovery may differ from the site of drowning. This is particularly applicable to decomposed bodies found in water that are often recovered from locations that are remote from the site of drowning. In these cases, the discordance of diatom type may be explained by regional variations in diatom species distribution and multiple water samples from a variety of local areas may facilitate the identification of concordant diatoms. If this sampling is comprehensive, it may be possible to specify the locality of drowning on the basis of the concordance of diatoms in the water samples and bone marrow. The low discordance rate of positive test also suggests that laboratory based contamination of bone marrow extracts with diatoms from other sources (i.e., false positives) is not a major problem when the test is administered in a controlled environment. This is further substantiated by the low frequency of positive test outcomes when samples of the putative drowning were devoid of diatoms.

In part one, we demonstrated a monthly variation in the frequency of positive test outcomes that was related to diatom blooms in freshwater (1). Analysis of the current data indicates that several additional variables are important in determining the positive outcome of the diatom test. A significant determinant was the absence of diatoms in approximately 50% of all freshwater samples from the winter months. On this basis, the low incidence of positive test outcomes in the winter may, in part, be due to a sharp decline in the diatom population. However, the diatom content of freshwater is not the only important variable that determines the outcome of the test. This is illustrated in Fig. 5, which indicates that the monthly outcome rate for positive diatom test is highly periodic even when the influence of drowning in water devoid of diatoms is subtracted and the outcome rates recalculated. This indicates the monthly variation in the type of diatom present in Ontario freshwater may significantly alter the outcome of the test. Paradoxically, the positive outcome rate for the diatom test in December is high if drowning occurs in diatom-rich water, whereas the rate of positive outcome may be lower in other months. This implies that, although the diatom content of winter freshwater is low, the type of diatom present in the water are likely predominantly "drowning-associated" diatoms (see below).

In a previous study, we have argued that the size of diatom extracted from the femoral bone marrow in cases of drowning was permissible for embolism (1). In the current study, additional characteristics were identified that allowed the identification of typical "drowning-associated" diatoms. These data indicated that not all diatoms are equally likely to be found in the bone marrow of drowning case. In addition to a limitations on size (~30 μm in maximal dimension), the shape and relative abundance of different diatom types in the drowning medium seemed to determine if specific diatoms types would reach the bone marrow. The most important size-independent characteristic of the diatom frustules associated with drowning was the aspect ratio. The typical "drowning-associated" frustule had an aspect ratio of 1:2 to 1:5. Although radial frustules were also observed (aspect ratio, 1:1), these were less frequently observed in the bone marrow and in water samples. This likely reflects the greater abundance of longitudinally symmetrical frustules among diatoms, at least in Ontario freshwater. The longitudinally symmetrical frustules of the genera *Cocconeis*, *Navicula*, and *Cymbella* typified the type of diatom often found in the bone marrow in cases of freshwater drowning. These genera were highly abundant in samples of freshwater indicating that their common association with drowning is due to both physical features and ubiquitous distribution in watery environs. Future studies that assess the monthly variation of the abundance of these genera compared with other diatoms not frequently extracted from bone marrow may further clarify the monthly variation in the outcome of diatom tests.

One intriguing corollary of the highly stereotyped features of the "drowning-associated" diatom frustules is the use of alternative organs for the extraction of diatoms. The size limitation of diatoms recovered from bone marrow indicates that the larger diatoms derived from inhaled freshwater do not reach the bone marrow although it does not exclude the possibility that these larger diatoms may embolize in other organs in the circulation. Cerebral thrombotic and atheromatous microemboli are frequently in excess of 100 μm in size indicating that larger diatom frustules may embolize to the brain (8). This is significant because many diatoms in freshwater are larger than those typically found in the bone marrow. Therefore, these diatoms, in addition to the typical "drowning-associated" diatoms extracted from bone marrow, may be extracted in a greater proportion of cases of freshwater drowning. On this basis, use of the brain as the tissue to extract diatoms may increased the likelihood of a positive outcome of the diatom test.

The characterization of diatom population cycles in the present study are similar to the observations of other investigators (9). Our analysis indicates the presence of diatoms in various bodies on water is not uniform over the seasons (Fig. 3) and that the three common genera studied were well represented over the seasons (Fig. 4). In addition, to seasonal alterations in diatom concentration, Ludes et al. also found that different genera of diatoms predominated at geographical distinct sites (9) which likely relates to intrinsic factors in the local water in those areas. From a forensic viewpoint, these observations underscore the significance of the monthly periodicity of positive test outcomes (Fig. 5) as a validation of the diatom test. In addition, these data also indicate that a survey of the common genera of diatoms involved in drowning may allow the deduction of the time of year that drowning occurred in cases of decomposed bodies.

In summary, in many cases of freshwater drowning, the same diatoms can be found in tissues and in the putative drowning medium. In addition, we have the characterized the features of typical "drowning-associated diatoms" in freshwater and have implicated certain physical characteristics that typify these diatom frustules. The seasonal differences in the diatom content of freshwater is a determinant of test outcome. We conclude that the presence of diatom frustules in extracts of bone marrow may be used as proof of drowning particularly when the frustules match those in the drowning medium.

Acknowledgments

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