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Experimental studies on diagnosis of death from drowning by means of detection of vegetative planktons (diatoms) II. Detection of diatoms from putrefied and cremated bones of drowned bodies

Masami Okuyama*

*Okayama University,

Experimental studies on diagnosis of death from drowning by means of detection of vegetative planktons (diatoms) II. Detection of diatoms from putrefied and cremated bones of drowned bodies*

Masami Okuyama

Abstract

A series of experiments have been conducted with ten adult rabbits, drowning them to death in a ditch those water contains diatoms in abundance. The bones (selected ones are the femur, humerus, riHand vertebra) of these drowned rabbits have been buried underground, wrapped tightly in cellophane bags and left there for three years, and the detection of diatoms has been conducted with these bones either as they are or after cremating them in the electric'oven at 300°C, 500°C, 800°C or 1,000°C, for 20 minutes. As the results it has been clarified that diatoms can be detected in a considerable number in the bones of four limbs, and of these detectable diatoms some of them can be found even after cremation at 1,000°C for 20 minutes. This clearly proves diatoms are detectable from the bones even after a long period of time·after burial and even after cremation at high temperatures.

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EXPERIMENTAL STUDIES ON DIAGNOSIS OF DEATH FROM DROWNING BY MEANS OF DETECTION OF VEGETATIVE PLANKTONS (DIATOMS)

II. DETECTION OF DIATOMS FROM PUTREFIED AND CREMATED BONES OF DROWNED BODIES

Masami OKUYAMA

Department of Legal Medicine, Okayama University Medical School
Okayama (Director: Prof. Y. Mikami)

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As introduced briefly in the first report, for the diagnosis and the determination of the place of death from drowning, the detection of vegetative planktons (diatoms) from the organs and bones now serves the purpose in legal medicine. The theoretical basis for the detection of diatoms has been established by many investigators (1895—1955)[1–9]. In Japan TOMONAGA *et al.* (1954)[10–20], and MIKAMI and his co-workes (1959)[21,22] made some detailed studies on this problem. However, similar studies on the possibility of detecting diatoms from the cremated bones and the bones that had been buried for a considerable period of time have not yet been studied much in details.

In the previous experiments attempts were made to detect diatoms from the incinerated bones of drowned rabbits and also it was possible to detect diatoms even from the bones cremated in the electric oven at high temperature. It was then pointed out that the diagnosis of death from drowning was possible by examining the bones of the corpse (1961)[23]. In the present paper the results of the detection of diatoms are described about a series of experiments conducted with the bones of drowned rabbits, buried for a relatively long period of time and also with the bones cremated or incinerated.

MATERIALS AND METHODS

The site of drowning is the same as in the previous report (1). Ten adult rabbits weighing about 2.5 kg. served as the experimental animals. The water of the ditch at time of drowning rabbits was used for the detection of diatoms, i. e. about 100 ml. water was centrifuged and the sediment placed on object glass was examined under microscope. Thus the kinds and approximate number of diatoms were estimated, and these served as the control.

The drowned rabbits were immediately dissected for extracting the bones (the femur, humerus, rib and vertebra), and these bones of each rabbit were

wrapped tightly in a cellophane bag and buried in the ground about a meter deep. Three years later these bones were unearthened, and the detection of diatoms was conducted. These were divided into five groups of a, b, c, d and e (each group consisted of the bones of two rabbits). The bones in each group were washed with distilled water and the surface of the bones dried prior to the use. The amounts of each bone used were about 11 g. of the femur, about 6 g. of the humerus, about 5 g. of the rib and about 10 g. of the vertebra. In the case of Group "a", the bones were used as they were just as in the previous experiments. Each bone is thoroughly disintegrated with 30 ml. fuming nitric acid in the identically same manner as in the previous experiments. To this 30 per cent hydrogen peroxide (10—20 ml.) is added until the liquid becomes completely colorless. This transparent liquid is transferred to spitz glasses and centrifuged at 3,000 r. p. m. for 20 minutes. The number and kinds of diatoms in the entire field are examined in the same manner as in the previous report.

The bones of Groups "b", "c", "d" and "e", are cremated it the electric oven at 300°C, 500°C, 800°C and 1,000°C, respectively for 20 minutes as in the previous experiments, and these are disintegrated, centrifuged and examined for the detection of diatoms.

RESULTS

The diatoms detected in the water of the ditch where the rabbits were drowned: These proved to be the same 11 kinds as in the previous report. Namely, *Cymbella*, *Navicula*, and *Nitzshcia*, 2 kinds each, *Melosira*, *Cyclotella*, *Rhopalodia*, *Diploneis* and *Tabellaria*, one kind each. Of them the most numerous kinds are *Melosira islandica*, *Navicula placentula*, *Navicula radiosa*, *Cymbella parva* and *Cyclotella comta*.

The rabbit bones (Group "a") that had been buried underground for three years demonstrated several kinds of the diatoms that had been detected from the ditch water at the time of drowning them. Namely, it was possible to detect 4 kinds from the femur and 8 diatoms in the entire field of 5 samples; and this number proved to be greatest. Next, 4 kinds from 5 samples of the humerus to the total of 7 diatoms, followed by 3 kinds and 4 diatoms, from the rib samples, 2 kinds and 3 diatoms from the vertebra samples, the number and kinds of diatoms decreasing in the order mentioned (Table 1).

In the case of the rabbit bones (Group "b"), buried underground for 3 years and cremated in the electric oven at 300°C for 20 minutes, likewise 5 samples each revealed 4 kinds and 7 diatoms in the femur; 3 kinds and 5 diatoms in the humerus; 2 kinds and 2 diatoms in the rib; and 3 kinds and 4 diatoms in the vertebra (Table 2).

In the case of those bones (Group "c"), cremated at 500°C in the electric

Table 1. The number and kinds of diatoms detected from drowned rabbit bones after being buried in the ground for three years (Group "a")

Diatoms \ Organs	Femur	Humerus	Rib	Vertebra
Cymbella naviculiformis	0	0	0	0
Cymbella parva	2	2	1	0
Navicula placentula	0	0	0	0
Navicula radiosa	1	3	1	2
Nitzschia filiformis	0	0	0	0
Nitzschia vermicularis	0	0	0	0
Melosira islandica	2	1	2	0
Cyclotella comta	3	1	0	1
Rhopalodia gibba	0	0	0	0
Diploneis elliptica	0	0	0	0
Tabellaria fenestrata	0	0	0	0

Table 2. The number and kinds of diatoms detected from the drowned rabbit bones after being buried for three years (Group "b")

Diatoms \ Organs	Femur	Humerus	Rib	Vertebra
Cymbella naviculiformis	0	0	0	0
Cymbella parva	2	1	0	0
Navicula placentula	0	0	0	0
Navicula radiosa	3	2	1	1
Nitzschia filiformis	0	0	0	0
Nitzschia vermicularis	0	0	0	0
Melosira islandica	1	2	1	2
Cyclotella comta	1	0	0	1
Rhopalodia gibba	0	0	0	0
Diploneis elliptica	0	0	0	0
Tabellaria fenestrata	0	0	0	0

Table 3. The number and kinds of diatoms detected in the bones of Group "c"

Diatoms \ Organs	Femur	Humerus	Rib	Vertebra
Cymbella naviculiformis	0	0	0	0
Cymbella parva	0	1	0	1
Navicula placentula	0	0	0	0
Navicula radiosa	2	1	0	2
Nitzschia filiformis	0	0	0	0
Nizschia vermicularis	0	0	0	0
Melosira islandica	3	2	3	1
Cyclotella comta	1	0	1	0
Rhopalodia gibba	0	0	0	0
Diploneis elliptica	0	0	0	0
Tabellaria fenestrata	0	0	0	0

oven for 20 minutes, 5 samples each, revealed 3 kinds and 6 diatoms in the femur, 3 kinds and 4 diatoms in the humerus, 2 kinds and 4 diatoms in the rib, and 3 kinds and 4 diatoms in the vertedra. (Table 3).

In the case of the bones (Group "d") cremated in the electric oven at 800°C for 20 minutes, 3 kinds, 6 diatoms in the femur samples; 3 kinds, 5 diatoms in the humerus; 3 kinds, 3 diatoms in the rib; and 2 kinds, 2 diatoms in the vertebra (Table 4).

Table 4. The number and kinds of diatoms detected in the bones of Group "d"

Organs / Diatoms	Femur	Humerus	Rib	Vertebra
Cymbella naviculiformis	0	0	0	0
Cymbella parva	0	0	1	0
Navicula placentula	0	0	0	0
Navicula radiosa	2	2	1	1
Nitzschia filiformis	0	0	0	0
Nitzschia vermicularis	0	0	0	0
Melosira islandica	2	1	1	1
Cyclotella comta	2	2	0	0
Rhopalodia gibba	0	0	0	0
Diploneis elliptica	0	0	0	0
Tabellaria fenestrata	0	0	0	0

In the case of those bones (Group "e") cremated in the electric oven at 1, 000°C for 20 minutes, 4 kinds and 5 diatoms in the femur samples; 2 kinds and 4 diatoms in the humerus; 2 kinds and 3 diatoms in the rib; and 2 kinds and 3 diatoms in the vertebra (Table 5).

Table 5. The number and kinds of diatoms detected in the bones of Group "e"

Organs / Diatoms	Femur	Humerus	Rib	Vertebra
Cymbella naviculiformis	0	0	0	0
Cymbella parva	1	2	0	0
Navicula placentula	0	0	0	0
Navicula radiosa	2	0	2	0
Nitzschia filiformis	0	0	0	0
Nitzschia vermicularis	0	0	0	0
Melosira islandica	1	2	0	2
Cyclotella comta	1	0	1	1
Rhopalodia gibba	0	0	0	0
Diploneis elliptica	0	0	0	0
Tabellaria fenestrata	0	0	0	0

In everyone of these cases the greater number of diatoms can be detected in the femur followed by that in the humerus. In addition, as the temperature

of the cremation is higher, there is a tendency to show a greater number of broken diatoms.

COMMENTS

In the case of death from drowning it is well established that diatoms of the river water can be detected in the organs of the drowned corpse. However, Mikami and others have contributed considerably for the legal medicine in the diagnosis of the death from drowning, especially where the corpse or remains had been buried in the ground.

In the present experinents it has been possible to detect such diatoms as *Navicula placentula*, *Melosira islandica*, *Navicula radiosa*, and *Cyclotella comta*, which are numerically greater among such diatoms as *Cymbella naviculiformis*, *Cymbella parva*, *Navicula placentula*, *Navicula radiosa*, *Nitzschia filiformis*, *Nitzschia vermicularis*, *Melosira islandica*, *Cyclotella comta*, *Rhopalodia gibba*, *Diploneis elliptica* and *Tabellaria fenestrata*, the same found in the ditch water where the rabbits had been drowned 3 years previously.

It is assumed that these diatoms have been detected because they are more numerous and also because they are the kinds that are more resistant to putrefaction. In addition, those six kinds of diatoms, such as *Cymbella parva*, *Cymbella naviculiformis*, *Navicula placentula*, *Nitzschia filiformis*, *Nitzschia vermicularis*, *Rhopalodia gibba*, *Diploneis elliptica* and *Tabellaria fenestrata* that could not be detected, seem to be the kinds of diatoms that are not resistant to putrefaction. These data agree with those of MIKAMI, *et al.* of our department.

These diatoms buried underground for three years did endure and could be detected even after the cremation in the elctric oven at 300°C, 500°C, 800°C and 1,000°C for 20 minutes, although the number of detectable diatoms is less than in the case of the buried but not-cremated bones (Group a). The decrease in the number of detectable diatoms after cremation seems to be due to the destruction of diatoms bv high temperature. Nonetheless, it is noteworthy that some diatoms do remain intact even after the cremation at 1,000C° for 20 minutes.

The fact that diatoms can be detected more numerously in the femur and humerus indicates that long bones of the four limbs should be selected for the detection of diatoms for such purpose in the legal medicine. These results also suggest that the detection of diatoms is possible for determining the actual cause of death from drowning so long as there remain some bones, even if a considerably long time has elapsed after the burial and even the bones have been exposed to a high temperature. This fact seems to have a great significance in the legal

medicine as well as in criminology for obtaining an evidence to prove death from drowning.

CONCLUSION

A series of experiments have been conducted with ten adult rabbits, drowning them to death in a ditch those water contains diatoms in abundance. The bones (selected ones are the femur, humerus, rib and vertebra) of these drowned rabbits have been buried underground, wrapped tightly in cellophane bags and left there for three years, and the detection of diatoms has been conducted with these bones either as they are or after cremating them in the electric oven at 300°C, 500°C, 800°C or 1,000°C, for 20 minutes.

As the results it has been clarified that diatoms can be detected in a considerable number in the bones of four limbs, and of these detectable diatoms some of them can be found even after cremation at 1,000°C for 20 minutes. This clearly proves diatoms are detectable from the bones even after a long period of time after burial and even after cremation at high temperatures.

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