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

Masami Okuyama\*

\*Okayama University,

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

Masami Okuyama

## Abstract

With an intention to establish definitely that detection of diatoms, vegetative planktons, in the remains of a corpse will offer an unequivocal proof of death from drowning, even in the case where the suspected corpse has been cremated, the author observed organs and bones of 16 healthy adult rabbits which were drowned in a ditch, and burned as a whole with wood fire or incinerated in the electric oven. As the results it was found that diatoms can be detected in the internal organs so long as they remain. Even in the case where the corpse was cremated at high temperature, it is also possible to detect several kinds of diatoms in bones, the femur showing the greatest number followed by the humerus suggesting that long bones are more suitable for the detection of diatoms than any other bones, as they contain more numbers and kinds of diatoms. Thus it is concluded that detection of diatoms in the remains of the corpse suspected of drowning can definitely give an unequivocal evidence that the death is from drowning.

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

**I. DETECTION OF DIATOMS FROM THE BONES OF  
DROWNED AND CREMATED BODIES**

Masami OKUYAMA

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

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The detection of vegetative planktons (diatoms) in the organs and bones is now a common practice in legal medicine for determining the cause of death as drowning. This stands on the basis of the reports of HOFFMANN (1895)<sup>1</sup>, REVENSTORF (1904)<sup>2</sup>, and BUHTZ and BURKHARDT (1938)<sup>3</sup>. The data have been confirmed by the works of KASPAREK (1937)<sup>4</sup>, MUEHNER (1949)<sup>5</sup>, INCZE et al. (1955)<sup>6</sup>, OGAWA (1941)<sup>7,8,9</sup>, and TOMONAGA and his co-workers (1954)<sup>10-20</sup>. Especially TOMONAGA and others carried out exhaustive investigations on the distribution of vegetative planktons (diatoms) in various organs of the drowned person, proving its great significance in the diagnosis of death from drowning.

MIKAMI and associates performed an autopsy of the child's body some two and a half years since her death, the cause of which had been suspected to be drowning, and they successfully detected diatoms in the bone marrow of the corpse. Later MIKAMI and co-workers (1959)<sup>21,22</sup> proved this fact, showing the importance of experimental studies concerning the detection of diatoms. But unfortunately no experimental studies on the putrefied bodies have been done excepting the report of MIKAMI and his colleagues. Supposing the cases where the corpse is unfortunately cremated after drowning the author conducted a series of experiments with rabbits, and succeeded in verifying that the detection of diatoms is possible even from the remains of cremated bodies with these animals. The results of such experiments are presented in this paper.

MATERIALS AND METHODS

Healthy adult 16 rabbits weighing around 2.5 kg. served as experimental animals. These animals were placed in 4 cages completely covered with metal net 4 animals in each cage, and were drowned in water of a small irrigation ditch nearby our Medical School Campus, which contains vegetative planktons in abundance. After confirming the death of animals by white, minute foamy

liquid oozing out of nostrils and mouth of animals, they were kept there for about 10 minutes longer. These drowned animals were brought to a sunny place near our laboratory until the body surface dried. They were divided into two groups of A and B, eight in each. These two groups were subdivided into 4 small groups of "a", "b", "c" and "d", 2 animals in each small group. The animals belonging to group A were cremated on the metal net stands constructed out-doors with wood fire kept around 300°C for various periods; for 10 minutes in group "a", 30 minutes in group "b", 60 minutes in group "c" and 2 hours in group "d".

The animal bodies of group "a" were burnt black but the general appearance were kept well excepting a slight exposure of intestine from the ruptured abdomen. In group "b" epiphysis of fore and hind legs fell off and a part of the femur or humerus was exposed. In group "c" femurs and humeri were burnt black and partially exposed, some of epiphysis fell off and intestine was almost completely consumed. In group "d" animal bodies were burnt down almost to the bones with only a little muscle remaining on the vertebra. After the treatment the internal organs (lungs, heart, liver and kidneys) and bones (the femur, humerus, rib and vertebra) were taken. These were washed thoroughly with distilled water and used for further treatment described later.

In the animals of group B femurs, humeri, ribs and vertebrae were taken out without precremation. Bones were washed with distilled water and incinerated in an electric oven, at 300°C in Group "a", 500°C in Group "b", 800°C in "c", and 1,000°C in "d", all for 20 minutes.

Each of organs from the animals belonging to A is thoroughly washed with distilled water, about 10 gm. of each organ in wet was placed in Kjeldahl flask and boiled with 20 ml. of fuming nitric acid until brownish nitric acid fume turns white, and then 10 ml. of conc. sulfuric acid is added and boiled for about 14 to 15 minutes. At termination of boiling 30 per cent hydrogen peroxide solution is added until the liquid turns completely colorless.

Each of the bones from both Groups of A and B is boiled in 30 ml. fuming nitric acid until the bone is completely disintegrated and then 30 per cent hydrogen peroxide solution is added until the liquid loses its color completely in the same manner as above.

Besides these, the water of the ditch, where the rabbits were drowned, was taken for detection of diatoms, 100 cc of this water was treated with 20 cc of fuming nitric acid and 10 cc of conc. sulfuric acid in the same manner as disintegration of organs.

The colorless liquids thus obtained after the disintegration processes above mentioned with internal organs, bones and ditch water are then centrifuged for 20 minutes at 3,000 r. p. m. A droplet of the sediment is placed on the object glass, mounted with cover slide and is examined under microscope for the detec-

tion of vegetative planktons (diatoms).

### RESULTS

The ditch water contained 11 species of diatoms: i. e. *Cymbella*, *Navicula*, and *Nitzschia* 2 kinds each, *Melosira*, *Cyclotella*, *Rhopalodia*, *Diploneis* and *Tabellaria*, one kind each. Of them those that were detected most numerous are *Melosira islandica*, *Navicula placentula*, *Navicula radiosa*, *Cymbella parva* and *Cyclotella comta*.

In the case of the drowned rabbits burned over the wood fire at about 300°C for 10 minutes (Group A-“a”). 15 gm. of lungs, 30 gm. of liver, and entire kidneys and heart; femurs, humeri, ribs and 20 gm. of vertebrae were used for disintegration, and many vegetative planktons (diatoms) of the same kinds as detected in the ditch water were found in each sample, as shown in Table 1. In the internal organs, the lung contained most numerous diatoms, namely, 10 kinds and 40 diatoms in one drop; 8 kinds and 13 diatoms in the liver, 7 kinds and 12 diatoms in the kidney but only 3 kinds of them and 4 diatoms in the heart. As for the bones, the femur revealed 7 kinds and 11 diatoms, the humerus 5 kinds and 8 diatoms and the vertebra 5 kinds and 7 diatoms, and the rib 4 kinds and 4 diatoms, proving that both species and numbers of diatoms detected in the femur are greatest (Table 1).

Table 1. Group “a” incinerated for 10 min. at about 300°C

Diatoms	Organs								
	Lung	Heart	Liver	Kidney	Femur	Humerus	Rib	Vertebra	
<i>Cymbella naviculiformis</i>	4	0	1	1	1	2	1	1	
<i>Cymbella parva</i>	6	1	1	2	3	0	1	0	
<i>Navicula placentula</i>	5	2	2	1	2	1	0	2	
<i>Navicula radiosa</i>	7	1	2	0	1	2	1	1	
<i>Nitzschia filiformis</i>	2	0	0	1	1	0	0	0	
<i>Nitzschia vermicularis</i>	0	0	1	1	0	0	0	0	
<i>Melosira islandica</i>	8	0	3	4	2	2	0	2	
<i>Cyclotella comta</i>	5	0	2	2	0	1	1	0	
<i>Rhopalodia gibba</i>	1	0	0	0	0	0	0	0	
<i>Diploneis elliptica</i>	1	0	1	0	1	0	0	0	
<i>Tabellaria fenestrata</i>	1	0	0	0	0	0	0	1	

In the Group A-“b” the amounts of organs and bones used for disintegration were the same as in Group A-“a”, and the results were almost the same as in A-“a”, i. e. the lung revealed 9 kinds and 36 diatoms, followed by 8 kinds and 15 diatoms in the liver, 7 kinds and 14 diatoms in the kidney, but only 2 kinds and 3 diatoms in the heart, as illustrated in Table 2. In the bones, the femur likewise

contained the greatest numbers of 6 kinds and 14 diatoms, followed by 5 kinds and 10 diatoms in the humerus, and 4 kinds and 6 diatoms in the rib and the vertebra (Table 2).

Table 2. Group "b" incinerated for 30 min. at about 300°C

Diatoms \ Organs	Lung	Heart	Liver	Kidney	Femur	Humerus	Rib	Vertebra
<i>Cymbella naviculiformis</i>	4	2	2	1	2	1	0	1
<i>Cymbella parva</i>	5	1	1	2	2	1	2	0
<i>Navicula placentula</i>	5	0	3	2	5	0	2	0
<i>Navicula radiosa</i>	4	0	2	3	3	4	1	2
<i>Nitzschia filiformis</i>	1	0	1	0	1	1	1	0
<i>Nitzschia vermicularis</i>	0	0	0	0	0	0	0	0
<i>Melosira islandica</i>	9	0	4	3	0	0	0	2
<i>Cyclotella comta</i>	6	0	1	2	0	3	0	1
<i>Rhopalodia gibba</i>	0	0	0	0	0	0	0	0
<i>Diploneis elliptica</i>	1	0	1	1	1	0	0	0
<i>Tabellaria fenestrata</i>	1	0	0	0	0	0	0	0

In Group A-"c" the amounts of organs and bones used for integration were the same as in Group A-"a" and A-"b", but something reduced detection rate of diatoms. The lung contained 8 kinds and 32 diatoms, followed by 6 kinds and 11 diatoms in the liver, 3 kinds and 6 diatoms in the kidney, and 2 kinds and 2 diatoms in the heart. As for the bones, the femur revealed 7 kinds and 12 diatoms, the humerus 5 kinds and 7 diatoms, the rib 4 kinds and 4 diatoms, and the vertebra 3 kinds and 4 diatoms as demonstrated in Table 3.

Table 3. Group "c" incinerated for 1 hour at about 300°C

Diatoms \ Organs	Lung	Heart	Liver	Kidney	Femur	Humerus	Rib	vertebra
<i>Cymbella naviculiformis</i>	2	0	1	0	1	2	0	0
<i>Cymbella parva</i>	5	1	2	2	2	1	1	2
<i>Navicula placentula</i>	7	0	2	1	2	0	1	0
<i>Navicula radiosa</i>	5	1	2	0	1	2	1	1
<i>Nitzschia filiformis</i>	0	0	1	0	0	1	0	0
<i>Nitzschia vermicularis</i>	0	0	0	0	1	0	0	0
<i>Melosira islandica</i>	7	0	0	3	3	0	1	1
<i>Cyclotella comta</i>	4	0	3	0	2	6	0	0
<i>Rhopalodia gibba</i>	0	0	0	0	0	0	0	0
<i>Diploneis elliptica</i>	1	0	0	0	0	1	0	0
<i>Tabellaria fenestrata</i>	1	0	0	0	0	0	0	0

In Group A-d the animal bodies were burnt almost to the bone, and no internal organs could be collected. 12 gm. of the the femur 8 gm. of the humerus,

19 gm. of the the vertebra and 6 gm. of the rib were disintegrated and observed. Observations revealed 8 kinds and 14 diatoms in the femur, 5 kinds and 6 diatoms in the humerus, 5 kinds and 5 diatoms in the rib, and 4 kinds and 7 diatoms in the vertebra. In this experiment the sample showed suspension of numerous black carbon particles but not detection of diatoms was not so difficult.

Table 4. Group "d" incinerated for 2 hours at about 300°C

Diatoms \ Organs	Femur	Humerus	Rib	Vertebra
<i>Cymbella naviculiformis</i>	2	1	0	1
<i>Cymbella parva</i>	1	0	1	2
<i>Navicula placentula</i>	3	2	0	0
<i>Navicula radiosa</i>	1	1	1	2
<i>Nitzschia filiformis</i>	1	1	1	0
<i>Nitzschia vermicularis</i>	0	0	0	0
<i>Melosira islandica</i>	4	1	1	2
<i>Cyclotella comta</i>	1	0	1	0
<i>Rhopalodia gibba</i>	0	0	0	0
<i>Diploneis elliptica</i>	1	0	0	0
<i>Tabellaria fenestrata</i>	0	0	0	0

In the animals belonging to Group B entire femurs, humeri and ribs, and 20 gm. of vertebrae were used as the materials. The bones in Group B-a which were cremated in an electric oven at about 300°C for 20 minutes, the bones are burnt almost carbon black but still there remains some fat (Plate 1). The numbers and kinds of diatoms detected are greatest in the femur, namely, 8 kinds and 17 diatoms, followed by 6 kinds and 10 diatoms in the humerus, and 5 kinds and 6 diatoms in the rib and vertebra 5 kinds and 5 diatoms, as shown in Table 5.

Table 5. Group "a" cremated for 20 min. at 300°C in an electric oven

Diatoms \ Organs	Femur	Humerus	Rib	Vertebra
<i>Cymbella naviculiformis</i>	2	1	1	0
<i>Cymbella parva</i>	2	1	2	1
<i>Navicula placentula</i>	5	4	0	1
<i>Navicula radiosa</i>	2	1	1	1
<i>Nitzschia filiformis</i>	1	0	0	1
<i>Nitzschia vermicularis</i>	1	1	0	0
<i>Melosira islandica</i>	2	2	1	1
<i>Cyclotella comta</i>	2	0	1	0
<i>Rhopalodia gibba</i>	0	0	0	0
<i>Diploneis elliptica</i>	0	0	0	0
<i>Tabellaria fenestrata</i>	0	0	0	0

In the cases incinerated at 500°C for 20 minutes (B-“b”). All the bones are completely carbonized but the shape remains intact (Plate 1<sub>2</sub>). The femur contains 6 kinds and 13 diatoms, while the humerus 5 kinds, 5 diatoms, the vertebra 4 kinds, 6 diatoms and the rib 3 kinds, 3 diatoms, as illustrated in Table 6.

Table 6. Group “b” cremated for 20 min. at 500°C in an electric oven

Diatoms \ Organs	Femur	Humerus	Rib	Vertebra
<i>Cymbella naviculiformis</i>	2	1	0	1
<i>Cymbella parva</i>	1	0	1	2
<i>Navicula placentula</i>	3	2	0	1
<i>Navicula radiosa</i>	1	1	1	0
<i>Nitzschia filiformis</i>	0	0	0	0
<i>Nitzschia vermicularis</i>	0	1	0	0
<i>Melosira islandica</i>	5	1	1	2
<i>Cyclotella comta</i>	1	0	0	0
<i>Rhopalodia gibba</i>	0	0	0	0
<i>Diploneis elliptica</i>	0	0	0	0
<i>Tabellaria fenestrata</i>	0	0	0	0

In the Group B-“c” whose bones were incinerated at 800°C for 20 minutes the bones are carbonized completely and are broken (Plate 1<sub>3</sub>). Observation revealed 6 kinds and 11 diatoms in the femur, 6 kinds and 9 diatoms in the humerus as shown in Table 7. The vertebra reveals 3 kinds and 4 diatoms while the rib 2 kinds, 4 diatoms (Table 7).

Table 7. Group “c” cremated for 20 min. at 800°C in an electric oven

Diatoms \ Organs	Femur	Humerus	Rib	Vertebra
<i>Cymbella naviculiformis</i>	1	1	2	1
<i>Cymbella parva</i>	2	3	0	0
<i>Navicula placentula</i>	2	0	2	2
<i>Navicula radiosa</i>	1	2	0	0
<i>Nitzschia filiformis</i>	0	1	0	0
<i>Nitzschia vermicularis</i>	0	0	0	0
<i>Melosira islandica</i>	4	1	0	0
<i>Cyclotella comta</i>	0	1	0	1
<i>Rhopalodia gibba</i>	0	0	0	0
<i>Diploneis elliptica</i>	1	0	0	0
<i>Tabellaria fenestrata</i>	0	0	0	0

Legends for photos:

Plate 1. Bones of the drowned rabbits, cremated for 20 minutes at 300°C (1), 500°C (2), 800°C (3) and 1,000°C (4).



Plate I.

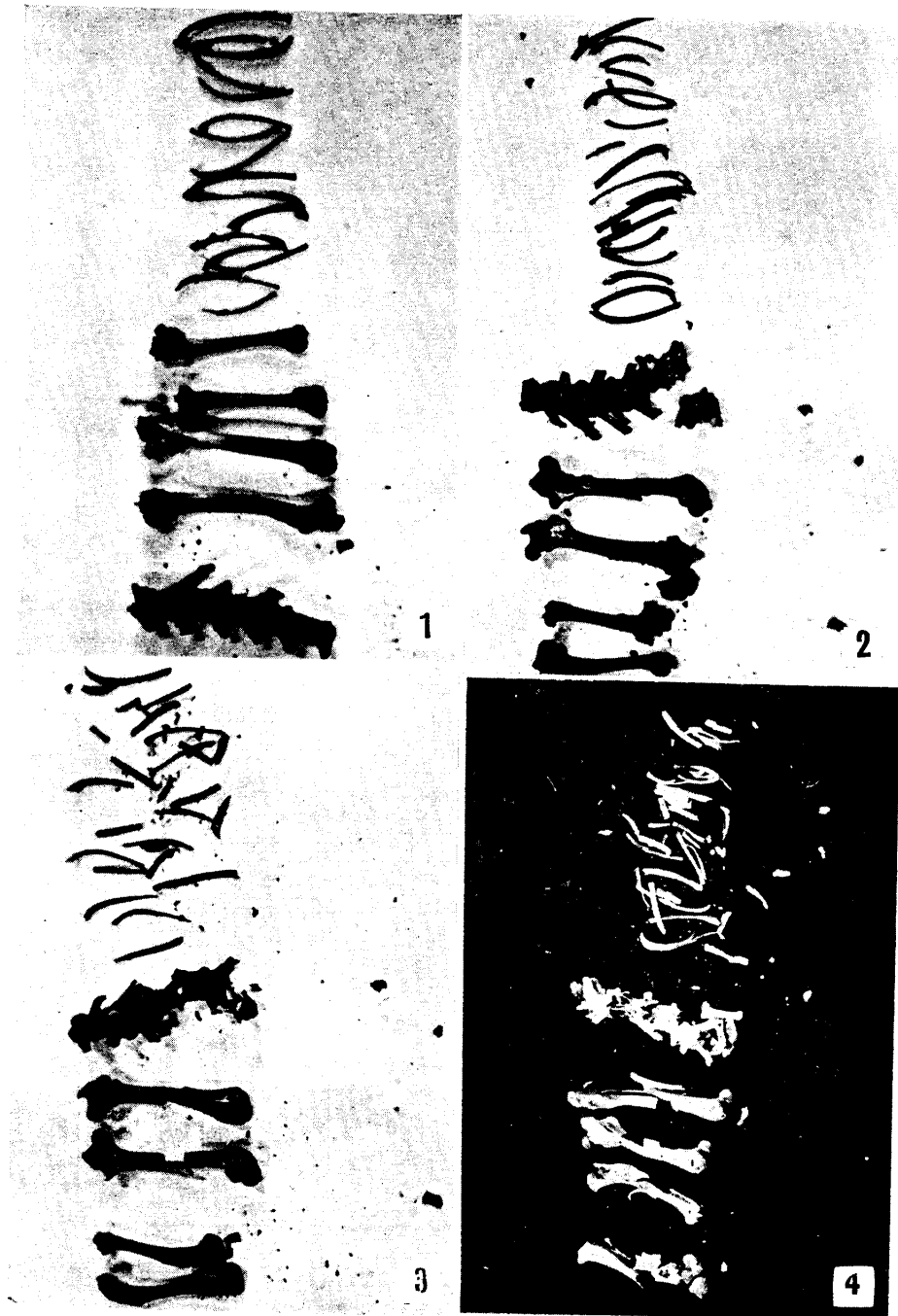
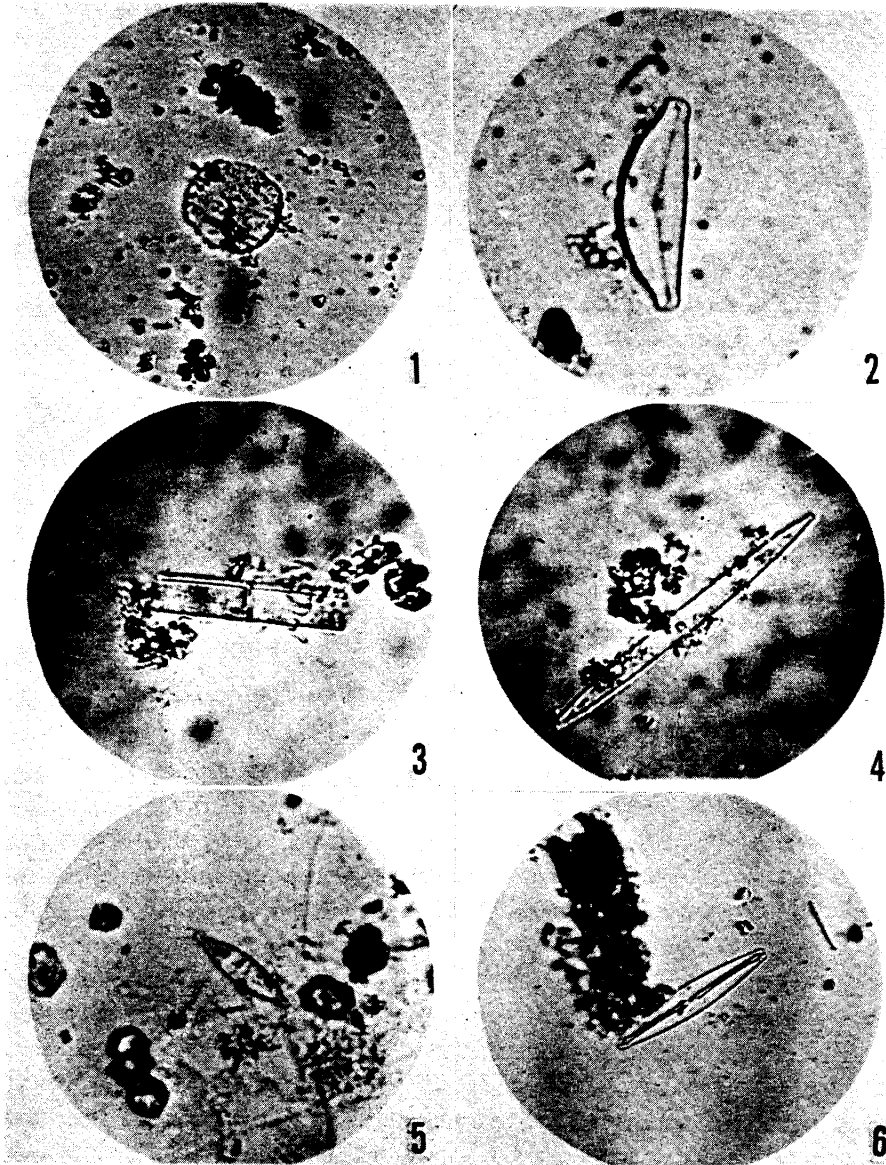


Plate 2



Legends for photos :

Plate 2. Shells of diatoms detected in the bones cremated at 1,000°C for 20 minutes (Plate 14). Pictures show those of *Cyclotella comta* (1), *Cymbella parva* (2), *Melosira islandica* (3), *Nitzschia filiformis* (4), *Navicula placentula* (5), *Navicula radiosa* (6).

In the bones incinerated at 1,000°C for 20 minutes, the bones turn completely white and almost all of them can easily be broken (Plate 1<sub>4</sub>). However, a greater amount of fuming nitric acid and a longer time are required for the disintegration of these bones. In this case, too, the femur contained the greatest numbers and kinds of diatoms, namely, 6 kinds, 9 diatoms, followed by 4 kinds and 5 diatoms each in the humerus, and vertebra, and 3 kinds, 3 diatoms in the rib. However, the numbers of diatoms detectable have somewhat decreased as compared with those in the foregoing groups, and in addition, there are more broken pieces of diatoms, as illustrated in Table 8.

Table 8. Group "d" cremated for 20 min. at 1,000°C in an electric oven

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

#### COMMENTS

Diatoms usually found in the water of the irrigation ditch nearby our Medical School, originating from the Asahi River, are 11 kinds consisting of *Cymbella naviculiformis*, *Cymbella parva*, *Navicula placentula*, *Navicula radiosa*, *Nitzschia filiformis*, *Nitzschia vermicularis*, *Melosira islandica*, *Cyclotella comta*, *Rhopalodia gibba*, *Diploneis elliptica* and *Tabellaria fenestrata*. Of them those that are found most numerous are *Navicula placentula*, *Melosira islandica*, *Navicula radiosa*, *Cymbella parva* and *Cyclotella comta*. Several kinds out of these eleven diatoms have been detected in the internal organs (lungs, heart, liver and kidneys) of the rabbits drowned in the ditch and burned with wood fire. And numbers and kinds of diatoms detected in the organs of these cremated rabbits are found greatest in the lung, followed by those in the liver and kidney but only a few in the heart. All these findings show a good coincidence with the data reported on the drowned rabbits but observed without burning. The number of diatoms is greatest in femur followed by that in humerus and least but about in an equal quantity in rib and vertebra,

in both those incinerated over the wood fire until the animals were burnt to the bones and those cremated bones in the electric oven.

As to the destruction of the shape of diatoms due to heat, the diatoms maintain a fairly good shape in those incinerated at 300°C over the wood fire but in the cases where the bones were cremated in an electric oven at high temperature (800—1,000°C) a greater number of diatoms are broken in proportion to the rise in temperature. It has been found that *Nitzschia vermicularis* and *Tabellaria fenestrata* are especially weak to heat whereas *Melosira islandica*, *Navicula placentula* and *Navicula radiosa* appear to be strong to heat.

These findings indicate that it is possible to determine the death from drowning so long as there are some remains of bones even the corpse was cremated at a high temperature, by virtue of detecting diatoms from the remaining bones.

#### CONCLUSION

With an intention to establish definitely that detection of diatoms, vegetative planktons, in the remains of a corpse will offer an unequivocal proof of death from drowning, even in the case where the suspected corpse has been cremated, the author observed organs and bones of 16 healthy adult rabbits which were drowned in a ditch, and burned as a whole with wood fire or incinerated in the electric oven.

As the results it was found that diatoms can be detected in the internal organs so long as they remain. Even in the case where the corpse was cremated at high temperature, it is also possible to detect several kinds of diatoms in bones, the femur showing the greatest number followed by the humerus suggesting that long bones are more suitable for the detection of diatoms than any other bones, as they contain more numbers and kinds of diatoms. Thus it is concluded that detection of diatoms in the remains of the corpse suspected of drowning can definitely give an unequivocal evidence that the death is from drowning.

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