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ORIGINAL ARTICLE

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Scanning and transmission electron microscopical evidence of the capacity of diatoms to penetrate the alveolo-capillary barrier in drowning[1]

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Abstract The diagnostic value of diatom analysis for drowning is considered to be one of the most controversial arguments in forensic medicine. However, the theoretical assumption of the method, i.e. the capacity of diatoms to penetrate the alveolo-capillary barrier during drowning, has never been addressed. Using scanning (SEM) and transmission electron microscopy (TEM), we have investigated the interaction of a natural population of diatoms and an unialgal culture of *Phaeodactylum tricornutum* (PT) with the alveolo-capillary barrier in an experimental model of drowning. The SEM analysis allowed the identification of several diatom species along the whole airways and their close interaction with the alveolar wall, but was poorly informative about the effective penetration of diatoms into pulmonary vessels. The TEM analysis was more informative and allowed a precise identification of the PT cells in alveolar spaces and to detect their phagocytosis by alveolar macrophages. PT penetrated into the pulmonary vessels through the thinnest portions of the alveolo-capillary barrier and through the interstitial spaces and were identified in pulmonary capillaries and venules. The morphological demonstration of the capacity of diatoms to penetrate the alveolo-capillary barrier is a step forward in assessing the potentiality, reliability and limitations of diatom analysis on a new basis as a tool for the diagnosis of drowning.

Key words Drowning · Diatoms · Lung · Alveolar macrophages · Scanning electron microscopy · Transmission electron microscopy

Introduction

The utility of diatoms for the diagnosis of drowning was debated soon after they were first found in lung exudates of drowned people towards the end of the 19th century [1]. In 1910 Ascarelli [2] and Stockis [3] emphasized the limited value of diatoms because of the postmortem penetration of the "drowning" medium into the respiratory system and because of the presumed incapacity of diatoms to penetrate the pulmonary vessels. These opinions were supported during the first decades of this century by the difficulties to detect diatoms in blood [4], although in a largely unknown work Bruna demonstrated as early as 1911 the presence of diatoms in heart blood after experimental drowning [5].

The modern concepts of the diatom method for the diagnosis of drowning, based upon the observation of diatoms in blood and peripheral tissues in the 1940s [6–8], were soon also challenged, by the detection of diatoms in peripheral organs of non-drowned [7] and by the absence of diatoms in the organism of drowned individuals [9–11]. The development of the diatom method, especially in Europe and Japan, influenced by the recovery of diatoms in bone marrow [8, 12–14], led to an extensive re-examination during the 1960s and the 1970s and at times severe critique of the method [15–27].

At the present few forensic pathologists use the diatom test. Many have abandoned it on the basis of their opinions or research, of the literature and/or of the evaluation of costs. Authors in favour of the method have developed more sophisticated methods for the detection and analysis of diatoms in tissues [28–35].

More than 250 articles published in the forensic literature during this century on diatoms and drowning have

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not given any morphological answer to the theoretical assumption of the method, i.e. the capacity of diatoms to penetrate the alveolar wall into the pulmonary vessels during the drowning process. In this study we have investigated the interaction of different diatom species with the alveolo-capillary barrier using scanning (SEM) and transmission electron microscopy (TEM).

Material and methods*

Drowning media

Two different drowning media have been used. The first (a) was a dense clonal culture of the fast-growing biradiate *Phaeodactylum tricornutum* (PT) (3–8 × 8–15 μm) imported from Italy (Joint Research Centre of the European Union of Ispra – Courtesy Dr. G. Premazzi) to Finland in a F/2 medium (salinity = 25 ppt) and transferred in a Tv2 medium (salinity = 20 ppt) [36] at Tvärminne Zoological Station, University of Helsinki. The second (b) was an epiphytic population of diatoms collected in the Tvärminne archipelago, Gulf of Finland, during July 1994, which was stored at –20° C, acid-cleaned and washed with deionized water 24 h before the experiments, to eliminate organic matter. This epiphytic population consisted of at least 41 species listed in Table 1. The diatom concentration was – at the time of the experiments – 357954 ± 23153 cells/ml in the drowning media (a) and 453487 ± 28808 cells/ml in the drowning media (b).

Animals

Sprague-Dawley adult male rats (n = 5) weighing 538 ± 26.8 g (SD) anesthetized by intraperitoneal injection of 3% thiopental sodium (4 ml/kg) were drowned with medium (a), and 5 Sprague-Dawley adult male rats weighing 536 ± 27.9 g (SD) and anesthetized with the same procedure were drowned with medium (b).

Procedures of drowning

A tracheal intubation at the upper third was performed using a plastic canula connected to a recipient filled with the drowning medium and adjusted to a constant height. The canule had a valve located 7 cm from the tracheostoma opening to allow respiration until the arrival of the drowning medium. The perfusion pressure was 40 cm H_2O.

Prefixation, sampling, postfixation, preparation of samples

Immediately after the last cardiac beat a tracheal perfusion at 40 cm H_2O pressure with 3 ml 25% glutaraldehyde in 0.1% phosphate buffer (pH 7.0) preceded the clumping of the trachea. The heart-lungs block was sectioned and immersed in 3% glutaraldehyde for 1 h. One sample measuring about 10 × 10 mm for SEM and 10 samples (5 subpleural and 5 central) measuring about 1 × 1 mm for TEM were taken from each lobe of the right lung (cranial, middle, caudal, postcaval) and from the left lung lobe. These samples were prefixed in 3% glutaraldehyde in 0.1% phosphate buffer (pH 7.0) for 3 h and washed 3 times with the same phosphate buffer. The samples for SEM were first dehydrated in a graded ethanol series and dried in a Balzer CPD 020 critical point dryer, mounted on specimens stubs and coated with gold in a Jeol Fine Coat – Ion sputter JFC 1100 for 10 min. All the samples were systematically studied with a Jeol JSEM-820 scanning electron microscope operating at 10–40 Kv. The samples for TEM were postfixed in a phosphate buffer 1% OsO4 at 6° C for 3 h, dehydrated in a graded ethanol series, infiltrated with propylene oxide and embedded in Ladd's epon LX-112. From both edges of all blocks (n = 250) 20 semiserial (interval 20 μm) semifine sections (0.5 μm) were prepared and stained with toluidine blue for light microscopical (LM) analysis. According to the LM findings (presence and number of diatoms in alveolar space and/or close to the alveolar wall, positive findings, etc.) a strike selection of the blocks (exclusion rate = 98%) was performed and 5 were analysed. From the selected samples, serial and semiserial (interval 0–40 μm) semifine sections were prepared and a step-by-step decision was made whether or

*The experimental procedures followed the "Principles of laboratory animal care" (NIH publication No 85-23, revised 1985) and were approved by the local ethical committee for animal experiments.

Table 1 Diatom species in drowning medium (b)

Species	Abund[1]
Achnantes delicatula (Kütz.) Grun.	1
Amphora coffeaeformis (C.A.Ag.) Kütz	2
A. delicatissima Krasske	5
A. holsatica Hust.	3
A. lineolata Ehr.	1
A. pediculus (Kütz.) Grun.	2
Berkeleya rutilans (Trent.) Grun.	1
Catacombas obtusa (Pant.) Snoeijs	2
Cocconeis pediculus Ehr.	2
Cylindrotheca closterium (Ehr.) Reimann & Lewin	1
Diatoma moniliformis Kütz.	3
Entomoneis paludosa (W.Sm.) Reimer	3
Epitzemia sorex Kütz.	4
E. turgida (Ehr.) Kütz.	2
Fragilaria elliptica Schum.	3
F. hyalina (Kütz.) Grun.	1
Gomphonema exiguum Kütz.	1
G. olivaceum (Lyngb.) Kütz.	2
Licmophora gracilis (Ehr.) Grun.	3
Martyana atomous (Hust.) Snoeijs	1
Mastogloia elliptica (C.A.Ag.) Cleve	2
M. pumila (Cleve & Möller) Cleve	2
M. smithii Thwaites	2
Melosira lineata (Dillw.) C.A.Ag.	1
Navicula gregaria Donkin	1
N. perminuta Grun.	4
N. phyllepta Kütz.	2
N. spicula (Hickie) Cleve	4
N. tripunctata (O.F.Müll.) Bory	1
Nitzschia inconspicua Grun.	2
N. cf. liebetruthii Rabenh.	4
N. microcephala Grun.	2
N. paleacea Grun.	2
Opephora olsenii M.Möller	1
cf. Pinnularia krockii Grun.	1
Rhoidosphenia abbreviata (C.A.Ag.) Lange-Bert.	3
Rhopalodia gibba (Ehr.) O.Müll.	2
Surirella brebissonii Krammer % Lange-Bert.	1
Synedra pulchella (Ralfs) Kütz.	2
Tabularia fasciculata (C.A.Ag.) Williams & Round	4
T. waemii Snoeijs	1

[1] Abundance values; 1 very sparse, 2 sparse, 3 scattered, 4 abundant, 5 very abundant

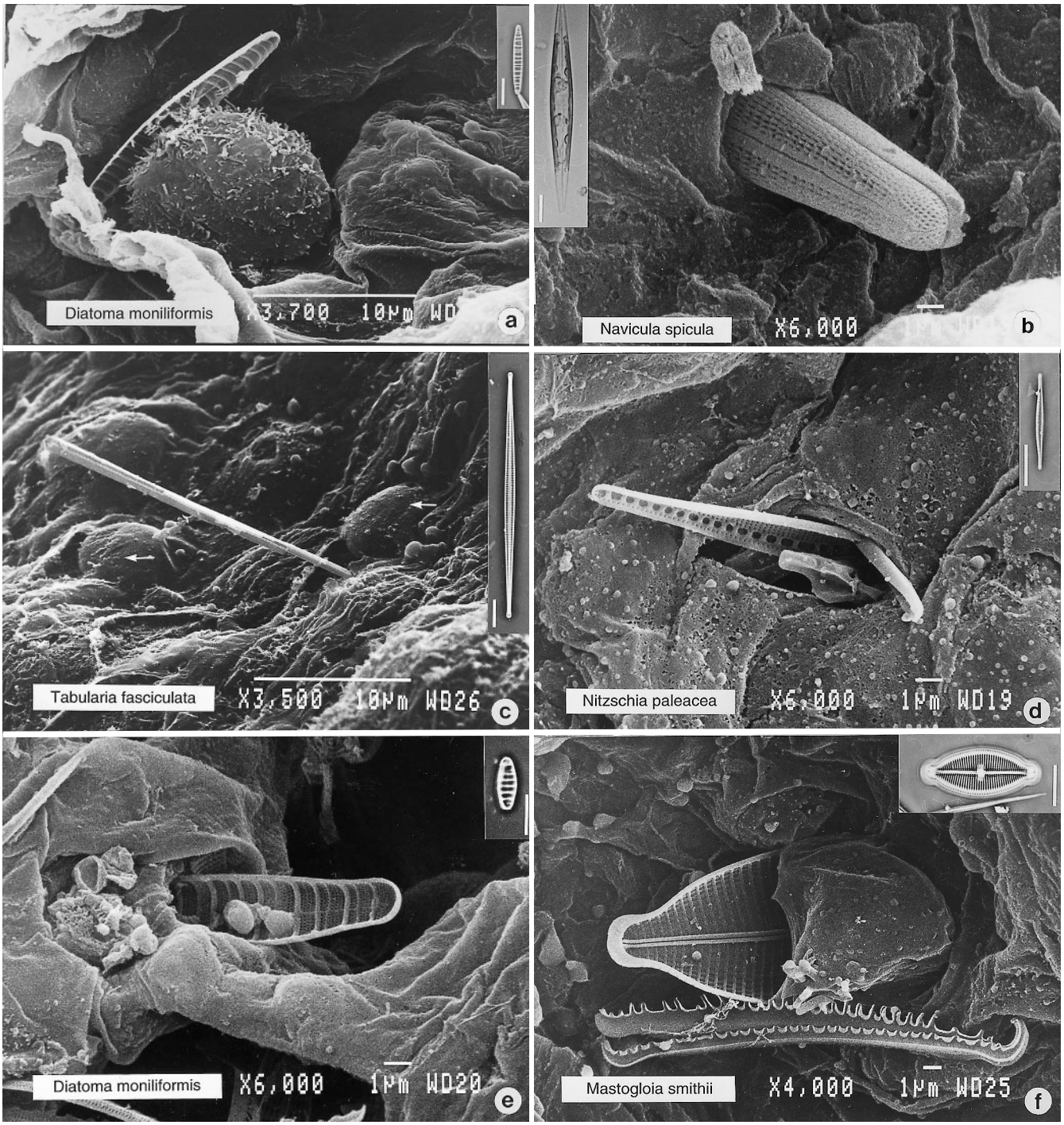

Fig. 1 a–f Diatoms in distal airways (SEM) **a** *Diatoma moniliformis* near an alveolar macrophage. **b** *Navicula spicula* penetrating a Kohn's pore. **c** A primarily fragmented *Tabularia fasciculata* partially penetrating into a laceration of the epithelial and endothelial lining of a distended alveolar septum; the arrows indicate intravascular erythrocytes. **d** A secondary in situ fragmented *Nitzschia paleacea* partially penetrating a laceration of the alveolar wall. **e** *Diatoma moniliformis* penetrating the wall of a distal airway. **f** *Mastogloia smithii* penetrating the alveolar wall through a clearly visible laceration. Scale bar of the LM micrographs in the upper corners = 10 µm

not to prepare 5–10 ultrathin serial sections. The ultrathin sections were stained on grids using uranyl acetate and lead citrate. For the ultrastructural study and micrography a Jeol 1200 Ex electron microscope was used.

Controls

For each of the four control groups (I–IV) two Sprague-Dawley adult male rats were killed by an overdose of thiopental sodium. For the control groups (III) and (IV) a passive penetration of the drowning media (a) and (b) at 40 cm H_2O was performed. The heart-lungs blocks of the rats of the control groups (I) and (III) were immersed in 3% glutaraldehyde for 1 h. The pre-fixation of

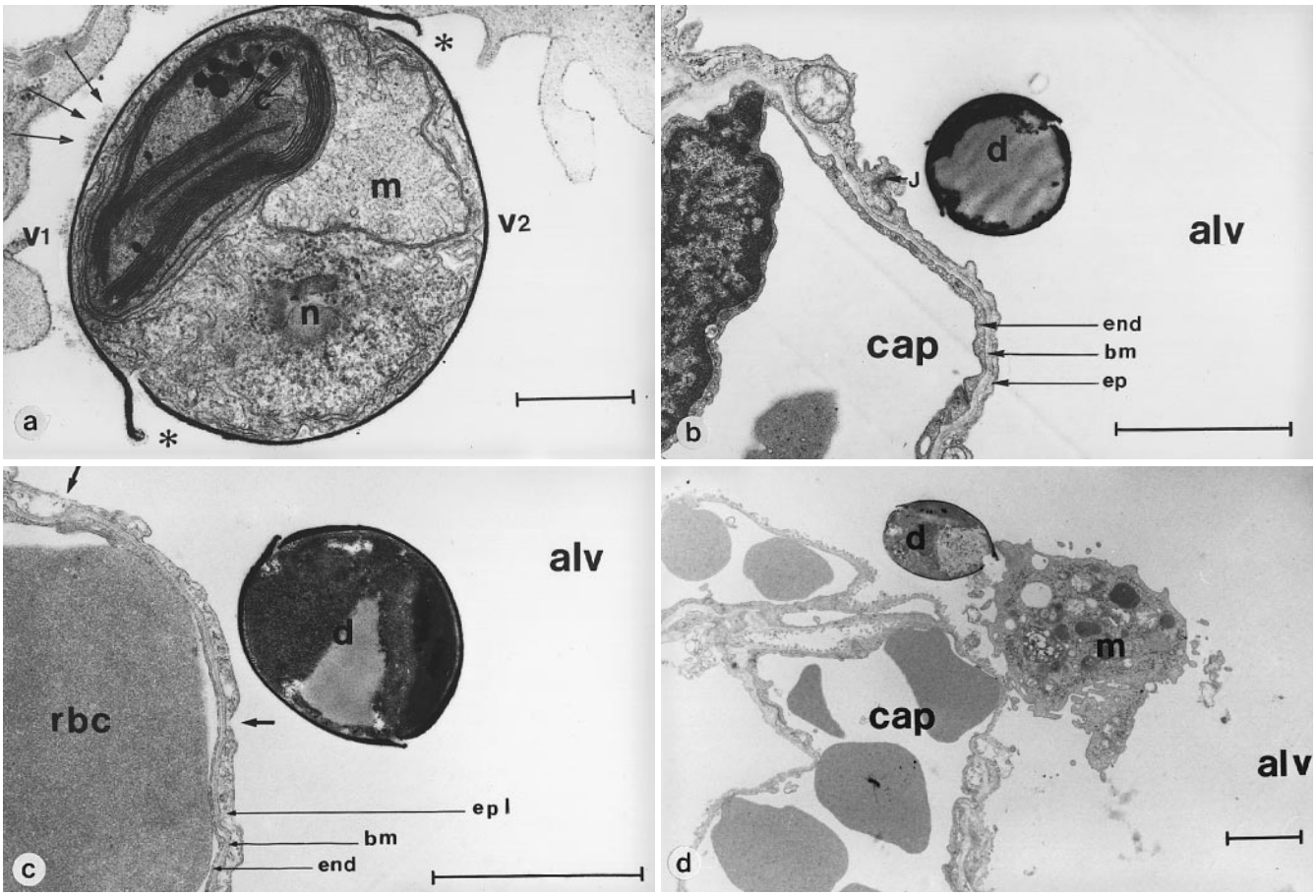

Fig. 2 a–d PT in alveolar spaces (TEM). **a** PT cell cross-section: valves (v1, v2), membrane polysaccharides (↑), cingulum regions (*), nucleus (n), mitochondria (m), chloroplast (c). Scale bar = 0.5 μm. **b–c** Eccentric cross-sections of PT close to the alveolar wall. Note in "b" the integrity of the epithelial lining and that of the intercellular junction and in "c" the partial and slight ruptures of the epithelial lining (arrows). **d** PT near an AM. Fig. 2 a–c scale bar = 2 μm. Fig. 2 d scale bar = 4 μm. *Explanation of the symbols: alv* = alveolar space, *bm* = basal membrane, *cap* = alveolar capillary, *d* = diatom, *end* = endothelial lining, *ep* = epithelial lining, *j* = intercellular junction, *m* = macrophage, *rbc* = red blood cell

lungs of control groups (II) and (IV) was performed with tracheal perfusion of 3 ml 25% glutaraldehyde followed by immersion of the heart-lungs blocks in 3% glutaraldehyde for 1 h. For each lung, 5 samples (one per lobe) were analysed with SEM and 10 samples (2 per lobe, 1 subpleural and 1 central) were analysed with TEM.

Peripheral organs

During the zoopsy following the experiments and controls performed with drowning medium (a) brain, heart, liver, spleen, right kidney and femoral bone marrow were sampled and, after fixation in formaldehyde, 20 semiserial sections for each of these organs (interval 15 mm; thickness 5 mm) were prepared and stained with hematoxylin-eosin. During the zoopsy following the experiments and controls performed with drowning medium (b), the same organs were entirely acid digested using the method of Auer and Möttönen [37] and the whole sediment was analysed by LM.

Results

The drowning process was characterized by a short period of persisting normal respiration (8–10 s), by an apnoic phase (1–1 min 15 s), by a dyspnoic phase (35 s–1 min) with 20–33 respiratory movements and by terminal breathing (4 min 30 s–5 min 30 s) followed by heart block. The average volume of drowning medium reaching the respiratory tract in each experiment was 24.9 ± 2.0 ml (SD). After diaphragm puncture and thoracotomy, the lungs filled the entire thoracic cavity with a marked overlapping of the anterior margins. The lungs were light pink with small focal areas of atelectasia and haemorrages. The pleural cavity contained 1.0–1.5 ml of transparent fluid. The fixation procedures for SEM and TEM were evaluated comparing the samples of the control groups (I–IV). No evident morphological differences were observed between the samples fixed with immersion (control groups I and III) and those fixed by tracheal perfusion/immersion (control groups II and IV). Moreover the comparison between the findings of the control groups III–IV and those of the experiments discriminated the changes caused by the perfusion pressure of the drowning media alone (moderate and uniform distension of the alveoli together with a discrete density of small tears of the epithelial lining and moderate disarray of the type II pneumocytes) from those caused by the drowning process.

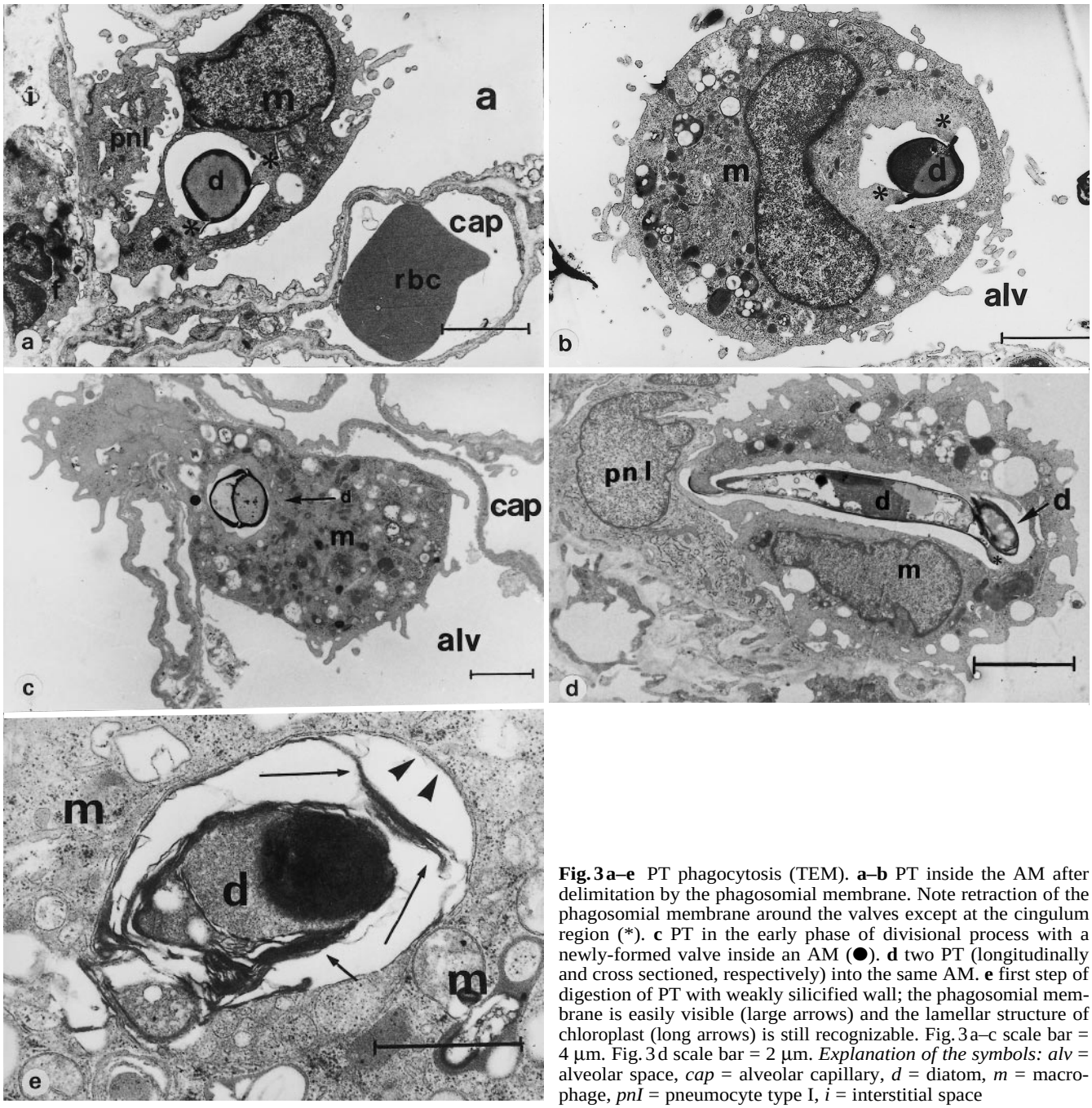

Fig. 3 a–e PT phagocytosis (TEM). **a–b** PT inside the AM after delimitation by the phagosomial membrane. Note retraction of the phagosomial membrane around the valves except at the cingulum region (*). **c** PT in the early phase of divisional process with a newly-formed valve inside an AM (●). **d** two PT (longitudinally and cross sectioned, respectively) into the same AM. **e** first step of digestion of PT with weakly silicified wall; the phagosomial membrane is easily visible (large arrows) and the lamellar structure of chloroplast (long arrows) is still recognizable. Fig. 3 a–c scale bar = 4 μm. Fig. 3 d scale bar = 2 μm. *Explanation of the symbols: alv* = alveolar space, *cap* = alveolar capillary, *d* = diatom, *m* = macrophage, *pnI* = pneumocyte type I, *i* = interstitial space

The use of living diatoms in the medium (a) facilitated the TEM and histological identification in ultrathin and semifine sections by cytology. Conversely the acid treatment used to eliminate the organic matter in the medium (b) removed the cytoplasm content of diatoms, restricting the sample analysis to SEM and LM diatom preparations because of the difficulties to identify cytoplasm-free diatoms in histological, semifine and ultrathin sections.

The SEM analysis of rats drowned in medium (b) disclosed marked changes in lung parenchyma and tissue similar to those previously described in the literature for drowning [38, 39]. The pleural surface showed a diffuse emphysematous pattern without any disruptures. Most of the diatoms species of the epiphytic population were detected along the respiratory tract. Proximally the number of diatoms was lower than distally and most of them were entire with only a minority showing fragmentation. Distally the diatoms were more fragmented. In the alveolar spaces the diatoms were centrally or eccentrically located and occasionally they were observed near alveolar macrophages (AM) or penetrating a Kohn's pore (Fig. 1 a, b). In some fields, the diatoms, generally large pennates, perforated the entire width of the alveolar wall and were visible in two adjacent alveolar spaces. Moreover, a close interaction between different diatom species e.g. *Amphora* spp., *Cocconeis pediculus, Diatoma moniliformis, Diatoma*

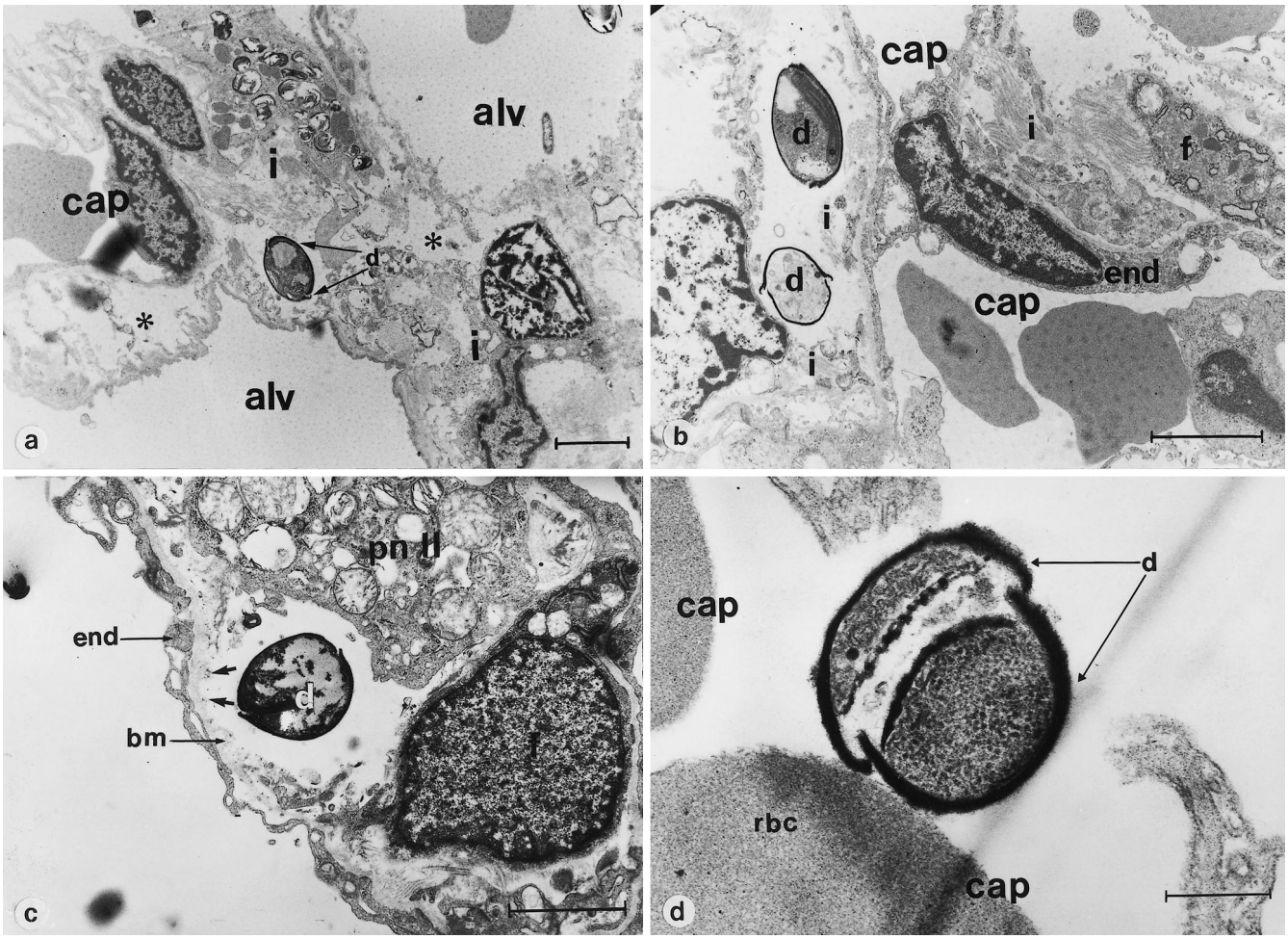

Fig. 4 a–d PT in interstitial space (TEM) **a** interstitial oedema (*) around a diatom. **b** Two PT close to each other in the oedematous interstitial space. **c** Incipient rupture of the endothelial basal membrane (↑). **d** Penetration of PT through a ruptured thin portion of the alveolo-capillary barrier. Scale bar = 4 µm. *Explanation of the symbols: alv* = alveolar space, *bm* = basal membrane, *cap* = alveolar capillary, *d* = diatom, *end* = endothelial lining, *f* = fibrocyte, *i* = interstitial space, *pnII* = pneumocyte type II, *rbc* = red blood cell

tenuis, Gomphonema olivaceum, Mastogloia smithii, Melosira lineata, Nitzschia microcephala, Nitzschia paleacea, Nitzschia perminuta, Tabularia fasciculata and the alveolar wall lacerations was observed (Fig. 1 c–f). These diatoms have dimensions of the order of magnitude < 10–> 100 mm. LM analysis of the acid-digested peripheral organs detected ca. 20% (8 species) of the original sample. Our material is still too small to prove any significant size and shape selective penetration and distribution of diatoms between the organs. The dimensions of unbroken diatom valves were in the range 3 × 12–5 × 110–8 × 56 × 12 × 29 mm. In addition, a number of fragments could also be identified. The rather robust species *Tabularia fasciculata* and *Diatoma moniliformis* dominated in the diatom preparations. The dominant species, *Amphora delicatissima*, may have been too delicate to be detected in our preparations after two acid treatments.

TEM analysis was preceded by a semiserial histological analysis of the peripheral organs. The histological analysis revealed the presence of at least one PT in the small vessels of the brain, myocardium, liver and kidney in about 1 out of 8–12 sections. In the semifine sections of the lung used to guide the TEM analysis the PT in alveolar spaces were clearly detectable in longitudinal and certain oblique sections whereas their identification in cross-sections was at times more difficult. In a few fields it was possible to observe diatom-like particles in alveolar macrophage (AM) and in pulmonary vessels.

The TEM analysis of lungs revealed changes qualitatively similar to those previously described in an experimental model of drowning performed with saline hypertonic medium [40]. PT was identified by TEM which allowed also the different components of the cells to be precisely identified (Fig. 2a). Occasionally PT were phagocytosed by AM (Fig. 3 a–e). We always found a postmortem retraction of the phagosomial membrane around the phagocytosed PT whereas the adhesion was persistent at the cingulum region which was often disruptured (Fig. 3 a–b). A PT in a type I pneumocyte was observed only once. The TEM study demonstrated the penetration of PT into the pulmonary vessels. Such penetration occurred through the interstitial spaces (Fig. 4 a–c) which were markedly oedematous and at the thinnest portions of the alveolo-capil-

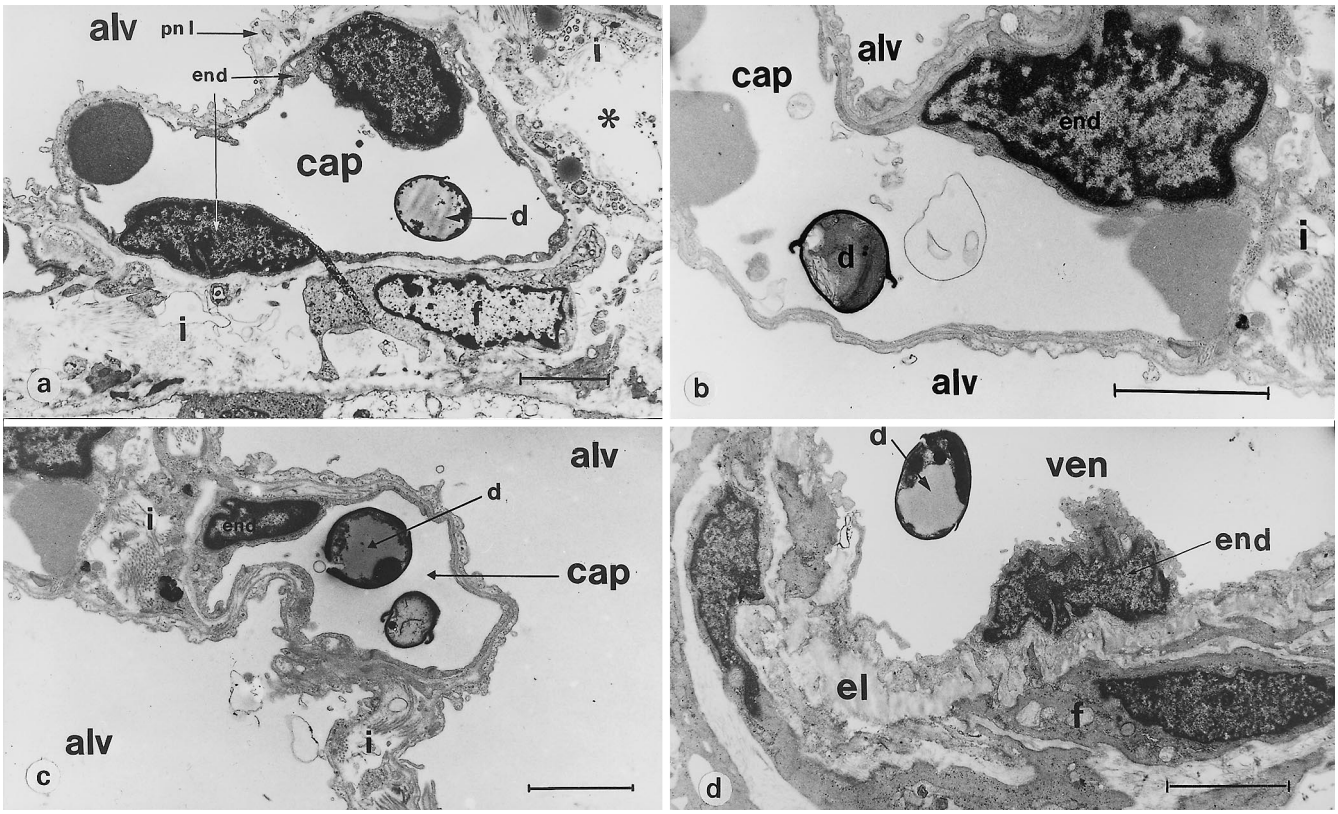

Fig. 5 a–d PT in pulmonary vessels (TEM). **a–c** PT in capillaries. **d** PT in pulmonary venule. Scale bar = 6 μm. *Explanation of the symbols: alv* = alveolar space, *cap* = alveolar capillary, *d* = diatom, *end* = endothelial lining, *f* = fibrocyte, *i* = interstitial space, *pnI* = pneumocyte type I, *ven* = venule, *el* = elastin

lary barrier (Fig. 4 d). The PT were also identified in pulmonary capillaries (Fig. 5 a–c) and venules (Fig. 5 d).

Discussion

The diatom test is based on the theoretical assumption that during the drowning process diatoms penetrate the alveolar walls and that they are transported by the heart beat and the bloodstream to the small vessels of peripheral organs, where they can be detected e.g. by microscopic examination of the sediment obtained from the chemical dissolution of tissues. Despite the long-lasting controversies on the reliability of the diatom method for the diagnosis of drowning no morphological demonstration of the capacity of diatoms to penetrate the alveolar wall into the pulmonary vessels during the drowning process has been given.

Our study focused on the interaction of different species of diatoms with the alveolo-capillary barrier and aimed at identifiying the process by which diatoms penetrate into the pulmonary vessels under experimental conditions. The high diatom concentrations in the drowning media – falling in or somewhat above the uppermost level of the concentration range in sea water (e.g. near the shore in eutrophic areas) – was not relevant in our study, where the purpose was primarily the qualitative assessment of the capacity of diatoms to penetrate into the pulmonary vessels.

With SEM it was possible to precisely identify along the whole airways several of the diatom species contained in the drowning medium (b) and to disclose some of them closely interacting with the alveolar wall (Fig. 1 a–f). The SEM analysis was poorly informative about the effective penetration of diatoms into the pulmonary vessels. The finding of several species of diatoms in chemically digested peripheral organs suggests, however, that some of the diatoms contained in the drowning medium penetrate into the circulation and that some of our figures illustrate the early phases of their passage into pulmonary vessels.

The TEM study was more informative. The identification of PT was straightforward as was the vitality and division stages of the cells. Because of the high reproductive rate of PT and the presence of a small number of dead cells in the Tv2 medium, it was not possible to determine whether or not the PT continued to divide inside the lungs and whether these cells were damaged during the agonal period. The phagocytosis of diatoms by AM has never been described or postulated. It occurred during the interval between the beginning of the drowning process and the fixation procedures (about 6–7 min). The interaction between PT and AM took place preferentially in the cingulum regions, probably because of their higher antigenicity, and/or the exposition of new cytoplasmic antigens following their disrupture. Some investigators have recently studied AM in drowning and asphyxial conditions. In freshwater experimental drowning, Brinkmann and Butenuth [41] observed a considerable increase of

AM, whereas Betz et al. [42] morphometrically demonstrated a decrease of the AM possibly related to a "wash out-effect" of the drowning medium. Whether asphyxia alone may or may not induce an AM activation is debatable [43–45]. A recent immunohistochemical study [46] failed to demonstrate a prefinal migration, mobilization or proliferation of AM during asphyxial deaths. The AM activation could be determined by the particulate matter contained in the drowning media e.g. latex as demonstrated by Fechner et al. [47] or diatoms as shown in our experiment. The comparison with AM activation after inhalation of ultrafine and larger particles is obviously of little value. It should be, however, noted that such response is chronologically and quantitatively different among different rodent species and other animals [48]. The mechanisms of AM recruitment by diatoms in exogenous liquid, the role of the composition and tonicity of the drowning medium in the process of endocytosis and the possibility to transfer our observations to humans, thus remain a matter of pure speculation.

The penetration of PT into the pulmonary vessels occurred via the lacerations of the thinner portions of the alveolo-capillary barrier, as well as via the septal interstitium. The primary site of penetration of PT into the alveolar wall could be a disrupture of the epithelial lining as a consequence of overdistension during the asphyxial process or to the impact of diatoms; it could also be a temporary or permanent relaxation of the epithelial tight junctions. The penetration of PT may enhance the passage of other diatoms through the same primary site as demonstrated by the simultaneous finding of two diatoms close to each other in the interstitial space (Fig. 4c) and in the same pulmonary vessel (Fig. 5c). The passage of the diatoms into the circulation is possibly determined by the perfusion pressure together with the increased respiratory pressure, whereas during the later stage the negative pressure between left heart and pulmonary vessels, as well as the kinetic energy of the bloodstream could play an additional role.

In the control group (II) no PT was observed either in the interstial space or in the pulmonary vessels. The statistical value of such controls is obviously very low. However, it seems probable that diatoms can penetrate postmortem into the pulmonary vessels and heart blood only under particular circumstances, e.g. high water pressure and advanced postmortem changes. The only situation where a small number of diatoms have been found in pulmonary vessels and heart blood of submersed bodies was that of a body submersed at a depth of 23 m; a few diatoms were also observed in internal organs of a submersed cadaver at a depth of 130 m [49,50]; these studies do not mention any control or strict protocol allowing the exclusion of contamination.

In our experiments the histological demonstration of PT in small vessels of brain, myocardium, liver, kidney, together with the negative results of the controls, confirmed the penetration into the circulation during the drowning process. Although such a demonstration can be considered a definite proof of penetration into the bloodstream during drowning, an intravascular localization of diatoms after vital inhalation or gastro-enteric absorption is still theoretically possible. In our experiments the utilization of the biradiate form of *Phaeodactylum tricornutum*, never isolated in Finland, allowed us to exclude such a possibility.

The morphological and formal demonstration of the penetration of diatoms in pulmonary and peripheral vessels substantially contradicts the research work of Spitz and Schneider [23] which although previously criticized [25], has been and still is a milestone and frequently cited critical work on the reliability of the diatom test. Using an experimental model similar to ours (tracheal perfusion at 40 cm H_2O, unialgal culture of diatoms measuring 8–18 μm in diameter at a concentration of 100 000 cells/ml), Spitz did not find any diatoms present in the drowning media by microscopical examination of preparations obtained after acid digestion of liver tissue.

The transfer of our results to human drowning is obviously not directly possible. The differences between natural drowning and the experimental model used, the diatom concentration in nature and the anatomo-histological-physiological differences between rats and humans – e.g. the total surface of the lung (400 g rat: 7.5 m^2; 70 kg man: 75 m^2) and the mean alveolar diameter (rat: 70 μm; man: 200–250 μm), the average thickness of the air-blood barrier (proportional to lung size), the thinning of pulmonary arteries and veins – are all variables that should be appropriately considered.

In conclusion the diagnostic value of diatoms for drowning is controversial. The relevance and the potential implications of the diatom test for drowning justify a prudent and critical approach, as well as a renewed effort in basic research. For these reasons we investigated the previously unquestioned theoretical assumption of the diatom method, i.e. the capacity of diatoms to penetrate the alveolar wall into the pulmonary vessels during the drowning process. We have demonstrated the "terminal" phagocytosis of PT by AM and the capacity of PT to penetrate the interstitial spaces and the pulmonary vessels under experimental conditions. This research does not provide new arguments for or against the reliability of the diatom test for the diagnosis of drowning because of the impossibility to transfer our results to human drowning. We expect, however, that the formal morphological demonstration of the capacity of diatoms to penetrate the alveolo-capillary barrier under experimental conditions is an important step towards a new basis for the assessment of the potentiality, reliability and limits of the diatom method for the diagnosis of drowning.

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