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Detection and analysis of tracers in experimental drowning

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Abstract In animal experiments, studies on the mechanisms involved in drowning were carried out using latex and gold tracers of defined size and concentration. The tracers were detectable by fluorescence microscopy (latex tracers) and by electron microscopy (gold tracers) in the lungs, kidneys and lymph nodes and were analysed further by X-ray microanalysis using a transmission scanning electron microscope. Tracers with small diameters were shown to penetrate intercellular gaps of the alveolar epithelium and the larger tracers were incorporated into the epithelial and endothelial cells by active pinocytotic mechanisms thus passing through the air-blood barrier. The detection and analysis of tracers in organs of the systemic circulation originating from the immersion fluid can assist in understanding the pathophysiology of drowning and in some selected cases, in making a more definitive diagnosis.

Key words Experimental drowning · Tracer study · Electron microscopy · X-ray microanalysis

Introduction

The diagnosis of drowning as the cause of death can be very difficult and problematic in individual cases, especially when typical morphological signs of drowning (e.g. upper airway froth, emphysema aquosum, subpleural and intrapulmonary haemorrhages) are not present or when the body is in an advanced stage of putrefaction [1]. In these cases other tests can be performed but the significance and interpretation of these findings (e.g. diatom analysis [2–4], detection of Chlorophyceae [5], blood electrolyte concentration [6, 7]) is controversial [1, 8]. The detection and analysis of other particles originating from the immersion fluid within organs of the systemic circulation could assist in the diagnosis. To estimate the possibility and the significance of this detection, animal experiments using different tracers were carried out (licence numbers: 26.III-0834 and 26.0834.20/81, RP Münster).

Materials and methods

Animal experiments

Male Wistar rats (n = 8, bodyweight 150–400 g) were anaesthetized by Ketanest (Ketamin-HCl, 0.9 ml/100 g), subjected to a tracheostomy and 5–15 ml (ca. 5 ml/150 g) of the immersion fluid containing defined amounts of tracers of different diameters was introduced into the airways under standardised conditions (pressure: 10 cm) by a microperfusion appliance [9]. The "survival period" (time between the beginning of fluid instillation and cardiac arrest) was shorter than 3 min. Immediately after death the lungs, kidneys, lumbar lymph nodes, and in one case the brain, were removed and fixed in 2.5% glutaraldehyde. Specimens were prepared for histology (HE, semithin section: toluidin-blue) and electron microscopy (uranylacetate/Pb-citrate) [10].

Tracers

Dow uniform latex particles (SERVA, Germany; Table 1) and colloidal gold (Table 2) in monodispersed and polydispersed solution (osmolarity 0.5 mosmol, pH 6.2; osmolarity of fresh water 0.7 mosmol) prepared according to Frens [11] and concentrated by centrifugation were used as tracers.

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Table 1 Parameters of the latex tracers used

Tracer	Diameter (μm)	Colour	Charge
CML*	0.06 0.845	none fluorescence	0.296 m eq COO^-/g

[* CML-carboxylate modified latex]

Table 2 Parameters of the gold tracers used

Tracer	Diameter (μm)	Mass (fg)	Expected concentration of particles in the lung (N/μm³)	
			non centrifuged colloid	centrifuged coll.
Gold	12	0.02	2.4	240
	24	0.15	0.32	32
	36	1.2	0.04	4
	48	9.2	0.005	0.5

Tracer detection

The tracers were detected by fluorescence microscopy (latex tracers of 845 nm diameter, magnification: 1,000) and by electron microscopy (Zeiss EM 109, Germany; latex particles: magnification 3,380–23,500, gold particles: 3,380–91,000).

Tracer analysis

X-ray microanalysis of specimens containing gold tracers was carried out using the transmission scanning technique (Philips EM 301) and the EDAX system. For each analysis the window had a size of 125×125 nm and the diameter of the electron beam was 32 nm.

Results

The morphology of the lungs of the experimental animals showed changes typical of drowning comparable with those seen in humans. At autopsy an overinflation of the lungs, froth in the air passages and subpleural petechial haemorrhages could be found. Hydropic swelling of the epithelial cells of the terminal airspaces with dilatation and rupture of alveoli, thinning of the interalveolar walls and empty pulmonary capillaries were observed in the histological preparations [12, 13].

Latex tracers

In the semithin and ultrathin sections of the lung, latex tracers could be seen only on the alveolar surface of pneumocytes in the form of tracer chains (Fig. 1). The identification of the tracers used was possible by the specific relation of diameter and thickness of the walls (Fig. 2). Using fluorescence microscopy these tracers were also detectable in alveolar macrophages but not in other alveolar lining cells, blood or lymphatic vessels. By electron microscopy the tracers were arranged in pools near the alveolar surface (Fig. 1). At a higher magnification it can be seen that the minimal distance between the cell surface and the tracers is about 10–15 nm. After phagocytosis by alveolar macrophages the tracers were detectable in small vesicles with a double membrane wall (Fig. 3).

Gold tracers

The smallest gold tracers (∅: 12 nm, 24 nm) could be detected intraalveolarly (Fig. 4), but they were also able to penetrate intercellular gaps. Furthermore the tracers were also observed in vesicular structures penetrating epithelial and endothelial cells (Fig. 5) and in alveolar macrophages after phagocytosis. At high magnification small gold tracers (∅: 12 nm) were seen in the proximal tubule (Fig. 6) and in capillaries of the proximal tubules of the kidneys. In one case the detection of 36 nm diameter tracers was possible in lymph nodes and lymphatic vessels while the other organs were completely negative for tracers.

X-ray microanalysis

In this method, each chemical element shows specific energy lines. Gold is characterised by four different energy lines ($L\alpha$ = 9.712 keV, $L\beta_1$ = 11.440 keV, $L\beta_2$ = 11.583 keV, Lg = 13.379 keV) producing the typical spectrum (Fig. 7). In addition to the lines of the gold tracers, energy lines from lead and osmium (originating from the processing of the specimens) were obtained, which could be easily distinguished (Fig. 8).

Discussion

Although other larger mammals (e.g. dogs, rabbits) have been used as experimental models in previous studies on drowning [14, 15], no perfect model completely mimicking the human situation has been described. The rat was chosen as the experimental model in this particular study as the authors have previous vast experience of the use of this animal in experimental work and the physiological mechanisms of breathing and the pulmonary histological architecture in rats are similar to those of humans [16]. The experiments carried out in this study were designed solely to study the mechanics of the uptake and transport of particles from within the pulmonary alveoli into the systemic circulation. Although some hydrostatic pressure would be exerted on the alveoli by the column of water containing the tracers, any trauma to the alveoli so in-

Fig. 1 Latex tracers (d = 0.845 μm) in lung alveoli. Magnification 4,400. ALV-alveoli, CAP-alveolar capillary, L-latex tracers ▶

Fig. 2 Latex tracers in high magnification (50,000). The tracers are characterized by a typical relation between diameter and thickness of the wall

Fig. 3 Alveolar macrophage (MACR) with two incorporated latex tracers (L). Magnification 16,300. ALV-alveoli

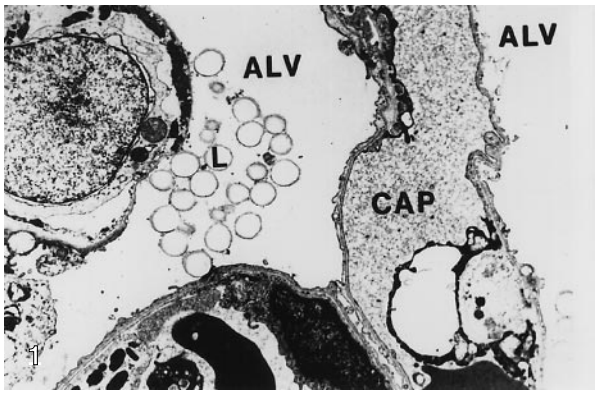
Fig. 4 Tracer transport through alveolar epithelial cells. Tracers (TR) are visible in the alveoli (ALV), the in cytoplasm of pneumocytes type I and in the capillary. Magnification 32,000

Fig. 5 Gold tracers (d = 24 and 48nm) in an evagination of the membrane of a pneumonocyte type I. ALV-alveoli. Magnification 149,000

Fig. 6 Kidney. Gold tracers (d = 12 nm) and cell remnants in the proximal tubule. TR-tracer. Magnification 89,900

Fig. 7 Typical whole energy spectrum for gold

Fig. 8 Energy lines of gold and lead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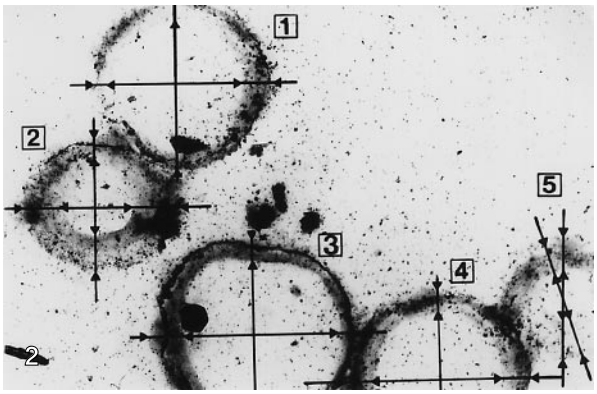
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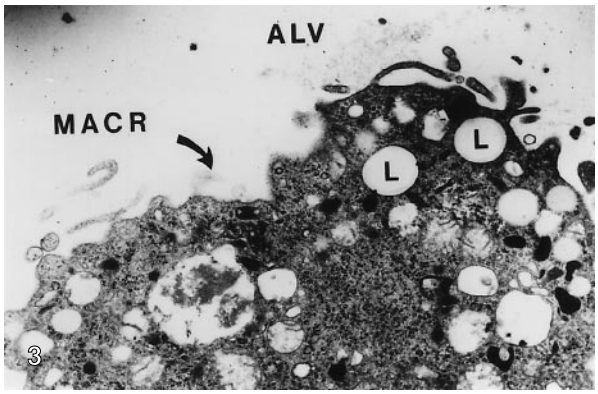
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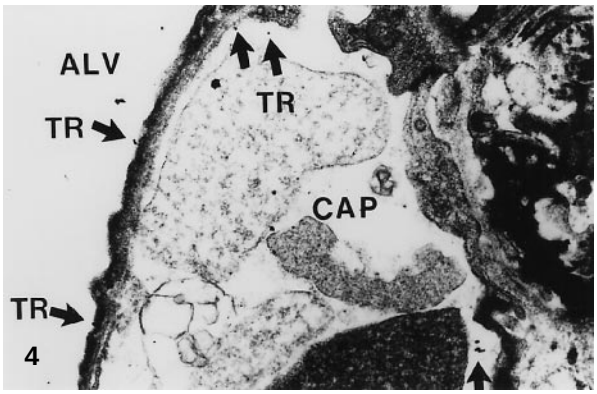
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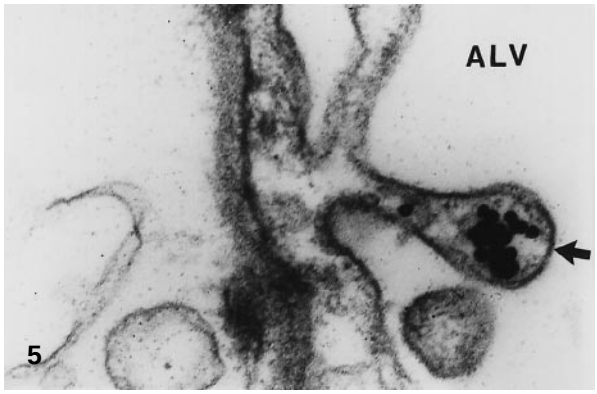
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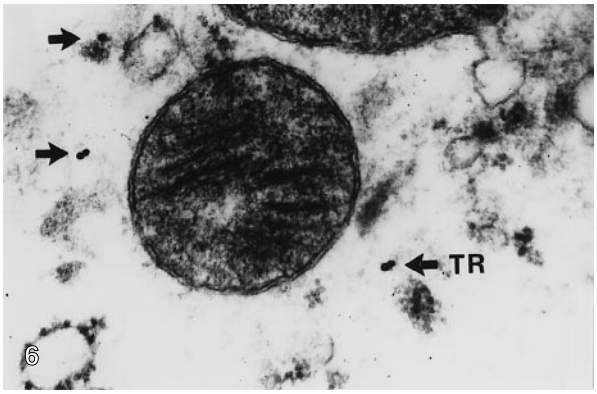
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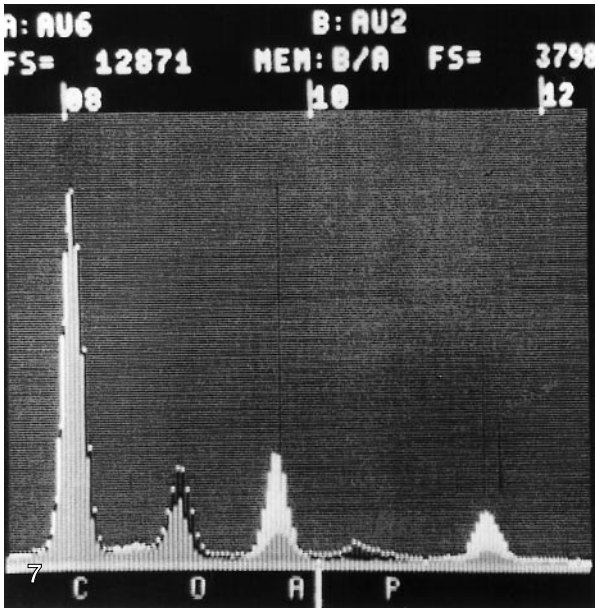
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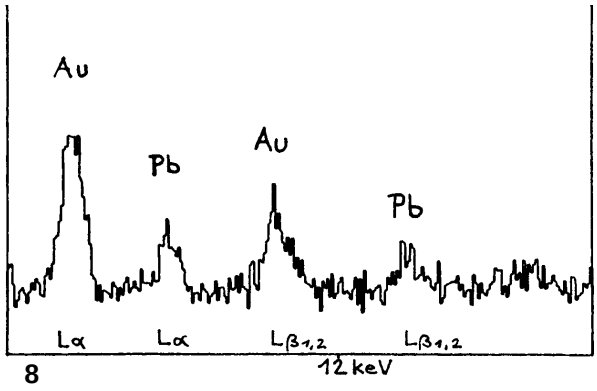
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duced – particularly to their membranes and pores – would not be excessive, as the major and principal force responsible for the exchanges of fluid through the alveolar/endothelial barrier is osmotic [17, 18].

In 1969 Bensch et al. [19] reported that in dogs, albumin and globulin labeled with radioactive iodine (I^{131}) which had been inhaled passed through the air-blood barrier in the lungs and could be detected in the blood. Chinard [20, 21] investigated the transport function of the alveolar-capillar barrier in dogs, from the capillary to the alveolar surface, using radioactive labeled butandiol. His results led to the so-called Bohr-Katy-Crone-Renkin-Perl – equation where the entry of a substance (X) into the tissue is proportional to the concentration of this substance (W_x) in the blood which is defined by an exponential function that is dependent on the permeability (Px) of the blood barrier, the surface (S) and the flow (F) [20, 21]. Pietra et al. [22] found haemoglobin tracers in pinocytotic vesicles of endothelial cells in similar experiments. Rubin [23] developed a kinetic-mechanical model describing the diffusion of vesicles and determined the vesicle-transfer time required for a vesicle to pass through a cell to be 6.3 s. Renkin [24] also postulated a vesicle-dependent transport of proteins through endothelial cells in addition to the large and small pore systems, and Simionescu et al. [25–27] proved their existence in endothelial cells of muscle capillaries. Schneeberger and Karnowsky [28] calculated that the effective diameter of endothelial pores was 4.5–5.8 nm and of epithelial pores between 0.6 and 1.0 nm in mice.

Fechner et al. [9] postulated that an active transport of small molecules (myoglobin) occurred allowing them to pass the gas-blood barrier and assumed that bigger tracer molecules could penetrate capillaries and alveolar cells in cases of drowning due to destruction of alveolar walls and capillaries.

The observations made in the studies quoted cannot be transposed unreservedly to the "drowning" model used. What, however, can be definitively shown by our study is that the large latex tracers (d = 0.845 μm) could not pass through the membrane of the pneumocytes: this type of tracer could be detected only in the alveoli. The smaller gold tracers (d = 12 and 24 nm) were able to penetrate intercellular gaps and the larger gold tracers (d < 50 nm) could be found in pneumocytes, endothelial cells and in capillaries. The diameter of endothelial and epithelial pores does not seem to be a factor which limits the uptake of particles into the alveolar cells or their passage from the alveoli through tissue barriers.

Grellner et al. [29] and Madea [30] reported that leucocytes and macrophages are able to migrate during the supravital period, i.e. after cardiac arrest, and show chemotactic activity. The results of the present study indicate that these cells also have pinocytotic activity and show active vesicular transport even after the cessation of circulation or in the short agonal and early perimortem period, while the detection of tracers in organs of the systemic circulation depends on an intact circulation during the agonal phase.

Together with the results of the X-ray analysis, this phenomenon may assist with the diagnosis of drowning in selected cases. In forensic case work, all elements contained in the immersion fluid but not physiologically present within the human body could be suitable as tracers. The method described is based on specialised techniques which are not available in every institute and they cannot be used routinely, but could be helpful to establish the diagnosis of drowning in selected cases in which special circumstances prevail. In such cases, heavy metals (Pb, Cr, Cd) or specific components of the immersion solution such as bath salts, may to be suitable as tracers.

This study demonstrates that there appear to be two phenomena resulting in the uptake of corpuscular substances from solutions from the alveoli into the tissues: a) a passive diffusion through the intercellular pores and release of these substances into the circulation in the agonal period while the circulation still is active; b) an active postmortem transport that proceeds in alveolar pneumonocytes and macrophages and continues to function for a short time period even after death and cessation of the circulation.

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