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Ultrastructural Aspects of Bubble Formation in Human Fatal Accidents After Exposure to Compressed Air

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Summary. Electron microscopic investigations were performed on samples of human tissue obtained from subjects following fatal decompression sickness, associated with hyperbaric air-therapy. Intra- and extracellular gas bubbles of varying size were identified throughout the entire body. Each bubble was covered by an osmiophilic non-homogeneous coat of cloudy and flocculent material, native to its specific locality. This envelope measured from 30 to 560 Angstroem-units in thickness. Association of this covering with an electrokinetic zonal activity, detected biophysically by Lee and Hairston (1971) is assumed. We consider this surface coat prevents nitrogen from being eliminated via the blood-lung-barrier.

Key words: Electron microscopic findings – Fatal decompression sickness.

Introduction

Since the establishment of physiologically derived decompression procedures by Boycott, Damant, and Haldane (1908), the incidence of fatal decompression has been significantly reduced and there has thus been a dearth of post mortem examination data from human subjects (Moetoenen and Karkola, 1971). In the beginning of a decompression sickness the various symptoms are mainly caused by a multiple and disseminated gas embolism. In former times the best knowledge has had concerned the venous air embolism of the lungs, pulmonary arteries and the right heart ventricles (Balogh, 1941; Froboese, 1947). Rössle (1944, 1947) emphasized to incriminate air embolism of the arteries as a cause for a considerable part of clinical symptoms, especially in the heart and in the brain. The phase like development in the brain of animals was described by Harter (1947). She assumed a similar process in human beings.

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This paper presents for the first time electron microscopy of human tissue obtained from subjects following fatal decompression sickness, associated with hyperbaric air therapy.

Table 1. Clinical data and autopsy findings in patients with fatal decompression sickness, following explosive and uncontrolled reductions of the high pressure in association with hyperbaric air-therapy

Case	Sex	Age (yr.)	Length (cm)	Weight (kg)	Preexisting disorders	Survival time after explosive decompression	Appearance of gas bubbles	Fat embolism
1. F.N. (SN 91/76)	♀	60	157	66	Hypertonus	6 h 22 min	Skin of trunk, upper arms and thighs right heart chamber ubiquitous in arteries and veins	no
2. K.B. (SN 92/76)	♂	62	176	87	Hypertonus Chronic emphysema of the lungs Cirrhosis of liver	2 h 22 min	Skin of trunk, arms and legs right heart chamber ubiquitous in arteries and veins, esp. in portal vein submucous in stomache and intestine	no
3. O.K. (SN 93/76)	♂	75	168	76	Hypertonus Arteriosclerosis Chronic emphysema of the lungs	1 h 52 min	Skin of left upper arm subpleural (interstitial emphysema) ubiquitous in arteries and veins right heart chamber Pneumothorax	no
4. H.T. (SN 94/76)	♀	62	154	61	Chronic emphysema of the lungs Bronchiectasis chronic bronchitis	21 h 27 min	Skin of lower abdominal wall Pneumothorax ubiquitous in arteries and veins	no
5. E.M. (SN 97/76)	♀	77	168	84	Carcinoma of the right breast Chronic emphysema of the lungs Hypertonus Arteriosclerosis Malignant lymphoma, low grade	38 h 47 min	ubiquitous in arteries and veins right heart chamber	brain lungs

Fig. 1. Fatal decompression sickness. Numerous gas bubbles, partially collapsed, within the interstitial tissue of heart muscle. Electron micrograph, following formaldehyde fixation. ×2500

Materials and Methods

In 1976, in Hannover, Germany, the "Society for Regenerative Hyperbaric Therapy" (GRT: Gesellschaft für Regenerative Überdruck-Therapie) was established. Treatment for multiple disorders in the elderly including disturbances of the cardio-respiratory system, nervous system, hepatic disease, and others was proposed. The founder and designer of the facility was not a licensed doctor of medicine, but a homeopath.

Within two coupled multiple position chambers, 20 patients were subjected to a hyperbaric environment equal to a maximum of 4 ata, for a duration of 15 min, followed by successive reductions in pressure. The entire simulated diving procedure should normally have lasted 1 h and 30 min. During one of the treatment sessions, a patient (K.B. SN 92/76, Table 1) suddenly

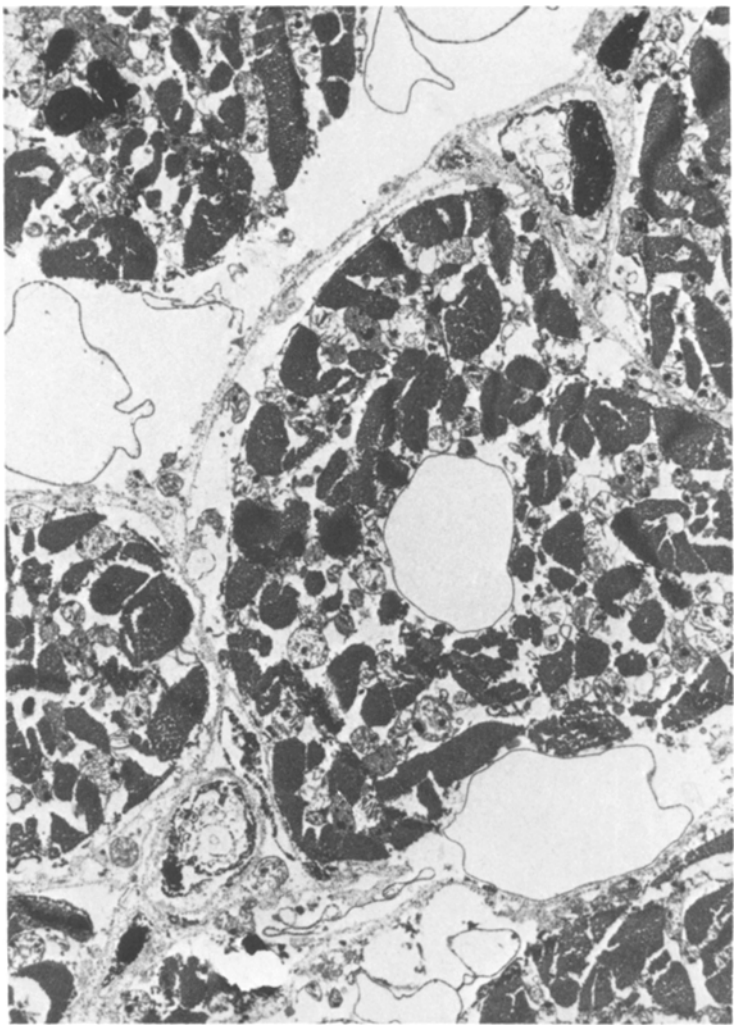


Fig. 2. Intra- and extracellular gas bubbles of varying size within myocardial tissue in fatal decompression sickness. Electron micrograph, following formaldehyde fixation. ×2500

experienced paresthesias of his hands and legs, progressing to hemiparesis. During the ensuing confusion, the chamber was suddenly opened at a pressure equivalent of a depth of 9 meters below the surface, resulting in an acute reduction of the environmental pressure. In the following two days, five of the twenty patients died of acute decompression sickness. All subjects were submitted for a particularly thorough post mortem examination.

The clinical data, containing noteworthy contraindications to this type of therapy and the light microscopic histological findings have already been presented (Richter and Löblich, 1978). Post mortem examination of the pleural cavity and the thoracic contents were performed under water in the usual manner (Richter, 1905). Samples from all organs were obtained for histopathology. These were fixed in neutral formaldehyde, embedded in paraplast and stained with hematoxylin-eosin and van Gieson staining. For electron microscopy, the formalin fixed samples were washed in cacodylate buffer (pH 7.2) 10–12 times over 8 h, cut into 1 mm pieces with a razor plate, postfixed

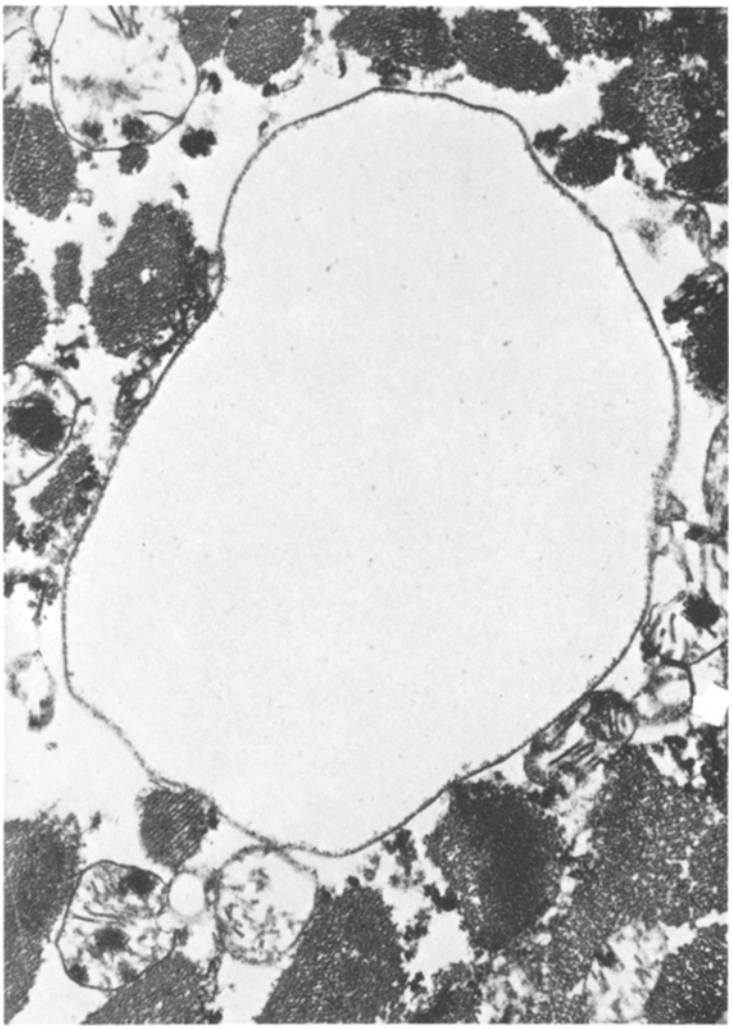


Fig. 3. Sharply outlined intracellular gas bubble adjacent to myocardial fibres in fatal decompression sickness. Electron micrograph, following formaldehyde fixation. × 5000

with 1% osmium tetroxide and embedded in Epon 812. Semi-thin and ultra-thin sections were cut on the ultramicrotome LKB 3. These ultra thin sections were then examined with a transmission electron microscope (Philipps EM 201) after lead citrate staining.

Results

Histologic examination revealed a diffuse distribution of gas bubbles in the entire cardiovascular system and profound emphysema of the skin. The bubbles were observed in almost all prepared sections of the brain, lungs, heart muscle (Figs. 1–4), kidneys and the liver (Fig. 5). Utilizing electron microscopic prepara-

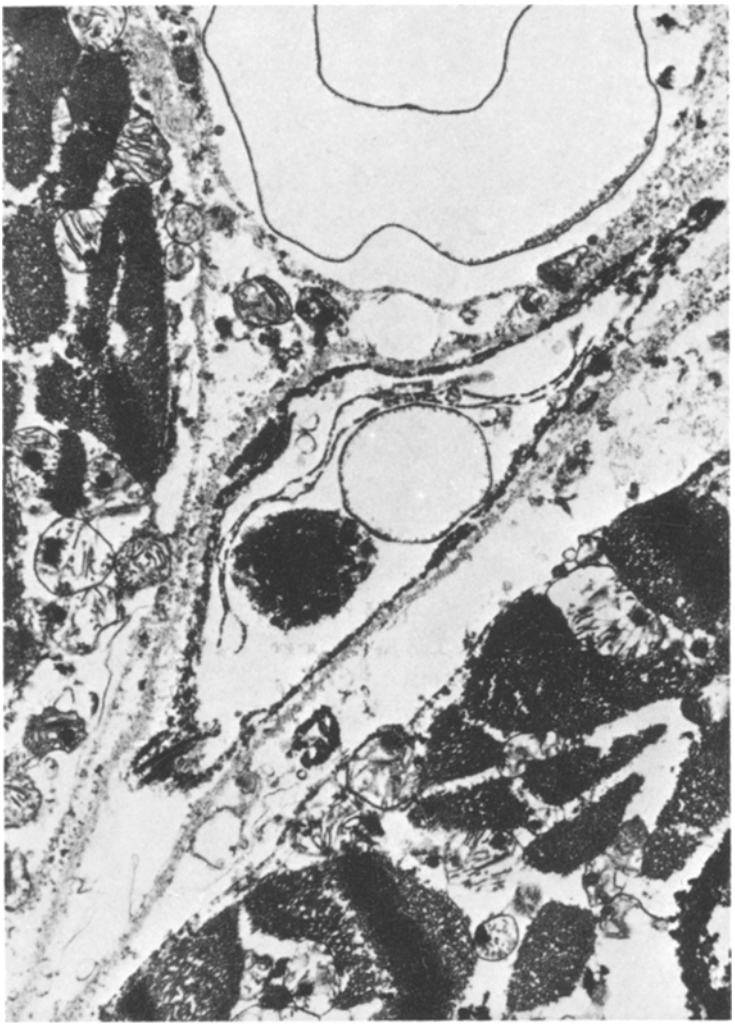


Fig. 4. Intra- and extravascular location of gas bubbles with deforming and partially compressing the wall of the vessel. Electron micrograph, following formaldehyde fixation. × 5000

tions, we found the bubbles to be of varying size. The smallest had a diameter of 30 Å. All the bubbles were covered by a non-homogeneous substance, consisting of osmiophilic material (Fig. 6). This covering measured from 30 Å to 560 Å in breadth (Figs. 7 and 8), and in some areas within the envelope there seemed to be parallel layers of membranes (Fig. 7), consisting of cloudy or flocculent material (Fig. 8). The bubbles were observed to be ubiquitiously distributed throughout all organs, within the interstitium and the cells themselves. They were also seen in the dense connective tissue obtained from the periarticular region of the knee (Fig. 9).

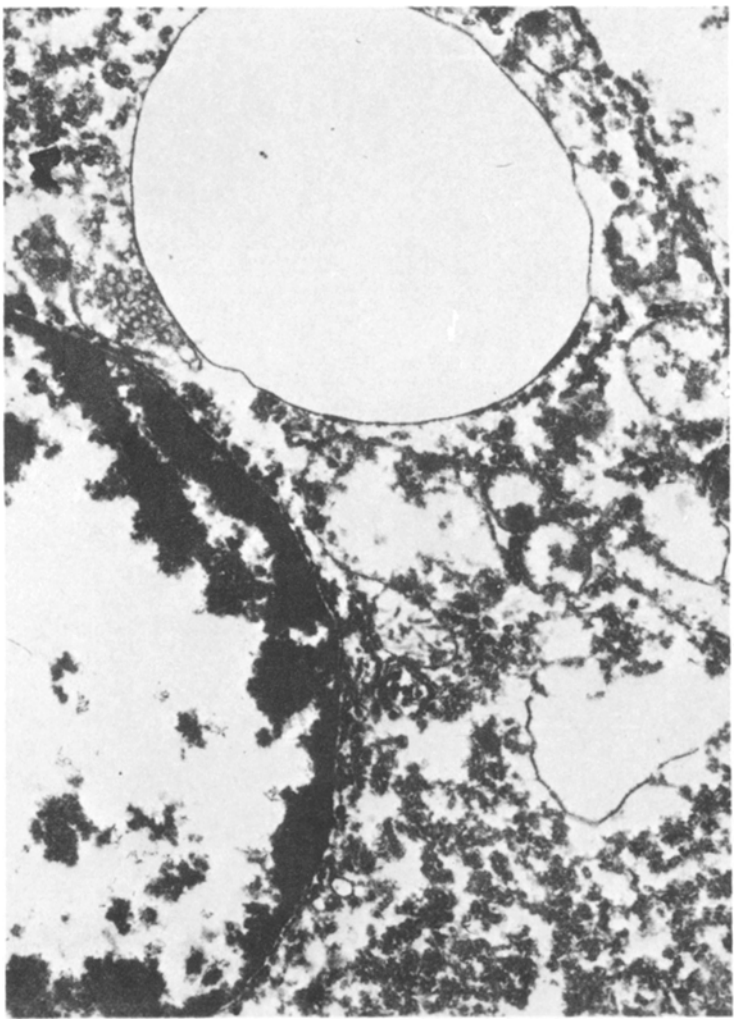
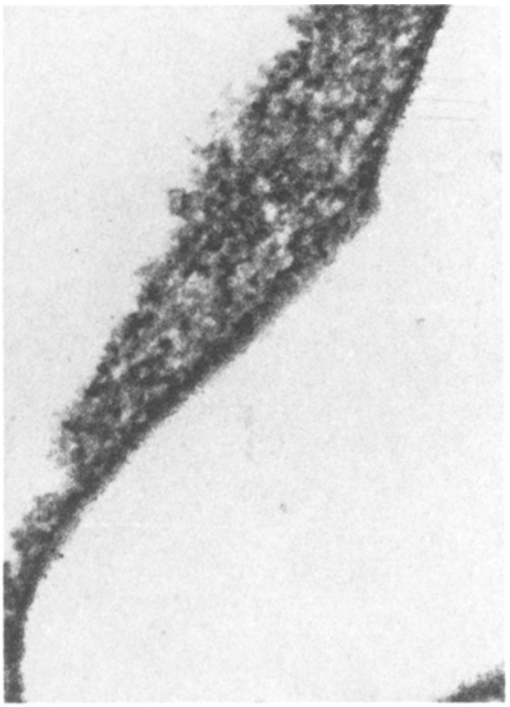


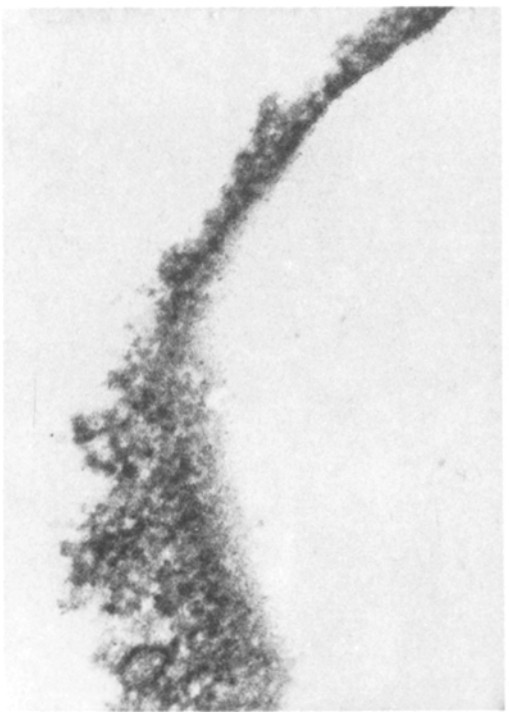
Fig. 5. Hepatocyte. Intracellular development of gas bubbles in fatal decompression sickness of human beings. Electron micrograph, following formaldehyde fixation. × 8000

Discussion

The clinical symptoms of decompression sickness, following acute reduction of environmental air pressure are well known (Boyle, 1670; Bert, 1878; Boycott, Damant and Haldane, 1908; Lewis, 1972; Hart, 1974; How and Edmonds, 1976; and others). However, the sources of and the contributing factors to the occurence of the gas bubbles are still unknown (Paganelli et al., 1977).

Electron microscopic observation of samples of human tissue obtained from subjects following fatal acute decompression sickness demonstrate a covering





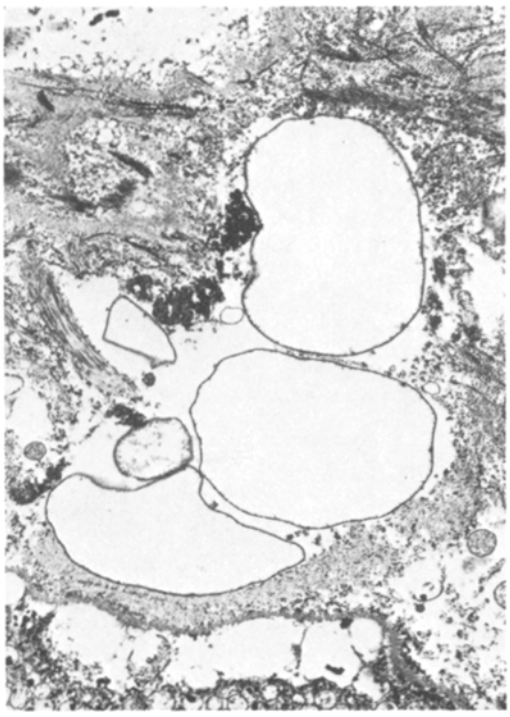


Fig. 6. Collapsed gas bubble with an osmiophilic covering coat in fatal decompression sickness. Electron micrograph, following formaldehyde fixation. ×4000

Figs. 7 and 8. Flocculent inhomogenous osmiophilic substances and incomplete parallel layers, forming surface coat at the gas-tissue-interface. Higher magnification of Figure 8. ×200,000

Fig. 9. Extensive gas bubbles between the collagen fibres in dense connective tissue from the periarticular region of the knee. Fibres were ruptured and distorted. Electron micrograph, following formaldehyde fixation. ×1500

of all gas bubbles. This covering consists of a non-homogeneous osmiophilic material appearing cloudy or flocculent and tending to be arranged in parallel layers of membranes. This envelope is of varying thickness ranging from 30 Å up to 560 Å. The apparent pathophysiological process leading to their formation results from the expansion of the bubbles and the effects of the resultant pressure on the surrounding tissue. Besides an enlargement of pre-existing bubbles, we also assume a de-novo development of gas bubbles and so called gas nuclei. We consider the osmiophilic material covering the bubbles to be a pseudo-coat; it is unreasonable to assume that gas could build its own jacket. This inability of bubbles to provide their own membrane, gives rise to the questions regarding its nature. The pseudomembrane seems to consist of material native to its specific locality, appearing to be made out of the matrix and components of the cells and interstitial substance. This would explain the variable thickness of the coat and its lack of homogeneity within the surface of each bubble.

Differing theories of the origin of the bubbles give rise to varying hypothesis (Paganelli et al., 1977). Presently so-called gas nuclei seem to be the focus of investigators interest. Harvey et al. (1944) tried to solve the problem by physical calculations, since in his opinion gas bubbles could not penetrate through an intact cell membrane. We observed large numbers of cells in various organs with intracellular gas bubbles in our material, without electron microscopic evidence of altered cell membranes. Harvey et al. (1946) performed experiments with high pressure (up to 80 ata) and were convinced that gas bubbles can only develop on the basis of preexisting gas nuclei. These nuclei, they postulated, do not need any pressure gradient for their development. 1975 a Lancet Editorial mentioned "nuclear fission" as a possible source for the development of such gas nuclei, and as possible explanation for the variable susceptibility of human beings to decompression sickness when allowance is made for obesity, cardiovascular disease and pre-existing illness (Fulton, 1951; Fryer, 1962; Richter and Löblich, 1978). Lee and Hairston (1971) found, at the gas-blood-interface, a zone of biophysically measured electrokinetic forces from 40 to 100 Å wide. This zonal activity may be identical with the electron microscopically visible surface coat. Thombocytes could cling to this cover, resulting in the brakdown of platelets, producing haematologic alterations (Martin and Nichols, 1972; Fratalli et al., 1977). Amoung others Vroman et al. (1973) focused the problems about aggregations of platelets and the conditions for their adhesion to surface protein layers. They used materials like special prepared glass, anodized tantalum sputtered glass slides ("TaO"), silicon crystal slices, hydrophobic glass and TaO and several human proteins. Differently conditioned interfaces were the subject of their investigations. In result of their observations they presume a monomolecular protein layer, probably consisting of fibrinogen and some globulins, that is forming against the air within 2 s. They suggest that this surface coat gives rise for adhesion of platelets resulting in a rapid thrombocytopenia. The intire process should be very complex and remains unclear more or less. The protein film forming at the bubble-blood interface following decompression is assumed to be approximate 200 Å thick. This suggestion is confirmed by our electronenmicroscopic observations intirely. Moreover, this coat, consisting as it does of indigeneous cellular material and

proteins would garantee a long survival period for the bubbles within the cells, the interstitial tissue, and the blood. In addition, the envelope could prevent the nitrogen from being eliminated via the blood-lungbarrier. If this hypothesis is valid, then new prospects for therapy may lie in developing methods by which these surfaces may be broken down and the bubbles eliminated.

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