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(Director: Prof. Dr. med. H. MEESSEN)

The Ultrastructural Effects of Carbon Monoxide Inhalation on the Rat Lung

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With 4 Figures in the Text

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The combination of carbon monoxide and hemoglobin (carboxyhemoglobin) causes a decrease in the oxygen transport capacity of blood resulting in tissue hypoxia that produces parenchymal necrosis in various organs. Carboxyhemoglobin also alters the oxyhemoglobin dissociation curve with impaired release of oxygen at the tissues further aggravating the hypoxia (ROUGHTON and DARLING). Since the early work of HALDANE (1), it has frequently been said that, except for this ability to combine with hemoglobin, carbon monoxide acts in man as a physiologically inert gas (LILIENTHAL).

Although the above statement would appear to be true from results of mammalian experiments, several investigators have demonstrated that carbon monoxide can produce inhibition of respiratory enzymes of lower, non-mammalian forms of life (LILIENTHAL, AMOORE). High pCO (2 atmospheres) was found to be toxic for rats even when an adequate supply of oxygen was available dissolved in plasma [HALDANE (2)]. In addition a recent report has demonstrated the ability of cytochrome-C to reverse the reduction of oxygen consumption in carbon monoxide poisoned mammalian tissue slices (DUTKIEWICZ, GWÓZDŹ, SPETT and SPIOCH 1960). Also many tetrapyrol respiratory pigments (hemes, oxidases, catalases) have an affinity for carbon monoxide.

Thus it is possible that selective effects of carbon monoxide on man may result not from hypoxemia alone, but from the direct poisoning of specific respiratory enzymes of the cell by carbon monoxide.

In order to investigate this possibility the following experiment, as suggested by Prof. MEESSEN, was conducted on rats poisoned by the inhalation of carbon monoxide. The ultrastructural pulmonary changes noted, although similar to the effect of hypoxemia on the lung, suggest that carbon monoxide does indeed have a direct, specific effect on mammalian pulmonary tissue.

Methods

Male Wistar rats weighing 160 to 215 gm were placed in large glass cylinders (I.D. 7.0 cm) through which 0.5% to 1.0% carbon monoxide in air was passed at a rate of approximately 300 to 400 ml per minute. Two rats (2 and 3) were allowed to die spontaneously from carbon monoxide poisoning. The remaining four rats were paired (No 4 and No 5, No 6 and No 7). Each rat of a pair was placed in a glass cylinder connected in parallel to one another. The

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parallel cylinders were joined to a single source of carbon monoxide so that identical concentrations and flow of carbon monoxide in air were delivered to each of the paired rats. One rat of each pair was killed by decapitation after fifteen and thirty minutes of carbon monoxide inhalation respectively, the other rat of each pair was allowed to die spontaneously from carbon monoxide poisoning. One normal rat served as a control.

Spectrophotometric determinations of blood carboxyhemoglobin were made on each rat at the time of death[1].

Samples of tissue from the dorsal and ventral (nondependent and dependent) portions of the lung were taken from each rat and prepared for electron microscopy by fixation for two hours in 1.0% osmium tetroxide solution buffered at pH 7.4 (PALADE 1952).

The tissue was dehydrated through a graded series of acetone and embedded in Vestopal-W. Ultra-thin sections were cut on a Porter-Blum microtome with glass knives and mounted on Formvar coated copper grids. Unstained and uranyl acetate stained sections were examined with an RCA-EMU-3 G electron microscope at 50 kV[2].

The remains of the left lung and the entire right lung were fixed in formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for examination with the light microscope.

Results

The results are summarized in the Table. Four of the rats died spontaneously after 13, 30, 137 and 42 min of breathing 1.0%, 0.75%, 0.5% and 0.5% carbon

Table. *Summary of the effects of carbon monoxide inhalation on rat lung*

Rat	CO inhaled	Duration	CO Hb (Blood)	Electron Microscopy			Light Microscopy See Text Results
				Edema Membrane Disrupt.	Cell-Mitochondrial Changes	Platelets, Platel. Thromb.	
1 (Control)	—	—	—	0	0	0	0
2	1.0%	13 min spontan. Death	70%	+++++	++++	+++++	+
3	0.75%	30 min spontan. Death	62%	++++	+++	++++	++
4	0.5%	15 min killed	59%	+	+	0	+
5	0.5%	137 min spontan. Death	67%	+++	++	+++ Platel. Accumulation, no Thrombosis	+++
6	0.5%	30 min killed	55%	+	+	+ Platelets only, no accumulation	+
7	0.5%	42 min spontan. Death	66%	++	+	++ rare platelet accumulation	+

[1] Analyses kindly performed by Dr. H. J. WULF, Gerichtsmedizin. Institut der Medizinischen Akademie Düsseldorf (Direktor: Prof. Dr. med. R. MANZ).

[2] We are grateful to Miss LIESELOTTE HENSEL for her technical assistance.

monoxide respectively. Two rats were killed after 15 and 30 min of ventilation with 0.5% carbon monoxide respectively. Two rats were killed after 15 and 30 min of ventilation with 0.5% carbon monoxide. Blood carboxyhemoglobin

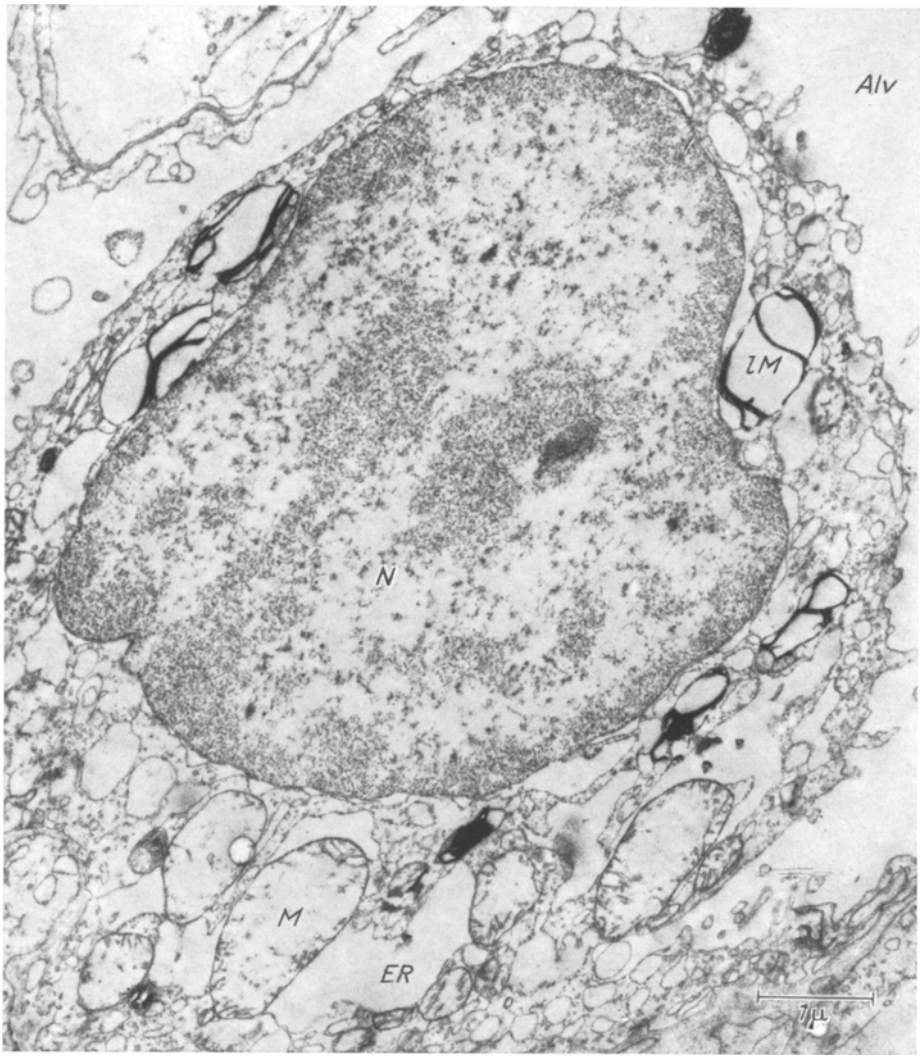

Fig. 1. Rat lung. Spontaneous death after 13 min of 1.0% carbon monoxide inhalation. Alveolar epithelial cell with swollen mitochondria (*M*), swollen endoplasmic reticulum (*ER*) and osmiophilic inclusions. *N* nucleus with swollen nucleoplasm. *Alv* pulmonary alveolus. *lM* lamellar transformed mitochondria. Archive No: II/556D. Electron microscopic magnification 4250:1, final magnification 15850:1. (Reduced to $^{19}/_{20}$)

ranged between 70% (after 13 min of 1.0% carbon monoxide) and 55% (after 30 min of 0.5% carbon monoxide).

Light microscopic findings of the lungs consisted of hyperemia, focal intra-alveolar edema, interstitial thickening and early focal infiltration with polymorphonuclear leukocytes. Vascular thromboses were not evident. The comparative results between the rats are summarized in the Table.

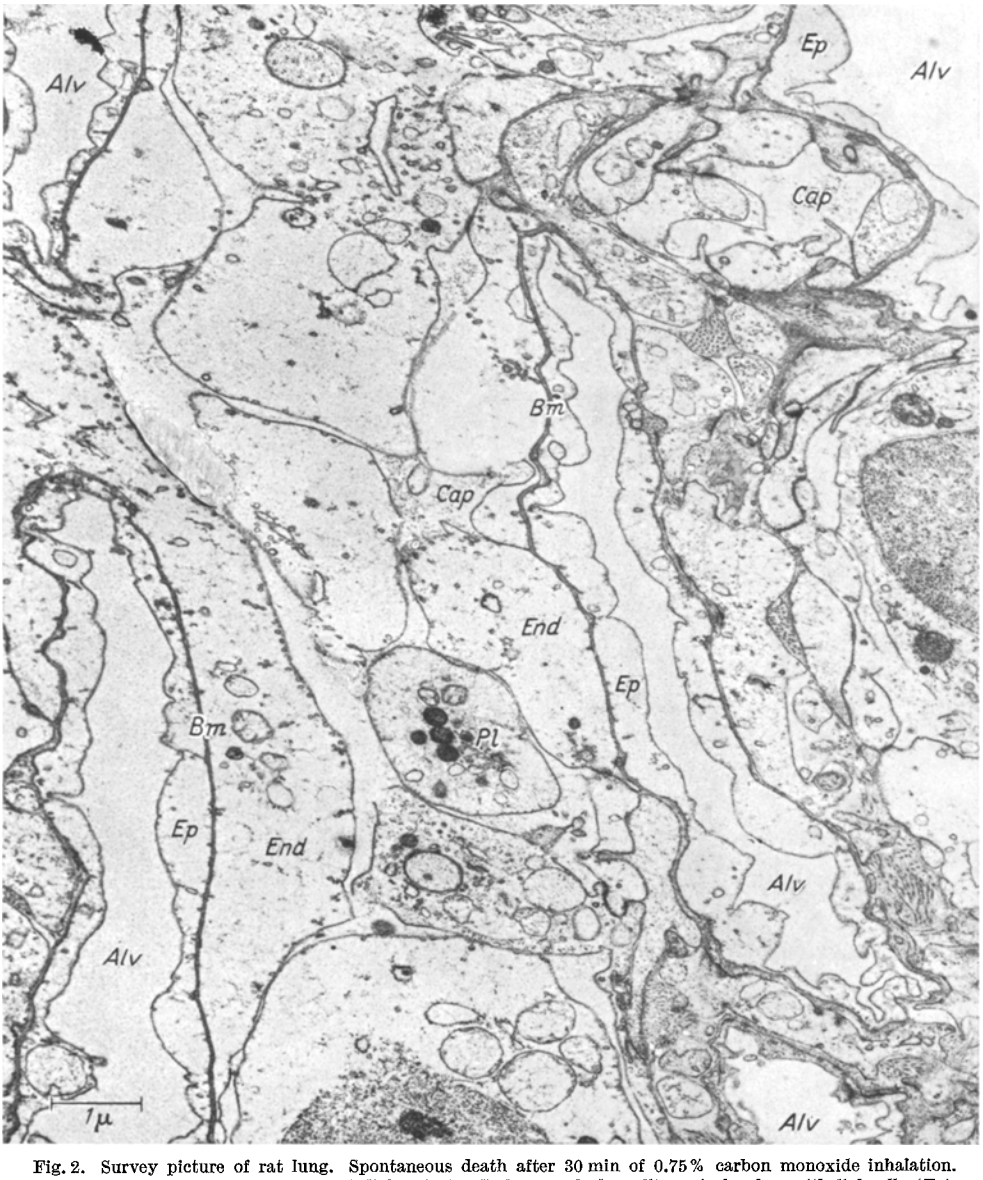

Fig. 2. Survey picture of rat lung. Spontaneous death after 30 min of 0.75% carbon monoxide inhalation. Extensive swelling of capillary endothelial cells (*End*), less marked swelling of alveolar epithelial cells (*Ep*). *Bm* basement membrane. Marked thickening of the blood-air pathway. *Pl* platelet. *Cap* pulmonary capillaries. *Alv* pulmonary alveoli. Archive No: II/636 A. Electron microscopic magnification 3450:1, final magnification 13000:1. (Reduced to $^{9}/_{10}$)

The *ultrastructural alterations* in the rats' lungs consisted of alveolar epithelial cell changes, marked capillary endothelial and alveolar epithelial edema, and capillary platelet thrombosis.

Epithelial cells (Fig. 1) revealed extensive swelling and vacuolization of the mitochondria and swelling of the endoplasmic reticulum. In addition swelling of the nucleoplasm and nuclear envelope were frequently noted with separation between the primary and secondary nuclear membranes.

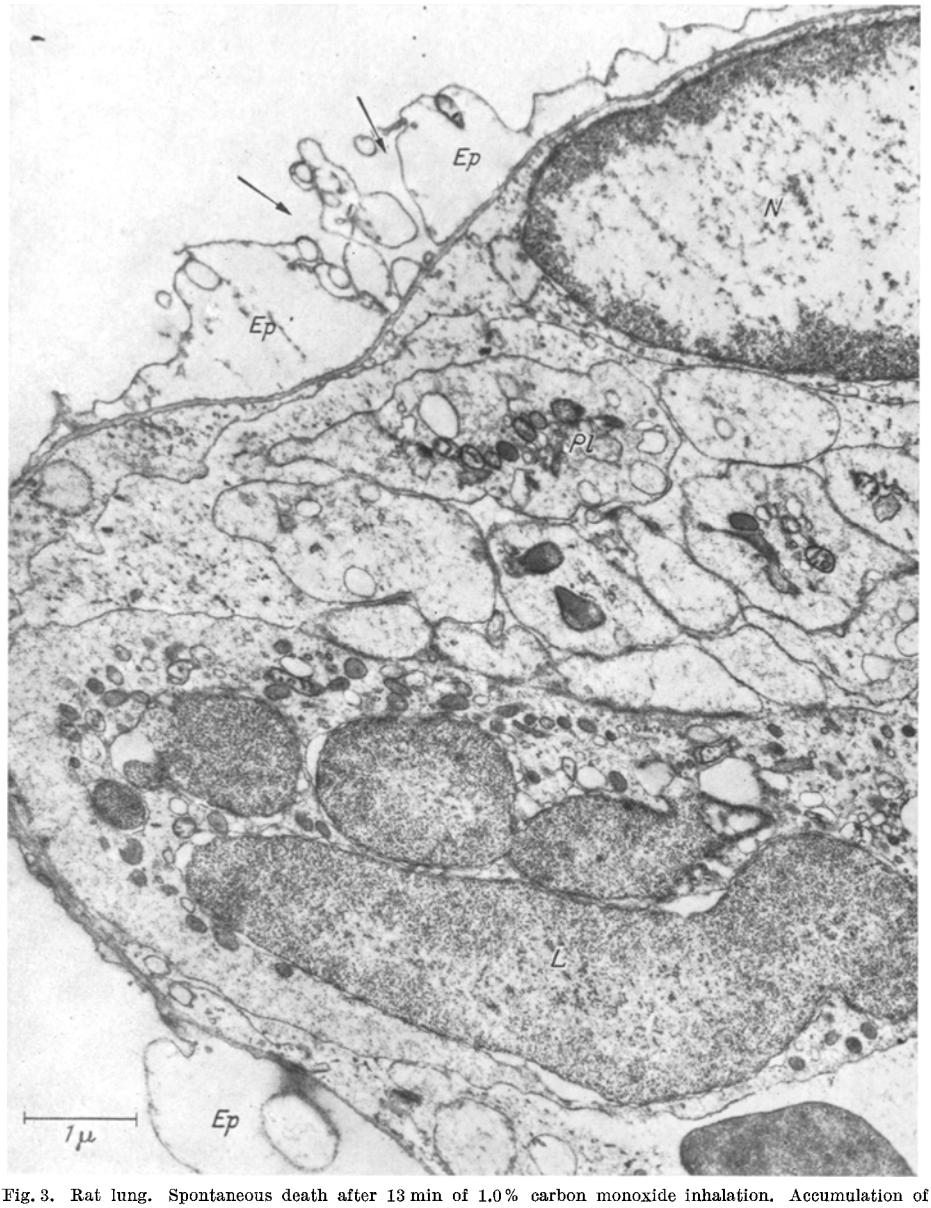

Fig. 3. Rat lung. Spontaneous death after 13 min of 1.0% carbon monoxide inhalation. Accumulation of platelets (*Pl*) in a pulmonary capillary. Some platelets are degranulated. *L* polymorphonuclear leukocyte. *N* swollen nucleus of an endothelial cell. *Ep* swollen epithelial cells with separations at the arrows (↓ ↓). Archive No: II/609 C. Electron microscopic magnification 4250:1, final magnification 16000:1. (Reduced to $^{19}/_{20}$)

Lamellar transformed mitochondria and osmiophilic inclusions of cell remnants were often present (SCHULZ 1959).

The degree of capillary endothelial and alveolar epithelial *edema* was often quite severe and extensive, particularly in rats No 2 and No 3 (Fig. 2 and 3). Swelling of the alveolar epithelium occurred which, at times, resulted in a rounding off and separation of the lateral borders of adjoining epithelial cells (Fig. 3). This disruption of the otherwise continuous alveolar lining exposed the basement

membrane to the alveolus. Marked capillary endothelial swelling and large vesicle formation reduced many capillary lumina to narrow slits. Occasionally these vesicles (2—4 μ in diameter) with double contoured membranes appeared to lie free in the capillary lumen. Defects in the endothelial membrane lining

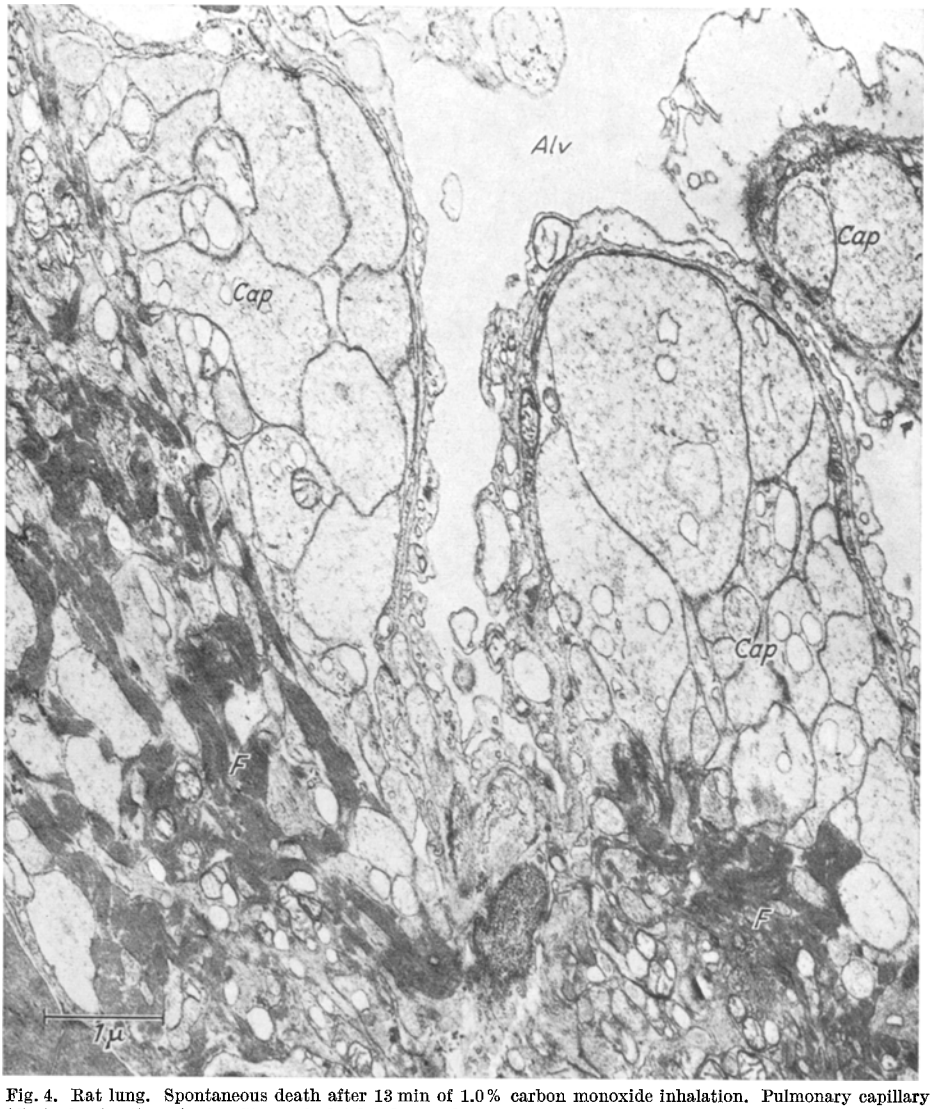

Fig. 4. Rat lung. Spontaneous death after 13 min of 1.0% carbon monoxide inhalation. Pulmonary capillary (*Cap*) platelet thrombosis. Stage of platelet degranulation and fibrin formation (*F*) at the periphery of the platelet mosaic. *Alv* pulmonary alveolus. Archive No: II/557 A. Electron microscopic magnification 4250:1, final magnification 15850:1. (Reduced to $^{19}/_{20}$)

the capillaries were not uncommon (Fig. 2). The blood-air-pathway was increased to 2,3 μ (normal range 0.13 to 0.26 μ). Most of this thickening was a result of swelling of the endothelium.

In two rats (No 2 and No 3) pulmonary capillary *platelet thromboses* were fairly widespread (Fig. 3 and 4). In another rat (No 5) platelet accumulation, an early

stage in the development of platelet thrombosis, was frequently encountered. All stages in the development of the capillary platelet thrombosis were observed: 1. platelet accumulation with an increased number and clumping of unaltered platelets in the capillaries (Fig. 3), 2. platelet agglutination in which the thrombocytes fitted together in a mosaic of platelets, 3. platelet degranulation in which there was a dissolution of the fine structures and vacuolization of the thrombocytes at the margins of the platelet mosaic (Fig. 4), and finally 4. fibrin formation at the periphery of the platelet mosaic, being seen only after the third stage of platelet degranulation.

In the Table, a comparison has been made of the ultrastructural alterations in the rats' lungs following inhalation of carbon monoxide. Although all of the changes described, with the exception of capillary platelet thrombosis, were noted in all of the experimental rat lungs, the differences in severity and frequency of the alterations between rat No 2 (and to a lesser extent rat No 3) and the remaining rats were striking (Table).

Mitochondria were observed in one immature red blood cell.

The severity and frequency of the changes noted do not appear related to the level of blood CO Hb (Table, column 4, cf. rats No 2 and No 3 with rats No 5 and No 7) nor to the duration of exposure (Table, column 3, cf. rats No 2 and No 3 with rat No 5). However the alterations observed do appear related to the concentration of carbon monoxide inhaled (Table, column 2, cf. rats No 2 and No 3 with rats No 5 and No 7).

Discussion

The primary alterations observed in rat lungs after the inhalation of 0.5% to 1.0% carbon monoxide for 13 to 137 min consist of: 1. epithelial cell changes, the most striking being swelling of the mitochondria, 2. edema of the capillary endothelium and alveolar epithelium, and 3. capillary platelet thrombosis.

Swelling and vacuolar alterations of the mitochondria as noted in this study indicate a non-specific injury to the cell demonstrating alterations in the multienzyme systems of the mitochondrial membranes. Lamellar transformation of the mitochondria reported here occurs almost exclusively in the alveolar epithelial cells of the lung. These pulmonary mitochondrial changes have been seen especially with hypercapnea (SCHULZ) and thus do not appear to be specific for carbon monoxide poisoning.

The ultrastructural manifestations of pulmonary edema after the inhalation of carbon monoxide consist of alveolar epithelial swelling, capillary endothelial swelling and vesicle formation. These findings are similar to the description of pulmonary edema produced experimentally by other means including hypoxia (MEESSEN and SCHULZ; GIESEKING; KISCH; MAGNUS, SCHEUNEMANN and SCHULZ). Submicroscopically, mechanical and chemical produced pulmonary edema appear the same. Thus the presence of lung edema can not in itself be said to be characteristic of a direct toxic effect of carbon monoxide.

The development of this intracellular swelling is quite rapid; it is already well advanced within 13 min. Many of the capillaries are partially or almost completely occluded by the swelling and vesicle formation in the capillary endothelium. Although the pulmonary vascular bed is quite distensible with many

capillaries normally closed, the dynamic changes noted above raise the question of the significance of this swelling in increasing pulmonary vascular resistance.

The occurrence of pulmonary capillary platelet thrombosis was a most interesting finding. Pulmonary capillary platelet thromboses have been reported following extracorporeal circulation, hypoxia, hyperoxia [SCHULZ (1)], whole lung irradiation, the intravenous injection of snake venom and fat infusion with Lipofundin [SCHULZ (2)].

The present observations in the development of capillary platelet thrombosis are in agreement with the above reports. Platelets accumulate in those capillaries with endothelial membrane alterations. Thus the initiating factor in the development of platelet thrombosis after the inhalation of carbon monoxide appears to be injury to the capillary wall. This observation supports the conclusion that carbon monoxide produces cerebral and coronary thromboses as a result of capillary wall damage (DRINKER). V. WACHTER and MARCACCI; GUARINO and IORIO reported on the hemorrhagic diathesis and on alterations in the blood clotting mechanism following carbon monoxide poisoning.

SCHULZ and HIEPLER have demonstrated that thrombokinase and the platelet accelerator factor are found in the granulomer fraction of the thrombocyte. In accordance with this finding, fibrin formation at the periphery of the platelet mosaic is not observed until the fine structures of the platelet are emptied out of the thrombocytes. The dense material in Fig. 4 consists of both a fibrin precursor and fibrin. Fibrin can be distinguished from its precursor by the presence of fine cross striations in the former indicative of the second stage of fibrin polymerization (KÖPPEL).

The rapidity of the above reaction is demonstrated by the presence of polymerized fibrin present in the capillary platelet thrombus within 13 min of carbon monoxide inhalation. This indicates that the development of the platelet thrombus must have started within the first few of minutes of carbon monoxide inhalation.

It is of interest that a bone marrow response was detected in the peripheral blood within 13 min of a carbon monoxide-hypoxic stimulation by the presence of a circulating immature red blood cell.

Qualitatively, all of the pulmary alterations described as a result of carbon monoxide poisoning have been observed with hypoxia. However the severity of the changes are out of proportion to an equivalent degree of hypoxemia alone. More important, a comparison of pulmonary changes in the rats reveals that the level of CO Hb, an index of the severity of hypoxia, and the duration of exposure to carbon monoxide are less important than the concentration of inhaled carbon monoxide in producing these alterations (Table). The findings suggest that carbon monoxide has a direct effect on the pulmonary tissue in addition to the indirect effect of tissue hypoxia. The severity of these changes occurring within 13 min implies that this effect, heretofore masked by the response to anoxia, may be more significant than previously suspected. Thus, the present study demonstrates the need for further experiments on the direct effect of carbon monoxide on the lung.

Summary

The effects of carbon monoxide inhalation on the ultrastructure of rat lungs were investigated. Rats were exposed to 0.5% to 1.0% carbon monoxide for 13 to 137 min.

The pulmonary ultrastructural alterations consisted of: 1. swelling of alveolar epithelial cell mitochondria and nucleoplasm, 2. capillary endothelial and alveolar epithelial swelling and 3. capillary platelet thrombosis. Due to the swelling of the epithelium defects are formed in the alveolar lining and the basement membranes of the capillaries are no longer covered with epithelium. As a result of the marked swelling of endothelium and epithelium the lumina of the capillaries and alveolar spaces appear to be narrow and slit-like.

The concentration of inhaled carbon monoxide was more important than the level of blood CO Hb and the duration of exposure to carbon monoxide in producing these changes. The results suggest that carbon monoxide has a direct effect on pulmonary tissue.

Die Wirkungen einer Kohlenmonoxyd-Atmung auf die Ultrastruktur der Rattenlunge

Zusammenfassung

Die Wirkungen einer Kohlenmonoxyd-Atmung auf die Ultrastruktur der Rattenlunge wurden elektronenmikroskopisch untersucht. Mehrere Ratten atmeten 13—137 min 0,5—1,0% CO, vermischt mit Luft. Die wichtigsten Veränderungen des Lungengewebes bestehen in einer Schwellung der Mitochondrien der Alveolarepithelien, in einer deutlichen Schwellung des Capillarendothels und des Alveolarepithels sowie in einer capillaren Plättchenthrombose. Durch die Schwellung der Epithelien entstehen Defekte in der Auskleidung der Lungenalveolen. Durch die erhebliche Schwellung der Endothelien und Epithelien werden die Lichtungen der Capillaren und der Alveolen eng und spaltförmig.

Für das Auftreten dieser Veränderungen ist die Konzentration des eingeatmeten CO wichtiger als die Konzentration des CO-Hb und als die Dauer der Gaseinwirkung. Die Ergebnisse lassen vermuten, daß CO unmittelbar auf das Lungengewebe einwirkt und Veränderungen am Lungengewebe hervorruft.

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