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Alpha-methyl-homocysteine thiolactone protects trachea and lung of BALB/c mice irradiated with 6 Gy – A qualitative morphological study

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Summary. Alpha-methyl-homocysteine thiolactone (AMHCTL) was shown to increase survival of irradiated bacteria. Based upon these observations we administered 100 mg/kg body weight intraperitoneally female BALB/c mice prior to whole body irradiation from a Co-source at a dose of 6 Gy. Unirradiated mice as well as irradiated and L-cysteine and saline pretreated mice served as controls. Light microscopical, scanning electron microscopical and transmission electron microscopical examinations showed a radioprotective effect: Tracheal epithelium including ciliae were preserved in the experimental group, studies on the lung revealed radiationprotection as no transudation or exsudation and no proliferation of capillaries was detected.

Keywords: Amino acids – Radiation protection – Alpha-methyl-homocysteine thiolactone – Mice – Lung

Introduction

In a recent report we described the effect of alpha-methyl-homocysteine thiolactone (AMHCTL) on the survival of irradiated bacteria. This homocysteine derivative was the most effective radiationprotector in our study (Mao et al., 1994).

AMHCTL is an alpha alkylated amino acid and therefore not being metabolized in mammalian systems (Noall et al., 1957). One of the advantages is that low doses are required, another is that the compound is more lipophilic and thus able to cross biological barriers. The mechanism of radiationprotection of homocysteines and other sulfur containing amino acids is not fully elucidated yet. According to current concepts they act as free oxygen radical scavengers but as they protect bacteria against high doses of irradiation also in the absence

of oxygen i.e. under argon, proton donation and action on repair mechanisms is more likely (Mao et al., 1994; Roberts, 1992; Prager et al., 1993; Clark, 1989).

We were interested to test the radiationprotection activity in the mammalian system and decided to study morphology of the effects of AMHCTL on lungs of whole body irradiated mice; AMHCTL proved to be a potent radiation protector.

Materials and methods

32 female mice, BALB/c, mean weight 20.2 g, mean age 8 weeks, were used in the experiments. These animals were divided into four groups; the first group obtained 1 ml isotonic saline intraperitoneally, the second 100 mg/kg body weight of alpha-methyl-homocysteine thiolactone (synthesized by one of our group), the third group 100 mg/kg body weight L-cysteine (from Sigma) in 1 ml intraperitoneally one hour prior to irradiation. The fourth group obtained 1 ml of isotonic saline intraperitoneally without irradiation. Whole body irradiation was performed administering 6.02 Gy using a Crisobald Co source. Animals were sacrificed by neck dislocation and exsanguinated.

Lung specimen for morphological examinations were taken by thoracotomy. Specimen for light microscopical examinations were fixed by ethanol-formaldehyde-acetic acid in the ratio of 12 : 6 : 1. 5 μm paraffin sections were stained by a) hematoxylin-eosin, b) after van Gieson and c) by azocarmine-aniline blue method.

Biopsies for scanning electron microscopy were fixed by double fixation with phosphate buffered 200 mM glutardialdehyde and 40 mM osmiumtetroxide according to a standard procedure. The scanning electron microscope used was a REM BS300 (Tesla Brno) with accelerating voltage of 25 kV using magnifications from 6000–15000x.

Biopsies for transmission electron microscopical studies were doublefixed as for scanning electron microscopy and handled by a routine method. The Transmission electron microscope JEM/EX 1200 (Jeol, Japan) was used with an accelerating voltage of 80 kV and magnifications from 2000–25000x.

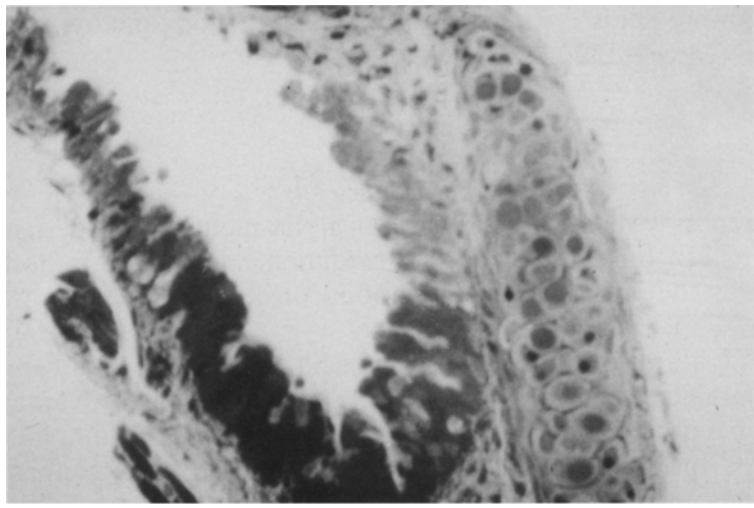


Fig. 1. Trachea of the control group as shown by toluidinblue stained semithin section, normal histoarchitectonics (x360)

Results

Studies on tracheal tissue

a) Light microscopy

The normal histological pattern of a tracheal section out of 8 nonirradiated animals (group 4) is shown in Fig. 1. Treatments with either cysteine (group 3) or with isotonic saline (group 1) prior to irradiation showed necrotic areas of the tracheal epithelium as well as of cartilage (Fig. 2). Figure 3 reveals the

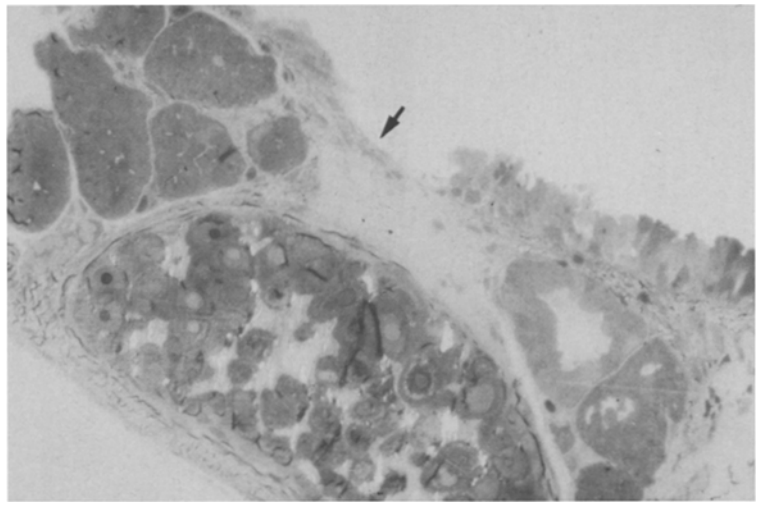


Fig. 2. Trachea after whole body irradiation. Note the damaged epithelium with an exulceration (arrow) and altered cartilage (x360)

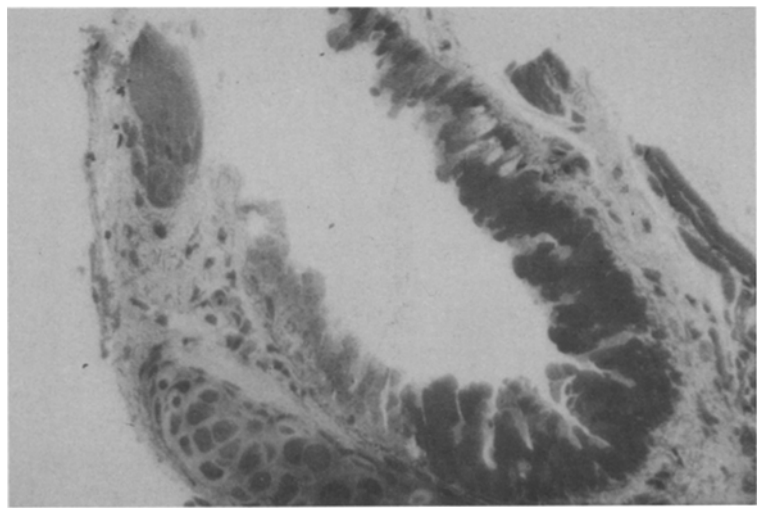


Fig. 3. Trachea, AMHCTL pretreatment resembling the normal control in Fig. 1

protective effect of AMHCTL in group 2 revealing a pattern comparable to the healthy nonirradiated controls in group 4.

b) Scanning electron microscopy

As shown in Fig. 4 control animals of group 4 showed intact ciliae. Irradiation with saline or cysteine as premedication led to severe degenerative alterations of the mucociliary epithelium in groups 1 and 3 (Fig. 5). Group 2 treated with AMHCTL showed a pattern comparable to healthy controls (Fig. 6).

Fig. 4. Scanning electron microscopical (SEM) appearance of normal tracheal ciliae (6000x)

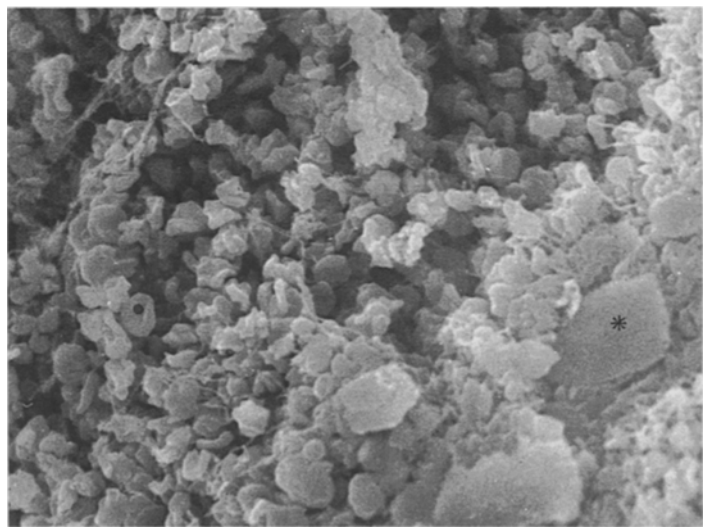


Fig. 5. Trachea, SEM appearance after irradiation. Destroyed ciliae, imbibition with blood corpuscles (·) and stagnation of mucociliary transport (*). x6000

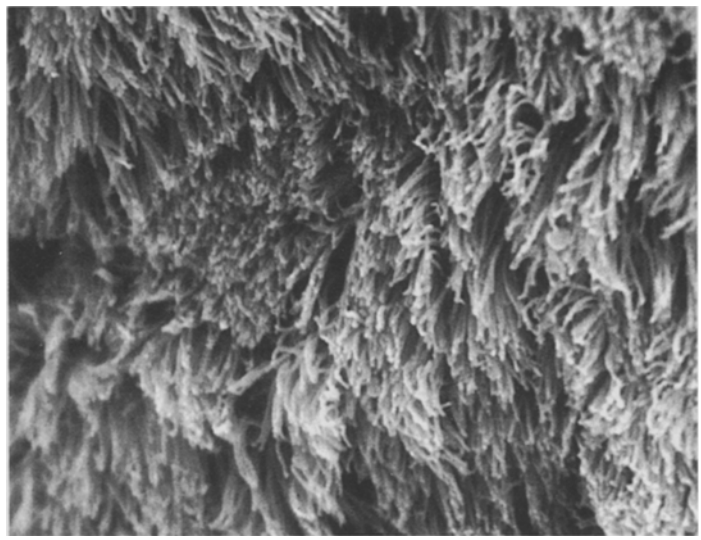


Fig. 6. Trachea, SEM appearance after irradiation but with AMHCTL pretreatment resembling the normal pattern (x6000)

Studies on lung tissue

a) Light microscopy

Figure 7A represents the findings in group 4, the control (nonirradiated) group demonstrating normal histological appearance of the lung.

Animals of group 1 and 3 showed severe alterations with dominating hyperemia, broadened alveolar septa, proliferation of capillaries, loss of histoarchitectonics, transudation into alveoli (Fig. 7B).

Pretreatment with AMHCTL in group 2 resembled the normal pattern but hyperemia is present (Fig. 7C).

b) Scanning electron microscopy

Group 4 revealed the normal pattern of healthy mouse lung (Fig. 8). In the AMHCTL treated group 2 alveolar septa are prominent, vessels congested but transudation or exudation is absent (Fig. 9).

Groups 1 and 3 showed prominent septa, vessels heavily congested but in addition regular and massive transudation occurred (Fig. 10).

c) Transmission electron microscopy

Animals of the control group 4 showed regular anatomical structures (Fig. 11). Animals of group 1 and 3 showed protruding of nuclei and cytoplasm of endothelial cells into the capillary lumen. The major finding is the proliferation of capillaries (Figs. 12A,B). Group 2 with AMHCTL treatment showed the protrusion of endothelial cells into the lumen as well but no signs of capillary proliferation (Fig. 13).

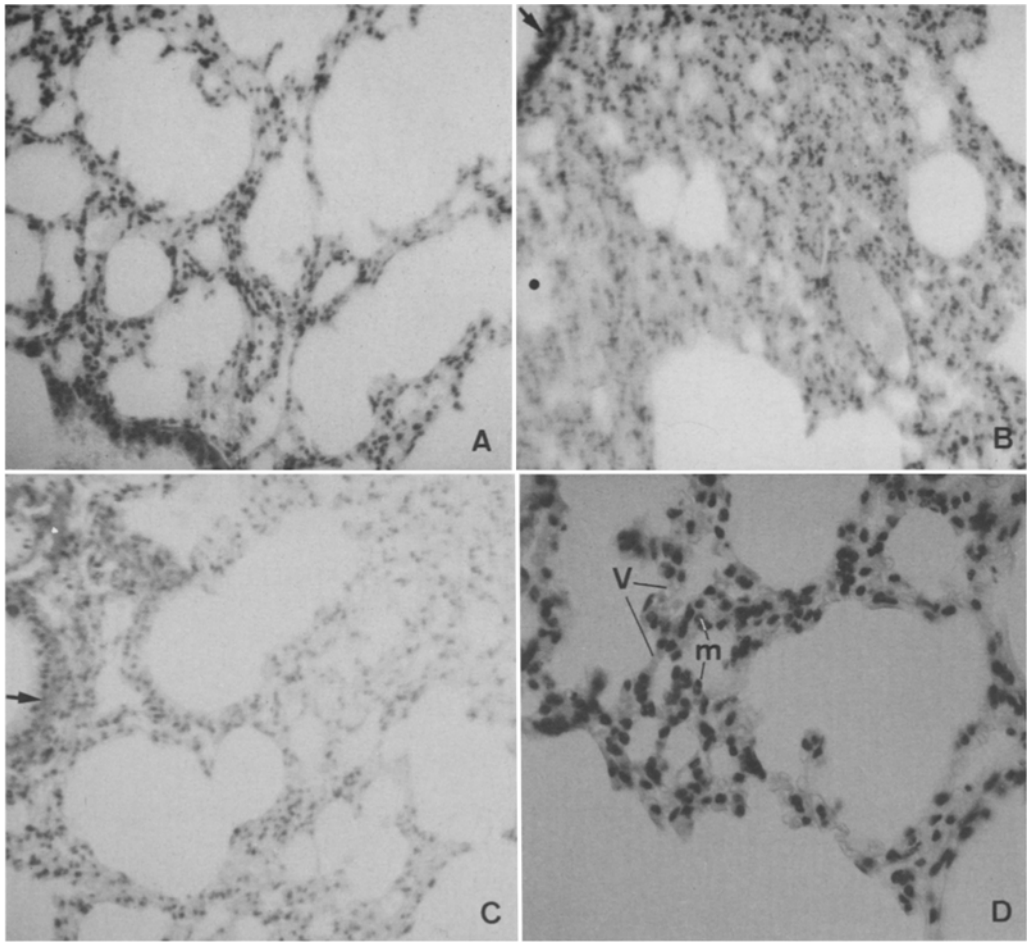


Fig. 7. A Microscopical features of the unirradiated control (stained by van Gieson, 200x). **B** Microscopical features after irradiation, group 1. Note the destruction of epithelia, necrotic areas, vanishing shape of boundaries between cells (arrow). Vessels congested (!), transudation into alveoli (·). **C** Microscopical features after irradiation and pretreatment with AMHCTL. Epithelial lining preserved as are boundaries (arrow). **D** Higher magnification from **C**, x360 showing prominent vessels (*V*) without transudation into the alveoli. Frequent mitotic activity (*m*)

Discussion

As shown in the results, untreated and cysteine treated animals revealed severe and typical (Coggle et al., 1986) histological changes 11 days after irradiation. Pretreatment with low dose alpha-methyl-homocysteine thiolactone was highly effective with respect to several criteria. The tracheal epithelium including ciliae and cartilage were unchanged.

In lungs boundaries between cells and histoarchitectonics were unchanged, no transudation into alveoli was found and no proliferation of capillaries was observed. These morphological changes can be assigned to the effect of treatment with AMHCTL. L-cysteine, the first described radiationprotector and a sulfur containing amino acid (Roberts, 1992) as well failed to protect trachea and lung

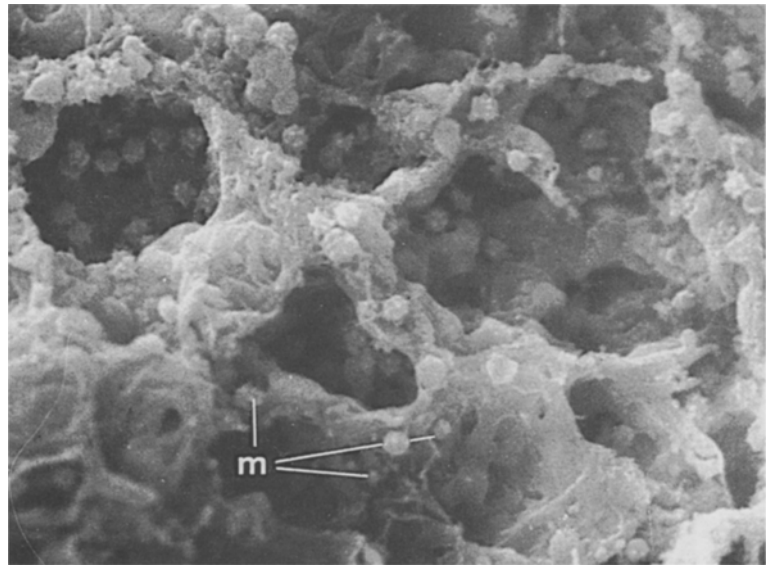


Fig. 8. SEM features of healthy control lung with normal structure and macrophages migrating through the alveolar wall (*m*), x6000

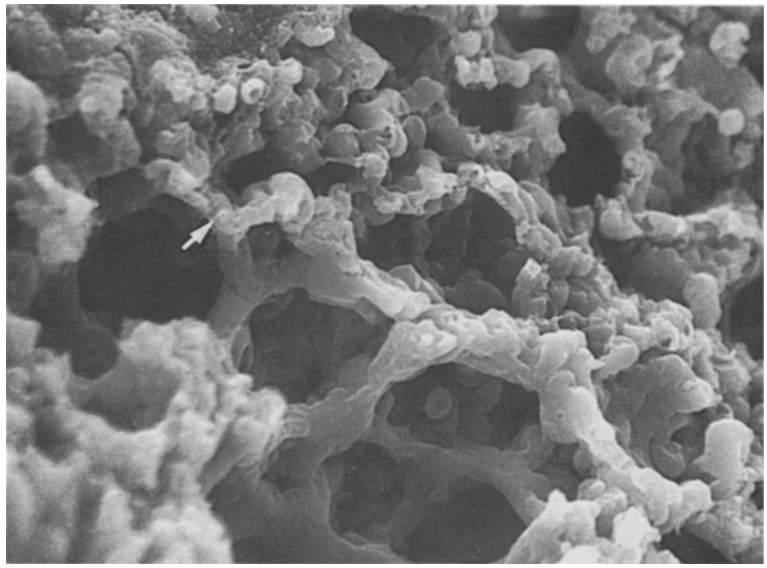


Fig. 9. SEM features of lung after irradiation and AMHCTL pretreatment. Alveolar septa are pronounced, no transudation or exsudation, arrow indicates a congested blood vessel (x6000)

from irradiation with the high dose of 6Gy. This clearly shows that the thiol residue alone, common to both compounds used is not the effective principle. AMHCTL could have been acting due to a higher biological half life time as described for alpha-alkylated amino acids, by rapidly crossing biomembranes due to its lipophilicity or by its structural properties (Roberts, 1992). The

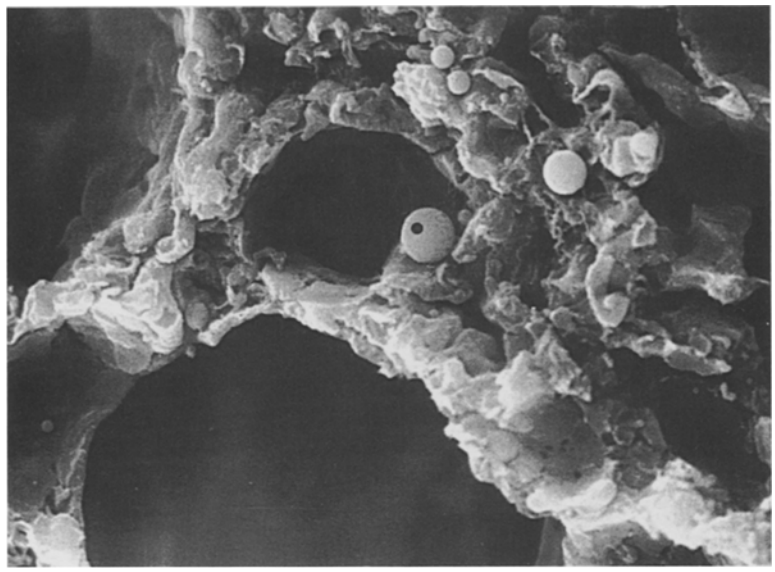


Fig. 10. SEM features of lung after irradiation from group 1. Heavy congestion, remarkable transudation. Transudation droplets (·)

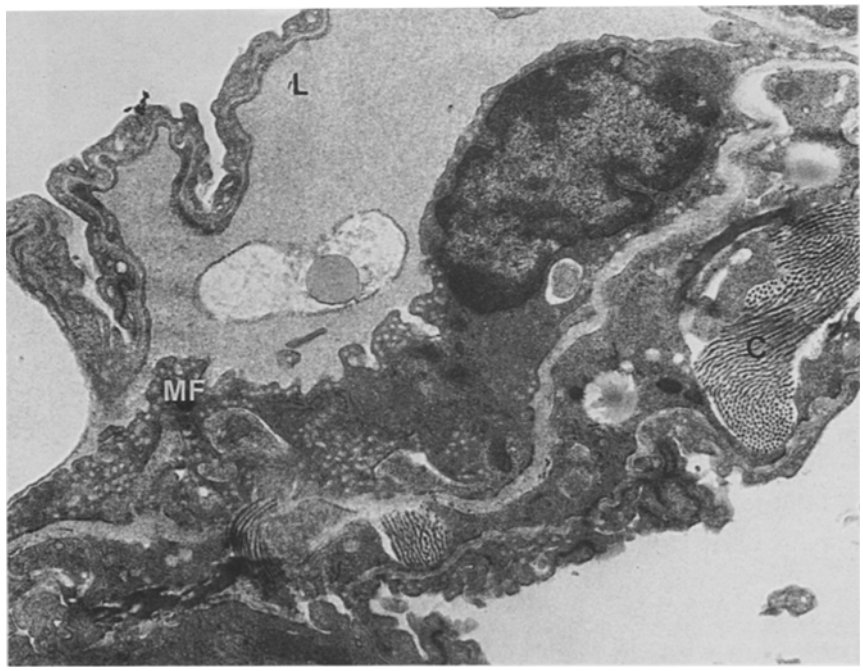
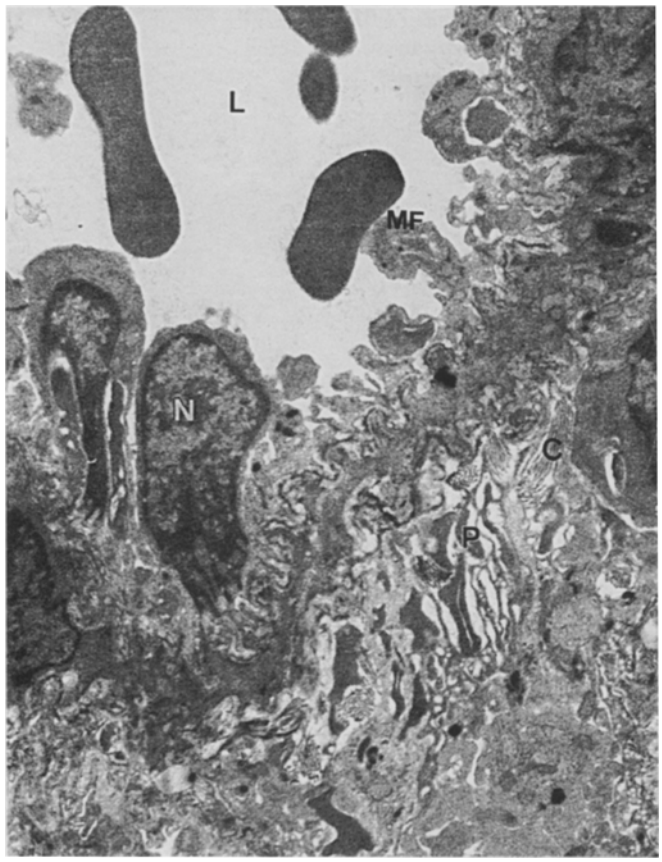
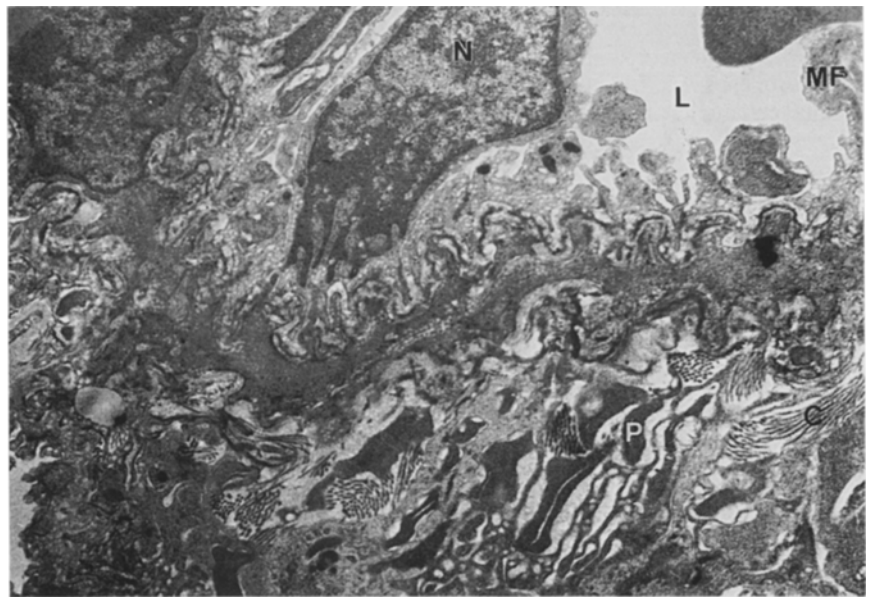


Fig. 11. Transmission electron microscopical (TEM) findings showing regular structures of unirradiated lung. A part of the alveolar septum is shown and a cross section of the capillary wall. The marginal fold (*MF*), the lumen of the capillary (*L*) and collagen fibres (*C*) are presented. x12000



A



B

Fig. 12. A TEM of lung after irradiation of group 1. Part of the alveolar septum, cross section of the capillary wall. Nuclei (*N*) protruding into the capillary lumen (*L*) cytoplasmic protrusions into the lumen (*MF*). Most prominent finding is the proliferation of capillaries (*P*). Presence of collagen fibres (*C*). **B** Detail of Fig 12A showing the proliferation of capillaries (*P*), protruding nulceus of endothelial cells (*N*) into the lumen (*L*)

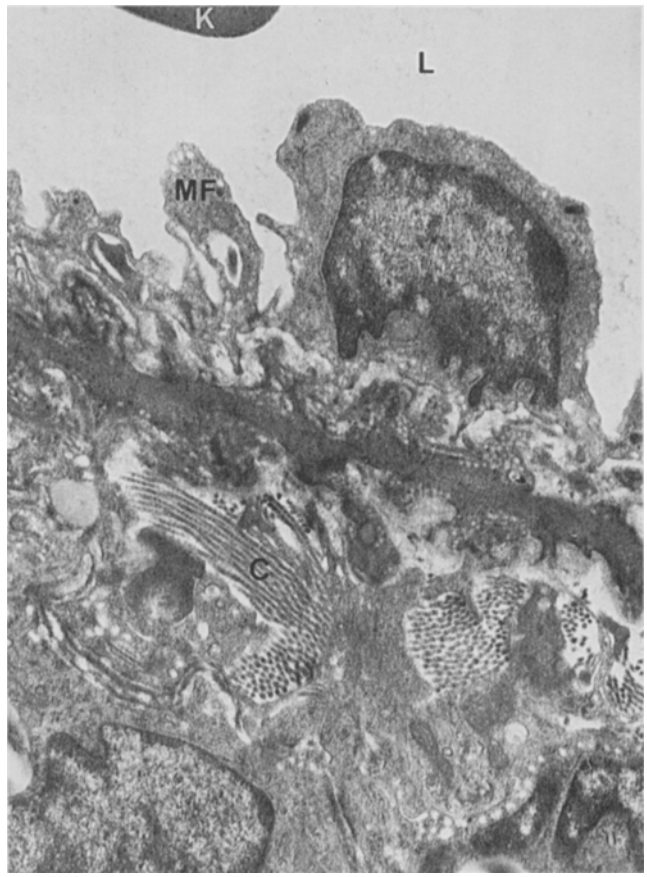


Fig. 13. TEM appearance of lungs irradiated and AMHCTL pretreatment. Part of the alveolar septum, cross section of the capillary wall. Lumen (*L*), collagen fibres (*C*) Protrusion of endothelial cell nuclei and cytoplasm (*MF*). Note the absence of proliferation of capillaries

molecular mechanism of action is still unknown. According to current concepts sulfur containing amino acids could act by free oxygen radical scavenging and activating repair mechanisms. We favorize the latter hypothesis as we found that AMHCTL increased the survival of irradiated bacteria E coli 1157 under argon atmosphere (Mao et al., 1994).

Our preliminary results are promising and confirm our studies showing significantly increased survival of BALB/c mice irradiated with 6Gy and AMHCTL treatment (manuscript in preparation). Biochemical studies carried out presently on the lungs of the present study will show if parameters for radiation injury as o-tyrosine and di-tyrosine are lower or absent in the AMHCTL treated animals of group 2.

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