9-15-1987

Response of Mouse Lung Air-Blood Barrier to X-Irradiation: Ultrastructural and Stereological Analysis

L. de Saint-Georges
Centre d'Etude de l'Energie Nucléaire

U. Van Gorp
Centre d'Etude de l'Energie Nucléaire

J. R. Maisin
Centre d'Etude de l'Energie Nucléaire

Recommended Citation

Utah State University
MERRILL-CAZIER LIBRARY

RESPONSE OF MOUSE LUNG AIR-BLOOD BARRIER TO X-IRRADIATION: ULTRASTRUCTURAL AND STEREOLOGICAL ANALYSIS

L. de Saint-Georges(*), U. Van Gorp, J.R. Maisin

Centre d'Etude de l'Energie Nucléaire, Department of Biology,
B-2400 Mol, Belgium

Abstract

Male mice of the Balb/c strain were exposed, at an age of three months, to a single dose of 10 or 20 Gy on the right hemithorax. At 3, 4, 6, 9 and 12 months after exposure, lungs were processed for electron microscopy following a standardized procedure in order to allow stereological analysis. By this method, the arithmetical mean thickness and, the air-blood barrier mean thickness in the lung parenchyma was shown to increase quickly with time by oedemization and fibrinization of the septal space. The ratio endothelium/epithelium surfaces (S_T/S_E) gradually decreased by reduction of both surfaces but this was more marked for Si. The endothelium and epithelium were both highly damaged. Quantitative results indicate that damage to the epithelial cells and mainly to type II, appear at the same time as damage to the endothelium. From the time lapse quantitation it is not possible to determine which one plays the predominant role in the radiation pneumonitis. The strong reaction of the basement membrane and mainly of the interstitial cells could play a decisive role in the evolution of the illness.

*Address for correspondence:
L. de Saint-Georges
Biology Department, C.E.N./S.C.K.
Boeretang 200, B-2400 Mol, Belgium
Phone no.: (014) 31.18.01. ext. 5161

Introduction

The damage to the lung parenchyma after irradiation, leading to radiation pneumonitis and interstitial fibrosis, was intensively studied by morphological and biochemical methods (3,14,15,17). However, the pathogenesis of radiation injury is still a matter for discussion (1,4,7,13,16). The critical target cells in irradiated lung were proposed to be either the epithelial or the endothelial cells (1,2,6,9,10,11,12). Although there are now more results available to emphasize the more important qualitative aspects of the problem, the lack of quantitative data concerning the cell distribution changes after irradiation has been regularly underlined.

In the present study the most relevant ultrastructural features regarding the endothelium and epithelium are pointed out and the quantitative evolution of the air-blood barrier mean thickness is followed from 3 to 12 months after X-ray exposure.

Material and Methods

Groups of 5, adequately shielded, 3 months old Balb/c male mice (Balb/c Cnb f Han: NMRI) were X-irradiated (Philips RT-250, 1 mm Cu filter, 0.86 Gy/min.) at 10 and 20 Gy only on the right lung and then sacrificed at 3, 4, 6, 9 and 12 months. Non irradiated mice were kept in the same room as a control for infectious lung diseases. At the time for tissue processing, mice were anesthetized by injection i.p. of 1 ml/100 gr mouse of Avertine solution (2-2-2 tribromoethanol 0.25% in 2.5% amyl alcohol) and the lungs were fixed with glutaraldehyde 3%, paraformaldehyde 1% in cacodylate-HCl buffer (0.1 M -pH 7.4) by cardiac perfusion at a constant pressure of 200 mm H_2O (0.75 ml/min.). The lungs were then removed, immediately cut into small pieces and degassed in the fixative under progressive vacuum for 15 min. The fixation continued thereafter at atm. P. for another 2 h. The tissue was rinsed in veronal acetate (V.A.) (pH 7.4) buffered 7.5% sucrose overnight, postfixed in 1% osmium tetroxide buffered 4% sucrose for 1 h., rinsed twice in 7% sucrose buffered V.A. for 5 min. dehydrated in a graded series of alcohol followed by a propylene oxide step

L. de Saint-Georges, U. Van Gorp, J.R. Maisin

(2 x 15 min). The tissue was then embedded in Epon epoxy resin, and ultrathin sections were cut with an LKB 4801-A ultramicrotome and mounted on naked copper grids, double stained with uranyl acetate and lead citrate and examined in a JEOL 1200-EX electron microscope.

Quantification : the investigation of the air blood barrier mean thickness evolution after irradiation, was carried out on ultra thin sections from randomly distributed samples of the mouse lung parenchyma from the different lobes, referring essentially to the method described by Weibel (18,19). All the different lobes from the right and the left lung separately were cut into small pieces. Two pieces from both the right and the left lung were prepared and 5 random electron micrographs per sample were examined by a point counting method using lattices of point engraved on a sheet of plexiglass and applied to the photograph (Fig.1.).

The arithmetical mean thickness T is an estimation of the amount of tissue lying between unit areas of the alveolar and the capillary surface. T cap. is an estimation of the mean thickness related to capillary surface only.

The method shows that if we use a set of Q lines of length Z with 2 Q end points :

$$T = \frac{Z.p}{2(ni+ne)} \quad (1)$$

$$T \, cap. = \frac{Z.p}{4ni} \quad (2)$$

where Z, expressed in µm and corrected for the final magnification of the photograph, is the length of the lines (2 µm)
* p is the number of end points lying on a section of the tissue.
* ni and ne are the numbers of intersections of the sampling lines with the internal (capillary) and external (epithelium) surfaces of the tissues, respectively.
* S_I/S_E given by ni/ne is the dimensionless factor giving the ratio between the endothelial and epithelial surface.
* p(x)/p gives the volume ratio of the x tissue component in the total volume of lung parenchyma tissue.

Results

The histology and ultrastructure of the normal mouse lung parenchyma has been described in detail (5-8). It is typically constituted of alveolae separated from each other by the alveolar wall which consists essentially of one capillary network covered on both sides by epithelial cells (Figs.2,3). The epithelium shows two cell types : the type I, flattened and covering most of the alveolar surface, and the type II, with typical lamellar inclusions, producing the surfactant. Between the capillary endothelium and the epithelium is a thin layer of fibrillar material to which these cells adhere by their basement membrane. The

interstitial cells are occasionally found in this fibrillar layer (septal space). The air-blood barrier is thus, schematically, always a succession of three sheets, namely, the endothelium, the septal space (with or without interstitial cells) and the epithelium (Fig.3.). As early as the fourth month after irradiation, all the different possible damage mentioned here was seen for both doses but it constituted very occasional features in the early stages which became widespread events as time elapsed. Type I epithelial cells, after irradiation, kept a smooth and rather regular alveolar surface but the basement membrane became very undulated (Figs. 4,5,6,7). The type II epithelial cells were very sensitive to irradiation but the intensity of the damage varied from place to place within the same lung. When higly damaged, the typical lamellar bodies were more numerous, larger and sometimes appeared empty (Fig.8.).

Figure 1 - Non irradiated lung parenchyma section covered by the type of lattice used for point and intersection counting. (X 1/4 of actual working magnification) (Control, 4 months, right lung).

Figure 2 - Scanning electron image of the lung parenchyma showing several alveolae (A) separated by the alveolar walls with their capillary network. The arrows indicate the air blood barrier. Box : see figure 3 (Control, right lung).

Figure 3 - Transmission electron image of a corresponding zone as seen in figure 2. The three typical layers of the air-blood barrier are : the epithelium (1), the septal space (2), and the endothelium (3). C : Capillary Lumen, A : Alveola, E : Erythrocyte, Pt : Platelet (10 Gy, 24 months, Shielded left lung).

Figure 4 - The basement membrane of the epithelium is very undulated (⟶). The capillaries are scarce (10 Gy, 6 months, Right lung). A: Alveola, C: Capillary lumen.

Figure 5 - Detail of figure 4. The inner membrane of the capillary protrudes inside the lumen (◄). (⟶) : basement membrane. Sp: Septal space, C: Capillary lumen.

Figure 6 - Typical appearance of an alveolar wall after irradiation. The septal space is oedemized. Large areas of the alveolar wall are devoid of capillaries. Epi: T_I epithelial cell. C: Capillary, A: Alveola, (20 Gy, 6 months, Right lung).

Figure 7 - Detail of figure 6 showing the basement membrane (BM) and the septal space (SP).

Figure 8 - Alveolar wall showing collagen fibers (⟶). T_{II} epith. cell with inclusions (⟶). Some appear empty (20 Gy, 12 months, Right lung). A: Alveola, C: Capillary lumen, SP: Septal space.
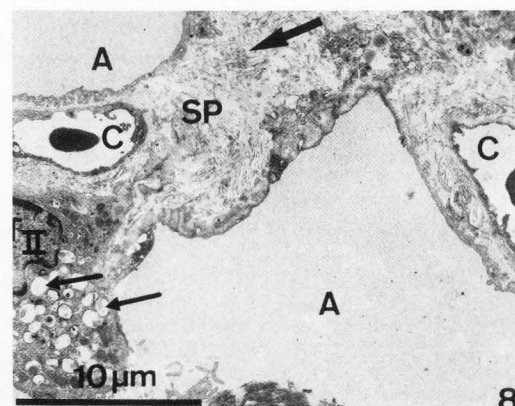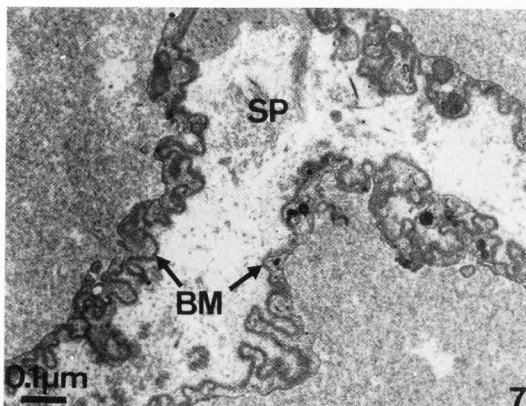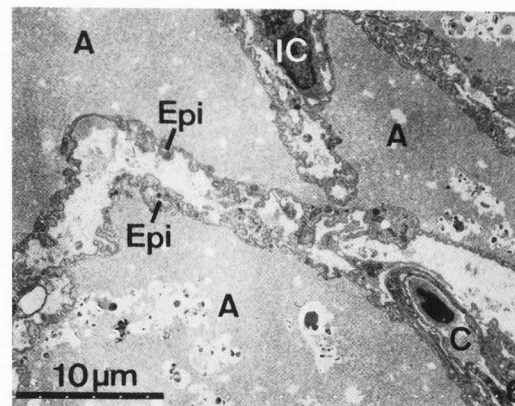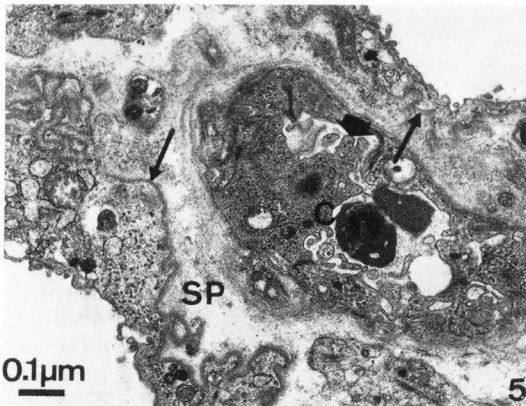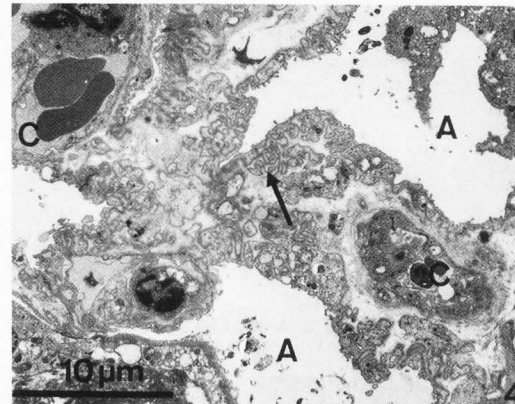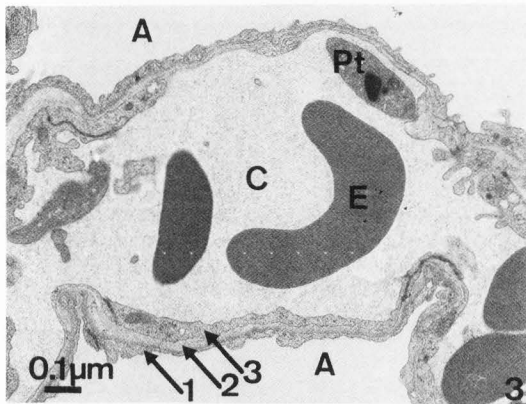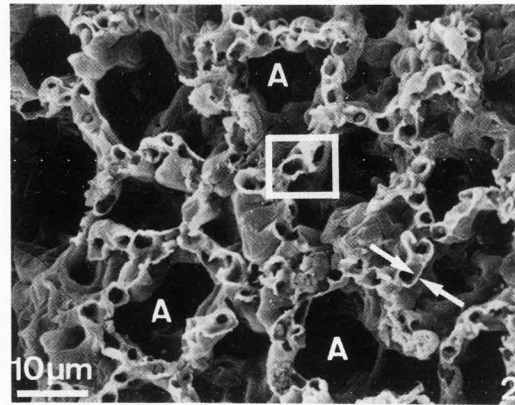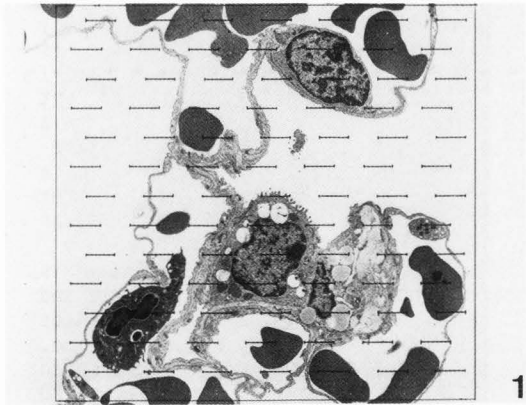
The alveolae were often completely filled with cell debris and macrophages (Fig.9.).

The appearance of the capillaries and their distribution in the tissue underwent deep modification. They were occasionally broken but more often their total diameter became smaller with a strong narrowing or collapsing of the lumen. The cytoplasm formed an irregular and thick layer around the lumen where it protruded in several places (Fig.5.).

The septal space became oedematous and highly fibrillar (Figs.5,7,8). It was obviously responsible for most of the increase (%) of the air-blood barrier. Collagen increased in quantity and various cell types, i.e., fibroblast, plasma cells, polymorphonuclear leucocytes, and lymphocytes became more numerous. The typical position of the septal space which, in healthy mice, is restrained between epithelial and endothelial cells often disappeared in irradiated mice. The withdrawal of the capillaries to more limited spots either by their size reduction or by their destruction, allowed the septal space to be limited on both sides by epithelial cells belonging to the surfaces of two neighbouring alveolae, giving a large portion of alveolar wall without capillary structure (Figs.6-8). The thickening of the septal space by fibrous material provokes a dramatic reduction of the alveolar cavities which can be reduced to a slit (Fig.10.) or to very small pockets (Figs.11-12.).

The arithmetical mean thickness T, the air blood barrier T cap. and the Si/Se ratio are indicated in table 1.

The control values of sham exposed mice indicate that the normal T cap. of Balb/c mice is 1.33 ± 0.16 µm. This is not significantly different from the T estimation 1.29 ± 0.12 µm and it is worthwhile noticing that in such mice the epithelial and the endothelial surfaces in the parenchyma are identical as deduced from Si/Se = 1.01 ± 0.08.

Three months after irradiation there are no significant differences between either the 10 and 20 Gy exposed right lungs. T and T cap are already higher than on the shielded left lungs and they gradually increase thereafter. Four months after exposure the air blood barrier is noticeably thicker and it reaches, after one year, 6 to 7 times the normal value.

In terms of physiology consideration the thickening factor determined by stereological method is underestimated. Indeed, considering the whole lung there are, mainly after 6 months, many alveolae completely filled with debris and macrophages and the total epithelial gas exchange surface available is dramatically reduced by such a loss of functional alveolae.

Both endothelial (S_I) and epithelial (S_E) surfaces are reduced but the reduction is more important for S_I, and the S_I/S_E factor decreases strongly to approximately half of the initial value. Figure 13 shows the evolution of the volume percentage of the various parenchymal components. It is shown clearly that the largest contribution to the increase in the tissue volume is due to the septal material and mainly to its fibrillar component which becomes

oedematous and progressively filled by collagen fibrils. The endothelial tissue is dramatically reduced and the total epithelial tissue is slightly increased. The balance between type I and type II cells is deeply modified but apparently more by a 10 Gy exposure than by a 20 Gy. Type I and type II cells show a diametrically opposed reaction. The former show a reduction in the total volume and the latter show a strong increase in response by both an increase in cell number and hypertrophia.

The intensity of the observed damage is well correlated with time. For endothelium and septum there are no significant differences between the 10 Gy and the 20 Gy groups of mice.

The type II cells seem to be more affected by the dose. The lower observed increase, at 20 Gy, of the volume percentage of those cells could be explained by a lack of regeneration capacity at this dose or by a shorter life span, explaining also the high amount of alveolar debris.

Conclusions

The present conclusions drawn from stereological analysis, meet and complete those from ultrastructural studies. The results emphasize the strong development of the interstitium, the air blood barrier increase, the filling of the alveolae with debris and the subsequent modifications of the functional capacity of the lung that this damage provokes.

The intensity of the observed damage is well correlated with time but there is no significant dose-response relationship for the 10 and 20 Gy groups of mice considered.

The gradual thickening of the alveolar wall and the air-blood barrier after irradiation is due to an oedemization and filling of the interstitial space, essentially by fibrous

Figure 9 - The alveolae can be filled with cell debris (CD) and macrophages (M). (10 Gy, 6 months, Right lung).

Figure 10 - The intense fibrinisation in the septal space reduces the alveola to a very thin non-functional layer. A : Alveola, F : Fibrin, Epi : T_I epith. cell., T_{II} : Type II epith. cell., (10 Gy, 12 months, Right lung).

Figure 11 - Highly damaged lung 12 months after irradiation showing hepatization of the structure. The alveolae are reduced to very small cavities (A). The capillaries are scarce and the lumen is generally narrowed (C). T_{II} : Type II epithelial cell., (20 Gy, 12 months, Right lung).

Figure 12 - SEM micrograph of lung parenchyma after a 20 Gy exposure. The alveolae (A) are reduced and the capillaries are collapsed ($\longrightarrow$)

Table 1 : Gives the mean values ($\pm$ sdv) of T, T_C and S_I/S_E, for the 10, 20 Gy doses and sham exposed mice. Upper : Right lung (irradiated), Lower : Left lung (shielded) sham exposed mice (cont).
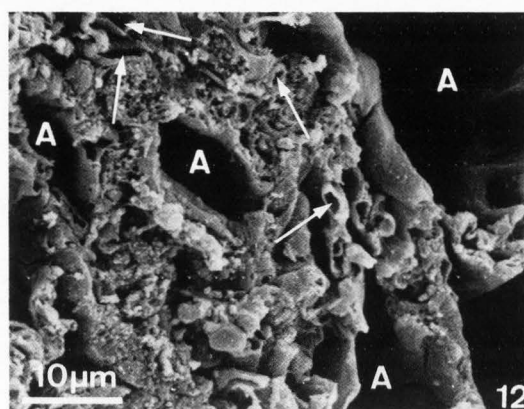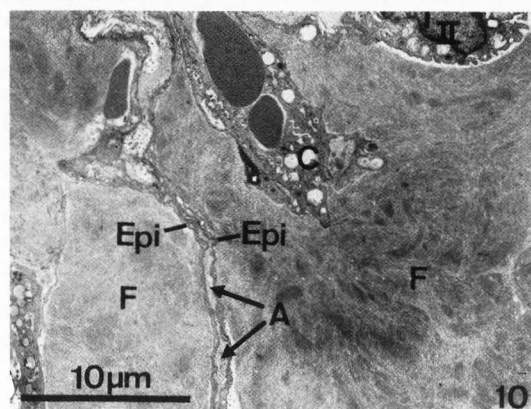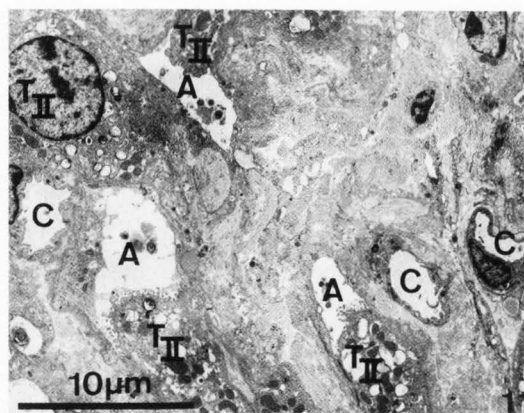
TABLE 1

	months	Right Lung (irradiated) T_t (μm)	T_{cap} (μm)	S_I/S_E		months	Left Lung (shielded) T_t (μm)	T_c (μm)	S_I/S_E
10 Gy	3	1.60 ± 0.31	1.56 ± 0.28	1.05 ± 0.12	10 Gy	3	1.30 ± 0.23	1.31 ± 0.32	1.13 ± 0.12
	4	1.87 ± 0.42	2.14 ± 0.67	0.78 ± 0.11		4	1.29 ± 0.23	1.43 ± 0.35	0.97 ± 0.13
	6	2.89 ± 1.79	3.13 ± 2.66	0.86 ± 0.12		6	1.41 ± 0.14	1.39 ± 0.11	1.07 ± 0.09
	9	3.96 ± 1.32	5.56 ± 1.95	0.55 ± 0.15		9	1.11 ± 0.08	1.19 ± 0.07	0.88 ± 0.04
	12	6.51 ± 1.76	9.26 ± 4.71	0.54 ± 0.09		12	1.50 ± 0.80	1.54 ± 0.87	0.99 ± 0.12
							1.32 ± 0.15	1.37 ± 0.13	1.01 ± 0.10
20 Gy	3	1.54 ± 0.25	1.62 ± 0.89	0.90 ± 0.13	20 Gy	3	1.18 ± 0.09	1.20 ± 0.13	1.02 ± 0.10
	4	1.95 ± 0.38	2.28 ± 0.92	0.75 ± 0.09		4	1.27 ± 0.18	1.29 ± 0.21	1.03 ± 0.08
	6	4.26 ± 0.92	7.81 ± 2.55	0.38 ± 0.06		6	1.51 ± 0.31	1.73 ± 0.46	0.92 ± 0.13
	9	6.11 ± 1.59	10.63 ± 2.20	0.40 ± 0.07		9	0.99 ± 0.08	0.99 ± 0.10	1.02 ± 0.04
	12	7.07 ± 4.44	9.03 ± 7.30	0.65 ± 0.13		12	1.44 ± 0.64	1.76 ± 0.33	0.94 ± 0.41
							1.28 ± 0.21	1.39 ± 0.34	0.99 ± 0.05
		Right Lung (control mice)					Left Lung (control mice)		
CONT	3	1.16 ± 0.11	1.09 ± 0.12	1.14 ± 0.04	CONT	3	1.13 ± 0.06	1.08 ± 0.08	1.14 ± 0.14
	4	1.25 ± 0.19	1.32 ± 0.25	0.97 ± 0.10		4	1.17 ± 0.09	1.26 ± 0.15	0.90 ± 0.07
	6	1.22 ± 0.19	1.29 ± 0.23	0.93 ± 0.05		6	1.25 ± 0.08	1.34 ± 0.15	0.90 ± 0.06
	9	1.46 ± 0.19	1.44 ± 0.19	1.03 ± 0.04		9	1.11 ± 0.12	1.09 ± 0.16	1.09 ± 0.08
	12	1.35 ± 0.24	1.51 ± 0.38	0.99 ± 0.17		12	1.31 ± 0.54	1.34 ± 0.65	1.02 ± 0.18
		1.29 ± 0.12	1.33 ± 0.16	1.01 ± 0.08			1.19 ± 0.08	1.22 ± 0.13	1.01 ± 0.11
							1.26 ± 0.07	1.33 ± 0.09	1.00 ± 0.01

material and also by an increased number of the interstitial cells.

Endothelial to epithelial surface ratio per volume unit is reduced to approximately half of the normal value. Both Si and Se are reduced but the phenomenon is more marked for Si.

The total volume of the epithelium increases slightly. Type II cells show an increase in number and hypertrophia whereas Type I are reduced in volume.

The quantitative study shows strong simultaneous modifications of both endothelium and epithelium and can therefore not indicate a predominant role of one or the other in radiation pneumonitis.
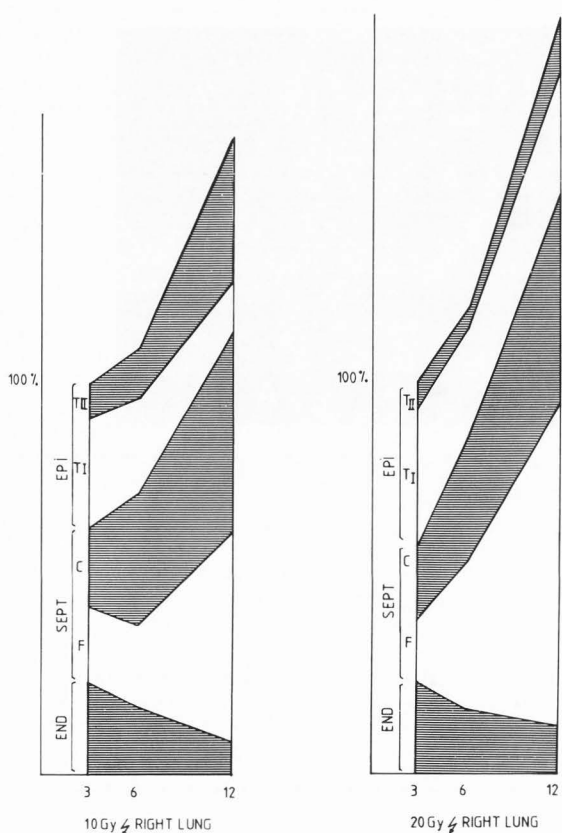
13

Figure 13 - The relative volume for each component in the total parenchyma tissue. End : endothelium sept : septal space, Epi : epithelium, C : cellular component of sept (intertitial cell volume), F : Fibrillar part of sept, T_I : Type I epithelial cell, T_{II} : Type II epithelial cell.

References

(1) Adamson IYR, Bowen DH. (1968). The Type 2 cell as progenitor of alveolar epithelial regeneration. A cytodynamic study in mice after exposure to oxygen. Lab. Invest. 30, 1, 35-42.

(2) Goldenberg VE, Warren S, Chute R, Besen M. (1968). Radiation pneumonitis in single or parabiotic rats. I. Short term effects of supralethal total body irradiation. Lab. Invest. 18, 3, 215-226.

(3) Hill RP. (1983). Response of mouse lung to irradiation at different dose rates. Int. J. Radiation Oncology Biol. Phys. 9, 1043-1047.

(4) Jennings FL, Arden A. (1961). Development of experimental radiation pneumonitis. Arch. Path. 77, 437-446.

(5) Kilburn KH. (1974). Functional morphology of the distal lung. Rev. Cytol. 37, 153-270.

(6) Maisin JR. (1970). The ultrastructure of the lung of mice exposed to a supralethal dose of ionizing radiation on the thorax. Radiation Research 44, 2, 545-564.

(7) Maisin JR. (1973). Radiosensitivity of the lung, In : Strahlenschutz in Forschung und Praxis, Band XIII, G. Thieme Verlag, Stuttgart, 49-64.

(8) Maisin JR, Van Gorp U, de Saint-Georges L. (1982). The ultrastructure of the lung after exposure to ionizing radiation as seen by transmission and scanning electron microscopy. Scanning Electron Microsc. 1982/ 1, 403-412.

(9) Penney DP, Rubin P. (1977). Specific early fine structural changes in the lung following irradiation. Int. J. Rad. Onc. Biol. Phys. 2, 1123-1132.

(10) Penney DP, Shapiro DL, Rubin F, Finkel-stein J, Siemon DW. (1981). Effects of radiation on the mouse lung and potential induction of radiation pneumonitis. Virchows Arch. Cell Path. 37, 327-336.

(11) Phillips TL. (1966). An ultrastructural study on the development of injury in the lung. Radiology 87, 49-54.

(12) Phillips TL, Benak S, Ross G. (1972). Ultrastructural and cellular effects of ionizing radiation. In : Frontiers Radiation Therapy and Oncology, Vaeth H (ed). Karger, Basel, 6, 23-41.

(13) Travis EL. (1979). The sequence of histological changes in mouse lung after single dose of X-rays. Int. J. Radiat. Onc. Biol. Phys. 6, 345-347.

(14) Ts'Ao CH, Ward WF, Port CD. (1983). Radiation injury in rat lung : prostacy-clin production, arterial perfusion and ultrastructure. Radiation Research 96, 284-293.

(15) Ts'Ao CH, Ward WF, Port CD. (1983). Radiation injury in rat lung : plasminogen activator and fibrinolytic inhibition activities. Radiation Research 96, 301-308.

(16) Van den Brank HAS. (1971). Radiation effects on the pulmonary system. In : Pathology of Irradiation, Berdjis CG (ed). The Williams & Wilkins Co., Baltimore MD., 569-591.

(17) Ward WF, Solliday NH, Molteni A, Port CD. (1983). Radiation injury in rat lung. Angiotensin converting activity. Radiation Research 96, 294-300.

(18) Weibel ER. (1963). Morphometry of the Human Lung. Springer Verlag Berlin, 151 pp.
(19) Weibel ER. (1979). Stereological methods. Vol.1. Practical Methods for Biological Morphometry. Academic Press Inc., London, 415 pp.

Discussion with Reviewers

Reviewer I : It is stated that although the response of type I and type II cells is modified, the lower dose of 10 Gy caused a "larger modification" than high dose of 20 Gy. This is quite the opposite of what would be expected.
Authors : The results presented in this paper are quantitative and indeed the total volume of type II cells seems to be higher at 12 months after 10 Gy than after 20 Gy but it does not imply a "larger modification". The lower value of the 20 Gy exposed cells could be due to a greater damage, i.e., a lack of regeneration capacity, a shortening of the life span. This would explain the lack of a clear quantitative dose response.

Reviewer III : In your 12 months study following 10 or 20 Gy exposures, did you encounter any morbidity or mortality of the animal group ? If so what was the mortality rate for the respective exposures ?
Authors : The mortality rates (The cumulative % of dead mice at given months after right lung exposure) were the following :

	cont.mice	10 Gy	20 Gy
3 months	5.25%	2.51%	33.97%
4 months	7.00%	11.09%	46.33%
6 months	10.60%	28.26%	70.76£
12 months	16.00%	54.00%	89.55%