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Research Article

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Time Course of and Stimuli to Compensatory Growth of the Lung after Pneumonectomy

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ABSTRACT Left pneumonectomy in the mature rat led to an increase of [³H]thymidine incorporation into DNA of the remaining lung in the first 3 postoperative days, and resulted in a subsequent 38% increase of lung weight and 41% increase of lung tissue volume measured 1 wk after surgery. Despite these early changes, total lung volume (TLV) did not increase until the 2nd postoperative wk, reaching values 33% greater than in controls. Analysis of lung pressure-volume curves revealed that lung recoil was increased at low lung volumes 1 wk after surgery, but returned to normal by the 2nd postoperative wk, suggesting that synthesis of both lung elastin and collagen had occurred by this time. Increased inspired oxygen concentration (28% or 35%) during the 1st but not the 2nd postoperative wk abolished the change in TLV without influencing the increase in lung weight, while diminished inspired oxygen (17% or 14%) accentuated the postoperative increase in TLV. Lung pressure-volume curves demonstrated changes in distensibility at low lung volumes, suggesting that oxygen may have influenced synthesis or cross-linking of lung elastin. Alterations of minute ventilation in the postoperative period produced by 3% CO₂ did not influence the compensatory growth process, nor did administration of cyclophosphamide. These studies suggest that postpneumonectomy lung growth is a two-phase process, beginning with cell proliferation and increased tissue volume, followed by increasing lung volume associated with formation of lung structural proteins. The latter process is profoundly influenced by inspired oxygen concentration in the early postoperative period.

INTRODUCTION

Compensatory growth of visceral organs has been described and characterized experimentally in the kidney after unilateral nephrectomy (1), in the heart after

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aortic constriction (2), in the liver after partial hepatectomy (3), and most recently in the lung after pneumonectomy (4, 5). In the first three organs, biochemical events associated with growth begin within 24 h, while functional compensation occurs over several weeks.

Little attention has been paid to the early compensatory changes in the lung after pneumonectomy. Cohn's studies in 1939 demonstrated a 40% increase in lung weight 2 wk after pneumonectomy in the dog (6), and recent studies by Nattie et al. have shown an increase in lung weight 1 wk after pneumonectomy in the rat (7), suggesting that a rapid compensatory growth response might occur in the lung. Our initial studies of postpneumonectomy lung growth in the adult rat showed that volume and weight of the remaining lung had increased by 25% and 35%, respectively, at the time of initial study 4 wk after surgery, and that no further increase in lung size occurred over the succeeding 4 wk, thus suggesting that the majority of compensatory growth changes occurred within the 1st postoperative mo (4).

The stimuli to compensatory organ growth remain unclear. Biochemical work, organ-specific chemical substances (chalone), humoral factors, and mechanical stresses have been mentioned as possible factors (8-10). The stimuli to postpneumonectomy lung growth remain unexplored since Cohn's conclusion that all lung growth results from stretch of the lung (6).

The purpose of the present study was to define better the time course of postpneumonectomy compensatory lung growth and to explore conditions in the postoperative period that might influence this growth process, and that in turn might provide insight into the factors responsible for this form of compensatory growth.

METHODS

Design. Experiments were designed to define the time course of postpneumonectomy lung growth and then investigate the effect of several potential modulating factors on this process. Adult Long-Evans hooded rats (Blue Spruce Farms, Inc., Altamont, N. Y.) were sacrificed and studied

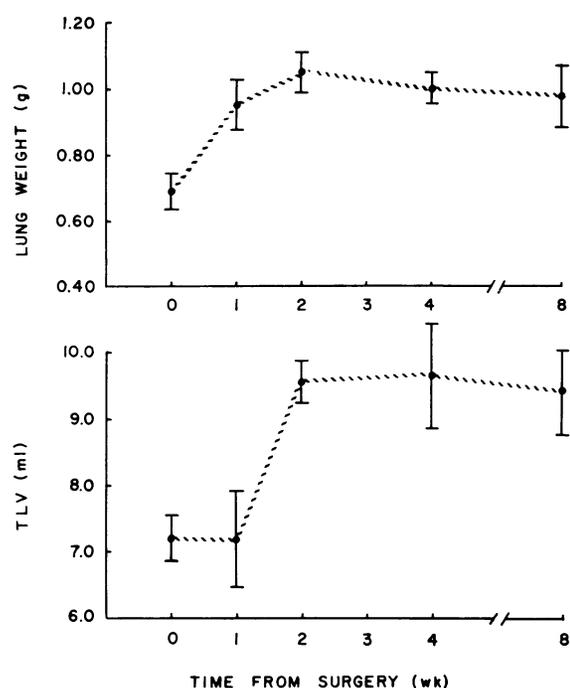


FIGURE 1 Time course of change in lung weight and TLV after pneumonectomy. Zero time represents control animals. Data from 4- and 8-wk time periods are extrapolated from previous studies (4, 5) corrected for body size with the regression formula $y = 0.044$ length (in centimeters) $- 0.821$ for lung weight, and $y = 0.0085$ length (in centimeters)^{1,2} for TLV. Data points represent mean values ± 2 SEM.

1 and 2 wk after left pneumonectomy, and results were compared with control animals of similar size and age. Four animals underwent sham pneumonectomy, which involved all procedures except removal of the left lung. Two each were sacrificed at 1 and 2 wk after surgery. These results were in turn compared with data from previous studies performed 4 and 8 wk after pneumonectomy (4) by correcting for the effect of body size on lung weight and lung size in the latter animals (see legend of Fig. 1).

The early effects of pneumonectomy on lung DNA synthesis were studied by injecting 0.4 $\mu\text{Ci/g}$ of [³H]thymidine (sp act 6.7 Ci/mmol) i.p. every 8 h, beginning at the time the animals had recovered from anesthesia. After sacrifice of control and postpneumonectomy rats, lung DNA was extracted and the incorporation of labeled thymidine into DNA was measured.

The factors explored that might influence the postpneumonectomy growth process included inspired oxygen concentration, ventilation, and a commonly used cytotoxic agent. In each instance, experimental conditions were applied during the 1st postoperative wk; the animals were allowed to live under control conditions during the 2nd postoperative wk and were then sacrificed 14 days after the initial surgery. Results were compared to those from control animals subjected to the same stimuli and to animals sacrificed 2 wk after pneumonectomy.

Procedures. Left pneumonectomy was performed in adult, 8-9-wk-old female Long-Evans rats, as previously described in detail (4, 5). The left lung, which accounts for 35% of total lung weight, was removed after induction of

anesthesia with i.p. sodium methohexital, 85 mg/kg. Operative time ranged from 10 to 15 min and the rats recovered from anesthesia in 15-30 min. Rats were maintained on commercial food and water ad libitum after pneumonectomy.

Upon recovery from anesthesia, rats were placed in a 170-liter Plexiglas box, two animals per cage, with water and food ad libitum. Control rats were exposed in the same fashion. Compressed air and 100% nitrogen, oxygen, or carbon dioxide were mixed after passage through separate flow meters which were adjusted to create chamber concentrations of 35% O₂, 28% O₂, 17% O₂, 14% O₂, or 3% CO₂.

During exposures, oxygen concentration was measured three to four times per day with a paramagnetic oxygen analyzer (Model OA 250, Servomex Controls, Ltd., Sussex, England). Chamber oxygen concentration varied no more than 2% at high oxygen levels and 1% at low levels, while CO₂ measured with a CO₂ electrode (Model IL-113, Instrumentation Laboratory, Inc., Lexington, Mass.) was always less than 0.4%. Carbon dioxide levels during the CO₂ exposure experiment varied between 2.5 and 3.5%. Excess water vapor was absorbed from the chamber with anhydrous calcium sulfate and, except for the CO₂ exposure experiment, CO₂ was absorbed with soda lime. During the exposure period, the box was opened daily for 5-15 min to clean cages and replenish food and water.

To evaluate the effect of cytotoxic agents on the compensatory growth process, cyclophosphamide was given to postpneumonectomy and control rats in a dose of 15 mg/kg by i.p. injection every other day for a total of five doses. This level of cyclophosphamide has been shown to produce peripheral leukopenia without producing histologic damage in the lung (11), and is analogous to dose levels used in human chemotherapy (12).

Measurements. 1 or 2 wk after pneumonectomy, the rats were anesthetized and the trachea was cannulated. The chest cage was opened and the animals were sacrificed by exsanguination. After vacuum extraction, transpulmonary pressure was monitored at the trachea while the right lung was inflated to 30 cm H₂O transpulmonary pressure and then deflated to 0 cm H₂O with a motor-driven syringe at a speed of 3.86 ml/min. The lung was then removed and vacuum-extracted, and the volume of the collapsed lung (tissue volume) was measured by water displacement. Tissue volume was measured only in lungs completely gas-free, as evidenced by their sinking in water. Total lung volume (TLV)¹ was defined as the volume of air infused into the lung to reach 30 cm H₂O tracheal pressure plus the tissue volume. Lung pressure-volume curves were constructed and lung compliance (C_L) was measured as the volume change between 5 and 10 cm H₂O pressure during deflation. Trapped gas volume (TGV) was defined as the volume of air remaining in the lung at 0 cm H₂O during deflation. In control rats, after sacrifice, the left hilum was ligated and the left lung removed before the above measurements. Thus, values for only the right lung are compared in operated and control animals. After physiologic studies, in most animals, the lung was blotted dry, weighed, and dried in an oven at 100°C for 2-4 days, and then its weight was again determined.

Two control animals and four postpneumonectomy animals were sacrificed by exsanguination 1 h after an i.p. injection of 0.4 $\mu\text{Ci/g}$ [³H]thymidine (specific activity 6.7

¹ Abbreviations used in this paper: C_L, lung compliance; TGV, trapped gas volume; TLV, total lung volume.

TABLE I
Influence of Variables in Pneumonectomy Animals

Experiment		Body wt	Length	Lung wt	Lung wt/ body wt	TLV	% predicted
		<i>g</i>	<i>cm</i>	<i>g</i>	<i>g/100 g</i>	<i>ml</i>	
PN - 2 wks (<i>n</i> = 10)	\bar{x}	167	35.0	1.05	0.60	9.6	123.1
	SD	37	0.8	0.10	0.05	0.4	10.3
PN + O ₂ (<i>n</i> = 7)	\bar{x}	173	34.7	1.34‡	0.79‡	7.2‡	94.5‡
	SD	18	0.5	0.13	0.12	0.8	9.9
PN + N ₂ (<i>n</i> = 8)	\bar{x}	174	33.9	1.14	0.66	10.5‡	143.1*
	SD	15	1.4	0.18	0.12	1.1	15.5
PN + CO ₂ (<i>n</i> = 4)	\bar{x}	193	34.9	1.05	0.54	9.1	118.1
	SD	5	1.2	0.17	0.08	0.2	10.4
PN + CYP (<i>n</i> = 4)	\bar{x}	167	34.6	0.98	0.59	9.1	118.3
	SD	12	0.9	0.09	0.08	0.8	4.2

Length, nose-tail length; % predicted, percent of predicted TLV value determined from control animals in this and two previous studies (4, 5) with regression formula $y = 0.0085$ length (cm)^{1.92}; PN, pneumonectomy; O₂, N₂, and CO₂ refer to exposure of postpneumonectomy animals to 35% or 25% O₂, 17% or 14% O₂, or 3% CO₂, respectively, for 1 wk at room air before sacrifice. Cyclophosphamide (CYP) was given in a dose of 15 mg/kg every other day for five doses. Lung weight and lung volume measurements in all PN animals were greater than in experiment-matched controls, except for TLV in PN + O₂ animals.

* $P < 0.01$.

‡ $P < 0.001$.

Ci/mmol), administered semicontinuously (i.e., every 8 h) for periods ranging from 24 to 96 h after pneumonectomy. The right lung was removed and blotted dry. Separate lobes were weighed and homogenized in iced 0.9% sodium chloride (Polytron PT-10, Brinkmann Instruments, Inc., Westbury, N. Y.). Lung DNA was extracted by the method of Schneider (13) and measured by Burton's modification of the diphenylamine reaction (14). Highly purified calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.) was used as a standard. Radioactivity in an aliquot of DNA was determined in a scintillation counter (Tri-Carb Model 4322, Packard Instrument Co., Inc., Downers Grove, Ill.) with an aqueous scintillation solution. All samples were equally quenched with counting efficiency for ³H of 28%, and results are expressed as counts per minute per milligram DNA.

After physiologic studies, several lungs from each experimental group were inflated to 20 cm H₂O tracheal pressure with 2.5% glutaraldehyde and embedded in methacrylate. 2- μ m sections were cut from several blocks of each lung and stained with hematoxylin and eosin for subsequent evaluation.

RESULTS

Time course. 1 wk after pneumonectomy, lung weight had increased to a value 38% greater than in control animals ($P < 0.001$), yet TLV at this time was unchanged (Fig. 1). There were no further increases in lung weight over periods up to 8 wk after surgery [4- and 8-wk points were extrapolated from previous studies (4)]. Lung dry-weight-to-wet-weight ratios did not change significantly after pneumonectomy, being 0.199 ± 0.016

in control animals, 0.196 (only two animals measured) 1 wk after pneumonectomy, and 0.191 ± 0.009 2 wk after pneumonectomy. Between the 1st and 2nd wk after pneumonectomy, there was a dramatic increase in TLV to values 33% greater than those of control animals (Fig. 1) and 23% greater than those predicted on the basis of body size (Table I). Thereafter, there was no further increase in TLV measured up to 8 wk after surgery. The early changes in lung weight without concomitant changes in TLV were reflected by the fact that tissue volume accounted for 10.3% of TLV in control animals, increased to represent 13.9% of TLV 1 wk after pneumonectomy ($P < 0.001$), and since TLV increased in the 2nd wk, represented 11.3% of TLV 2 wk after pneumonectomy (Table II). 1 wk after pneumonectomy, both C_L and TGV fell significantly, suggesting an increase in lung elastic recoil forces (see Table II). Lung compliance rose to values greater than normal 2 wk after pneumonectomy, while TGV returned to normal.

1 wk after sham surgery, TLV averaged 86% and 104% of predicted, and 2 wk after surgery TLV averaged 81% and 88% of predicted. Lung weight and lung-weight to body-weight ratios were similar to controls.

Random histologic sections of 1-wk- and 2-wk-post-pneumonectomy animals appeared to be similar except for obvious variations in inflated lung size.

TABLE II
Lung Elastic Recoil and Tissue Volume

Experiment		TLV	TV	TGV	CL	
		ml	% TLV	ml/5 cm H ₂ O	% TLV	
Control (n = 10)	\bar{x}	7.4	10.3	10.8	1.9	25.9
	SD	0.6	1.5	2.2	0.2	1.3
PN + 1 wk (n = 4)	\bar{x}	7.4	13.9*	8.1*†	1.6*†	21.9*
	SD	0.8	2.6	1.6	0.2	1.1
PN + 2 wk (n = 5)	\bar{x}	9.6*	11.3	12.7	2.2*	23.0*
	SD	0.4	0.8	2.1	0.1	0.9
PN + O ₂ (n = 5)	\bar{x}	7.6	17.5*†	8.9‡	1.7‡	21.9*
	SD	0.5	1.1	2.8	0.1	1.1
PN + N ₂ (n = 5)	\bar{x}	10.7*	12.7*	17.6*†	2.8*†	26.5
	SD	1.4	1.4	2.7	0.4	3.3

TV, tissue volume; CL, lung volume change between 5 and 10 cm H₂O on deflation limb of pressure-volume curve.

* $P < 0.05$ compared to control.

† $P < 0.05$ compared to PN + 2 wk rats.

Incorporation of [³H]thymidine into lung DNA of control animals occurred at a relatively low rate over the 72-h study (Fig. 2). At all time periods studied after pneumonectomy, from 24 to 96 h, and in all lobes, incorporation of [³H]thymidine was greater than in control animals, with a seeming plateau of [³H]thymidine incorporation after 72 h.

Effect of inspired oxygen. Exposure to increased concentrations of inspired oxygen (28% or 35%) for one wk produced a slight but significant decrease in lung weight and lung weight per 100 g body weight in control animals ($P < 0.05$), but had no effect on TLV

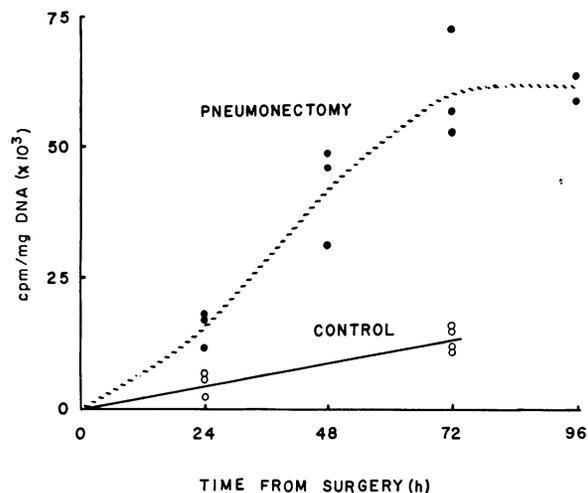


FIGURE 2 Incorporation of [³H]thymidine given at 8-h intervals into lung DNA in two control and four post-pneumonectomy animals. Individual points represent two to four lobes from the right lung of one animal sacrificed at each time interval. Time is measured from awakening after pneumonectomy.

expressed in milliliters or as percent of predicted TLV (Table III). In contrast, these levels of inspired oxygen given during the 1st wk after pneumonectomy did not influence the increase in lung weight but completely abolished any increase in postpneumonectomy TLV (Table I). Lung dry-to-wet weight ratios were 0.192 ± 0.019 and did not differ from control or 2-wk-postpneumonectomy animals. In these animals, tissue volume increased to account for 17.5% of TLV. CL

TABLE III
Influence of Variables in Control Animals

Experiment		Body wt	Length	Lung wt	Lung wt/ body wt	TLV	% predicted
		g	cm	g	g/100 g	ml	
Control (n = 14)	\bar{x}	182	34.2	0.69	0.38	7.2	96.2
	SD	20	1.0	0.10	0.07	0.6	6.9
C + O ₂ (n = 6)	\bar{x}	191	35.9§	0.58*	0.31*	7.5	91.8
	SD	12	0.9	0.09	0.05	0.5	6.7
C + N ₂ (n = 10)	\bar{x}	200*	36.2*	0.81*	0.41	8.3‡	98.6
	SD	9	1.3	0.14	0.06	1.0	14.3
C + CO ₂ (n = 5)	\bar{x}	195	35.0	1.71	0.37	7.5	97.9
	SD	13	1.2	0.08	0.05	0.9	10.6
C + CYP (n = 4)	\bar{x}	184	35.7	0.76	0.41	7.4	91.2
	SD	18	1.4	0.18	0.06	0.3	9.8

C, control. Other abbreviations and experiments as in Table I.

* $P < 0.05$.

† $P < 0.01$.

§ $P < 0.001$.

and TGV were significantly less than in 2-wk-post-pneumonectomy animals, suggesting a persistence of the early postpneumonectomy increase in elastic recoil (Table II and Fig. 3). Exposure to 28% oxygen during the 2nd wk after pneumonectomy did not alter the normal postpneumonectomy increase in lung weight or TLV (Fig. 4).

Control animals exposed to diminished concentrations of inspired oxygen (17% and 14%) had a slightly greater lung weight than air-breathing controls, although this may have been due in part to their greater size (Table III). There was a slight but insignificant increase in lung weight of postpneumonectomy animals subjected to hypoxia, and a significant increase in actual and predicted TLV (Table I). There was a loss of lung elastic recoil at low lung volumes with increased C_L and TGV, the latter now accounting for 17.6% of TLV (Table II and Fig. 3).

The influence of exposure to each level of inspired oxygen in the 1st wk after pneumonectomy and the lack of effect of oxygen during the 2nd postoperative wk are illustrated in Fig. 4. The relationship between oxygen level and TLV is significant at all concentrations. There were no specific histologic differences noted between any groups studied.

Effect of other variables. Exposure to 3% CO_2 had no effect on any of the parameters measured in either control or postpneumonectomy rats (Tables I and III). Similarly, cyclophosphamide did not influence any of the parameters measured in control or postpneumonectomy rats, although several postpneumonectomy rats either died or were eliminated from experiments because of grossly apparent pulmonary infections.

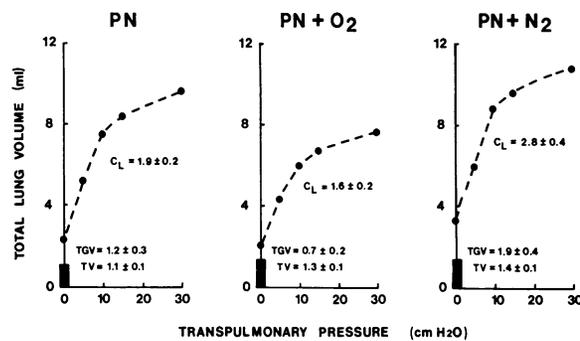


FIGURE 3 Lung deflation pressure-volume curves in 2-wk postpneumonectomy rats (PN), rats exposed to 28% O_2 during the 1st wk after surgery (PN + O_2), and rats exposed to 14 or 17% O_2 during the 1st wk after surgery (PN + N_2). C_L , change in lung volume in milliliters between 5 and 10 cm H_2O ; TV, tissue volume. Mean values ± 1 SD. All values noted in PN + O_2 and PN + N_2 curves differ significantly from PN results, and C_L and TGV differ significantly between PN + O_2 and PN + N_2 animals.

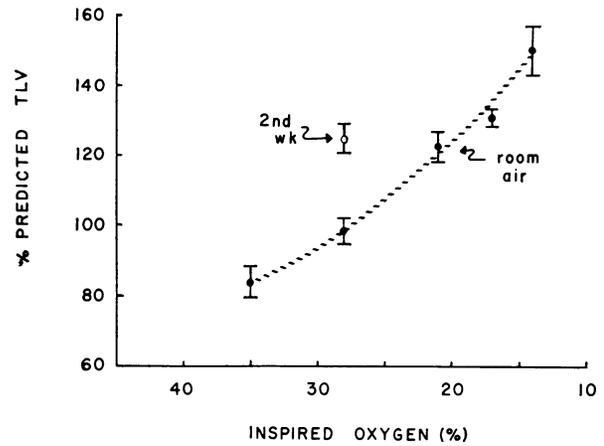


FIGURE 4 Relation of postpneumonectomy TLV to inspired oxygen concentration. Predicted TLV was determined with the regression $y = 0.0085$ length (in centimeters)^{1.62}. Closed circles represent mean values ± 1 SEM in animals exposed to indicated oxygen concentrations during the 1st postoperative wk and sacrificed at the end of the 2nd postoperative wk. The open circle represents five animals exposed to 28% oxygen during the second postoperative week. TLV averaged $96.2 \pm 1.9\%$ (SEM) of predicted in control animals.

DISCUSSION

The time course of compensatory lung growth after pneumonectomy generally follows that of other types of compensatory organ growth. There is an early phase of cellular proliferation within the first several days after surgery, followed by a later increase in function of the organ, defined in these experiments by measurements of lung volume. In the rat, these morphologic and functional changes appear to be complete by the 2nd postoperative wk, with little further change in lung weight or lung size occurring over the 8-wk period studied. Nattie and co-workers have recently reported a similar plateau in these parameters after the 2nd wk after surgery (7).

The early increase of thymidine incorporation into lung DNA, coupled with our previous findings of increased lung DNA after pneumonectomy (4, 5), suggest that a wave of cell proliferation in the lung begins within the first 24 h and continues over at least the first 3 postoperative days. Fisher and Simnett have recently demonstrated a similar early increase in mitotic activity of alveolar cells in the rat after pneumonectomy (15). This early increase in DNA synthesis is similar in timing to that seen in the kidney after unilateral nephrectomy and in the liver after partial hepatectomy (1, 3, 16). Despite the early cell proliferation and increase in tissue volume, the inflation volume of the lung (i.e., TLV) did not change until the 2nd postoperative wk. This delay in lung volume change differs somewhat from

a previous study showing slight but significant increases in lung volumes determined by nonphysiological methods, but these authors also noted that the major increase in lung volume occurred during the 2nd postoperative wk (7).

The divergence between early DNA synthesis and presumed cell proliferation and later increases in functional lung size could be explained in several ways. It seems probable that much of the cell proliferation after pneumonectomy is not directly associated with increased lung volume. In previous studies with growth hormone, we found that blunting of the postpneumonectomy increase in lung DNA did not impair the postpneumonectomy increase in lung volume (5). We have also shown that lung volume does not increase in postpneumonectomy animals that have undergone prior hypophysectomy, despite a normal postoperative increase in lung weight and tissue volume (5). It is possible that a small but critical population of poorly differentiated cells may proliferate during the 1st postoperative wk, and in turn, differentiate into alveolar lining cells during the 2nd postoperative wk. Several investigators have demonstrated such a process after exposure to toxic concentrations of oxygen (17, 18) and in experimental proteus pneumonia (19), where proliferating alveolar type II cells evolve into type I lining cells during the repair process.

Another explanation for the divergence between cell proliferation and lung volume change relates to the possible late postoperative synthesis of ground substances that might allow the lung to enlarge. Recent studies by Cowan and Crystal (20) have shown that lung collagen synthesis increases dramatically in the 2nd wk after pneumonectomy in the rabbit. Examination of the deflation pressure-volume curve of the lung provides some indirect evidence of the status of lung ground substances such as elastin and collagen. Koo et al. (21) have recently provided additional support for Setnikar's original hypothesis that elastin is the primary determinant of lung distensibility at low lung volumes, while collagen determines lung distention characteristics near total lung capacity (22). 1 wk after pneumonectomy, C_L and TGV had both decreased, suggesting that the recoil properties of the lung had increased. Lung volume change at high transpulmonary pressures did not differ from normal. While these lung recoil changes might have represented cellular proliferation within interstitial tissues not grossly evident on microscopic examination, or changes in lung surfactant associated with the postoperative state, the abnormalities in the elastin range of the pressure-volume curve might reflect early synthesis of an elastin precursor, which might be less distensible than the final amorphous cross-linked product (23). 2 wk after pneumonectomy, TLV had increased propor-

tionally to the increase in tissue volume, and recoil at high and low lung volumes had returned toward normal. These findings are compatible with synthesis of both elastin and collagen and structural reconstitution of the lung by this time.

Inspired oxygen tension has been shown to markedly affect lung size in immature (24, 25) and adult animals (25). Alveolar hypoxia stimulates lung growth, while hyperoxia in the range of 40% inspired oxygen impairs normal lung growth (24). In this study, variations of inspired oxygen during the 1st postoperative wk had a profound influence on the normal compensatory increase in lung volume. Oxygen concentrations of as little as 28% prevented any compensatory increase in TLV, while inspired oxygen concentrations of 14% resulted in an increase in TLV 23% greater than in pneumonectomy animals exposed to room air. Indeed, as Fig. 4 illustrates, over the range tested there was a direct relationship between inspired oxygen concentration and the postpneumonectomy increase in lung volume.

In contrast, inspired oxygen had little or no effect on postoperative changes in lung weight or tissue volume. If one assumes that these latter changes reflect postoperative cell proliferation, one might conclude that oxygen has little influence on postpneumonectomy cell proliferation in the lung, but has a marked influence on the process that ultimately results in lung enlargement.

The lung pressure-volume curves in postpneumonectomy animals exposed to oxygen revealed an increase in lung elastic recoil and a decrease in volume change over the elastin-related volume range, while in postpneumonectomy animals exposed to hypoxia there was evidence of diminished lung elastic recoil at low lung volumes, with increased C_L and TGV. Thus, inspired oxygen concentration appeared to have had an important regulating effect on the distensile characteristics of the lung in the elastin-dependent portion of the pressure-volume curve. It is unlikely that these pressure-volume changes represent alterations in the lung surfactant system, since oxygen concentrations in this range have been nontoxic in man and animals (26) and since our control animals were not affected. These findings might be explained by an oxygen effect on cell differentiation, with hyperoxia preventing cell differentiation with persistent interstitial accumulation of cells (even though histologic sections did not confirm this possibility) and hypoxia accelerating cell differentiation. Another possible explanation relates to the effect that oxygen might have on synthesis or cross-linking of lung elastin. The initial stages of elastin cross-link formation involve oxidative deamination of lysine in tropoelastin by the enzyme lysyl oxidase (23, 27). Chvapil et al. have shown this enzyme to be elevated in early granuloma formation and to decrease in activity with maturation of the granuloma (28). Pre-

liminary studies from our laboratory have shown a rapid rise in lung lysyl oxidase activity that reached a peak twice control values on the 1st day after pneumonectomy with a return to normal values by the 5th postoperative day (29). In these experiments, 30% oxygen blunted the postoperative increase in lysyl oxidase activity, while 13% oxygen accentuated changes in lysyl oxidase activity. Thus, during the postpneumonectomy growth process, inspired oxygen may influence lung volume by its effect on the structure or composition of lung connective tissue.

It is of interest regarding timing of postpneumonectomy events that 28% oxygen given during the 2nd postoperative wk had no effect on postpneumonectomy lung weight, tissue volume, or TLV. Thus, although the lung volume changes occur during the 2nd wk after surgery, the oxygen-sensitive events leading to this compensatory increase in lung size occur in the 1st postoperative wk. The early postpneumonectomy period, therefore, appears to be crucial in determining the ultimate degree of compensatory lung growth.

The oxygen-related changes discussed above may have resulted from oxygen-induced changes in ventilation. There are suggestions in both experimental and clinical literature that implicate ventilation as an important stimulus to normal lung growth (30), and it seems reasonable to suspect that increased ventilation of the remaining lung might be an important stimulus to postpneumonectomy lung growth. However, exposure to 3% CO₂, which might be expected to change minute ventilation by increasing both rate and tidal volume (31), had no effect on postpneumonectomy lung weight or TLV, eliminating alterations in ventilation as an explanation for the oxygen observations, and most likely for normal postpneumonectomy compensatory growth.

Doses of cyclophosphamide sufficient to produce leukopenia in rats yet not interfere with macrophage ingestion of bacteria (11) did not adversely influence postpneumonectomy compensatory lung growth in our study. Cyclophosphamide is known to interfere with mitosis and cell division via nonspecific cell cycle inhibition of DNA synthesis (32). The reason for the apparent absence of effect on compensatory increase in lung weight or tissue volume is unclear, but our results suggest that this commonly used adjunct to surgery in carcinoma of the lung (12) should not impair the ability of the remaining lung to grow after pneumonectomy.

Although these studies do not answer the fundamental question of why and how compensatory lung growth occurs after pneumonectomy, they do provide some useful insights into this process. The compensatory process seems to involve two phases: the first associated with an increase in lung weight, tissue volume, and cell proliferation, the second associated with increasing lung

volume. None of the variables employed in this or preceding experiments influenced the magnitude of phase one; but several factors, most notably growth hormone in previous experiments (5) and inspired oxygen concentration applied in the early postoperative period in these experiments dramatically influenced phase two of the process. Increased blood flow to or stretch of the remaining lung, factors common to all of our experiments, might be the major determinants of the early proliferation response. Fisher and Simnett found that atelectasis, which might be expected to shift blood flow to the remaining lung, was an active stimulant to increased mitosis in the contralateral lung; and they postulate that a transient increase in lung blood flow may influence the level of a tissue-specific controller of mitosis (15, 33). Lung pressure-volume changes in postpneumonectomy animals with hyperoxia or hypoxia and postoperative changes in lung lysyl oxidase activity illustrate the possible importance of connective tissue elements in determining the magnitude of the second phase of compensatory lung growth.

The clinical significance of these studies relates to the critical role that inspired oxygen concentration played in postoperative compensatory growth. Relatively small increments in inspired oxygen during the early postoperative period prevented any subsequent increase in lung volume. Concentrations of inspired oxygen that do not adversely influence pulmonary function have been found to impair more subtle pulmonary processes, such as alveolar macrophage function (34) and mucociliary clearance (35). The potential detrimental influence of increased levels of oxygen on compensatory lung growth must be added to the growing list of toxic but subclinical effects of oxygen. Oxygen is commonly administered to patients after pneumonectomy, and our studies provide another reason for keeping the inspired concentration of oxygen at the lowest level necessary to control hypoxemia.

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