Lung Structure and Function with Age in Normal Rats and Rats With Papain Emphysema

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ABSTRACT Intrapulmonary deposition of the proteolytic enzyme papain produces a lesion resembling emphysema in experimental animals. The natural history of this lesion has not been well defined. The present study was performed to evaluate changes in lung structure and function with aging in normal rats and rats exposed to an aerosol of papain at 2 mo of age. Groups of control and papain-exposed animals were studied at 4, 8, and 18 mo of age. The parameters of lung function studied were specific airways' conductance (G_{aw}/TGV), diffusing capacity per unit of alveolar volume (D_{Lco}/V_A), diffusing capacity (D_{Lco}), and functional residual capacity (FRC). Morphometric parameters were the postfixation lung volume (V_L) and mean chord length (L_M); internal surface area (ISA) and ISA extrapolated to both the mean V_L of the corresponding papain group and a V_L of 10 ml (ISA_{10}) were calculated.

At 4 mo of age L_M and FRC were significantly increased and ISA, D_{Lco}/V_A, and D_{Lco} were significantly reduced in the papain group. At 8 mo of age L_M was significantly increased and ISA was significantly decreased in the papain group; physiologic studies were not performed in this group. At 18 mo of age L_M was significantly increased and D_{Lco}/V_A, D_{Lco}, and ISA were significantly decreased. Neither progression nor healing of the lesion was observed despite similar lung growth in both groups.

This study demonstrates that a single proteolytic lung injury produces a fixed deficit of lung parenchyma. Progressive lung destruction may require repeated or continuous lung injury.

INTRODUCTION

Exposure of the lungs of experimental animals to the enzyme papain produces a lesion which morphologically resembles emphysema (1–4). Pulmonary function has been studied at a single interval after papain exposure in several species (5–9). These studies have shown increased functional residual capacity, decreased lung elastic recoil, increased flow resistance in small airways, and decreased diffusing capacity in papain-exposed animals, indicating that papain produces changes in pulmonary function similar to those observed in human emphysema. It is not clear whether a single exposure to papain produces a stable lesion or whether progressive alterations in lung structure or function occur. Nonquantitative histologic studies have suggested that significant repair of the lesion does not occur (4). The purpose of the present study was to evaluate changes in lung structure and function with aging in normal rats and rats exposed to a papain aerosol at 2 mo of age.

METHODS

Groups of 10–20 male white Sprague-Dawley rats were exposed to an aerosol of 10% papain[1] in saline for 4 h at 2 mo of age (weight approximately 200 g). A comparable control group received no aerosol exposure. Groups of control and papain-exposed animals were studied at 4, 8, and 18 mo of age.

Following thiamylal anesthesia (30 mg/kg intraperitoneally) airways' conductance was measured by a double-plethysmographic technique (10). Diffusing capacity of the lung was estimated during forced rebreathing. The trachea was cannulated with a polyethylene catheter (length 1.0 cm, internal diameter 0.16 cm) connected through a three-way stopcock to a syringe containing 5.0 ml of gas (0.3% CO, 0.3% neon in air). A water-filled sidearm of the cannula was connected to a Statham PM 23 transducer, the signal from which was recorded by a Hewlett-Packard series 1100 recorder[2] to allow accurate timing of the rebreathing maneuver. At the end of a normal expiration, the stopcock was opened to the syringe and rebreathing at approximately 2 cycles/s was initiated. The entire 5 ml (two to three times the normal tidal volume of the rat) were injected and withdrawn with each cycle. Rebreathing

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[1] Papain, Difco Laboratories, Detroit, Mich.
[2] Hewlett-Packard Co., Palo Alto, Calif.

was continued for 2–10 s. At least three rebreathing maneuvers of different times, separated by 10 min, were performed in each animal. The CO and neon content of 1 ml aliquots of expired gas were measured by gas chromatography.[3] Functional residual capacity (FRC) [4] was calculated from the dilution of neon after subtraction of apparatus dead space (0.3 ml). The apparent diffusing capacity for CO per unit of alveolar volume (D_{LCO}/V_A) was calculated for each animal from the slope of $\ln(F_{ACO}/F_{ACO_0})$ against time, where F_{ACO} equals the fractional concentration of alveolar CO at the end of a rebreathing cycle and F_{ACO_0} equals the initial fractional concentration of alveolar CO, determined for each rebreathing cycle from the dilution of inert gas. An estimate of D_{LCO} was obtained by multiplying D_{LCO}/V_A by the alveolar volume obtained from that rebreathing cycle, corrected to STPD. The diffusing capacity measurements were performed in 4- and 18-mo old animals only.

Following the rebreathing maneuvers, a lethal dose of thiamylal was administered intraperitoneally and the animal's chest was opened to prevent vigorous agonal respiratory efforts. The trachea, heart, and lungs were resected en bloc and the lungs inflated to 25 cm H_2O pressure with 10% buffered formalin or 3% glutaraldehyde. An open sump system maintained this pressure for 12–14 h before the tissues were processed further. Following dissection of the heart, thymus, and adipose tissue, the postfixation volume of the lungs (V_L) was determined by water displacement. Midcoronal sections of both lungs were removed, dehydrated, and embedded in paraffin. Sections 6 μm thick were stained with hematoxylin and eosin for histologic study. For determination of the mean chord length, or average distance between alveolar walls (L_M), a grid was drawn on the slides with lines approximately 3 mm apart. Alveolar wall intercepts were counted in one microscopic field in each of 10 randomly selected squares using an eyepiece with four parallel lines. L_M was calculated by: $L_M = n \cdot L/\Sigma i$, where n equals the number of lines counted, L equals the length of the line, and Σi equals the sum of alveolar intercepts (11). No correction for tissue shrinkage or for the percent of the lung occupied by parenchyma was employed. ISA was determined by the relationship $ISA = 4 \cdot V_L/L_M$ (11, 12). Since the ISA measured in this fashion is highly dependent on lung volume, or V_L, the measured ISA of each control lung was extrapolated to that which would have existed at a V_L equal to the mean V_L of the corresponding papain group. This extrapolation was based on the assumption that ISA varies as $V_L^{2/3}$ (13). As a further means of comparison, the measured ISA of each control and each papain-exposed lung was extrapolated to that which would have existed at a V_L of 10 ml (ISA_{10}), using the same assumption.

Statistical evaluation of the data was performed with Student's t test for grouped data. Probabilities equal to or less than 0.05 were considered significant.

The battery of physiologic and morphometric assessments described above was developed during the course of this investigation. Consequently, each animal studied at the earliest time period (4 mo of age) was evaluated by some, but not all, of these techniques, resulting in differing numbers of animals studied with each technique during this period.

[3] Carle Instruments, Inc., model 8000, Fullerton, Calif.

[4] *Abbreviations used in this paper:* D_{LCO}, diffusing capacity; D_{LCO}/V_A, diffusing capacity per unit of alveolar volume; F_{ACO_0}, initial fractional concentration of alveolar CO; F_{ACO_t}, fractional concentration of alveolar CO at end of rebreathing cycle; FRC, functional residual capacity; G_{aw}/TGV, specific airways' conductance; ISA, internal surface area of lung; L_M, mean chord length; V_L, postfixation lung volume.

RESULTS

The acute mortality following a single papain exposure ranged from 10 to 20% and was due to diffuse alveolar hemorrhage. Later deaths due to chronic pulmonary infection occurred in both groups, but were more frequent in the control group. Histologically the lungs of papain-exposed animals showed markedly enlarged smooth-walled centrilobular airspaces. The airways appeared normal (Figs. 1 and 2).

The mean body weights of control and papain-exposed animals were similar at all ages (Table I). Specific airways' conduction decreased with age in both groups; however, neither the differences between the control and papain groups nor the decrease with age within either group was significant.

FRC and FRC/kg were significantly larger in the papain group than in the control group at 4 mo, but the differences at 18 mo were not significant. The increase in FRC/kg with age in the control group was not significant.

D_{LCO}/V_A and D_{LCO} were significantly smaller in the papain group at 4 and 18 mo of age. The slight increases in D_{LCO}/V_A observed in both groups between ages 4 and 18 mo were not significant.

The morphometric data are presented in Table II. Both V_L and V_L/kg in the papain group were significantly larger than that of controls at 4 mo but not at 8 or 18 mo of age. V_L/kg increased significantly with age in the control group but not in the papain group. L_M was greater in the papain group at all ages. L_M increased significantly with age in control animals but not in the papain group. The regression of L_M on age in controls was: $L_M = 51 + 2.07 \times \text{age (months)} \pm 0.48$ ($r = 0.79$).

ISA was significantly smaller in the papain group compared with controls at each age. Among papain-exposed animals ISA increased significantly during each age interval; among controls the increase between ages 4 and 8 mo was significant, while that between 8 and 18 mo was not. Because of the larger V_L in the papain group at 4 mo, extrapolation of the measured ISA of the control lungs to that which would have existed at the mean V_L of the papain group amplified the difference in ISA between control and papain groups. This correction had little influence at 8 or 18 mo where lung volumes were similar in control and papain groups. When corrected for differences in lung volume the change in ISA with increasing age was similar in both groups (Fig. 3). The surface area of the lung extrapolated to that which would have existed at a V_L of 10 ml (ISA_{10}) did not

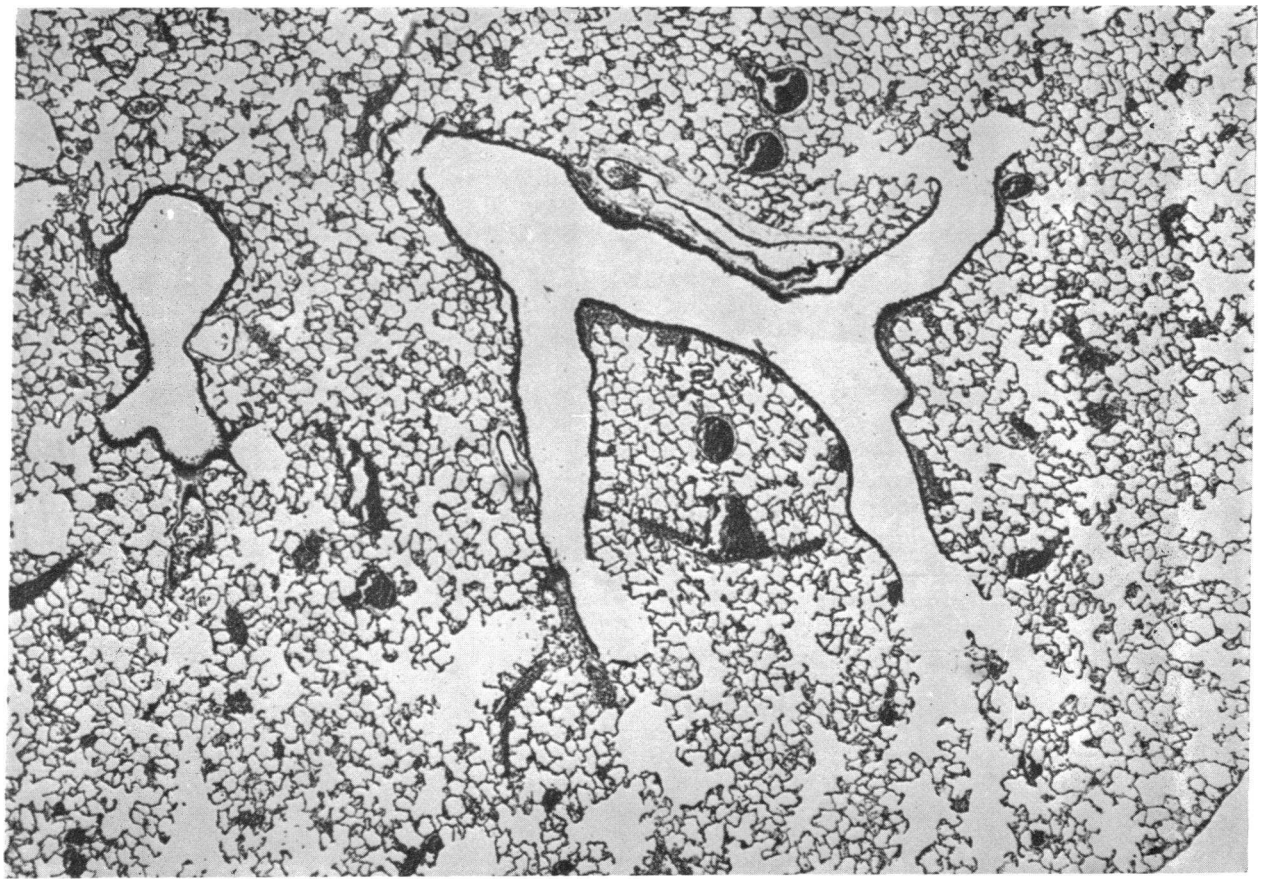

FIGURE 1 The lung of a control animal 8 mo of age fixed inflated at 25 cm formalin pressure. A terminal bronchiole with branching alveolar ducts is shown. Hematoxylin and eosin stain. Original magnification, × 50.

change with age in either group and was significantly smaller in the papain group at all ages (Table II). We were unable to correlate ISA and D_{Lco} for individual animals since both were not measured in all animals. However, a strong correlation between these parameters was found using the mean data for each group: ISA (m^2) = $0.0792 + 3.366 \times D_{Lco} \pm 0.0296$, $r = 0.983$.

DISCUSSION

Aging of the human lung is associated with alterations in its elastic properties, demonstrated most commonly as decreased lung elastic recoil in elderly subjects (14). Functional residual capacity increases with age, most likely related to changes in elastic properties of the lungs and thorax (15). Similar changes appear to characterize the rat lung between 4 and 18 mo of age. While we did not measure lung elastic recoil pressures directly, the measurement of V_L of lungs fixed inflated at a constant transpulmonary pressure provides a good estimate of the elastic properties of lung tissue (16). In control animals V_L normalized for body weight progressively increased although the change between 8 and 18 mo of age was not statistically significant. FRC/kg similarly increased, although not significantly, between 4 and 18 mo. These directional changes suggest that aging affects the elastic properties of rat and human lungs in similar fashion.

Previous studies have shown that papain damages lung elastic tissue acutely and reduces lung elastic recoil (16). Such changes were demonstrated 2 mo after papain in the present study by increased FRC, FRC/kg, V_L, and V_L/kg. However, no differences in these parameters between the control and papain groups were found at 8 or 18 mo of age. These findings suggest that, while papain altered the elastic properties of some regions of the lung, the lung tissue added during subsequent growth had normal elastic properties. Thus, the proportion of the lung with abnormal elastic behavior became smaller and the difference between control and papain-exposed groups became less marked.

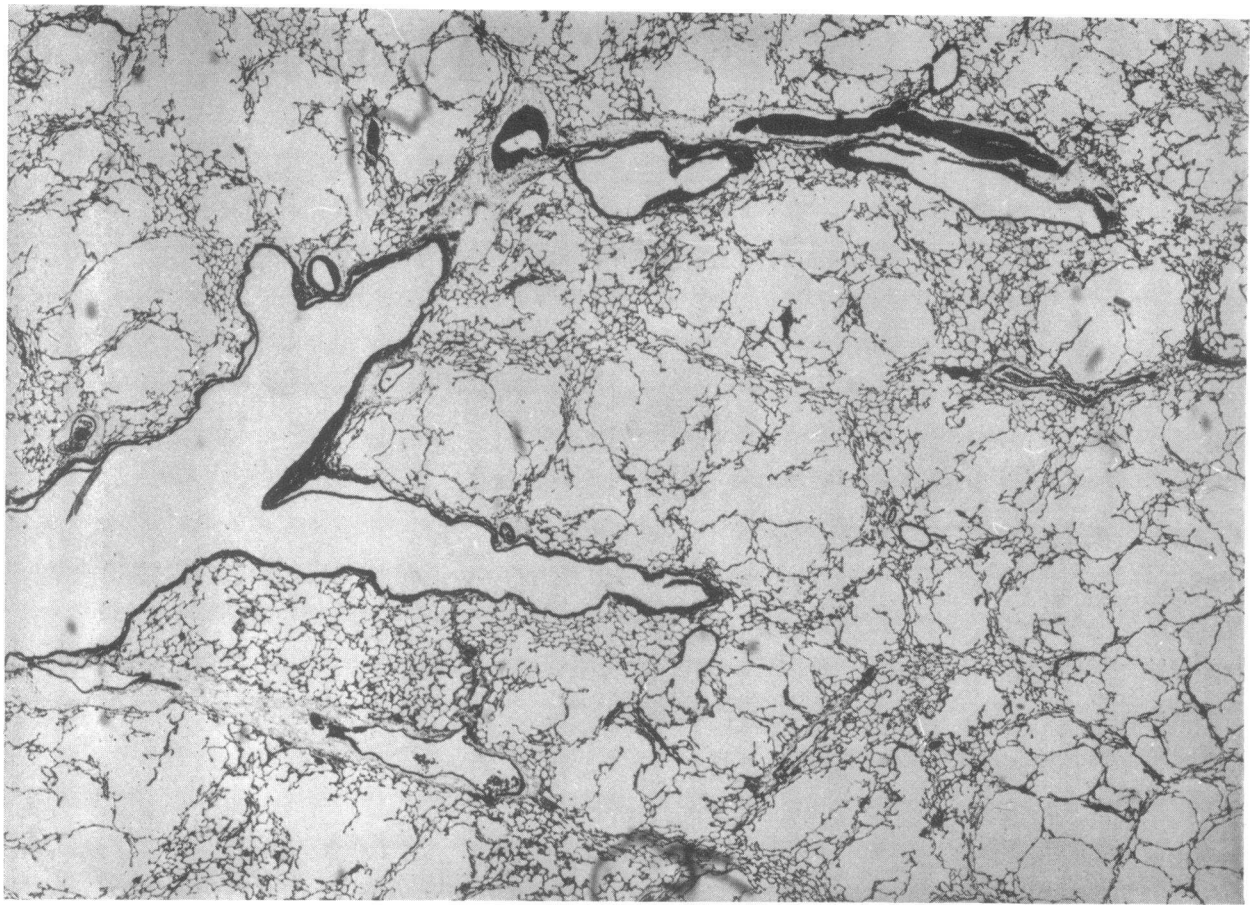

FIGURE 2 The lung of an 8-mo old rat which was exposed to an aerosol of papain at 2 mo of age. Markedly enlarged airspaces which are devoid of invaginating alveolar walls are shown. The terminal bronchiole appears to be normal. Hematoxylin and eosin stain. Original magnification, × 50.

The increased FRC of papain-exposed animals also could be explained by "small airways' disease," the presence of which might not be detected by the measurement of G_{aw}/TGV (17). Pushpakom et al. found increased peripheral airways' resistance in dogs following papain administration (6). However, Park et al. analyzed flow-

TABLE I

Body Weight and Lung Function Measurements in Control Rats and Rats Exposed to a Papain Aerosol at 2 mo of Age (Mean ±SE)

Age	Weight	G_{aw}/TGV	FRC	FRC/kg	D_{LCO}/V_A	D_{LCO}
	g	*(ml/s)/(cm H_2O/ml)*	*ml*	*ml/kg*	*(ml/min)/(mm Hg/ml)*	*ml/min/mm Hg*
4 mo						
Control	338±7 (32)	0.65±0.14 (14)	3.31±0.19 (18)	10.03±0.79 (18)	0.0219±0.0007 (18)	0.1463±0.0058 (18)
Papain	318±7 (39)	0.78±0.12 (20)	4.36±0.37* (19)	14.37±1.21* (19)	0.0152±0.0010* (19)	0.1146±0.0089* (19)
8 mo						
Control	488±22‡ (9)	0.62±0.10 (9)				
Papain	449±16‡ (5)	0.64±0.16 (5)				
18 mo						
Control	493±21‡ (5)	0.34±0.12 (5)	6.11±0.52‡ (5)	12.64±1.47 (5)	0.0279±0.0041 (5)	0.2411±0.0272‡ (5)
Papain	482±19‡ (12)	0.51±0.11 (12)	7.04±0.51‡ (12)	14.65±1.00 (12)	0.0175±0.0017* (12)	0.1667±0.0169*‡ (12)

G_{aw}/TGV, specific airways' conductance; FRC, functional residual capacity; FRC/kg, FRC per kilogram of body weight; D_{LCO}/V_A, diffusing capacity for carbon monoxide per unit of alveolar volume (STPD); D_{LCO}, diffusing capacity for carbon monoxide. *n* is in parentheses.

* Significant difference ($P \leq 0.05$) from controls.

‡ Significant difference from 4 mo.

TABLE II

Lung Morphometrics in Control Rats and Rats Exposed to a Papain Aerosol at 2 mo of Age (Mean ±SE)

Age	VL	VL/kg	LM	ISA Measured	ISA Corrected	ISA_{10}
	ml	*ml/kg*	*μm*	*cm²*		*cm²*
4 mo						
Control (n = 7)	7.8±0.4	21.7±1.0	54±2	5,571±445	6,923±317‡	6,540±298
Papain (n = 4)	10.9±0.9*	32.0±4.4*	97±2*	4,458±250*		4,219±49*
8 mo						
Control (n = 9)	14.9±0.4§	30.9±1.5‡	71±2‡	7,979±318§	7,620±261‡	6,118±210
Papain (n = 4)	13.9±1.1	31.8±4.0	92±4*	5,706±344*§		4,598±128*
18 mo						
Control (n = 5)	19.7±2.7§	38.4±2.8‡	87±7§	8,733±721‡	8,628±467‡	5,751±311
Papain (n = 13)	18.4±1.1§	38.7±2.7	107±4*	6,915±375*§		4,625±166*

VL, lung volume after fixation at 25 cm H_2O distending pressure; VL/kg, VL per kilogram of body weight; LM, mean linear intercept; ISA, internal surface area of the lung; Corrected, ISA corrected to the VL of the corresponding papain group; ISA_{10}, internal surface area extrapolated to a lung volume of 10 ml.

* Significant difference from control.

‡ Significant difference from measured ISA of papain group.

§ Significant difference from 4 mo.

‖ Significant difference from 8 mo.

volume curves over a range of driving pressures in the isolated lungs of hamsters previously exposed to papain and concluded that the reduced expiratory flow from lungs of animals exposed to papain was due principally to reduced elastic recoil and not to airway obstruction (7). The airways of our papain-exposed animals appeared normal histologically and it seems unlikely that the change in FRC was due to intrinsic disease of the small airways themselves.

The major effect of papain inhalation appears to be a loss of alveolar tissue as suggested by the presence of large, smooth-walled centrilobular airspaces in the lungs of papain-exposed animals. Such alveolar loss is confirmed by the significant reduction in measured ISA found in all papain groups. The deficit in measured ISA found at 4 mo of age in the papain group remained essentially constant throughout the study and measured ISA increased similarly with age in control and papain groups. This finding suggests that the lung injury following papain exposure neither stimulated the development of new alveoli nor led to progressive deterioration in lung structure. In the early postnatal period the ISA of the rat lung increases far more rapidly than lung volume, indicating the formation of new alveoli (18) and this increase in ISA can be further augmented if the animals are raised under hypobaric conditions (19). The lack of an increase in the number of alveoli in our animals is probably explained by the timing of the papain exposure.

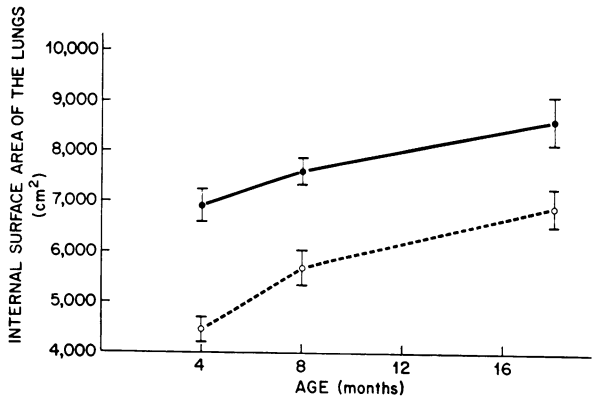

FIGURE 3 The change in internal surface area of the lungs (ISA) with age is shown for control (●——●) and papain-exposed (○- - -○) animals. The ISA indicated for the papain group is the measured ISA; that shown for controls is the measured ISA corrected to the lung volume of the corresponding papain group. Thus, the ISA's of control and papain groups are compared at the same lung volume in each time period. Differences were significant in all time periods (mean±SEM).

New alveoli are apparently not formed in response to stress later in life, such as pneumonectomy, following which compensatory lung growth is due to enlargement of existing airspaces (20).

However, the magnitude of alveolar loss following papain is underestimated by the measured ISA in the 4-mo old animals because of the coincident increase in the V_L of the papain group. A similar problem arises in the comparison of ISA in human lungs of different sizes. Thurlbeck used the internal surface area of the lung extrapolated to a constant lung volume to demonstrate a loss of alveolar tissue with age in human lungs (13). This approach assumes that the number of alveoli in the adult lung is essentially constant and that differences in lung volume between individuals are due to differences in alveolar dimensions so that surface area varies as the 2/3 power of volume. This assumption is consistent with the work of Dunnill (21) and of Weibel (12) which indicates the number of alveoli in human lungs remains constant beyond the age of 8 yr. It is not clear that the extrapolation of ISA to a constant lung volume is justified in the case of abnormal lungs. While the change in surface area of an individual unit of the lung is related to the change in volume of that unit to the 2/3 power, the surface area of the whole lung may not be if individual units vary widely in dimensions and compliance. The correction of ISA by the relationship of $V_L^{2/3}$ assumes that all linear dimensions, *i.e.,* diameters of all airspaces, change proportionately, and this may well not be true in emphysematous lungs.

The avoid this potential error we compared the ISA of control and papain animals by extrapolation of the ISA of control lungs to that which would have existed at the mean V_L of the corresponding papain group, assuming that differences for dimensions and compliance between lung units would be less in normal lungs. This correction amplified the difference between the ISA of control and papain animals at 4 mo but resulted in only small changes from the measured ISA at 8 and 18 mo where V_L was similar in the two groups.

However, the extrapolation of ISA to that which would have existed at a constant lung volume also appears to be a useful technique, particularly to compare normal lungs of varying sizes. Thus, the ISA_{10} of control animals did not change significantly between 4 and 18 mo of age while the measured ISA increased 57%. This indicates that the increase in surface area is explained solely by an increase in size of lung units. Although ISA_{10} tended to decrease slightly in control animals, we did not observe the significant decrement in surface area with age found by Thurlbeck in human lungs (13). Whether this difference is due to inherent species differences, or is due to the wide variety of alveolar insults inflicted on the human lung is unknown.

Extensive injury to alveolar walls occurs within hours of papain exposure (4). While this observation coupled with our present findings suggests that loss of alveolar tissue accounts for the decrease in lung surface area, changes in compliance of some lung regions alone could explain our results. If lung volume, or V_L, remains constant enlargement of some airspaces must be associated with a decrease in the dimensions of others. Such changes are suggested in the lung shown in Fig. 2. It can be shown that the maximum surface area for a particular V_L occurs when airspaces have the same linear dimensions; surface area at that V_L decreases as the radii of individual units become more disparate. Thus, the effects of papain on lung elastic tissue alone could explain the decrease in surface area, if V_L remained constant. Since the maximal inflation of the lung is probably determined by collagenous elements, which are relatively less affected by papain (6), this explanation remains possible.

D_{Lco} was linearly related to ISA in both the control and papain groups, and thus D_{Lco} increased in proportion to the increase in ISA as lung volume increased with age. This finding also differs from results in humans where D_{Lco} decreases with age (22), due at least in part to decreasing internal surface area of the lung. In our study, D_{Lco}/V_A remained constant despite the increased alveolar size found in 18-mo old animals. This finding could be explained by the opening of existing but previously nonfunctional capillaries as volume increased, or by the growth of new capillaries. We cannot differentiate between these possibilities on the basis of our data.

Neither progression of the papain lesion nor healing was demonstrated with any of the physiologic or morphologic parameters studied. Progression of the lesion might have been expected if the altered mechanical forces resulting from the remodeling of the lung were alone responsible for further lung damage. Conversely, healing of the lesion would have suggested that structural damage produced early in life may be repaired. In contrast, our results suggest that a single proteolytic insult with papain produces a fixed deficit in lung parenchyma. Since proteolysis of lung tissue may be responsible for at least the forms of human emphysema associated with alpha-*1*-antitrypsin deficiency, the papain model provides a basis for understanding the natural history of this process. Our data imply that the progression of such disease is the result of repeated or continuous episodes of lung injury and is not explained by a single lung insult even in early life.

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