Marked Longevity of Human Lung Parenchymal Elastic Fibers Deduced from Prevalence of D-Aspartate and Nuclear Weapons–related Radiocarbon

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Abstract

Normal structure and function of the lung parenchyma depend upon elastic fibers. Amorphous elastin is biochemically stable in vitro, and may provide a metabolically stable structural framework for the lung parenchyma. To test the metabolic stability of elastin in the normal human lung parenchyma, we have (*a*) estimated the time elapsed since the synthesis of the protein through measurement of aspartic acid racemization and (*b*) modeled the elastin turnover through measurement of the prevalence of nuclear weapons–related ^{14}C. Elastin purified by a new technique from normal lung parenchyma was hydrolyzed; then the prevalences of D-aspartate and ^{14}C were measured by gas chromatography and accelerator-mass spectrometry, respectively. D-aspartate increased linearly with age; K_{asp} ($1.76 \times 10^{-3} yr^{-1}$) was similar to that previously found for extraordinarily stable human tissues, indicating that the age of lung parenchymal elastin corresponded with the age of the subject. Radiocarbon prevalence data also were consistent with extraordinary metabolic stability of elastin; the calculated mean carbon residence time in elastin was 74 yr (95% confidence limits, 40–174 yr). These results indicate that airspace enlargement characteristic of "aging lung" is not associated with appreciable new synthesis of lung parenchymal elastin. The present study provides the first tissue-specific evaluation of turnover of an extracellular matrix component in humans and underscores the potential importance of elastin for maintenance of normal lung structure. Most importantly, the present work provides a foundation for strategies to directly evaluate extracellular matrix injury and repair in diseases of lung (especially pulmonary emphysema), vascular tissue, and skin. (*J. Clin. Invest.* 1991. 87:1828–1834.) Key words: elastin • emphysema • lung • microfibril • turnover

Introduction

The elastic properties of a variety of pliant tissues, including lung, are due in large part to the presence of elastic fibers in the extracellular space (1–3). The metabolic stability of elastic fibers may also be critical to the maintenance of normal tissue structure (4). Indeed, elastin injury and subsequent imperfect repair are held to be pivotal events in the pathogenesis of pulmonary emphysema (4–6).

Amorphous elastin is very insoluble and biochemically stable, and thus has been presumed to be long-lived (1). The other major components of the mature elastic fiber, tightly associated microfibrils, are composed of incompletely characterized glycoproteins (7–9). Little is known about human elastin turnover (2, 4), and virtually nothing is known about metabolic activity of microfibrils.

Elastin injury and repair in human diseases involving lung (especially pulmonary emphysema), vascular tissue, and skin has been little studied because of methodologic difficulties. Prior studies in rodents and birds have suggested that turnover of metabolically labeled elastin occurs over a period of years (10–13); however, the short lifespan of these animals has precluded extrapolation of such results to humans. Conventional metabolic labeling is not applicable to humans. Other techniques which are applicable to humans, such as measurement of urinary desmosines or plasma elastin-derived peptides (4–6), are not organ specific. Organ specificity of the analytical techniques is required to answer biologically important questions regarding lung elastin turnover in health and disease, because lung contains only a small fraction of total body elastin.

We now report an evaluation of the longevity of elastic fibers in the normal human lung parenchyma, making use of two independent and mutually confirmatory methods which are tissue specific: (*a*) estimating the time elapsed since the synthesis of the protein through measurement of aspartic acid racemization (14) and (*b*) modeling the elastin turnover through measurement of the prevalence of nuclear weapons–related ^{14}C (15), taking advantage of metabolic labeling of the biosphere in the last three decades that has resulted from atmospheric nuclear weapons testing. Our data indicate minimal synthesis, and persistence for a human lifespan, of parenchymal elastic fibers in normal adult lungs.

Methods

Reagents. D- and L-aspartic acid, pentafluoropropionic acid anhydride, (PFAA),[1] phenylisothiocyanate, and amino acid standards (standard H, to which hydroxyproline in equimolar amounts was added) were from Pierce Chemical Co., Rockford, IL. Trypsin, bacterial collagenase (type 7), guanidine HCl, urea, cyanogen bromide, and α-aminobutyric acid were from Sigma Chemical Co., St. Louis, MO. Trimethylamine was from Aldrich Chemical Co., Milwaukee, WI. All other chemicals were reagent grade.

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1. *Abbreviations used in this paper:* AMS, accelerator-mass spectrometry; K_{asp}, first-order rate constant for aspartate racemization; PFAA, pentafluoropropionic acid.

Subjects. Specimens (intact lungs or lobes) were obtained at postmortem examination from 14 individuals who had attained widely varying ages (range 6–78 yr) at the time of death. Death in all cases was either traumatic or from causes unrelated to the respiratory system.

Lung inflation, scoring for emphysema, and dissection. Specimens were either processed immediately after procurement or were frozen at $\leq -70°C$ until they were further processed. To begin the processing, unfixed lobes or lungs were inflated through the proximal airway at constant 25-cm H_2O air pressure, then frozen in inflation on dry ice.

After inflation and freezing but before dissection, a mid-saggital section of the lungs was photographed and scored for the presence of emphysema by comparison with standard photographs (16). Emphysema scores are based on a scale of 0–100, with 0 being normal and 100 representing the most severely emphysematous lungs.

Parenchyma of the frozen, inflated lungs was sharply dissected free from pleura and from vessels and airways greater than 1 mm diam. The specimens of parenchyma were stored at −90°C.

Elastin purification. We evaluated a variety of established purification strategies (17–22). We found it necessary to avoid prolonged exposure of the specimens to high temperatures, so as to minimize procedure-related aspartate racemization. For example, our preliminary experiments revealed that autoclaving authentic L-aspartic acid (in H_2O) for 15, 45, and 60 min resulted in 2.8%, 6.5%, and 7.3% racemization to D-aspartate, respectively. Our optimized purification, described below, was a substantial modification of the technique of Starcher and Galione (18), which had previously been found to be excellent for purification of lung elastin (23).

For purification of elastin, the frozen tissue was pulverized on dry ice in a blender, then subjected to the following purification steps: (*a*) prolonged (6 d) extraction at 4°C with repeated changes of 0.05 M Na_2HPO_4, pH 7.6, containing 1% (wt/vol) NaCl and 0.1% EDTA; (*b*) 5 M guanidine HCl (24 h, 25°C); (*c*) trypsin digestion (133 μg/ml, 37°C, 18 h, in 0.1 M Tris, 0.02 M $CaCl_2$, pH 8.2); (*d*) cyanogen bromide (in excess) in formic acid (5 h), followed by 0.05 M Tris, pH 8.0, containing 6 M urea and 0.5% β-mercaptoethanol (18 h); and (*e*) bacterial collagenase (Sigma type 7, 4,000 U/20 ml final volume, 37°C, 24 h). Variations from the procedure of Starcher and Galione included prolongation of salt extraction, elimination of autoclaving, addition of guanidine extraction, and collagenase digestion.

Hydrolysates of the purified elastin preparations were then prepared. Elastin is customarily hydrolyzed for up to 72 h before analysis; however, preliminary experiments revealed that heating authentic L-aspartic acid (6 M HCl, 105°C) for 6, 24, and 72 h resulted in 0.2%, 0.6%, and 4.2% racemization to D-aspartate, respectively. Therefore, the time to complete hydrolysis (achieved in 6 h; 6 M HCl, 105°C in vacuo) was shortened by first solubilizing elastin with porcine pancreatic elastase (1/1,000 by wt). Elastin hydrolysates were stored at −90°C.

Amino acid analyses. Analyses were performed either by ion-exchange chromatography on a model 110C amino acid analyzer (Beckman Instruments, Inc., Palo Alto, CA) or by reversed-phase HPLC after phenylisothiocyanate derivatization (24). The latter was performed on a model 1090 liquid chromatograph with model 1040A diode array detector and model 3392A integrator (Hewlett-Packard Co., Palo Alto, CA), using "solvent system III" of Henrikson and Meredith (24) and a 250 × 2-mm Hypersil octadecyl silica column, 5-μm bead size (Keystone Scientific, State College, PA). Each 100-μg sample of hydrolysate was spiked with 25 nmol of α-aminobutyric acid as internal standard.

Rationale: aspartic acid racemization. Eukaryotic organisms utilize only L-enantiomers of amino acids in protein synthesis because of the stereochemical constraints of polypeptide synthesis; post-translational racemization to D-amino acids occurs slowly but predictably over time, following reversible first-order kinetics. Racemization rates depend greatly upon temperature and the R-group of the amino acid, and are additionally influenced (but much less strongly) by pH and by ill-defined "local environmental effects" within the polypeptide (14). Content of D-amino acids in proteins has been correlated with the age of various archaeologic and paleontologic specimens (25–33). Racemization of aspartic acid occurs rapidly relative to that of the other amino acids. In the human body, D-aspartate accumulates at a rate of ~ 0.1% per year when tissue turnover is absent; measured prevalence of D-aspartate has been used to demonstrate that proteins in several tissues are metabolically stable over a human lifespan (34–37).

Quantification of prevalence of D-aspartate. Aspartate enantiomers were quantified by gas chromatography on a capillary column coated with a chiral stationary phase (38–40). Isopropyl esters of the elastin hydrolysates were produced, followed by acylation with PFAA.

For the esterification, a 3.5:1.5 solution of 2-propanol/3 M HCl was prepared immediately before use. Elastin hydrolysates (1 ml) were added to 500 μl of the isopropyl alcohol/HCl mixture in Reacti-Vials (Pierce Chemical Co.) and then incubated at 110°C for 1 h in an aluminum heating block. After cooling, the reagents were evaporated with a gentle stream of dry N_2. The residue was acylated by addition of 40 μl of PFAA and 200 μl of 0.05% trimethylamine in benzene, followed by heating for 10 min at 50°C. Contaminants were extracted by mixing the solution with 500 μl of water and then 500 μl of 5% ammonium hydroxide, followed by centrifugation. The aqueous layer was discarded, and the derivatized amino acids in benzene were injected onto a chiral column for gas chromatography as described below.

Aliquots (1 μl) of derivatized amino acids were injected into a model 5890A gas chromatograph fitted with a model 7673A autoinjector and autosampler (Hewlett-Packard Co.), and with a split/splitless injector which was packed with glass wool and 3% OV101 (Alltech Associates, Inc., Deerfield, IL). A split ratio of 10:1 and an injection temperature of 220°C were used. The column was a Chirasil-Val capillary column (50 m long, 0.25 mm i.d. supplied by Alltech Associates, Inc. A column head pressure of 18 psi and oven temperature of 125°C were optimal. Under these conditions, trimethylamine eluted at 4.5 min. After 30 min, the oven temperature was raised to 230°C to clear the column for the next injection. Detection of eluted gases was accomplished with a nitrogen-phosphorus detector (230°C; Hewlett-Packard Co.), and peaks were integrated with a model 3393A integrator (Hewlett-Packard Co.). Areas under the peaks were calculated from baseline to baseline, and integration parameters were invariant between all runs. Calculations were only made on baseline separations, which were consistently achieved.

Rationale and model: nuclear weapons–related ^{14}C. Excess ^{14}C in the atmosphere, a result of atomic weapons testing in the late 1950s and early 1960s, serves as a kinetic tracer for biogenic material (15). Weapons-related $^{14}CO_2$ reaches the biosphere through photosynthesis, and reaches human tissues through the food chain, with a lag time of 1.0–1.5 yr (41–43). Thus, ^{14}C prevalence in modern carbonaceous samples, including human tissues, provides a well-validated dating tool (15, 43, 44); ^{14}C prevalence in human proteins can be assumed to reflect prevalence of ^{14}C in the diet at the time of synthesis.

Quantification of ^{14}C by accelerator-mass spectrometry (AMS). Because of the small amounts of sample available, ^{14}C measurements were made by AMS (45). Purified elastin (10–30 mg per sample) was combusted to CO_2 in an enclosed system. The CO_2 collected was purified, then reacted with hydrogen on iron catalysts to produce graphite. The graphite was applied to copper targets. AMS measurements were made in triplicate at the Eidgenossische Technische Hochschule in Zurich. The chemical pretreatments and target material conversions were performed at Beta Analytical, Coral Gables, FL. Reported values have been corrected by ^{13}C for total isotope effects generated in both nature and during the physical and chemical laboratory procedures; 95% of the activity of National Bureau of Standards oxalic acid was used as the modern standard.

Mean carbon residence time in elastin. A one-compartment box-model was used to estimate mean carbon residence time in elastin (15), assuming no tissue growth (i.e., carbon input equals output). In this model, the mean residence (turnover) time is the average time required by a tissue for replacement of an amount of carbon equivalent to the tissue itself (15). Dietary ^{14}C during the interval 1962–1970 was estimated from prevalence in human blood and hair samples (see Fig. 4). For the periods 1953–1962 and 1970–1981, we used atmospheric ^{14}C

data (see Fig. 4), taking the dietary ^{14}C prevalence to be a weighted average of the current and two previous half-year periods with weights of 1, 2, and 1, respectively. Finally, for the post-1983 period, we extrapolated to the present assuming a simple exponential decay. Local Suess effects resulting from combustion of fossil fuels were assumed to be negligible. Estimates of mean carbon residence time and 95% confidence limits were made by the method of least squares.

Results

Emphysema scores for lung specimens from all subjects were 0 (normal) on a scale of 0–100, with the exception of one specimen (from the 70-yr-old individual) with a score of 10.

Amino acid analyses of the purified elastins were quite similar to those found by others (18, 23). Aspartate prevalence (8.1±[SD] 2.9 residues/1,000) was essentially identical to the 9 residues per 1,000 found by Starcher and Galione (18). Glutamate, hydroxyproline, glycine, alanine, and valine comprised 24.2±5.6, 15.4±7.8, 317±37, 231±18, and 89±15 residues per 1,000. Methionine residues were not detected. Since purified elastin is operationally defined based upon its unique amino acid composition (2), we concluded that our new purification strategy was comparable to the best methods previously published.

Gas chromatographic measurement of D-aspartic acid. Fig. 1 *a* shows a representative chromatogram of a 1:1 mixture of authentic D- and L-aspartic acid. Baseline separation was achieved ($R_s > 2.5$ [46]). Fig. 1 *b* shows a representative chromatogram of lung parenchymal elastin hydrolysate. Note that the aspartic acid enantiomers were well separated from all other amino acids by the capillary column; therefore, no purification of aspartic acid was required prior to gas chromatography. Fig. 1 *c* shows results of analysis of various mixtures of authentic D- and L-aspartic acid; note both the repetitive accuracy and the small fractions of D-aspartate that could be accurately determined.

Variation of D-aspartic acid prevalence during elastin purification. Fig. 2 shows percent D-aspartate in lung parenchyma from the youngest (6 yr old) and oldest (78 yr old) specimens. Definite but analytically acceptable amounts of procedure-related aspartate racemization occur during both the hydrolysis and the elastin purification steps, as shown by D-aspartate in samples from the young subject (only ~ 0.6% D-aspartate could have accumulated in vivo in 6 yr at body temperature). The amount of procedure-related racemization we have found in elastin is similar to that observed in other studies (34–37).

Fig. 2 also shows that D-aspartate is low in whole-lung hydrolysates from both specimens, indicating that the lung parenchyma is composed of predominantly short-lived (and recently synthesized) globular proteins. However, during successive elastin purification steps a progressively increasing discrepancy in D-aspartate prevalence is noted when specimens from the oldest and youngest lungs are compared. This observation can only be explained by accumulation of D-aspartate during life in the 78-yr-old individual. The excess of D-aspartate in elastin purified from the 78-yr-old represents in vivo racemization and

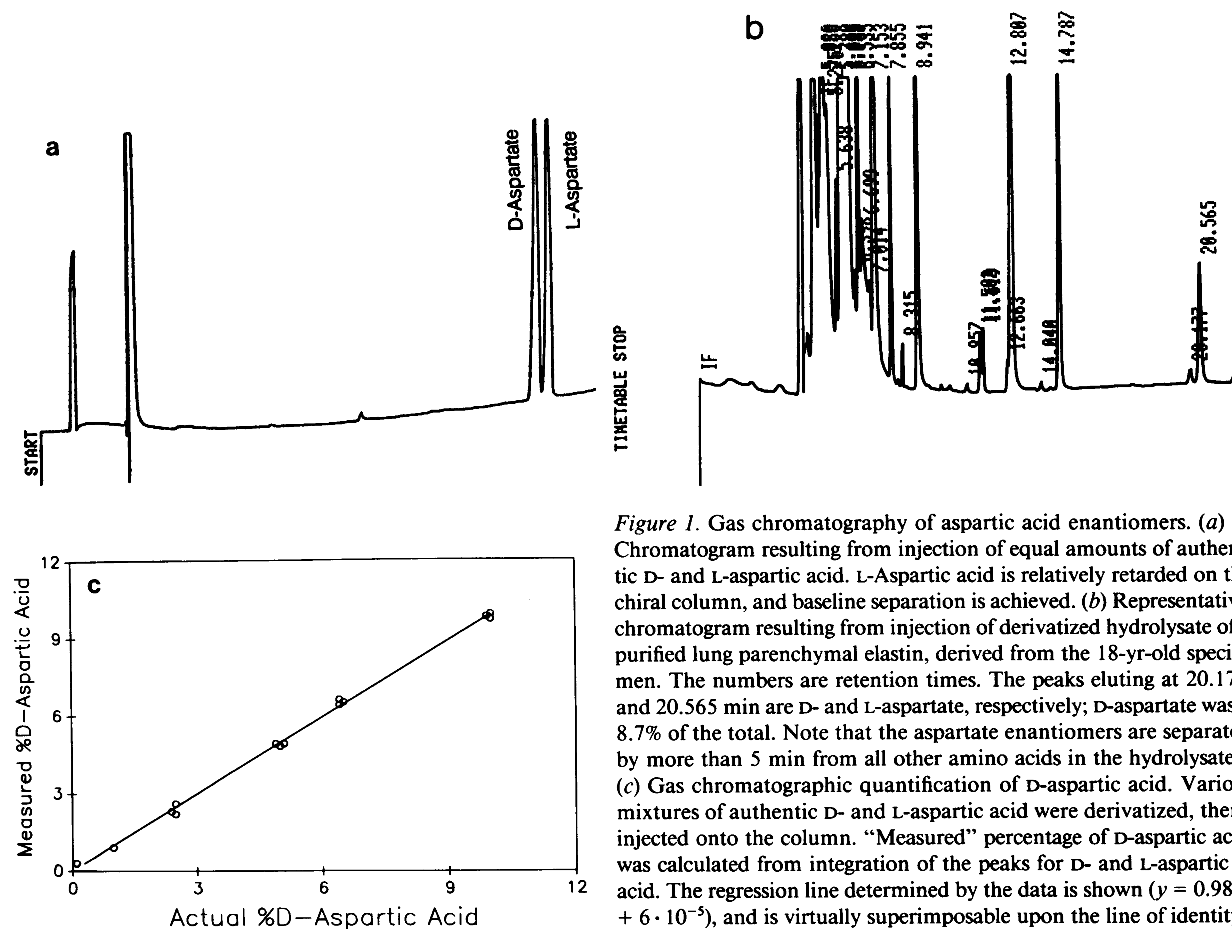

Figure 1. Gas chromatography of aspartic acid enantiomers. (*a*) Chromatogram resulting from injection of equal amounts of authentic D- and L-aspartic acid. L-Aspartic acid is relatively retarded on the chiral column, and baseline separation is achieved. (*b*) Representative chromatogram resulting from injection of derivatized hydrolysate of purified lung parenchymal elastin, derived from the 18-yr-old specimen. The numbers are retention times. The peaks eluting at 20.177 and 20.565 min are D- and L-aspartate, respectively; D-aspartate was 8.7% of the total. Note that the aspartate enantiomers are separated by more than 5 min from all other amino acids in the hydrolysate. (*c*) Gas chromatographic quantification of D-aspartic acid. Various mixtures of authentic D- and L-aspartic acid were derivatized, then injected onto the column. "Measured" percentage of D-aspartic acid was calculated from integration of the peaks for D- and L-aspartic acid. The regression line determined by the data is shown ($y = 0.987x + 6 \cdot 10^{-5}$), and is virtually superimposable upon the line of identity.

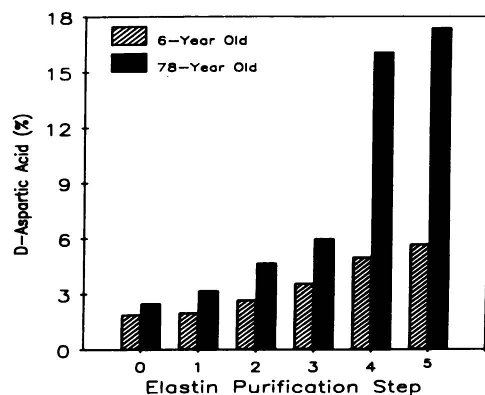

Figure 2. Evidence for in vivo racemization of aspartic acid. Variation in prevalence of D-aspartate during elastin purification is shown for our youngest and oldest specimens. Step 0 represents whole lung parenchyma; step 5 is purified elastin. For details of our purification strategy, see Methods. D-Aspartic acid detected in the youngest specimen can be attributed almost completely to procedure-related racemization. The excess of D-aspartic acid at each step in the other specimen, however, has resulted from racemization in vivo. Note that results from the whole-lung hydrolysates are similar for both specimens, reflecting their composition of proteins having predominantly rapid turnover. However, purified elastin from the oldest specimen has racemized extensively in vivo.

provides evidence of striking longevity of human lung elastic fibers.

Variation of D-aspartic acid prevalence with increasing age. Fig. 3 shows the correlation of % D-aspartic acid in lung parenchymal elastin with age attained at death for adult lungs (≥ 18 yr old). Prevalence of D-aspartate was linearly related to age. The data can be transformed to allow application of a reversible first-order rate equation (47), allowing calculation of the rate of formation of D-aspartate in human lung elastic fibers: $K_{asp} = 1.76 \times 10^{-3}\ yr^{-1}$ ($r = 0.98$); other extraordinarily metabolically stable human tissues have yielded similar results (34–37). Our data indicate that the age of lung parenchymal elastin corresponds with the age attained by the subject.

Prevalence of nuclear weapons-related radiocarbon. ^{14}C prevalence was determined in 8 of the 14 purified elastin specimens (Table I). Fig. 4 shows the relationship between the results obtained for lung parenchymal elastin, previously published results for metabolically active human proteins, and results of atmospheric sampling.

The specimens analyzed were carefully chosen to represent three groups. The 78-yr-old individual died in 1965; during the last 7 yr of life, newly synthesized proteins would have shown strikingly high prevalence of ^{14}C, yet the measured prevalence corresponded to that which existed during her youth. The second group (59–74 yr old at time of death in 1987) had completed postnatal lung growth prior to atmospheric weapons testing. Although the measured ^{14}C prevalence was higher than historical levels, it was lower than that which existed during the last 29 yr of their lives. The final group (18–35 yr old at time of death in 1987–88) was experiencing lung growth during the period of highest ^{14}C prevalence in the biosphere; the ^{14}C prevalence in their elastin reflected levels extant at the time of fetal and postnatal lung growth. The measured ^{14}C prevalence in their lung elastin remained higher than the levels in the biosphere at the time of their deaths.

Mean carbon residence time in elastin. Utilizing an extension of the analyses of Stenhouse and Baxter (15), we calculated the mean carbon residence time in lung elastin from individuals who completed postnatal lung growth prior to weapons testing (Table I); the least-squares estimate of the mean carbon residence time for all specimens was 74 yr (95% confidence limits 40–174 yr). A confirmatory analysis using an extrapolation from the dietary estimates of Stenhouse and Baxter (15) gave similar results (mean carbon residence time 72 yr; 95% confidence limits 40–180 yr). For the remainder of the specimens, errors involved in basic assumptions about fetal and postnatal lung growth (48) are considered to be too great to justify modeling attempts. Taken together, the results detailed above indicate minimal synthesis and marked persistence of elastin in lung parenchyma of normal adult humans.

Discussion

The data reported here represent the first information regarding organ-specific turnover rate of an extracellular matrix component purified from a complex human tissue. Our results indicate remarkable longevity of elastic fibers in the normal human lung parenchyma.

At the constant and relatively high body temperatures of humans, post-translational racemization of aspartic acid occurs at a predictable rate which is rapid enough to allow accumulation of readily measurable D-aspartate during the human lifespan in metabolically stable proteins (14).

Unacceptable aspartate racemization induced by high temperatures required that we substantially modify previously described elastin purification strategies before proceeding with these studies. Our purified elastin was essentially identical in amino acid composition to that purified by the best previously existing strategies (18, 23). Our gas chromatographic analyses were sensitive and accurate (Fig. 1), and obviated the need for chiral reagents.

In human tissues, prevalence of D-aspartate correlates with time elapsed since synthesis of the protein. Our present data (Figs. 2 and 3) indicate that D-aspartate prevalence in lung parenchymal elastin was very closely related to the age attained by the subject at death. The first-order rate constant for aspar-

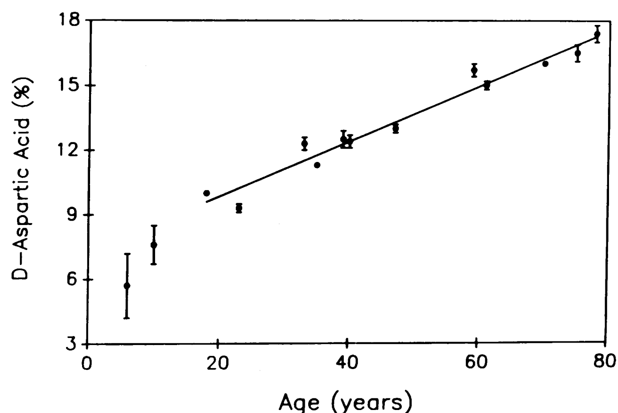

Figure 3. D-Aspartate in elastic fibers correlated with age at death. Data are means of three or more determinations; error bars represent SD (where SD is not shown, it was < 0.1%). The linear regression line determined by the data is shown ($r = 0.98$). As discussed in the text, the relationship of D-aspartic acid to the age of the subject argues that parenchymal elastin is markedly persistent after its synthesis in the lung.

Table I. Radiocarbon Abundance in Elastin from Normal Human Lung Parenchyma

Sample number*	Age at death	^{14}C prevalence	Mean carbon residence time
	yr	*% modern*	*yr*
Beta-27091/ETH-4412	18	135.0±1.0‡	
Beta-27092/ETH-4413	23	145.7±1.1	
Beta-27093/ETH-4414	33	127.9±1.0	
Beta-27094/ETH-4415	35	118.3±0.9	
Beta-27096/ETH-4416	59	114.2±0.8	54±5§
Beta-27097/ETH-4417	61	105.6±0.8	131±5
Beta-27098/ETH-4418	74	113.2±0.8	59±6
Beta-27099/ETH-4419	78	100.3±0.8	103±4

* Sample numbers were assigned by Beta Analytical and the Eidgenossische Technische Hochschule, Zurich, respectively.
‡ Results are means±SD.
§ Estimates, ±1 SD of the estimate (i.e., 95% confidence limits = ±1.96 SD).

tate racemization in elastin was 1.76×10^{-3} yr^{-1}. This result is similar to those reported for other metabolically stable human proteins (0.794×10^{-3} yr^{-1} for tooth dentine [35], 0.829×10^{-3} yr^{-1} for tooth enamel [34], 1.25×10^{-3} yr^{-1} for eye lens nucleus [36], and 1.5×10^{-3} yr^{-1} for myelinated white matter [37]). The higher rate constants for elastic fibers, lens, and myelin are not surprising given that they exist at slightly higher (core) temperature in vivo in comparison to enamel and dentine, and that racemization rates are strongly related to temperature (the Arrhenius activation energy for amino acid racemization is 33.4 kcal mol^{-1} [29]; at 20°C the half-time for aspartic acid racemization is 15,000 yr [34]).

Elastic fibers are composed not only of amorphous elastin but also microfibrils and other more poorly characterized proteins (1, 2, 7–9, 49). The microfibrillar components (10–12-nm fibrils) are located not only around the periphery of the amorphous component but are also interspersed within it (1). Aspartate prevalence in the amorphous component can be estimated, since the gene sequence of human tropoelastin (the precursor of amorphous elastin [1]) has been determined. Aspartate comprises 3 of 752 residues in the deduced amino acid sequence (50). Aspartate prevalence in lung tropoelastin is not known with precision because of unknown effects of RNA splicing, but it can be approximated to be 1.5–4 residues per 1,000 (51). This contrasts strikingly with the mean aspartate prevalence of eukaryotic nonenzymatic proteins (100 residues per 1,000 [52]), and also with microfibrillar proteins of elastic fibers, which are acidic glycoproteins containing up to 134 residues of aspartate per 1,000 (8).

Since our preparations contained 8±3 (SD) aspartate residues per 1,000 (a typical result for purified elastic fibers), it is clear that a considerable proportion of the total aspartate residues in our specimens was derived from small amounts of proteins in the elastic fiber with much higher aspartate prevalence than tropoelastin. However, the relationship between the extent of racemization and age was not affected by the relative abundance of aspartate residues. Linear regression analysis demonstrated no significant relationship between percent D-aspartate and the total number of aspartate residues per 1,000 in the various purified elastin samples. Furthermore, in multiple regression analysis of D-aspartate prevalence as a function of age and aspartate residues per 1,000, age remained highly predictive of D-aspartate ($P < 0.0001$). These results indicate that the age of microfibrils and other nonelastin components of the elastic fiber are highly correlated with that of amorphous elastin; both elastin and nonelastin components of the elastic fiber are equally long-lived.

Metabolic labeling of human tissues by atmospheric testing of nuclear weapons allowed an independent assessment of lung elastin turnover. Moreover, this method samples all carbons in the specimens, and thus is not biased toward contributions of relatively aspartate-rich proteins in the elastic fiber.

The technique of accelerator-mass spectrometry (45) allowed highly accurate quantification of ^{14}C prevalence in milligram-size samples of purified elastin. Analyses of all eight samples tested were consistent with the conclusion that lung parenchymal elastin synthesis occurs almost exclusively during fetal and postnatal lung growth. Modeling of ^{14}C prevalence data according to the method of Stenhouse and Baxter (15) revealed the mean carbon residence time in elastin of adult lung to be 74 yr (95% confidence limits 40–174 yr). Two different estimations of dietary histories gave nearly identical results.

Our present data, derived from two independent analytical approaches, thus indicate that elastic fibers are remarkably persistent in the human lung parenchyma. The components of the mature lung elastic fiber can be considered to be metabolically inseparable.

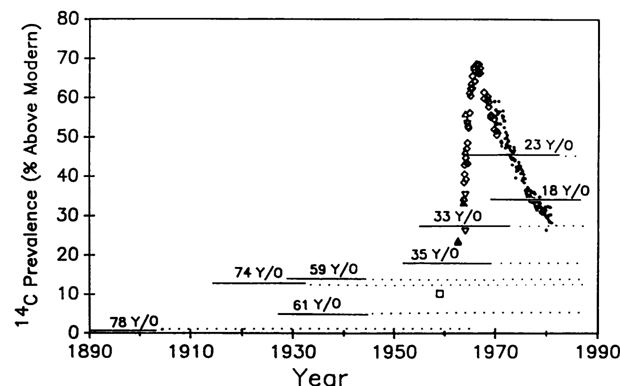

Figure 4. Synthesis and persistence of elastin in normal adult lung parenchyma, as deduced from prevalence of nuclear weapons–related ^{14}C. Large open symbols are previously published data from human blood samples collected in the Northern Hemisphere (representative of a carbon pool with rapid turnover—data from hair published by Nydal [43] were superimposable): Diamonds, serial samples from two individuals (43); square, a single blood sample (41); triangles and inverse triangles, plasma and erythrocytes, respectively (42). Small circles are representative atmospheric samples collected at Nordkapp, Norway (65). Radiocarbon prevalence in metabolically active human tissues and in the atmosphere become virtually indistinguishable after 1970. Each horizontal line corresponds to a result of analysis of human lung parenchymal elastin given in Table I. The age at time of death is shown for each subject. The lengths and positioning of the solid portions of the lines correspond to timing and duration of fetal and postnatal lung growth (48), whereas the interrupted portions of the lines represent the remainder of the individuals' lifespans. The vertical position of each line represents a ^{14}C prevalence result given in Table I. Note that the ^{14}C prevalence in the samples of purified elastin corresponds with the ^{14}C prevalence in the biosphere during lung growth. As further discussed in the text, the results indicate minimal synthesis, and marked persistence, of elastic tissue in the normal adult human lung.

It is instructive to compare the present results with earlier data regarding elastin turnover in animals and humans. Metabolic labeling of elastin in rodents (10, 11, 53) and birds (12) revealed that turnover of elastin in lungs and aorta occurred over a period of months to years. Because of the short lifespan of these animals, however, it was difficult to predict the amount of elastin turnover to be expected over several decades in human tissues. In the human, immunoreactive elastin peptides can be found in plasma (54–56), and are thought to be derived in large part from degradation of mature elastic fibers. The tissue of origin for these peptides is not known. Moreover, in the absence of precise information regarding clearance rate of elastin peptides, it is not possible to estimate even total-body elastin turnover. Low levels of elastin peptides have been found in human bronchoalveolar lavage fluid (57). The same ambiguities about these latter results apply, and in normals it is not clear what proportion of the elastin peptides in bronchoalveolar lavage fluid diffuse into the lung epithelial lining fluid from plasma. Desmosines, which are unique cross-linking amino acids in elastin (1, 2) that are not absorbed from dietary sources (58), are thought to be quantitatively excreted in human urine. The measurable desmosines found in all human urine samples (59–62) provide evidence of some amount of turnover of mature cross-linked elastin in humans, but do not indicate the tissue(s) in which this turnover is occurring. Our present work is consistent with these previous results, and has allowed the first tissue-specific evaluation of elastin turnover in the human.

In summary, we conclude from measurements of both D-aspartate and weapons-related ^{14}C prevalence that the mature lung parenchymal elastic fiber is normally a metabolically stable unit over the human lifespan. It may not be appropriate to extrapolate these results to elastin in other sites (such as pleura) or to other tissues; some animal data indicate that turnover of skin elastin is more rapid than that of elastin in lung or aorta (11). However, our working hypothesis is that elastic fibers, when compared to the metabolic activity of most other human proteins, are extraordinarily stable wherever they located.

The aging lung parenchyma exhibits airspace enlargement, particularly of alveolar ducts (63). Although this change has been called "senile emphysema," the consensus of a recent conference (64) was that the structural changes were not truly emphysematous in nature. Our data indicate that this change in lung structure is not associated with appreciable new synthesis of parenchymal elastin.

The work reported here should provide a strategic foundation for studies to directly evaluate turnover of a variety of extracellular matrix components in the human, both in health and in disease. Extension of these studies to the evaluation of elastin turnover in disease (especially human pulmonary emphysema [4–6]) will be of particular interest. Moreover, these methods are applicable to study of elastin injury and repair in vascular diseases (such as atherosclerosis) and skin (such as actinic effects).

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References

1. Rosenbloom, J. 1984. Biology of disease. Elastin: relation of protein and gene structure to disease. *Lab. Invest.* 51:605–623.

2. Starcher, B. C. 1986. Elastin and the lung. *Thorax.* 41:577–585.

3. Sandberg, L. B., N. T. Soskel, and J. G. Leslie. 1981. Elastin structure, biosynthesis, and relation to disease states. *N. Engl. J. Med.* 304:566–579.

4. Niewoehner, D. E. 1988. Cigarette smoking, lung inflammation, and the development of emphysema. *J. Lab. Clin. Med.* 111:15–27.

5. Janoff, A. 1985. State of the art. Elastases and emphysema. *Am. Rev. Respir. Dis.* 132:417–433.

6. Wewers, M. 1989. Pathogenesis of emphysema: Assessment of basic science concepts through clinical investigation. *Chest.* 95:190–195.

7. Mecham, R. P., A. Hinek, E. G. Cleary, U. Kucich, S. J. Lee, and J. Rosenbloom. 1988. Development of immunoreagents to ciliary zonules that react with protein components of elastic fiber microfibrils and with elastin-producing cells. *Biochem. Biophys. Res. Commun.* 151:822–826.

8. Gibson, M. A., J. S. Kumaratilake, and E. G. Cleary. 1989. The protein components of the 12-nanometer microfibrils of elastic and nonelastic tissues. *J. Biol. Chem.* 264:4590–4598.

9. Cleary, E. G., and M. A. Gibson. 1983. Elastin-associated microfibrils and microfibrillar proteins. *Int. Rev. Connect. Tissue Res.* 10:97–209.

10. Dubick, M. A., R. B. Rucker, C. E. Cross, and J. A. Last. 1981. Elastin metabolism in rodent lung. *Biochim. Biophys. Acta.* 672:303–306.

11. Pierce, J. A., H. Resnick, and P. H. Henry. 1967. Collagen and elastin metabolism in the lungs, skin, and bones of adult rats. *J. Lab. Clin. Med.* 69:485–492.

12. Lefevre, M., and R. B. Rucker. 1980. Aorta elastin turnover in normal and hypercholesterolemic Japanese quail. *Biochim. Biophys. Acta.* 630:519–529.

13. Walford, R. L., P. K. Carter, and R. B. Schneider. 1964. Stability of labeled aortic elastin tissue with age and pregnancy in the rat. *Arch. Pathol.* 78:43–45.

14. Helfman, P. M. 1976. Biological and Chronometric Applications of Amino Acid Racemization Reactions. Doctoral thesis. University of California, San Diego, San Diego.

15. Stenhouse, M. J., and M. S. Baxter. 1977. Bomb ^{14}C as a biological tracer. *Nature (Lond.).* 267:828–832.

16. Thurlbeck, W. M., M. S. Dunnill, W. Hartung, B. Heard, A. Heppleston, and R. Ryder. 1970. A comparison of three methods of measuring emphysema. *Hum. Pathol.* 1:215–226.

17. John, R., and J. Thomas. 1972. Chemical compositions of elastins isolated from aortas and pulmonary tissues of humans of different ages. *Biochem. J.* 127:261–269.

18. Starcher, B. C., and M. J. Galione. 1976. Purification and comparison of elastins from different animal species. *Anal. Biochem.* 74:441–447.

19. Lowry, O. H., D. R. Gilligan, and E. M. Katersky. 1941. The determination of collagen and elastin in tissues, with results obtained in various normal tissues from different species. *J. Biol. Chem.* 139:795–804.

20. Rasmussen, B. L., E. Bruenger, and L. B. Sandberg. 1975. A new method for purification of mature elastin. *Anal. Biochem.* 64:255–259.

21. Richmond, V. 1974. Lung parenchymal elastin isolated by non-degradative means. *Biochim. Biophys. Acta.* 351:173–177.

22. Paz, M. A., D. A. Keith, H. P. Traverso, and P. M. Gallop. 1976. Isolation, purification, and cross-linking profiles of elastin from lung and aorta. *Biochemistry.* 15:4912–4918.

23. Soskel, N. T., and L. B. Sandburg. 1983. A comparison of six methods of extracting elastin residue from hamster lungs. *Exp. Lung Res.* 4:109–119.

24. Henrikson, R. L., and S. Meredith. 1984. Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. *Anal. Biochem.* 136:65–74.

25. Bada, J. L., B. P. Luyendyk, and J. B. Maynard. 1970. Marine sediments: dating by the racemization of amino acids. *Science (Wash. DC).* 170:730–732.

26. Wehmiller, J. F., and P. E. Hare. 1971. Racemization of amino acids in marine sediments. *Science (Wash. DC).* 173:907–911.

27. Bada, J. L., R. A. Schroeder, and G. F. Carter. 1974. New evidence for the antiquity of man in North America deduced from aspartic acid racemization. *Science (Wash. DC).* 184:791–793.

28. Masters, P. M., and J. L. Bada. 1978. Amino acid racemization dating of bone and shell. *Archaeol. Chem.* 8:117–138.

29. Bada, J. L., K. A. Kvenvolden, and E. Peterson. 1973. Racemization of amino acids in bones. *Nature (Lond.).* 245:308–310.

30. Bada, J. L., and R. Protsch. 1973. Racemization reaction of aspartic acid and its use in dating fossil bones. *Proc. Natl. Acad. Sci. USA.* 70:1331–1334.

31. Bada, J. L., R. A. Schroeder, R. Protsch, and R. Berger. 1974. Concordance of collagen-based radiocarbon and aspartic-acid racemization ages. *Proc. Natl. Acad. Sci. USA.* 71:914–917.

32. Petit, M. G. 1974. The racemization rate constant for protein-bound aspartic acid in woodrat middens. *Quat. Res. (Duluth).* 4:340–345.

33. Brooks, A. S., P. E. Hare, J. E. Kokis, G. H. Miller, R. D. Ernst, and F. Wendorf. 1990. Dating pleistocene archeological sites by protein diagenesis in ostrich eggshell. *Science (Wash. DC).* 248:60–64.

34. Helfman, P. M., and J. L. Bada. 1975. Aspartic acid racemization in tooth enamel from living humans. *Proc. Natl. Acad. Sci. USA.* 72:2891–2894.

35. Helfman, P. M., and J. L. Bada. 1976. Aspartic acid racemisation in dentine as a measure of ageing. *Nature (Lond.).* 262:279–281.

36. Masters, P. M., J. L. Bada, and J. S. Zigler. 1977. Aspartic acid racemisation in the human lens during ageing and in cataract formation. *Nature (Lond.).* 268:71–73.

37. Man, E. H., M. E. Sandhouse, J. Burg, and G. H. Fisher. 1983. Accumulation of D-aspartic acid with age in the human brain. *Science (Wash. DC).* 220:1407–1408.

38. Frank, H., G. J. Nicholson, and E. Bayer. 1978. Enantiomer labelling, a method for the quantitative analysis of amino acids. *J. Chromatogr.* 167:187–196.

39. Frank, H., A. Rettenmeier, H. Weicker, G. J. Nicholson, and E. Bayer. 1980. A new gas chromatographic method for determination of amino acid levels in human serum. *Clin. Chim. Acta.* 105:201–211.

40. Bailey, E., P. B. Farmer, and J. H. Lamb. 1980. The enantiomer as internal standard for the quantitation of the alkylated amino acid *S*-methyl-L-cysteine in haemoglobin by gas chromatography-chemical ionosation mass spectrometry with single ion detection. *J. Chromatogr.* 200:145–152.

41. Broecker, W. S., A. Schulert, and E. A. Olson. 1959. Bomb carbon-14 in human beings. *Science (Wash. DC).* 130:331–332.

42. Libby, W. F., R. Berger, J. F. Mead, G. V. Alexander, and J. F. Ross. 1964. Replacement rates for human tissue from atmospheric radiocarbon. *Science (Wash. DC).* 146:1170–1172.

43. Nydal, R., K. Lovseth, and O. Syrstad. 1971. Bomb ^{14}C in the human population. *Nature (Lond.).* 232:418–421.

44. Harkness, D. D., and A. Walton. 1972. Further investigations of the transfer of bomb ^{14}C to man. *Nature (Lond.).* 240:302–303.

45. Elmore, D., and F. M. Phillips. 1987. Accelerator mass spectrometry for measurement of long-lived radioisotopes. *Science (Wash. DC).* 236:543–550.

46. Jennings, W. 1980. Gas Chromatography with Glass Capillary Columns. Academic Press, Inc., New York.

47. Bada, J. L. 1972. The dating of fossil bones using the racemization of isoleucine. *Earth Planet. Sci. Lett.* 15:223–231.

48. Burri, P. H. 1988. Development and regeneration of the lung. *In* Pulmonary Diseases and Disorders. A. P. Fishman, editor. McGraw-Hill Book Co., Inc., New York. 61–78.

49. Kucich, U., W. Abrams, G. Weinbaum, and J. Rosenbloom. 1988. Evidence that elastin is covalently crosslinked to other extracellular macromolecule(s). *J. Cell Biol.* 107:603a. (Abstr.)

50. Indik, Z., H. Yeh, N. Ornstein-Goldstein, P. Sheppard, N. Anderson, J. C. Rosenbloom, L. Peltonen, and J. Rosenbloom. 1987. Alternative splicing of human elastin mRNA indicated by sequence analysis of cloned genomic and complementary DNA. *Proc. Natl. Acad. Sci. USA.* 84:5680–5684.

51. Fazio, M. J., D. R. Olsen, H. Kuivaniemi, M.-L. Chu, J. M. Davidson, J. Rosenbloom, and J. Uitto. 1988. Isolation and characterization of human elastin cDNAs and age-associated variation in elastin gene expression in cultured skin fibroblasts. *Lab. Invest.* 58:270–277.

52. Reeck, G. R., and L. Fisher. 1973. A statistical analysis of the amino acid compositions of proteins. *Int. J. Pept. Protein Res.* 5:109–117.

53. Ohlsson, I. U., and F. A. N. Osadebe. 1974. Carbon isotope variations and fractionation corrections in ^{14}C dating. *Boreas (Oslo).* 3:139–146.

54. Kucich, U., P. Christner, M. Lippmann, A. Fein, A. Goldberg, P. Kimbel, G. Weinbaum, and J. Rosenbloom. 1983. Immunologic measurement of elastin-derived peptides in human serum. *Am. Rev. Respir. Dis.* 127:S28–S30.

55. Kucich, U., P. Christner, M. Lippmann, P. Kimbel, G. Williams, J. Rosenbloom, and G. Weinbaum. 1985. Utilization of a peroxidase antiperoxidase complex in an enzyme-linked immunosorbent assay of elastin-derived peptides in human plasma. *Am. Rev. Respir. Dis.* 131:709–713.

56. Darnule, T. V., M. McKee, A. T. Darnule, G. M. Turino, and I. Mandl. 1982. Solid-phase radioimmunoassay for estimation of elastin peptides in human sera. *Anal. Biochem.* 122:302–307.

57. Abrams, W. R., A. M. Fein, U. Kucich, F. Kueppers, H. Yamada, T. Kuzmowycz, L. Morgan, M. Lippmann, S. K. Goldberg, and G. Weinbaum. 1984. Proteinase inhibitory function in inflammatory lung disease. I. Acute bacterial pneumonia. *Am. Rev. Respir. Dis.* 129:735–741.

58. Starcher, B. C., and R. A. Goldstein. 1979. Studies on the absorption of desmosine and isodesmosine. *J. Lab. Clin. Med.* 94:848–852.

59. Johnson, D. A., B. Carter-Hamm, and W. M. Dralle. 1982. Inactivation of human bronchial mucosal proteinase inhibitor by *Pseudomonas aeruginosa* elastase. *Am. Rev. Respir. Dis.* 126:1070–1073.

60. Gunja-Smith, Z., and R. J. Boucek. 1981. Desmosines in human urine: amounts in early development and in Marfan's syndrome. *Biochem. J.* 193:915–918.

61. Pelham, F., M. Wewers, R. Crystal, A. S. Buist, and A. Janoff. 1985. Urinary excretion of desmosine (elastin cross-links) in subjects with PiZZ alpha-1-antitrypsin deficiency, a phenotype associated with hereditary predisposition to pulmonary emphysema. *Am. Rev. Respir. Dis.* 132:821–823.

62. Davies, S. F., K. P. Offord, M. G. Brown, H. Campe, and D. Niewoehner. 1983. Urine desmosine is unrelated to cigarette smoking or to spirometric function. *Am. Rev. Respir. Dis.* 128:473–475.

63. Campbell, E. J., and S. S. Lefrak. 1984. Physiologic processes of aging in the respiratory system. *In* Anesthesia and the Geriatric Patient. S. W. Krechel, editor. Grune & Stratton, Inc., Orlando, FL. 19–44.

64. Snider, G. L., J. Kleinerman, W. M. Thurlbeck, and Z. H. Bengali. 1985. The definition of emphysema: report of a National Heart, Lung, and Blood Institute, Division of Lung Diseases workshop. *Am. Rev. Respir. Dis.* 132:182–185.

65. Nydal, R., and K. Lovseth. 1983. Tracing bomb ^{14}C in the atmosphere 1962–1980. *J. Geophys. Res.* 88:3621–3642.