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Elastin Metabolism and Chemistry: Potential Roles in Lung Development and Structure

by Robert B. Rucker* and Michael A. Dubick*

Elastic fibers are important for elasticity and extensibility of lung tissue. In the developing lung, elastic fibers appear in greatest numbers during the process or period of alveolarization. A variety of mesenchymal cells in lung appear responsible for elastin synthesis. Elastin is a novel protein both from the standpoint of its processing into elastic fibers and chemical properties. For example, elastin undergoes posttranslational modification before its assembly into fibers. These steps include limited proteolysis, hydroxylation of prolyl residues and the oxidative deamination of lysyl residues prior to their incorporation into the crosslinks that covalently bond together polypeptide chains of elastin. The crosslinking amino acids include lysinonorleucine, merodesmosine and desmosine isomers. A key enzyme that controls this process is lysyl oxidase. Lysyl oxidase is a copper metalloprotein whose activity is responsive to and modulated by environmental insults, nutrition deficiencies and the administration of various pharmacological agents. Regarding chemical properties, elastin is one of the most apolar proteins secreted by mammalian cells. Moreover, elastin is one of the most long-lived proteins secreted into the extracellular matrix. In relationship to its processing into elastic fibers and chemical properties, details related to major aspects of elastin metabolism as well as speculation on its potential as a factor in lung development and disease are discussed.

Introduction

Although some of the first descriptions of elastin appeared in literature 100 years ago, it has only been in the last two decades that elastin's role in lung has been fully appreciated (*1–5*). Elastin is found in lung pleura, parenchyma, blood vessels, bronchi and trachea. Since elastic fibers are concentrated around the mouths of the alveoli, it is speculated that alveolar shape results from the molding influence of elastic fibers (*6–8*). It is generally accepted that elastic fibers are of crucial importance in the maintenance of lung structure and normal lung compliance, and that alteration in lung elastin metabolism can lead to a variety of pathological changes in the lung.

The role of elastin in normal lung development will be discussed in this article, as well as factors influencing the synthesis and degradation of elastin. Since the primary purpose of this article is to provide a general overview, the reader is advised also to consult more exhaustive reviews on specific aspects of elastin metabolism and structure. Franzblau (*9*), Sandberg (*5*), Rucker and Tinker (*10*), Foster (*11*), and Bailey and Etherington (*1*) have provided reviews that deal with selected aspects of elastin metabolism and structure. Gosline (*12*) and Urry (*8*) have reviewed work important to the understanding of elastin's biomechanical and physical properties. Information on the novel crosslinking amino acids in elastin may be found in reviews by Gallop and his co-workers (*13,14*). Recent information on control of elastin gene expression and synthesis has been summarized by Davidson and Crystal (*15*) and Burnett et al. (*16*).

It is also important to note that some of the information in this chapter is speculative in nature. Progress in elastin research still suffers from the fact that certain features of its metabolism and chemistry are defined operationally. However, where this is the case, attempts have been made to qualify statements, so that the general reader will recognize those areas in which there is still controversy or where problems in methodology have led to tentative conclusions. This is particularly true in the first section which deals with composition and quantitation of elastin in elastic fibers. Elastin in its mature state has yet to be fully characterized and the methods for elastin quantitation are still in need of refinement. The information developed in the sections on synthesis of elastin, events important to the post-translational modification of elastin, and the role of elastin in lung development are also subject to differing interpretations.

Components of Elastic Fiber: Isolation and Composition of Elastin

Elastin fibers contain two major components (*5,17,18*). Elastin is the predominant component (70–90%) in

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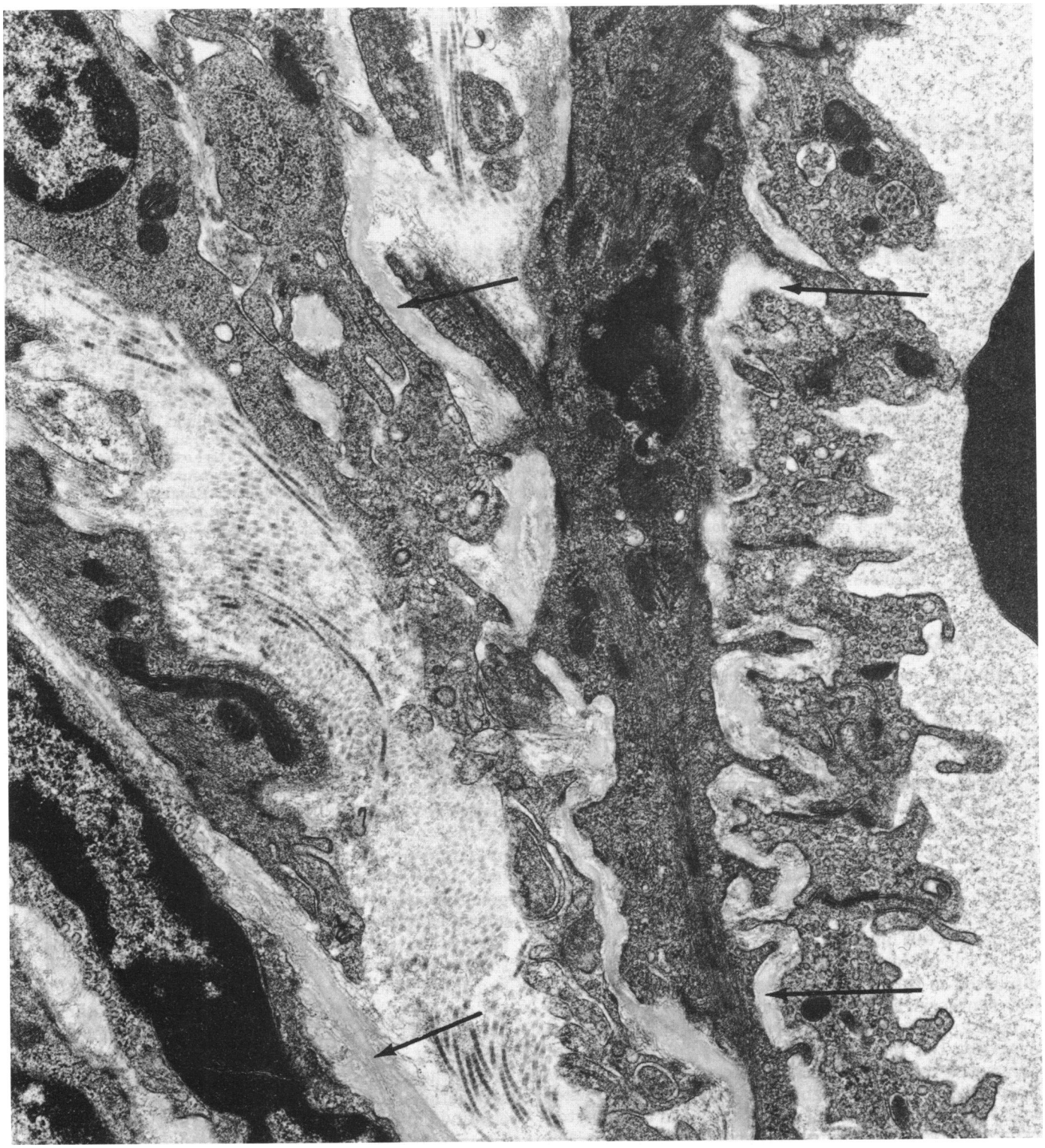

FIGURE 1. Mouse lung parenchymal tissue. Arrows indicate the location of elastic fibers.

mature elastic fibers. Immature or newly synthesized fibers are rich in an acidic glycoprotein component (*19*) often designated as microfibrillar protein (*17–20*). The microfibrils are found in the interstices and around the peripheries of elastic fibers. They are tubular in appearance and differ markedly from elastin fibrils with respect to amino acid composition (*17,18*). Morphological evidence (*18*) suggests that in some cases the microfibrils may serve as a nudus for the eventual deposition of elastin (*17,18,20*). Presumably elastic fiber formation is initiated by the secretion of microfibrils and as elastin synthesis proceeds the relative concentration of microfibrillar protein is decreased. In cartilage, however, current evidence suggests elastogenesis proceeds via "primary fibrils" that condense without the aid of microfibrils (*21*). Details pertaining to the chemistry of microfibrillar proteins and morphological features of the microfibrils derived from microfibrillar protein have been reviewed by Ross (*17*).

Elastin usually appears amorphous in electron micrographs (Fig. 1). Elastin is also one of nature's most apolar proteins, which is clearly evident from data on amino acid compositions of elastin or tropoelastin, an elastin precursor. Further, elastin is a very insoluble protein. Many of the novel amino acid sequences in the protein are resistant to mild hydrolytic conditions (*22*). This property, in addition to the large number of inter- and intrachain crosslinks in the protein, results in poor dissolution even when elastin is exposed to strong protein denaturants, hot alkali treatment, or repeated autoclaving.

Operationally, the definition of elastin often depends upon the nature of the chemical treatment used in isolating the protein. To quantify elastin in a tissue such as lung, it is common practice to extract tissue until the final residue resembles material with an amino acid composition similar to that given in Table 1. This residue is then dried and expressed as a unit of lung weight or other appropriate expression. General protocols for some of the commonly used methods to isolate and quantify mature elastin are summarized in Figure 2. Also, Starcher and Galione (*32*) give values for the amino acid compositions of lung elastins from various animal species.

It is perhaps obvious from examination of these protocols that precise estimation of elastin content is difficult. This is also reflected by data for values of lung elastin content from differing species (Table 2). Undoubtedly some of the discrepancy arises not only from variations due to methodology, but also from the influence of age or disease on elastin content (*4*). The so-called "milder" procedures for elastin isolation based on extraction with denaturants may not result in complete removal of residual microfibrillar components and other matrix proteins. The harsher procedures based on extraction with alkali or autoclaving may result in partial hydrolysis of elastin.

In general, the results from gravimetric methods for quantifying elastin are most easily interpreted when the relative amount of elastin as a percentage of total protein is high (greater than 10%). Further, the methods are best suited for whole organs from mature animals, in which most of the protein comprising the elastic fiber is most likely to be present as elastin.

Table 1. Amino acid composition of chick lung and aorta tropoelastin.

Amino acid	Lung	Aorta
Lys	43	40
His	2	—
Arg	8	5
Hyp	12	8
Asp	8	4
Thr	13	11
Ser	12	7
Glu	18	12
Pro	120	128
Gly	313	330
Ala	180	174
½ Cys	—	—
Val	163	177
Met	Trace	—
Ile	21	18
Leu	58	52
Tyr	15	10
Phe	14	20

Precursor of Elastin in Elastic Fibers: Properties and Cellular Sources

One of the precursors to the elastin component of elastic fibers has been designated as tropoelastin (*5,10,40*). Tropoelastin appears to be secreted from cells in the form of a 72,000 molecular weight protein (*5,10,11,15,41*). This protein has now been isolated from the aorta and ligamentum of various species (*1,5,10*), and chick lung (*33,42*). As an example, the composition of tropoelastin isolated from copper-deficient chick lung is given in Table 1.

Currently, there is interest in the possible existence of differing types of elastin, analogous to the differing types of collagen (*1*). Whether or not tropoelastin first appears as a high molecular weight gene product has also been investigated. Several early reports indicated that a proelastin is first synthesized with a molecular weight near to or greater than 100,000 daltons with a large carboxy-terminal extension (*11,43–45*). Later investigations, however, were unable to confirm the presence of soluble proelastin greater than 76,000–78,000 daltons (*11,15,16,41,46–49*). When translation systems are used utilizing messenger RNA preparations from aortae and lung, the major elastinlike products of translation appear to resemble tropoelastin with perhaps a short extension peptide (*2,15,16,41,42,48,49*).

That elastin-secreting cells make differing types of elastin also has not been fully resolved. Although Keith et al. (*50*) suggested cartilage contains a unique type of elastin, this observation could not be confirmed when

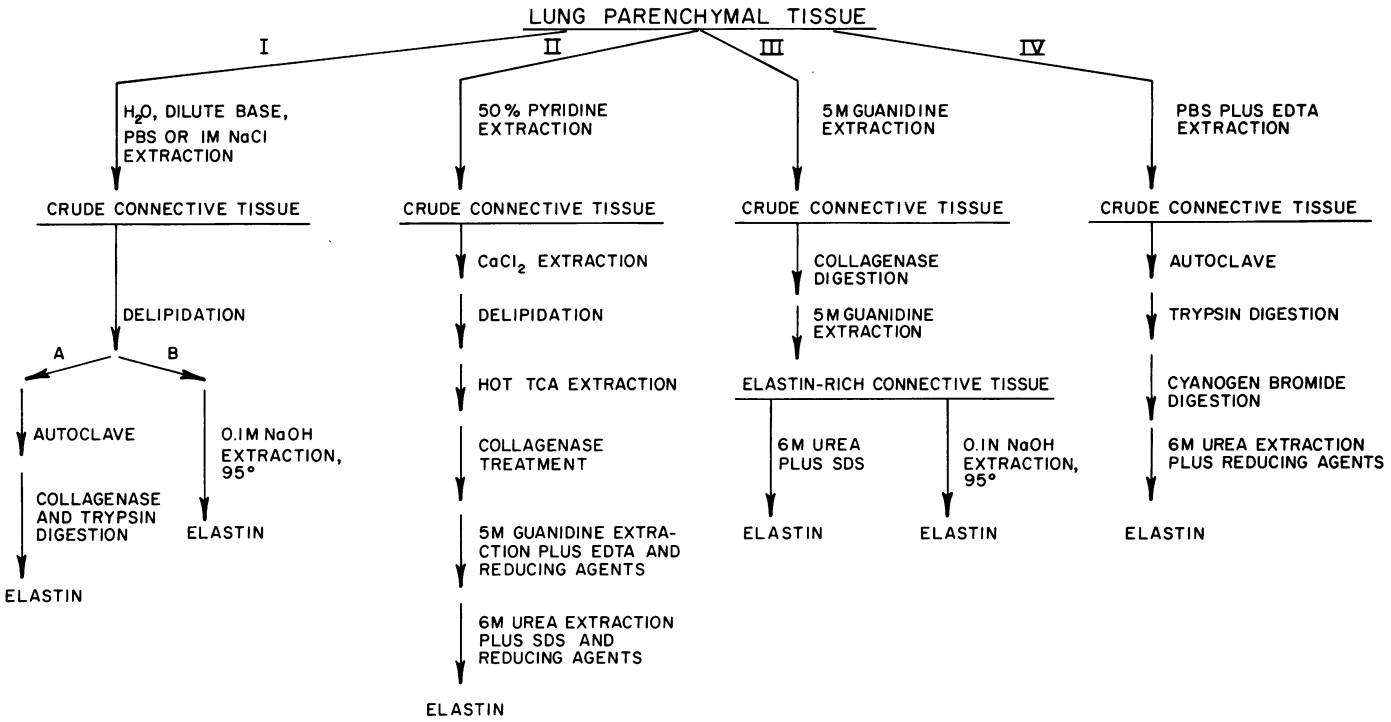

FIGURE 2. Protocols for the isolation of insoluble elastin. The protocols designated as IA and IB have been commonly used for the isolation of elastin from lung and other tissues. They are based on the resistance of elastin to autoclaving, the effects of alkali, and action by various proteases (*37–39*). Procedures have also been used that are presumably less hydrolytic (II and III), that result in an elastin-rich residue substantially free of other structural protein contaminants (*34,35*). Protocol IV, based on cyanogen bromide cleavage, is useful when it is certain that the elastin to be isolated contains no methionyl residues (*5,32*).

re-examined by Quintarelli et al. (*21*) and Foster (*11*). Further, Foster et al. (*42*) have noted that mRNA from both embryonic chick aorta and lung translates two apparent elastinlike polypeptide chains that differ in size and perhaps composition. However, the role of these two polypeptide chains in elastic fiber formation has yet to be resolved as well. More information is needed to demonstrate that the proposed elastin types do not arise from genetic polymorphisms. With respect to lung, the observations to date (*11,33,42*) suggest that chick aorta and lung tropoelastin are nearly identical in size and have similar chemical properties and composition (Table 1). There also appear to be close similarities between sheep nuchal ligament and sheep lung elastin (*15,41*).

Several cell types found in lung may be capable of synthesizing tropoelastin and microfibrillar protein. One of the primary candidates is the smooth muscle cell (*20*). Endothelial cells also appear capable of synthesizing elastin. Kantor et al. (*51*) have reported the synthesis of elastin by lung endothelial cells in culture. Elastin was suggested as a product of protein synthesis from cloned rat lung endothelial cells based on the presence of crosslinking amino acids common to elastin in protein secreted by the cells and immunofluorescence following incubation of cell matrix with fluorescent-labeled rat lung elastin antibody preparations. Carnes et al. (*52*) and Jaffe et al. (*53*) have also suggested that cultured endothelial cells synthesize elastin. In addition, during alveolar formation in the rat, interstitial cell fibroblasts have been observed to differentiate into myofibroblast-like cells. Histological evidence suggests a potential role of these cells in elastin synthesis (*54,55*). Including this fibroblastlike cell similar to those implicated in elastin synthesis in ligamentum (*15*) and chondrocytes (*21,50*), it becomes apparent that a variety of mesenchymal cells are capable of elastin synthesis. An important point is that involvement of these various cell types in turn offers the possibility for differing cell-mediated mechanisms in elastin-related lung disease.

Post-Translational Steps in Elastic Fiber Formation

The tropoelastin in lung undergoes a number of posttranslational modifications before it is assembled into the elastic fibers. First, it is clear from examination of composition data that 3 to 8% of the total prolyl residues in elastin are hydroxylated (*5,33,56*). However, unlike collagen the lysyl residues in elastin are not hydroxylated and the hydroxylation of proline does not

Table 2. Elastin content of lung parenchyma and pleura.

Source	Procedure[a]	Elastin content, % dry weight	Reference
Parenchyma			
Human	IB	26.5	(*23*)
	IB	25.0	(*24–26*)
Ox	IB	12.5	(*27*)
Mouse	IB	2–2.5	(*28*)
Rat	IB	3.7–4.4	(*7,28*)
	IB	1.8–3.0	(*29,30*)
Total desmosine		2.0–2.3	(*31*)
Chick	IB	2–10	(*32,33*)
Guinea Pig	III	20	(*34*)
Calf	II	14–24	(*35*)
Dog	IB	11–17	(*36*)
Pleura			
Human	IB	4–14	(*23*)
	IB	8–16	(*26*)
Ox	IB	28	(*27*)
Calf	II	25	(*35*)
Dog	II	25	(*35*)

[a] Values are for sexually mature animals. The procedures which most closely resemble the protocols outlined in Figure 2 are indicated. In some cases the total desmosine content of whole lung or lung residue was used for the final calculation.

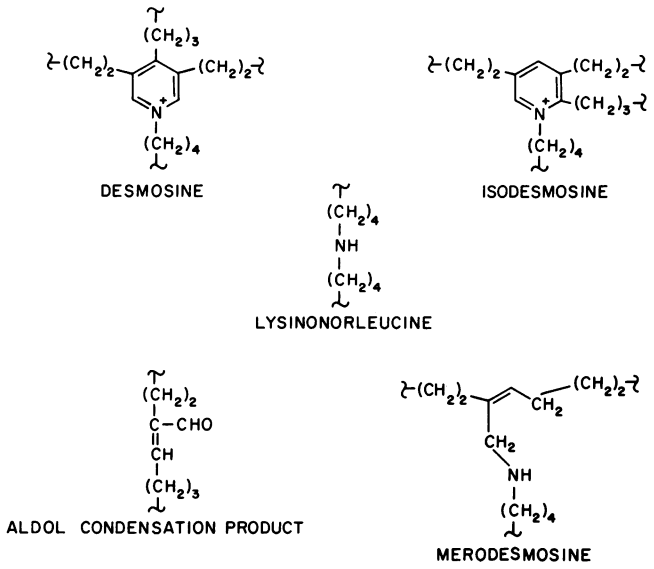

FIGURE 3. Structure of crosslinks common to mature elastins. These amino acids appear to function as both intra- and interchain crosslinks. Alanyl residues are usually found adjacent to the lysyl residues. However, an aromatic residue is often found adjacent to the lysyl residue that provides the ring nitrogen in the pyridinium moiety of desmosine and its isomers (*64,65*).

appear to be a prerequisite for tropoelastin secretion (*57,58*). Indeed, its accumulation is often enhanced in the absence of ascorbic acid (*58*). From the standpoint of methodology this point is important, since the estimation of elastin synthesis, turnover or net accumulation may be subject to considerable errors based on changes in hydroxyproline or hydroxyproline radioactivity. The problems of interpretation are the same as those pointed out in the article on collagen in this volume.

Another amino acid in elastin that is subject to modification is lysine. The oxidative deamination of lysyl residues in tropoelastin followed by subsequent condensation reactions results in a family of unusual crosslinking amino acids. It has now been established that the crosslinking in elastin is extensive and influences significantly the biophysical properties of the protein. The crosslinks are necessary to restrict the elastic fiber so that upon stretching, the individual polypeptide chains are constrained, thus allowing realignment and organization upon release of tension (*12*).

Elucidation of the major crosslinks in elastin came about largely because of the initial efforts by Partridge and his co-workers (*59–63*). They were first to recognize isomers of desmosine as major crosslinks in elastin. The structures of desmosine and isodesmosine in addition to other common lysine-derived amino acids in elastin are given in Figure 3.

The work by Partridge and his co-workers also coincided with the efforts by numerous investigators who were studying the formation of similar or related compounds in collagen. In turn this work coincided with numerous observations from nutritional and pharmacological studies that indicated nutritional copper deficiency and lathyrism resulted in an apparent increase in the amount of lysine in elastin and collagen (*66–68*). When all of the data were taken together it eventually became clear that the lysine in elastin and collagen served as a precursor to the crosslinks; and selected steps in crosslink formation could be inhibited by copper deficiency and lathyrogenic agents.

It is now known that in tropoelastin, there are polypeptide regions that are rich in alanyl and lysyl sequences (*5,64,65,69*). Specific lysyl residues are modified to peptidyl α-aminoadipic-δ-semialdehyde residues (allysine). Upon proper ordering and alignment, the allysine residues then condense to form various condensation products. Suggested reaction schemes for this process are given in Figure 4. It has been demonstrated that once allysine is formed, the formation of desmosine may occur spontaneously (*68*). However, equilibrium of allysine to desmosine is a relatively slow process (*70*). The time required to obtain ^{14}C-labeled desmosine with a specific activity the same as that of lysine often requires several days (*61,63*). Since it is difficult to conceptualize a metabolic pathway requiring days or weeks for eventual conversion of a substrate to its end product, Gray (*70*) has proposed that the crosslinks in elastin may be viewed as a mixture of chemically reversible products which slowly equilibrate to give rise to the more stable end products shown in Figure 4. It is important to appreciate that if desmosine is used as a marker for elastin synthesis its appearance may follow the initial synthesis and deposition of elastin by a significant period of time.

Typical data for the relative distribution of crosslinking amino acids in calf lung elastin is given in Table

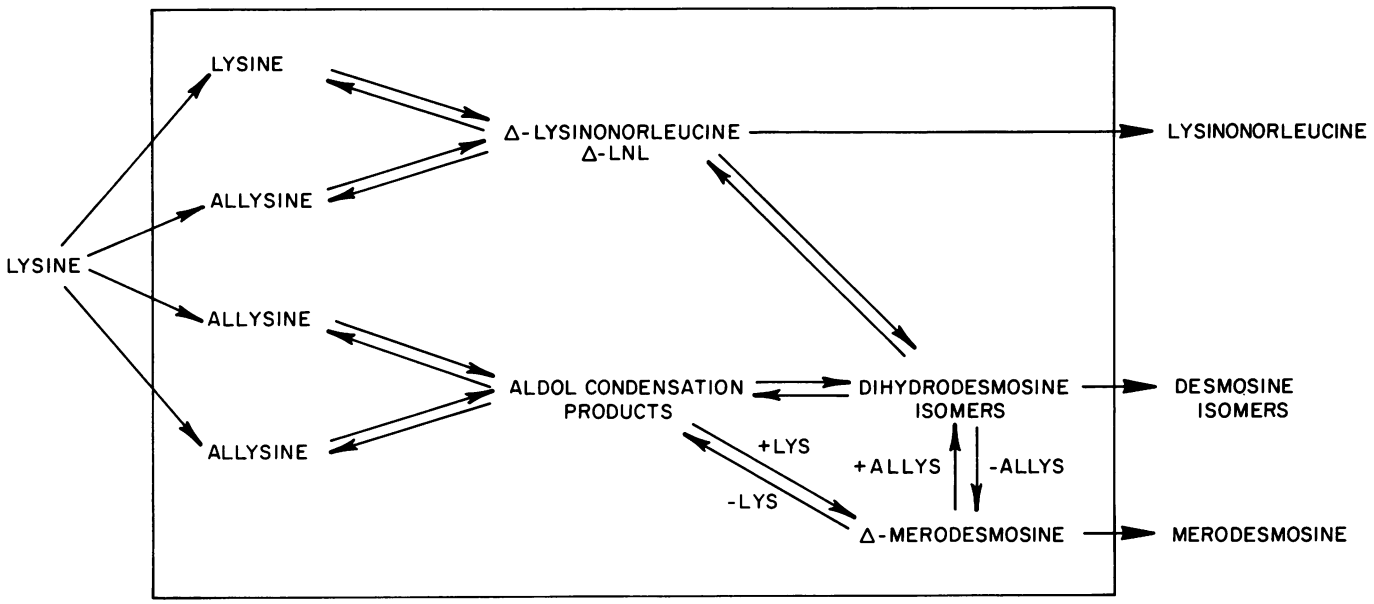

FIGURE 4. A generalized scheme for the formation of crosslinks in elastin. Lysyl oxidase acts to catalyze by oxidative deamination selected residues of peptidyl lysine in tropelastin or a tropelastin precursor. Lysyl and/or allysyl residues then condense to form dehydrolysinonorleucine (Δ-LNL) or various aldol condensation products. These reactions allow the formation of both intra- or interchain crosslinks. The aldol condensation product and Δ-LNL may then condense to form dihydrodesmosine isomers. Alternatively, it is possible that a form of the aldol condensation product reacts with peptidyl lysine to form dehydromerodesmosine, which in turn reacts with an allysine residue to form dihydrodesmosine. Eventually, these reactions lead to the formation of the chemically stable derivatives lysinonorleucine, merodesmosine, and the desmosines, respectively. Steps that occur in the box are considered reversible (*70*).

3. With aging, there are often shifts in the distribution of crosslinks in elastin so that the more chemically stable crosslinks are observed in greater proportions.

The enzyme, lysyl oxidase, that catalyzes the oxidative deamination of specific lysyl residues, has been characterized from bone, ligamentum, and arterial sources (*41,71–74*), but has yet to be well characterized from a lung tissue source. Lysyl oxidase activity is inhibited or reduced upon administration of lathyrogens (*73,75*). Nutritional copper deficiency or intoxication with zinc or cadmium may also effect a reduction or alteration in activity (*24,68,76*). It has also been shown that lysyl oxidase activity is increased in lung tissue following partial pneumothorax (*77*). Lysyl oxidase is a copper-dependent enzyme (*78*), which explains in part the effects of nutritional copper deficiency or zinc intoxication on the depression of lysyl oxidase activity. Another interesting feature of the relationship between copper and lysyl oxidase is the observation that copper ligand complexes appear to influence the actual synthesis of the enzyme (*78*). Certain genetic disorders are also characterized in part by low or suboptimal lysyl oxidase activity (*5,25,66,68*).

In addition to amino acid modification, it has also been observed that tropoelastin undergoes rather specific proteolytic modification (*79,80*). The protease is apparently tightly bound to tropoelastin and is inhibited by serum protease inhibitors (*80*). However, the degree to which the protease attacks crosslinked elastin is not known. Whether or not the protease is involved in a specific step in posttranslational modification is also not clear, although it has been suggested that the proteolysis of tropoelastin may occur when the concentration of tropoelastin is elevated (*81*), e.g., when the incorporation of tropoelastin into the elastic fiber is inhibited. In this regard, the function of the protease may be analogous to collagenase.

In Figure 5 are summarized some of the known posttranslation events important to elastic fiber formation. The most tentative steps are the initial ones, since the details of elastin and microfibrillar protein transcription and translation are only now being ac-

Table 3. Crosslinking amino acids commonly found in acid-hydrolyzed lung elastins.

Amino acid	Lysine equivalents[a]
Desmosine	6 - 9
Isodesmosine	3 - 5
Lysinonorleucine	0.5- 2.0
Allysine	0.5- 2.0
Aldol condensation products	0.5- 2.0
Dehydrolysinonorleucine	0.2- 0.5
Others	8 -25
Lysine	6 -12

[a] A range of values is given in keeping with the range of values often reported for elastins from the whole lung, pleura or parenchyma (*1,5,10,13,14,26,27,35,63,68*).

tively investigated. An interesting point is that, unlike collagen, no evidence exists for direct glycosylation of elastin (*82*).

Elastin Structure

The polypeptide chains of tropoelastin, once crosslinked, form a three-dimensional network. When elastic fibers are observed morphologically, they are often found branched and fused in the form of a complex matrix. In tissues such as ligamentum, the elastin fibers are organized in the direction of the stress, i.e., the orientation of the fibers is parallel to the direction of stress. In major blood vessels, the elastic fibers take on a lamellar arrangement in the form of concentric sheets. This may also be the case in mammalian parenchymal tissue where elastin is found as a lamellar sheet encapsulating the alveoli (*6,20,54,83–88*) or in avian lung (*33,89,90*), where much of the elastin is found as sheets or rings in the tertiary bronchial lumen. At the

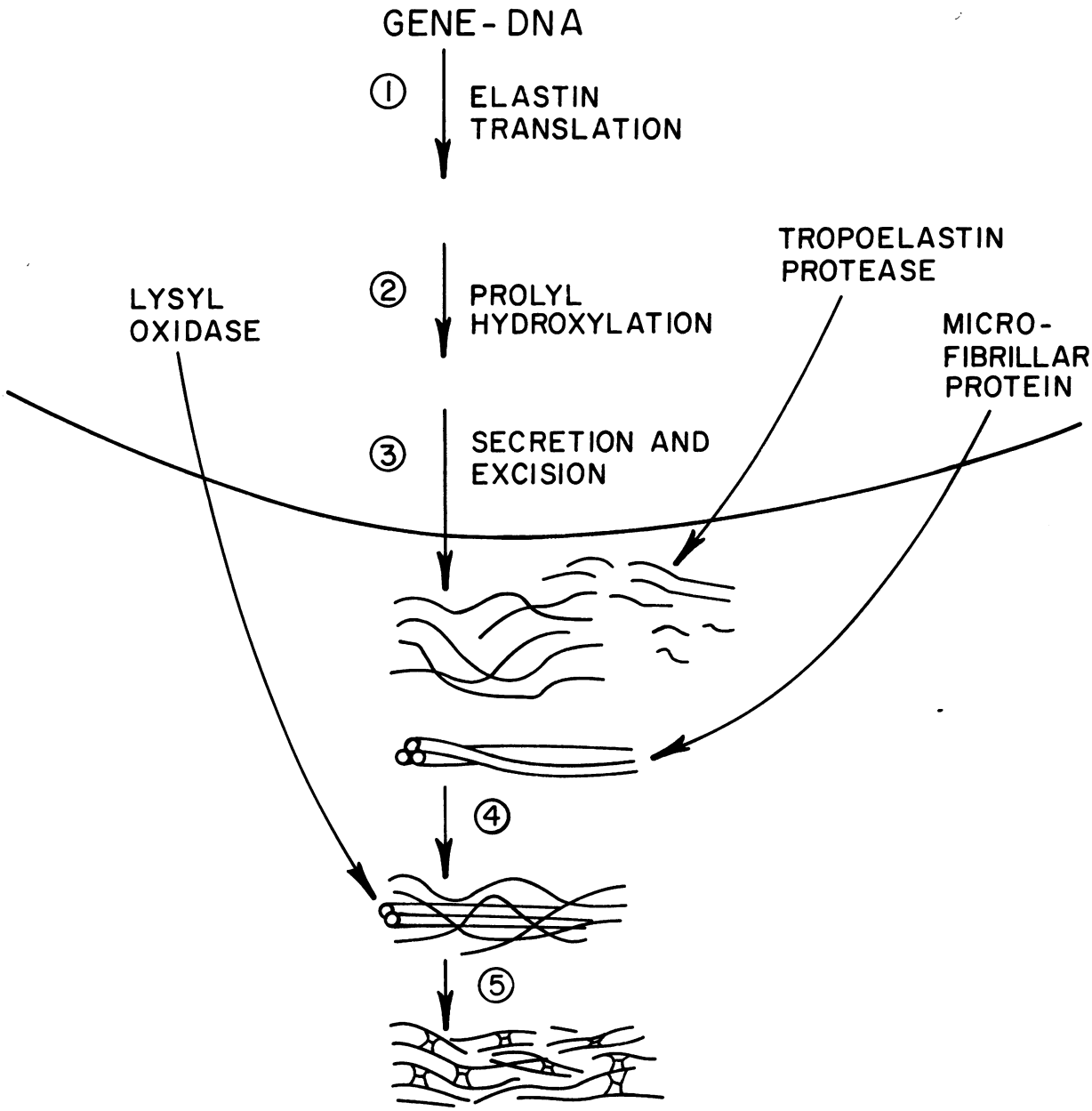

FIGURE 5. The processing of elastin into elastic fibers. Following translation (step 1) and prolyl hydroxylation (step 2) tropoelastin is secreted from the cell (step 3). Tropoelastin combines with microfibrillar protein (step 4) and is then crosslinked into mature fibers (step 5). Proteolysis of tropoelastin is also shown; however, the extent of which proteolysis influences the net accumulation is not known.

level of scanning electron microscopy, isolated elastin fibers often appear ropelike in appearance (*91*). The fibers appear to be branched and interconnected at higher magnification. At the level of fine resolution, elastic fibers often appear filamentous containing systemically spaced striations. However, the striations are only observed under certain conditions and have been suggested to be artifacts (*57,92*).

Serafini-Fracassini et al. (*93*) have suggested that the individual filaments contain internal ordered structure, but possess such small diameters that they may also behave as random chains. The latter is an important point if elastin fibers are to function in keeping with the entropic interpretation of elastic recoil (*94,95*). Although a degree of ordered structure is attractive from a biological point of view and there is ample evidence to suggest ordered structure (*74*), there is also considerable physical evidence that suggests elastin fibers behave as random chains (*12,92,95*).

A very special feature of elastin structure is its unique amino acid composition. The compositional data given in Table 1 indicate that elastin is a very apolar protein. It contains a very high concentration of val-pro sequences. Glycine accounts for 30 to 33% of the total residues in mammalian elastin. It has also been noted that a high percentage of the glutaryl and aspartyl residues in elastin are amidated (*96*). As mentioned previously, the alanyl and lysyl residues in tropoelastin are clustered together to comprise the crosslinking regions in elastin (*64,65*). Between the crosslinking regions are amino acid sequences rich in valine, glycine, and proline. Sequence data from chick and porcine tropoelastin would suggest that certain sequences appear to repeat (*5,64*). These sequences are gly-gly-val-pro, pro-gly-val-gly-val, and pro-gly-val-gly-val-ala. In chick tropoelastin, series of gly-val-pro repeats are also found (*97*). The length of the sequences that are rich in glycine, valine and proline has been estimated to have a molecular weight of 6000 to 8000.

Information on amino acid sequences has been very helpful in elucidating features of elastin structure. Urry and his co-workers have observed that the apolar polymeric sequences often take on the form of β-spirals containing repeating β-turn structures (*8*). It has also been suggested that some of the energy that results in elastic recoil possibly comes about because of apolar-polar interactions between water and the apolar residues comprising these regions (*12*).

A simple representation of elastin is shown in Figure 6. However, many of its features are tentative. The repeating sequences do not extend throughout the length of the valine, glycine and proline-rich polypeptide chains. Subsequently, suggestion of a high degree of ordered structure as shown in Figure 6 is subject to considerable debate. As Bailey and Etherington (*1*) have pointed out, models for elastin are based on particular types of physical measures. Although a random network model provides the best fit from stress-strain measurements on elastin, direct analogy

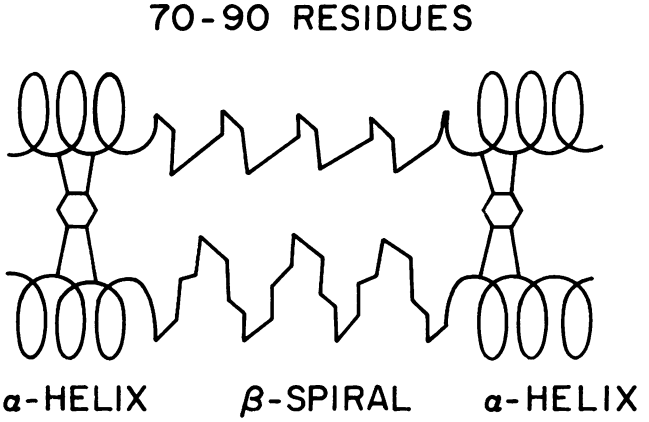

FIGURE 6. Fibrillar model for elastin (*74*). The crosslink regions are proposed to be α-helical. The glycine-, valine-, and proline-rich sequences appear as so-called β-spirals. The hydrophobic side chains of amino acids in the spirals extend outward. As noted in the text, elastin contains a high concentration of glycine. An important conformational feature of glycine is that it allows the insertion of near right-angle turns in a polypeptide sequence. If glycine is followed by an amino acid residue with bulky side chains, e.g., valine, the near right-angle turns become stable conformations which give rise to the β-spirals shown in the figure. The β-spiral regions have been suggested to be 70 to 90 amino acid residues in length.

to rubber or other elastic random network material is difficult to fully accept. The ability of soluble elastins to form fibrous structures upon changes in temperature or ionic strength and the capability of acting as an enzyme substrate, suggest a degree of organization. The figure represents the bias of the authors and may prove to be incorrect as more information becomes available.

Elastin and Lung Development

With an overview of elastin structure and chemistry, it is possible to deal more easily with the role of elastin in normal lung development and structure. Features important to both avian and mammalian lung development and function will be discussed, since aspects of both had been important in the assessment and understanding of elastin's role in lung tissue.

Avian Lung

There is now increasing evidence that elastic fibers are fundamental to tertiary bronchial maturation. One of the first morphological descriptions of elastin in the airways of lung was that by Fisher (*98*). More recently, Jones and Barson (*90*) have described elastogenesis in the developing chick lung, using both light and electron microscopic techniques. They have shown that elastogenesis in chick lung parenchyma commences near day 14. In keeping with the general view of elastic fiber development, the fibers first appear as sparse bundles of microfibrils close to smooth muscle cells. Later the

fiber appears with an amorphous core of elastin and with a peripheral mantle of microfibrils.

Elastin is found situated predominantly in the walls of the so-called "primary, secondary and tertiary" bronchi of avian lung. In comparison with mammals, only negligible quantities of elastin are present in the air exchange areas. Most of the elastin is found in the tertiary bronchial wall in close association with smooth muscle bundles. The initial accumulation of elastin fibers appears associated with the early stages of development of the parabronchi, the major functional unit in avian lung.

When the chick hatches, elastic fibers in varying stages of maturation are visible up to day 10 to 14. Jones and Barson (*90*) point out that the development of the elastic fiber may be described as asynchronous throughout this period. Also, throughout this period, elastic fiber formation may be observed in relationship to the development of the pulmonary vasculature. The function of the elastin in the tertiary bronchial units may be to provide tension against which the spiral smooth muscle cells may contract. Elastin probably prevents undue narrowing of the tertiary bronchial lumen because of contraction of the smooth muscle network, thus maintaining patency of the parabronchi in lung (*89,99*).

As the chick lung grows, the functional units, tertiary bronchi, elongate with the growth of the lung. With lung growth, there appears to be new elastin synthesis. For example, if young birds at different stages of development are injected with radiochemically labeled amino acids such as valine, the incorporation of valine into elastic fiber occurs throughout early development posthatching and well into adulthood (M. Lefevre and R. Rucker, unpublished data). A continual net synthesis is also inferred from data related to the net accumulation of elastin in avian lung (*33*). For example, the percentage of elastin in avian lung does not change significantly throughout early development and growth in spite of a 3-fold increase in lung size. The elastin in avian lung amounts to approximately 1 to 2% of the wet weight of the tissue (*33*).

Mammalian Lung

The appearance of elastic fibers in mammalian lung occurs early in prenatal and perinatal development. Fierer (*100*) has shown that in the developing fetal lamb, the microfibrillar components are present at day 90 of gestation. Elastin appears at day 110 and gradually increases relative to the amount of microfibrillar components until term, which occurs at day 150. The composition of pulmonary alveolar septal elastin at term resembles that of the adult ewe. The same pattern also appears to be true for the human as well in which the process may continue for as long as 7 years after birth (*6,25,26,54,73,101–103*).

In the rodent and fetal lamb, elastin synthesis has been highly correlated with selected features of lung development (*7,19,28,31,54,77,104*). Powell and Whitney (*31*) have demonstrated that there is a marked increase in the desmosine content of lung during the period of alveolarization. However, as shown in Figure 7, it is possible to demonstrate that the incorporation of amino acids into elastin fibers occurs prior to the appearance of desmosine (*28,54*). One interpretation is that there is an increase in elastin biosynthesis associated with the process of alveolarization. This may not be reflected, however, in a new accumulation of elastin (as it is usually defined operationally), since desmosine or the presence of highly insoluble elastin may reflect more the state of maturation of elastin than its actual amount. In early development, the desmosine content of the elastin need not correlate highly with the appearance or synthesis of amorphous elastin estimated using morphological or other biochemical techniques,

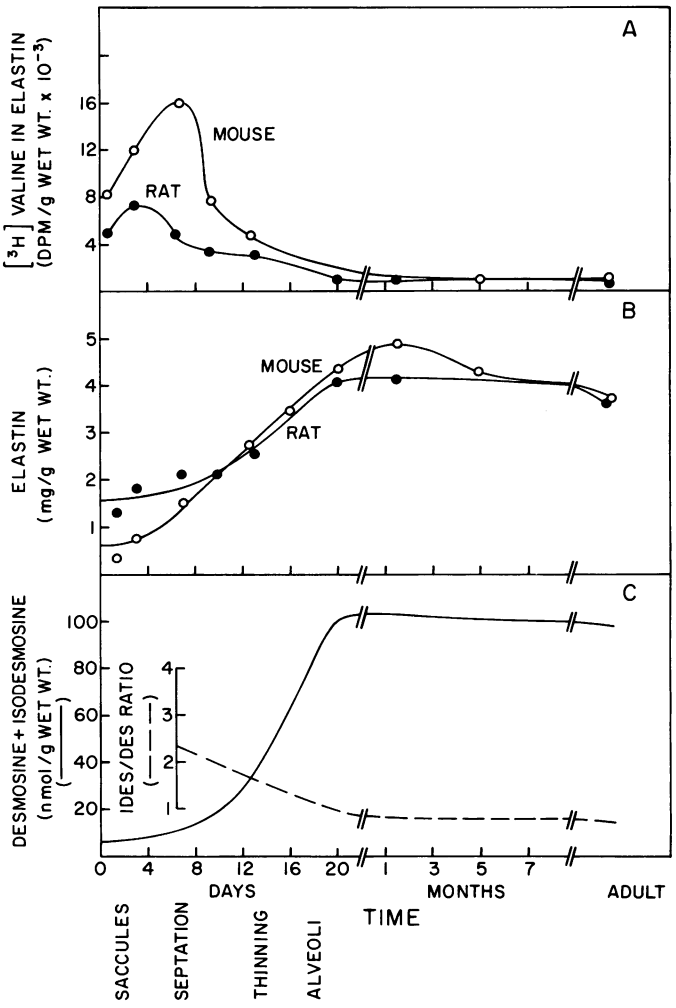

FIGURE 7. Changes in the net accumulation of rodent lung elastin during development. Based on amino acid incorporation data the bulk of the elastin in lung appears to be synthesized early in neonatal development. Later in neonatal development the elastin fibers mature as indicated by the marked increase in lung desmosine content. An interesting observation is in immature fibers the ratio of isodesmosine to desmosine is higher than in mature fibers (*28,31,87*).

since the appearance of desmosine may lag substantially behind initial incorporation of tropoelastin into the elastin matrix (*63*).

Turnover and Degradation of Elastin in Lung

Once elastin is synthesized and stabilized by crosslinks, it does not undergo rates of turnover common to other proteins (*10,23,28,97,105–108*). For example, the data in Figure 8 indicate that the elastin in whole lung turns over very slowly. Indeed, when corrections are made for new lung growth it is extremely difficult to demonstrate measurable turnover for elastin, except for the release of minute quantities of desmosine or elastin peptides by sensitive immunological techniques (*36,84,109*). Dubick et al. (*28*) have shown that when mice are injected with ^{14}C-labeled lysine and appropriate time is allowed for the lysine to be incorporated into mature elastin and its crosslinks, the turnover of elastin in lung is best estimated in years. Repeated exposure to ozone which causes an inflammatory response in lung does not alter turnover significantly (*28*). Consequently, for those diseases in which elastin destruction is of biological significance (*110*), the protein's "inertness" takes on considerable importance, particularly if the elastic fibers subjected to degradation are not resynthesized in a normal manner. For example, Kuhn et al. (*85*) and Goldstein and Starcher (*111*) have shown that instillation of elastase into hamster lung causes net destruction of lung elastin. This can be measured by both a decrease in the elastin content of the lung and the appearance of desmosine in urine or the presence of elastin-derived peptides in circulation (*84*). Following acute destruction, the elastin content of lung has been shown to return to its normal content; however, the newly synthesized elastin is in the form of highly disorganized fibers (*86,103,112*).

As pointed out elsewhere, one of the current concepts regarding induction of emphysema is the proteolytic destruction of elastin (*67,113,114*). This process apparently results from elastolytic proteinases derived from leukocytes and macrophages, or elastase from other sources, such as that secreted by the pancreas (*44,86, 103,110,111,115*). As in mice, the turnover of elastin in normal humans is also best estimated in years. Observations by Harel et al. (*105*) suggest that in man the excretion of desmosine is normally 40 to 50 μg/day, i.e., equivalent to turnover of less than 1% of the total body pool of elastin per year. In smokers with chronic obstructive lung disease, however, urinary excretion of desmosine may be as high as 400 μg per day; presumably the result of leukocytic and macrophage elastases. Certainly, such observations take on significance when one considers that if elastin is lost from adult lung it may not be replaced; or if it is replaced, its resynthesis may result in fibers inappropriate for normal function. Further, the suggestion that elastin-derived peptides

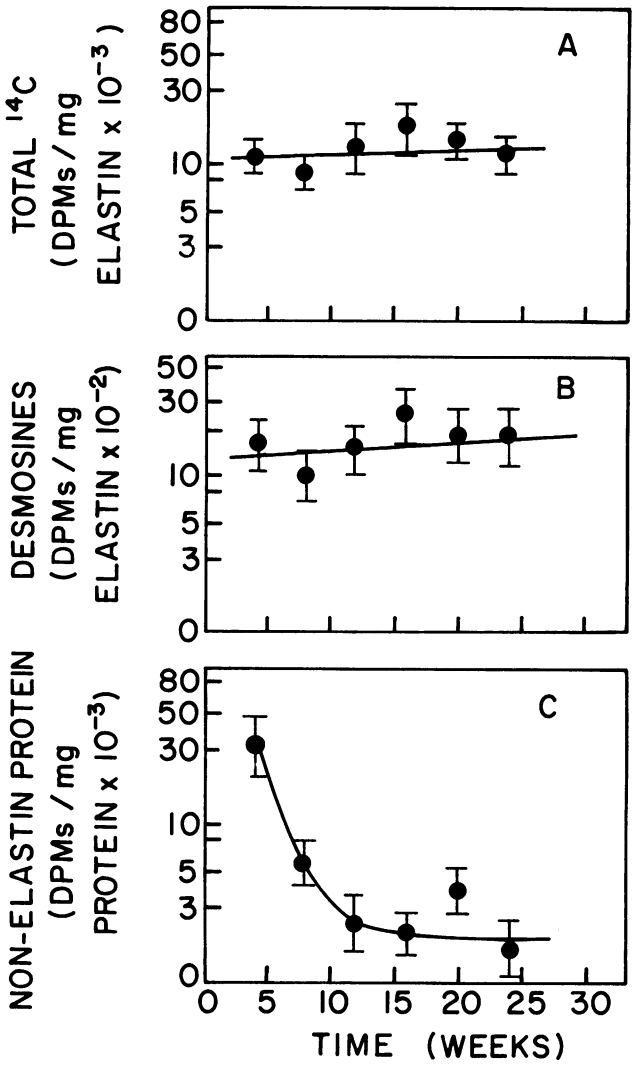

FIGURE 8. Turnover of elastin in murine lung. Mice were injected with ^{14}C-lysine at days 10 to 12 and radioactivity as ^{14}C-desmosine and isodesmosine in elastin was estimated over a 24 week period. As shown in (*A*) and (*B*) there is little change in radioactivity in the elastin and elastin–desmosine fractions indicating only limited turnover. Values that reflect the turnover of long-lived, nonelastin proteins are given in (*C*). Other experimental details may be found in the literature (*28,87*).

may be chemotactic to pulmonary macrophage precursors offers interesting possibilities regarding elastin's role in lung defense mechanisms (*37*).

With regard to elastin degradation and developmental features of lung, several reports suggest that a decrease in crosslinking or decreased net accumulation of elastin during the period of septation or alveolarization retards alveolar development, and the response persists into adulthood. For example, in the rodent, nutritional copper deficiency (*116*) administration of β-aminopropionitrile (*112,117,118*) or penicillamine (*29,30,119*) during neonatal development or early life causes defective alveolarization which persists throughout further lung development. In adult animals, introduction of

fibrotic agents, e.g., Cd, also influences elastin deposition, albeit the response is less dynamic than in young animals. Crosslinkage inhibitors again diminish the magnitude of deposition (*72,76*). Consequently, it appears essential that factors be identified that influence or modulate synthesis of lung elastin. That aorta elastin biosynthesis may be precociously accelerated during embryogenesis by administration of glucocorticoids or its accumulation or deposition may be altered by ascorbic acid has been demonstrated (*46,47*). Extension of this type of information to lung is necessary and should eventually lead to a better understanding of the role of elastin in lung development and function.

Summary

Elastin is an important protein to the overall development and structure of lung. Alterations in elastin content can compromise lung function and may be important to conditions which lead to chronic obstructive lung disease. From the standpoint of protein chemistry, elastin is of interest because of its unique properties and structure. It is one of the few proteins in nature that can act as a rubberlike elastomer. It possesses a novel amino acid composition and undergoes unusual posttranslational modifications in the process of its final maturation into a fiber. It is an unusual protein from a biological point of view because of its long biological half-life. It would appear that in normal situations elastin fibers are meant to function for exceedingly long periods. Consequently, the response of lung to pneumotoxic agents, particularly those that stimulate elastolytic processes, may also have long-lasting effects.

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