Review article

Elastin and the lung

It is essential that the framework of all multicellular organisms should include some materials with high tensile strength and rigidity, such as bone and collagen, to maintain shape and mechanical rigidity. In addition, there is a requirement for a component with intrinsic elasticity that can stretch and undergo elastic recoil when required. This property is supplied by an unusual fibrous protein, which over 150 years ago was given the name elastin.

Elastin fibres are present in virtually all vertebrate tissues, although it is only within a few, such as arteries, some ligaments, and the lung, that elastin comprises an appreciable percentage of the total protein. The ligamentum nuchae of grazing animals and the aorta of most vertebrates contain over 50% elastin on a dry weight basis. The elastin content of lungs on the other hand is quite variable, ranging from as low as 2% in rodents up to 28% in the cow and in man. For this reason most of the chemical and biological studies that have been conducted on elastin have used either the bovine neck ligament or the ascending aorta of several species. Although the primary aim of this article is to review the role and metabolism of elastin in the lung, most of the background information must come from elastin derived from other sources. The findings may, however, be extrapolated to the lung, since within a given species all elastins appear to be chemically the same, regardless of the tissue of origin. We may safely assume that the essentials of elastin metabolism are the same in all tissues, although apparently much influenced by other components in the extracellular matrix of that particular organ.

Identification and purification of elastin

The first century of elastin research was primarily directed towards the morphological and histological features of the elastin fibre. This early history of elastin research has been reviewed in detail by Hass.[1] The elastin fibre is composed of two distinct components. The elastin, or amorphous component, is the major fraction, comprising some 90% of a mature fibre. It derives its name from the absence of any repeating structure or banding pattern, when reviewed under an electron microscope. The microfibrillar component,

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most prominently displayed in newly synthesised or juvenile elastin, is a glycoprotein that stains with uranyl acetate and leads, which appears as small fibrils 10–12 nm in diameter concentrated around the periphery of the amorphous elastin. The microfibrils are chemically and morphologically quite distinct from the amorphous elastin. There is some evidence to suggest that the microfibrils are secreted into the extracellular matrix before elastin synthesis and function as a nucleation site for future elastin deposition. For more details on every aspect of the microfibrils, readers are referred to other articles.[2-4]

The purification of elastin depends predominantly on its remarkable insolubility, even under harsh, denaturing conditions. The protein has been defined operationally as the insoluble residue remaining after a tissue is autoclaved repeatedly or boiled in 0·1 N NaOH for up to one hour. This procedure was satisfactory for tissues comprised mostly of elastin and collagen. Isolation of a relatively intact and pure elastin is impossible using this method, however, in tissues that have a low elastin content or are particularly rich in glycoproteins or complex ground substance. In recent years milder yet considerably more complex methods have been used. These methods have been compared by Soskel[5] and it is now fairly well accepted that, even in such complex tissue as the lung, elastin can be purified in the absence of harsh alkali extraction. "Pure" elastin, however, still remains as an operationally defined protein residue that has an amino acid composition typical of elastin for that particular species. This has created some confusion since with slight contamination this protein may appear as a different elastin. There have been reports of two distinct types of elastin within cartilage[6] and synthesised by chick aorta in culture.[7] Advanced techniques analysing elastin messenger RNA do not support this theory.[8 9] Other studies have compared the amino compositions of elastins derived from various organs within the same species. These studies indicate that, once the glycoproteins and other proteins in close association with elastin have been completely removed, the compositions are the same within a species regardless of the tissue of origin.[10 11]

Structure of elastin

The amino acid analysis of elastin is consistent with its unique physical properties. Almost one third of the

residues are glycine, about 12% are proline, and over 40% of the remaining amino acids contain hydrophobic side chains, making this one of nature's most non-polar proteins. A survey of the amino acid compositions of elastin, from representative species of all vertebrate classes and some invertebrate phyla, indicates that elastin is present in all vertebrates except Agnatha.[11]

Renewed interest in elastin biochemistry began in the late 1950s and early 1960s with a series of interesting nutritional observations on the effects of copper deficiency in young growing animals, and the simultaneous studies on the chemistry of elastin and its crosslinks by the Cambridge group headed by Partridge.[12][13] The most dramatic signs of copper deficiency are the fragmented appearance and considerable decrease in the amorphous elastin content of major blood vessels, leading to aortic aneurysms and subsequent death of the animal.[14] It soon became apparent that lysyl oxidase, a copper metalloenzyme, was required for the formation of crosslinkages that stabilise the elastin fibre.[15] In the absence of crosslinking, a soluble, non-functional elastin molecule accumulates in the tissues in sufficient quantities for it to be isolated. This rather fortunate event (for the biochemist) has led to the characterisation of tropoelastin, the soluble monomer form of elastin, which has now been isolated from several tissues, including ligaments, aortas, and lungs.[16] Tropoelastin has been essential for the sequencing of this otherwise highly crosslinked and very difficult protein. Characteristic repeating sequences are found throughout the tropoelastin molecule. The sequences ala-ala-ala-lys and ala-ala-lys each repeat six times and are believed to be the sites for future oxidation by lysyl oxidase and subsequent crosslink formation.[17] Several hydrophobic repeat sequences are also found in elastin, including the pentapeptide pro-gly-val-gly-val and a hexapeptide pro-gly-cal-gly-val-ala. Some have proposed that the pentapeptide repeats form a β spiral structure consisting of β turns while the crosslinked regions form a more rigid α helical conformation. Other sharply contrasting views, derived from physical measurements, suggest a highly anisotropic or random arrangement for the polypeptide chains.[18−21]

Synthesis of elastin

Elastin synthesis has been documented in fibroblasts,[22] smooth muscle cells,[23] chondrocytes,[24] and endothelial cells.[25−26] The process is initiated by nuclear transcription from the elastin gene. The gene is quite extensive, containing many large intervening sequences, suggesting that the final elastin messenger RNA (mRNA$_E$) molecule has undergone major processing from the initial primary transcript.[8] In the tissues that have been examined, including the lung, the data indicate that elastin synthesis is regulated by the transcription and availability of mRNA$_E$.[27]

Translation of the mRNA$_E$ produces a tropoelastin molecule containing a short (24–26 residue) signal peptide.[8] As the chain elongation proceeds, select prolyl residues are hydroxylated by prolyl-hydroxylase.[8][14] The hydroxylation of prolyl residues in tropoelastin, unlike collagen, does not affect either synthesis or stability of the molecule.[28][29] Some investigators have even considered this a serendipitous event that occurs simply because the machinery is present and the molecule resembles collagen in many aspects of primary structure. The entire intracellular elastin synthetic process requires about 20 minutes.[21]

Tropoelastin is secreted from the cell into the extracellular matrix as a protein with a molecular weight of 72000. As tropoelastin molecules become aligned, perhaps in association with the microfibrils, hydrophobic interactions occur, presumably leading to coacervation. Lysyl oxidase subsequently converts the epsilon (ε) aminos of all but five or six of the total 37 lysine residues in tropoelastin to aldehydes. These very reactive residues (allysines) spontaneously condense to form various Schiff base and aldol condensation crosslinks. Within a few days most of the crosslinks have isomerised into the stable quaternary pyridinium ring structures of desmosine and isodesmosine. Several excellent reviews have discussed the crosslinking process in great detail.[30][31]

Lysyl oxidase has been isolated from several sources and, although shown to be present in the lung, it has not been isolated from that tissue. Copper is required for enzymatic activity, which explains the connective tissue defects observed in copper deficient animals. Additionally, lathrogens like BAPN inhibit the enzyme, producing the connective tissue defects that are seen in copper deficiency.[14] Genetic disorders have also been described that are characterised by low lysyl oxidase activity and severe connective tissue defects.[14][31]

Once the tropoelastin molecules are crosslinked they form a three dimensional fibrous network that often appears branched and fused into a very complex matrix. The elastin fibre can be organised in various configurations in different tissues. In ligaments they extend as long fibres parallel to the direction of stress. The fibres in the aorta appear to have a lamellar arrangement, forming concentric sheets. In the lung parenchyma the elastin fibres establish lamellar sheets surrounding the alveoli. It is apparent, in whatever tissue elastin is found, that its orientation takes advantage of the protein's main function of being able to stretch to large extensions with little force and then return to its original dimensions. The essence of

the physical properties of elastin has been recently reviewed.[21]

Degradation of elastin

Turnover studies have shown that, once the elastin fibre is formed, normal remodelling processes are extremely slow. Studies in man, using sensitive immunological techniques to measure elastin peptides in the blood or desmosines excreted in the urine,[8] suggest that less than 1% of the total body elastin pool turns over in a year. By means of the more direct method of in vivo radiolabelling of elastin, these observations have been confirmed with several animal models, including mice, rats, chicken, and quail.[8 32]

The mechanisms for elastin degradation in vivo may be divided into two categories—proteolytic enzymes that degrade uncrosslinked tropoelastin, and enzymes that degrade the fully mature crosslinked and insoluble elastin fibre.

Tropoelastin is probably susceptible to a wide range of proteolytic enzymes, yet in only a few instances has it been systematically studied as a substrate. On the basis of these experiments and the results obtained from sequencing data the known tropoelastinases are elastase, trypsin, chymotrypsin, pronase, thermolysin, cathepsin G, thrombin, and kallikrein.

Elastases

Fully crosslinked elastin, on the other hand, is susceptible to attack by a select group of enzymes classified as elastases. These enzymes are derived from several sources, including the pancreas, neutrophils, macrophages, monocytes, platelets, smooth muscle cells, and fibroblasts. Although they are designated as elastases, these enzymes are not specific in any sense and have been shown to cleave core proteins of proteoglycan molecules, types III and IV collagen, fibronectin, and many of the plasma proteins.[33]

The role of these enzymes in normal elastin metabolism is difficult to assess. It is assumed that elastin does have a normal turnover, albeit with a very long half life, and that the turnover observed is not just a reflection of some pathological degradation process; nevertheless no one has yet shown a requirement for elastase in normal turnover or restructuring of elastin in tissues as they develop.

Pancreatic elastase is secreted into the small intestine and it is unlikely to have any role in normal elastin turnover. There have been reports, however, of its detection in the blood,[34] and during severe pancreatitis it can damage the lung, inducing vascular injury.[35]

Circulating neutrophil leucocytes produce an elastase that is stored in the azurophil granules of maturing neutrophils at levels of up to 3 $\mu g/10^6$ cells. The current theory, however, relegates neutrophil elastase more to pathological processes than to any physiological turnover. The enzyme is released into tissues when the neutrophil dies or encounters molecules to be phagocytosed. The enzyme has the potential to destroy not only elastin but a wide variety of other extracellular matrix components as well as clotting factors and complement proteins.

Macrophages contain low levels of a metalloprotease, which is secreted into the extracellular matrix and has the ability to degrade elastin.[36] This elastase has the additional property of not being inhibited by α_1 antiprotease and can actually digest this important elastase inhibitor.[37] These cells are of particular importance to the lung connective tissue by the nature of their presence in the lung for purposes of defence.

Very little is known about the physiological role of elastases produced by other cells, such as fibroblasts or smooth muscle cells. Although the concentration of elastase is very low in these cells, it may be quite important in normal elastin metabolism since these are also the cells known to synthesise and secrete elastin on to the extracellular matrix. There are several excellent reviews devoted to the pathological capacity of elastases in the lung.[38-42]

Protease inhibitors

Protease inhibitors are a major weapon in the body's defence arsenal to protect against excessive proteolytic damage to the lung, and in particular to the elastin fibre. Of the many inhibitors circulating in the serum, α_1 antiprotease has the greatest impact on elastin metabolism. α_1 Antiprotease is a glycoprotein of molecular weight 54 000 that is synthesised in the liver and is present in the serum at concentrations ranging from 1·8 to 2·0 mg/l. The diffusion of this protein into the lung accounts for over 90% of the elastase inhibitory capacity of the alveolar epithelial fluid. Very stable, inactive complexes are formed with all elastases except macrophage elastase. Individuals with a heritable deficiency in α_1 antiprotease (Pi ZZ) often have circulating inhibitor concentrations only 10–20% of normal. This defect is often associated with early onset of panlobular emphysema and has been instrumental in the development of the hypothesis of protease-antiprotease imbalance in the pathogenesis of emphysema.[38]

Configuration and function of elastin fibres in the lung

During the past two decades perhaps no single organ in the body has generated more interest in the con-

nective tissue studies than the lung. This is fitting in some respects, since before this biochemists seemed to have a particular reluctance in applying their techniques to studies of this complex tissue. Moreover, during this period there evolved a realisation that no other organ in the body depended so heavily on the proper architecture and stability of connective tissues for proper function, or could be so influenced by environmental factors.

The elastin fibres of the lung are probably the most important determinant of lung elasticity under physiological pressures. They can stretch to 140% of their resting length before breaking, whereas collagen, which contributes tensile strength to the lung, can stretch only about 2%.

Elastic fibres are ubiquitous in the lung, and as with other organs they are closely associated with collagen and proteoglycans. Elastin and collagen fibres from alveoli, bronchi, interlobular septae, and the pleura all appear to have connections with fibres of pulmonary arteries. This gives a continuum of fibres throughout the lung such that any force exerted on the parenchyma is distributed throughout the organ. In the larger pulmonary arteries and airway elastin fibres are present both as circular and as longitudinal bundles. In the vessels they are more prominent in the media but are also evident in the adventitia and intima. The fibres are seen in the submucosal areas in the airways. Elastin fibres in respiratory bronchioles become more distinct distally, where the alveoli are also more developed. As the fibres continue distally, they take on a circular or helical arrangement. This observation was reported early by Pierce and Ebert.[43] They observed that collagen and elastin encircled respiratory bronchioles and alveolar ducts in a coiled or helical fashion. They proposed that the lung tissue unfolds to accommodate its increased volume on expansion, much as in the expansion of an old fashioned door spring. In other words, the tissue can expand with no substantial increase in the length of the elastin fibres. This is an important concept since elastin is so intimately associated with collagen that the fibres may be unable to undergo axial elongation owing to the inelasticity of collagen. The Setnikar-Mead model,[44] on the other hand, proposes that collagen and elastin operate in parallel, and independently of each other. At low lung volumes the elastin fibres are readily extendable, giving the steep part of the volume-pressure curve. As the lung volume increases, the coiled collagen fibres strengthen, producing a decrease in compliance of the combined collagen and elastin networks and increasing the stiffness of the lungs at maximal lung volumes. In vitro studies, using collagenase or elastase for selective disruption of connective tissue components, support the theory of parallel functioning of elastin and collagen fibres.

Elastin and early development of the lung

The chronological appearance of elastin in mammalian lung is similar to that seen in studies in other tissues.[45] Generally, it has been observed that around the third trimester of pregnancy microfibrillar components appear, followed within a few days or weeks, depending on the species, by deposition of amorphous elastin, which steadily increases in amount until parturition. During embryogenesis elastin synthesis in the lung is associated with specific developmental periods. Throughout the canular stage of fetal lung development the elastin concentration is very low. During the period of alveolarisation elastin synthesis is dramatically increased and the concentration in the lung rises. After parturition rapid elastin accumulation continues through the perinatal period, which in man may extend up to seven years. Elastin and lung development has been reviewed in more detail by Rucker and Dubick.[32]

Experimental disruption of elastin in the lung

There is general agreement that the mechanical properties of the normal lung are much influenced by elastin and other connective tissue components. The heterogeneity and close association of these components, however, make it very difficult to assess the contribution that each makes to lung function. The most successful approach to defining the mechanical properties of the lungs in terms of its individual connective tissue components is found in studies aimed at producing lesions directed towards specific lung proteins.

Numerous studies have been reported using various means to disrupt or degrade lung elastin. When the injury is considerable and affects the lower airways and alveoli, the lungs usually progress to some form of emphysema or fibrosis. These models have been reviewed at great length in several excellent articles.[38-42]

INTRATRACHEAL INSTILLATION OF ELASTASE

Perhaps the most dramatic model has been the intratracheal administration of pancreatic or neutrophil elastase to experimental animals, which results in a rapid loss of as much as 40% of lung elastin. Within minutes of elastase administration surfactant activity is altered, and the elastase has begun to enter the type I alveolar epithelium. On entering the lung interstitium, the elastase spreads rapidly and degradation of elastin fibres is initiated. A major portion of the enzyme is cleared from the lung either by entering the blood stream and combining with inhibitors or by being engulfed by macrophages. The remaining elas-

tase continues to attack the elastin fibre for at least several days. When the degradative process tapers off, there is a rapid increase in connective tissue synthesis as the cells attempt to repair the damaged tissue. Within a month total elastin content has been replaced but, of course, any severed alveolar walls cannot be rebuilt, which results in permanently enlarged air spaces. It is not clear whether the newly synthesised elastin actually repairs areas where a rupture would otherwise be imminent. Clearly if the repair process is impaired by inhibition of elastin crosslinking with BAPN or by cigarette smoke, the resulting emphysema is exacerbated.[46 47] Furthermore, young, rapidly growing animals appear to have less severe emphysema than older animals after elastase administration, which may also reflect the capability of young animals for accelerated elastin synthesis and repair.[48]

The severity of emphysema increases substantially between three weeks and several months after elastase administration. Studies with tritiated elastase show that only 1% of the initial dose remains in the lung after four days.[49 50] Radioactivity, however, is still detected in the lung 144 days after administration. It was suggested that a persistent low level of elastase might remain for months, giving rise to the progressive lesions in these animals. Other studies, however, suggest that functional elastase activity in the lung lasts only a few days after administration.[51] The outcome of studies with chloromethyl ketone inhibitors also argues against prolonged enzymatic activity.[52] Another suggestion is that the stress of breathing has an effect on the injured connective tissue framework, leading to further damage of alveolar walls.

Light and electron microscopic examination shows lesions resembling human panlobular emphysema, including the destruction of elastin fibres with decreased numbers of enlarged and distorted alveoli. Quantitative histological techniques reflect these changes; the mean linear intercept is increased and the internal surface area is decreased. Physiological studies show an increase in lung compliance, total lung capacity, residual volume, and functional residual capacity. Apparently there is also some destruction of lung collagen, but this lesion has not been localised.

NITROUS OXIDE
Another model of emphysema results from prolonged exposure of experimental animals to nitrous oxide, which may result in small airway lesions, an increase in neutrophil and macrophage populations in lavage fluid, and a loss of lung elastin.[53] Total elastin returns to normal with the cessation of exposure to nitrous oxide. Mean linear intercept and lung volumes increase and there is a corresponding decrease in the internal surface area. The age of the animal during exposure appears to be an important factor influencing the extent of lung injury, as nursing hamsters are more susceptible to permanent lung injury than three week old animals or adults.[42 54]

ENDOTOXIN
Mild lung destruction has been observed with repeated intravenous injections of endotoxin. If rats are treated with D-galactosamine to lower α_1 antiproteinase concentrations just before the administration of endotoxin, there is a considerable reduction in lung elastin with an increase in mean linear intercept and lung compliance.[55]

INTRATRACHEAL CADMIUM
Intratracheal administration of cadmium has been shown to cause enlargement of air spaces and, in some instances, fibrosis.[42] The mechanism of injury is not clear and may be modulated to a large degree by peripheral factors, such as the mode of administration or agents that block the elastin repair process. Aerosol administration produces a lesion resembling human centrilobular emphysema. Instillation of cadmium in saline, however, causes functional and morphological abnormalities characteristic of pulmonary fibrosis. When BAPN is administered simultaneously with cadmium instillation the animals develop changes typical of bullous emphysema, with no indication of fibrosis.[56] This again illustrates the importance of elastin and perhaps collagen repair processes not only in the degree of severity but also in the nature of the lesion. The role played by elastase in this model is not clear. Neutrophils are pulled into the lungs of hamsters after cadmium installation and have been shown to lose azurophilic granules and elastase.[57] If, however, the animals are depleted of neutrophils before being given cadmium and BAPN, the lesions are just as severe as those produced in hamsters with normal neutrophil levels.[58]

COPPER DEFICIENCY
Copper deficiency prevents normal crosslink formation in both elastin and collagen. When weanling rats from copper deficient dams were continued on a copper deficient diet for six to 10 weeks, their lungs showed a significant reduction in elastin content along with increased mean linear intercepts.[14 42] The emphysematous changes were not reversible with copper supplementation once the lesions were formed. Similar observations have been made with copper deficient pigs and hamsters.[42] Yet, interestingly, the lungs from these animals were not different from controls in total elastin content. It was postulated that low ceruloplasmin concentrations resulted in oxidative cleavage of the elastin fibres, producing emphysematous changes without appre-

ciable removal of elastin.

The mottled mouse has a genetic defect affecting copper metabolism, which results in low lysyl oxidase activity and lack of normal connective tissue cross-linking.[40] These animals develop progressive pan-lobular emphysema with increased compliance and decreased elastin recoil. Mean linear intercepts are increased and internal surface area is decreased. These animals develop more severe emphysema when exposed to nitrous oxide[59] and could provide a sensitive model system for testing compounds that might potentiate lung injury.

Repair of lung damage

Although there are some differences between the animal models, the importance of the integrity of the elastin fibre in maintaining normal lung function is apparent. Equally important is the ability of the lung to turn on elastin synthesis and remodel areas where lesions have occurred.

Often when the lung is injured and there is cellular destruction in the parenchyma, reparative processes go awry and the lungs become fibrotic. This process is usually equated with a considerable increase in collagen, yet there is an equally important increase in total cell mass, as well as in elastin and the other components of the extracellular matrix. Agents that may cause interstitial pulmonary fibrosis are diverse, including oxygen and oxides of nitrogen and sulphur, inorganic dusts, infectious agents, and drugs such as bleomycin. Many patients with adult respiratory distress syndrome who have survived develop what may be considered a form of rapidly progressing interstitial pulmonary fibrosis. The mechanism of fibrosis is unknown. It is apparent that the major pulmonary inflammatory and immune effector cells, neutrophils, macrophages, and lymphocytes have critical roles in this process through the release of enzymes, oxidants, chemotactic factors, growth factors, and secretagogues. Acting in concert with probably several types of lung cells, such as the septal cells of the alveolar walls, smooth muscle cells, fibroblasts, and endothelial and epithelial cells, there is a rapid move to repair the injury and restore normal lung architecture and function. An apparent loss of regulatory control, as well as problems in directing the replacement proteins to the appropriate sites of injury, may result in a connective tissue build up that may virtually obliterate the alveolar air spaces.

Effect of tobacco smoke

The effect of tobacco smoke on lung elastin is extremely complicated, affecting many facets of connective tissue metabolism. Inhalation of cigarette smoke causes an accumulation in the respiratory bronchi of alveolar macrophages, which appear to be filled with pigments and are metabolically and morphologically activated. The activated macrophage has the ability to secrete chemoattractants and secretagogues for neutrophils, as well as secrete a metalloprotease capable of digesting elastin and α_1 antiprotease. The end result is a clustering of large numbers of neutrophils and macrophages, poised to release considerable amounts of elastolytic enzymes at the site where the earliest signs of centrilobular emphysema are detected. In addition to this, the alveolar macrophages, as well as cigarette smoke, are rich sources of oxidising agents. One potential action of these oxidants would be to oxidise the methionine residue found at the active site of α_1 proteinase inhibitor. This has been shown by selective chemical oxidation to yield a relatively ineffective inhibitor that associates with elastase some 2000 times more slowly than the native protein.[38 60 61] There is also the potential for direct oxidant damage to lung cells or cellular components such as lipids, cofactors, and nucleic acids. Recently there have been suggestions that endogenous antioxidant systems within the lung, such as ceruloplasmin, vitamin C, or methionine sulphoxide-peptide reductase, may be adversely affected by cigarette smoke, lowering the lung's defence against oxidants.[61] The elastin maturation process may itself be impaired by cigarette smoke. Chronic exposure of hamsters to cigarette smoke, after a single intratracheal dose of elastase, reduced the rate of desmosine formation in resynthesised elastin.[47] Lung lysyl oxidase activity appeared significantly lowered in the animals exposed to cigarette smoke. Similar in vitro studies have also shown that the ability of lysyl oxidase to convert the lysine residues of tropoelastin to aldehydes is blocked by extracts from cigarette smoke.[62]

Effect of infection

Bacterial infection in the lung is another means of delivering destructive potential to the elastin fibres. On the one hand, infection can draw substantial numbers of neutrophils to the lung. Even more damaging may be an elastase released from organisms such as *Pseudomonas aeruginosa*. Patients with cystic fibrosis commonly have serious infection with this organism that proves impossible to eradicate. As the lifespan of these patients has dramatically increased in recent years, the incidence of emphysema has also gone up. Increased degradation of elastin has been documented in these patients by increased excretion of desmosine.[63] The assumption is made that the increased desmosines originate from the lung and are a reflection of the damage being done to elastin fibres,

but this has not been documented.

Effect of nutrition

One area of growing interest is the role of nutrition in chronic lung disease. This topic has been recently reviewed in detail.[60][64] For many years it has been known that excessive weight loss and starvation are positively correlated with the development of emphysema. Deprivation of essential nutrients, such as trace minerals and vitamins, could foster connective tissue abnormalities and predispose the lung to injury through several means. Unusually low concentrations of copper could affect lysyl oxidase activity, retarding crosslink formation in elastin and collagen. Superoxide dismutase and ceruloplasmin also require copper for activity and both may function as antioxidants. Selenium is required for glutathione peroxidase activity and iron is required for catalase. Vitamins E and C are effective scavengers of free radicals and deficiencies of these may also affect the lung cells' defence against oxidant damage.

A more direct effect on lung connective tissue may be seen with a deficiency in vitamin B_6. Several studies have indicated that elastin crosslinking is impaired in B_6 deficient animals, implying that pyridoxal phosphate is a cofactor for lysyl oxidase.[64] A recent study, however, presents evidence suggesting that the B_6 effect on elastin crosslinking is due to a block in the conversion of homocysteine to cystathionine.[65] The raised concentrations of homocysteine then block the conversion of allysine to the desmosines through the formation of thiazine derivatives with the aldehyde functional group.

Supplementing a copper deficient diet with a high content of vitamin C was shown many years ago to worsen the effects of copper deficiency and further reduce the elastin content of the aorta.[66] Recently it has been observed that smooth muscle cells in culture synthesise significantly less elastin when the medium is supplemented with vitamin C.[8][67] One could speculate that, with today's overindulgence in vitamin supplements, excessive vitamin C concentrations could slow down elastin synthesis in the lung during acute or chronic lung injury and thereby impair the crucial elastin replacement process.

Starvation in young growing rats produces a general loss of connective tissues, which may result in emphysematous like changes in lung structure.[42] This effect was not seen in older animals, where growth retardation did not occur.[68] A direct relationship between malnutrition and the elastin fibre component of the adult human lung will be difficult to show. Other lung elements, such as surfactant, collagen, and the respiratory muscles, which all play important parts in normal lung function, would probably feel the effects of a poor nutritional state sooner than a protein like elastin with its extremely slow turnover. Malnutrition in infants, however, particularly during the period of alveolarisation, could have pronounced and perhaps permanent harmful effects on elastin architecture and lung function.

Consequences of stability

In many respects elastin is a perfectly designed protein for its role in normal lung function. The unusual amino acid composition and lysine derived crosslinks provide the elastin fibre with great distensibility and recoil properties. They also lend chemical stability to the fibre, which is susceptible to few proteolytic enzymes and chemical injuries. Complications arise in conjunction with this inherent stability. Mature elastin has an extremely low turnover rate. Once the delicate architecture of the alveolar walls has been constructed and the continuum of connective tissue fibres is established, the components are meant to remain in that configuration. After the fetal and early perinatal stages of lung development we apparently have no contingency programme for initiating a new and architecturally correct alveolus if the original structure has been destroyed. So what has man done in his infinite wisdom but devise every means possible to bring tobacco smoke and other pollutants and drugs into direct contact with this very durable but not indestructible connective tissue matrix? Our defence system is magnificent, as we signal for macrophages and neutrophils to fight this unforeseen menace. As the neutrophils degranulate and release their enzymes there is disparity between the finely tuned ratio of elastase to antiprotease. Every injury sustained by alveolar elastin that is not repaired hastens the inevitable cleavage of the alveolar wall. If the injury is perpetuated, as is the case with cigarette smoke, alveolar walls are slowly cleaved, leaving greatly enlarged air spaces and a lung without elastic recoil. Apparently, during some phase of alveolar damage the resynthesis of elastin and the repair of fibres has an appreciable effect on the development of the lesion. The nutritional state and age at onset of injury may be of paramount importance during this period.

Protecting elastin

What avenues are being considered for preventing or at least curtailing the destruction of the elastin fibre in the lung? One approach is to increase the serum elastase inhibitory capacity. Replacement treatment with α_1 antiprotease is currently being evaluated with limited numbers of patients with a genetic deficiency in α_1 antiprotease.[69] Assessment of the efficacy of this method will be difficult and it is the topic of a recent

supplement.[41] Eglin C, a low molecular weight peptide inhibitor isolated from the medicinal leech, has also been shown to confer protection to experimental animals given elastase intratracheally, provided that it is given just before or shortly after the enzyme.[70]

A similar approach would be to administer low molecular weight inhibitors specific for elastase.[71] These molecules have many advantages, including availability, aerosol or oral administration, and non-antigenicity. So far, they have been used in animal models with varying degrees of success. As more effective inhibitors become available, we can expect to see their use in clinical trials. Short of destroying the tobacco crops, a "perfect" low molecular weight inhibitor could be the best bet for the future.

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