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A L Horwitz, R C Crystal

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Research Article

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Content and Synthesis of Glycosaminoglycans in the Developing Lung

ALLEN L. HORWITZ and RONALD G. CRYSTAL

*From the Section on Pulmonary Biochemistry, National Heart and Lung
Institute, Bethesda, Maryland 20014*

ABSTRACT The function of lung is fundamentally linked to the connective tissue composition of the alveolar interstitium. The composition and synthesis of one class of interstitial connective tissue components, the glycosaminoglycans (GAG), was determined in lung parenchyma of rabbits at different stages of development. Parenchymal GAG content ranged between 0.2 and 0.4% (wt/wt) of dry weight, with highest concentration in adult lung. There were significant changes in types of GAG present at different ages. Fetal lungs contained a relatively high proportion of chondroitin 4-sulfate while the GAG in lung parenchyma of older animals was predominantly dermatan sulfate, heparan sulfate, and heparin. Methods were developed for the study of rates of synthesis of GAG by incorporation of [1-¹⁴C]glucosamine into lung explants. The rate of synthesis of total GAG per cell increased with development to a maximum in lung from weanling rabbits and fell to low rates of synthesis in mature rabbits. Fetal rabbit lung parenchyma synthesized mostly hyaluronic acid and heparan sulfate, while in weanling rabbit parenchyma hyaluronic acid and chondroitin 4/6-sulfate synthesis was greatest. In mature animals, the rates of synthesis of all types of GAG were relatively low but there was a relatively greater emphasis on synthesis of dermatan sulfate and heparin. These results may have significance in changes in lung function during development and in effects on other connective tissue components.

INTRODUCTION

The interstitium of the lung alveolus is composed of mesenchymal cells and connective tissue. The latter is of fundamental importance in the maintenance of lung

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structure, determination of lung mechanical properties, interchange of nutrients and metabolites between the capillary and epithelial cells, and diffusion of O₂ and CO₂ between the alveolar gas and the blood. The two major constituents of interstitial connective tissue, collagen and elastin, comprise more than 90% of the non-cellular interstitial dry weight. The remainder has been termed the "amorphous ground substance" (1, 2).

The term "amorphous" is used because this material is poorly defined morphologically. It is known to include serum proteins, glycoproteins, cellular metabolites, small solutes (e.g., glucose, urea, salts), and proteoglycans (3). The latter are macromolecules composed of a protein backbone with multiple, large polysaccharide side chains termed glycosaminoglycans (GAG)¹ (4). The GAG also include hyaluronic acid, which may not be part of a protein-polysaccharide complex. In tissues other than lung and in simplified model systems with purified components, it has been shown that specific types of GAG will: (a) associate with other connective tissue elements (5, 6); (b) influence the rate of synthesis of connective tissue components (7, 8); (c) affect the hydration of connective tissue (9); and (d) influence the rate of collagen fibril formation and subsequent stability of these fibrils (5, 10). Thus, the quantity and type of GAG in the lung interstitium may have significant effects on the mechanical properties of the lung as well as on gas, solute, and fluid movements between the alveolar space and capillary (11). It is the purpose of this study to describe methods to quantitate the composition and synthesis of GAG in the lung interstitium and to define the normal pattern of GAG accumulation and synthesis in the growing lung.

METHODS

Materials. An inbred strain of New Zealand white rabbits (B and H Rabbitry, Rockville, Md.) were used as a

¹ Abbreviations used in this paper: CPC, cetylpyridinium chloride; GAG, glycosaminoglycans.

source of lung. The animals were exsanguinated by decapitation and the lungs were dissected free. No lungs had evidence of infection. The hila were widely excised and the parenchyma was minced into 1–2-mm pieces.

Enzymes utilized in preparation and quantitation of GAG included: papain (twice crystallized) and testicular hyaluronidase (Sigma Chemical Co., Inc., St. Louis, Mo.); partially purified streptococcal hyaluronidase isolated from Varidase (a gift of Dr. Paul Bell, Lederle Laboratories, Pearl River, N. Y.); chondroitinase ABC and chondroitinase AC (Miles Laboratories, Inc., Elkhart, Indiana). GAG standards of hyaluronic acid, chondroitin 6-sulfate and dermatan sulfate, heparin, and heparan sulfate were kindly supplied by Dr. J. A. Cifonelli (University of Chicago Standards, N.I.H. Contract 5 RO HE 11083). Chondroitin 4-sulfate was obtained from Sigma Chemical Co.

Isolation of GAG from lung. Lung minces were homogenized (Polytron, Brinkmann Instruments, Inc., Westbury, N. Y.) at 4°C and then placed on a boiling water bath for 3 min to denature proteins. The homogenate was then made to 0.1 M sodium acetate, 20 mM EDTA, and 20 mM cystine, and digested at 60°C by two separate additions (18 h apart) of papain (1 mg papain/20 mg dry wt lung). After papain digestion, trichloroacetic acid was added at 4°C to 6% concentration to precipitate nucleic acids and remaining protein. After 30 min, the precipitate was collected (10,000 g, 10 min); it contained less than 2% of the total uronic acid. The supernate was dialyzed against 0.03 M NaCl (4°C, 24 h) and the GAG were precipitated by addition of 10% cetylpyridinium chloride (CPC) in drops until no further precipitate formed. The CPC precipitate was pelleted (2,000 g, 10 min) and dissolved in 2.0 M NaCl. Occasionally, a small amount of material would not dissolve in 2 M NaCl; this residue contained no hexuronic acid and was discarded. The GAG were reprecipitated from the 2 M NaCl solution with ethanol and dried. Total GAG content was determined by measurement of uronic acid (12) or hexosamine (13) content after hydrolysis of the dried GAG with 4 N HCl for 16 h at 100°C.

Identification and quantitation of GAG types in lung. Total lung GAG was isolated by CPC precipitation as described above and dissolved in 0.4 M NaCl, and 10% CPC was added until no further precipitate formed. The precipitate (containing all GAG types except hyaluronic acid) was pelleted (2,000 g, 10 min), dissolved in 2 M NaCl, and reprecipitated with 80% ethanol to remove CPC and other salts. The supernate from the 0.4 M NaCl solution (containing hyaluronic acid) was made 70% in ethanol and the precipitate was pelleted, washed with ethanol, and dried. The two dried precipitates were then analyzed for total uronic acid (12). Identification and quantitation of hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, heparin, and heparan sulfate in these precipitates was done by enzymatic methods. Hyaluronic acid was determined by the method of Greiling (14), utilizing partially purified streptococcal hyaluronidase. Chondroitin 4-sulfate, chondroitin 6-sulfate, and dermatan sulfate were analyzed with chondroitinase ABC and chondroitinase AC by the methods of Saito et al. (15). The sum of heparin plus heparan sulfate was determined by subtracting total hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, and dermatan sulfate from total GAG hexuronic acid (16). The validity of this method for quantitating heparin plus heparan sulfate was proven by demonstrating that the total GAG hexuronic acid resistant to testicular hyaluronidase plus chondroitinase ABC was

degraded by nitrous acid. The products after nitrous acid were quantitated by chromatography on columns of Bio-Gel P10 (1 × 100 cm) (Bio-Rad Laboratories, Richmond, Calif.), and equaled the heparin plus heparan sulfate as measured by difference.

Rate of total GAG synthesis by lung explants. Minced rabbit lung parenchyma was placed in glass vials in 2 ml of Dulbecco's Modified Eagle's medium (prepared by the N.I.H. Media Unit) diluted 1:1 with phosphate-buffered saline (17). D-[1-¹⁴C]Glucosamine (2 μCi, 37.5 nmol/incubation, New England Nuclear, Boston, Mass.) was added and the incubation (37°C, 5% CO₂-95% O₂) was continued from 2 to 10 h. At different time periods, the contents of each vial were diluted with 0.4 ml of cold 0.4 M EDTA and homogenized. 1 mg each of hyaluronic acid, heparan sulfate, heparin, chondroitin 4-sulfate, and dermatan sulfate were added as carriers and aliquots were taken for total GAG synthesis, DNA, specific activity of ¹⁴C-labeled GAG precursors in the tissue, and specific GAG types synthesized.

Total GAG synthesis was measured at 2, 4, 6, 8, and 10 h of incubation by isolating total GAG as described above. The incorporation of D-[1-¹⁴C]glucosamine into total GAG was assayed by dissolving the isolated dried GAG in water and counting aliquots in Aquasol (New England Nuclear) with an efficiency of 85%.

DNA was used to quantitate cell number in the explant. Trichloroacetic acid was added to an aliquot of the homogenate to 5% concentration. After 10 min at 4°C, the precipitate was pelleted (6,000 g, 10 min) and total DNA in the precipitate was determined (18). The supernate from this precipitation step was used to measure the specific activity of GAG precursors in the tissue.

UDP-*N*-Acetylglucosamine and UDP-*N*-acetylgalactosamine are precursors of all lung GAG (19). The quantitation of {cpm [¹⁴C]UDP-*N*-acetylglucosamine + cpm [¹⁴C]-UDP-*N*-acetylgalactosamine}/ {pmol UDP-*N*-acetylglucosamine + pmol UDP-*N*-acetylgalactosamine} gives a measure of the specific activity of the immediate precursors of lung GAG, thus compensating for differing precursor pool sizes in each lung sample. The supernate from the trichloroacetic acid precipitation described above was neutralized with KOH, diluted with 10 vol of water, and applied to 0.7 × 7 cm columns of Dowex 1X8 (Dow Chemical Co., Midland, Mich.). The columns were washed with 12 ml of 0.1 M ammonium formate, pH 4.5, and the nucleotide sugars were eluted with 12 ml of 0.4 M ammonium formate. The eluants were lyophilized, dissolved in water, and chromatographed on Whatman 3 MM paper in ethanol-1 M ammonium acetate (7.5:3) (20). In this system, UDP-*N*-acetylglucosamine and UDP-*N*-acetylgalactosamine cochromatograph. The area chromatographing with the UDP-*N*-acetylhexosamine standards was eluted with water, the radioactivity of [¹⁴C]UDP-*N*-acetylhexosamines was determined by liquid scintillation counting in Aquasol, and the concentration of UDP-*N*-acetylhexosamines was quantitated either by absorbance at 260 nm or by hexosamine content after hydrolysis in 1 N HCl at 100°C for 2 h (13). The specific activity of the [¹⁴C]UDP-*N*-acetylhexosamines was expressed as counts per minute per picomole.

The 4, 6, 8, and 10-h values of ¹⁴C-total GAG per milligram DNA (expressed on the basis of the specific activity of the GAG precursor) were used to calculate the rate of total GAG synthesis (pmol [1-¹⁴C]glucosamine incorporated into total lung GAG/mg DNA·h) by least squares fit analysis. All rates measured had a correlation coefficient over 0.95.

Identification and quantitation of rates of synthesis of specific types of GAG in explants. The total labeled GAG isolated by CPC precipitation in 0.03 M NaCl were fractionated on 1.2 × 15 cm columns of Dowex 1 × 2 (Cl⁻, 200–400 mesh) by stepwise elution according to the method of Schiller et al (21). Fractions eluting with 0.5 M (hyaluronic acid), 1.25 M (heparin sulfate), 1.5 M (chondroitin 4-sulfate plus chondroitin 6-sulfate), 1.5 M (dermatan sulfate), and 2.0 M (heparin) NaCl were tested for degradability by streptococcal hyaluronidase, testicular hyaluronidase, chondroitinase ABC, or nitrous acid (22). The degree of degradation of each fraction was measured by quantitating the molecular weight spectrum on a 1 × 100 cm column of Bio-Gel P-10, eluted with 0.2 M NaCl. The degree of degradation was expressed as a percent [(cpm in the included volume × 100)/(cpm in the included volume + cpm in the excluded volume)]. The absolute rate of synthesis of each type of GAG was determined by multiplying the percent degradation times the total rate of GAG synthesis. The following criteria were used to identify each type of GAG:

(a) Hyaluronic acid: elute from Dowex 1 × 2 at 0.5 M NaCl, degraded by streptococcal hyaluronidase, testicular hyaluronidase, and chondroitinase ABC, but not by HNO₂.

(b) Heparan sulfate: elute from Dowex 1 × 2 at 1.25 M NaCl, degraded by nitrous acid but not by the enzymes. In some samples, 10–15% of the 1.25 M fraction was resistant to HNO₂ and susceptible to streptococcal hyaluronidase. Testicular hyaluronidase and chondroitinase ABC did not increase degradation of this material and it was thus assigned to the hyaluronic acid fraction.

(c) Chondroitin 4-sulfate plus chondroitin 6-sulfate: elute from Dowex 1 × 2 at 1.5 M NaCl, degraded by testicular hyaluronidase but not by streptococcal hyaluronidase or HNO₂.

(d) Dermatan sulfate: elute from Dowex 1 × 2 at 1.5 M NaCl, degraded by chondroitinase ABC but not by streptococcal hyaluronidase, testicular hyaluronidase, or HNO₂. Thus, synthesized dermatan sulfate was the difference between that portion of the 1.5 M fraction degraded by chondroitinase ABC and that degraded by testicular hyaluronidase alone.

(e) Heparin: elute from Dowex 1 × 2 at 2.0 M NaCl, degraded by HNO₂ but not by the enzymes. Approximately 15% of some 2.0 M fractions were resistant to HNO₂ but were degraded by testicular hyaluronidase and chondroitinase ABC. This material was assigned to the chondroitin 4-sulfate plus chondroitin 6-sulfate fraction.

By these methods, all of the [¹⁴C]GAG isolated by CPC could be accounted for by the sum of these five fractions. The data for rates of synthesis of the specific types of lung GAG were expressed as a percent of total GAG synthesized per milligram DNA·hour or as picomoles of [1-¹⁴C]glucosamine incorporated into each type of GAG per milligram DNA·hour.

Changes in lung GAG concentration and rates of synthesis with lung growth. Lungs from rabbits at 6 days before birth (labeled “–6 days” or “fetal”), and 7 (“newborn”), 45 (“weanling”), and 180 (“adult”) days after birth were used to determine: total GAG/dry weight, percent of total GAG per dry weight represented by each type of GAG, rate of incorporation of [1-¹⁴C]glucosamine into total GAG per milligram DNA·h and rate of incorporation of [1-¹⁴C]-glucosamine into each type of GAG per milligram DNA·hour. A total of 72 rabbits was used.

RESULTS

Concentration of lung parenchyma GAG. The average concentration of total GAG in rabbit lung parenchyma is relatively constant (3.5–3.9 μmol hexosamine/g dry wt) from late in gestation through the weanling period but rises approximately 60% as the animal reaches maturity. Based on an average GAG hexosamine content of 30% (22), this corresponds to 0.2–0.4% (wt/wt) of the lung parenchyma being composed of GAG. Since rabbit lung collagen concentration rises rapidly in the perinatal period and then levels off (17), the ratio of lung GAG concentration to collagen concentration decreases 150% between fetal and the weanling period (Fig. 1).

There are marked differences in the percent distribution of lung parenchyma GAG concentration with growth (Fig. 2). The late fetal lung has approximately equal concentrations of hyaluronic acid, chondroitin 6-sulfate, dermatan sulfate, and heparan sulfate plus heparin, but almost twice as much chondroitin 4-sulfate as any other GAG type. These relationships change abruptly after birth, when dermatan sulfate concentration has increased almost threefold while the relative concentrations of hyaluronic acid, chondroitin 6-sulfate and heparan sulfate plus heparin have fallen. In the weanling rabbit parenchyma, the relative chondroitin 4-sulfate concentration has decreased even more, with concomitant relative elevations of heparin sulfate + heparin. The increase in this latter fraction is even

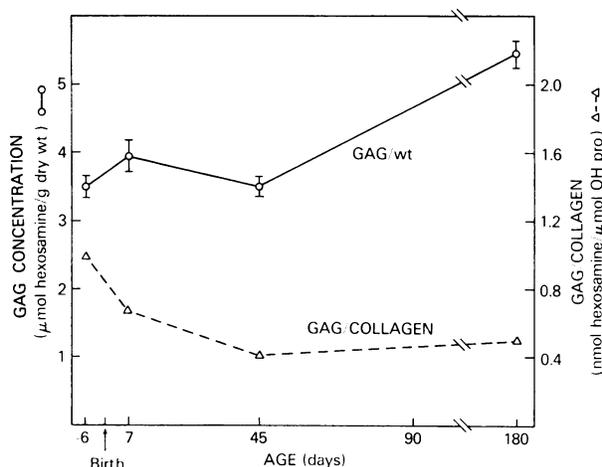


FIGURE 1 Changes in rabbit lung parenchymal GAG in the maturing rabbit. The concentration of total GAG per unit dry weight (○—○) was quantitated as described in Methods. Error estimates represent the SEM. Lungs from six rabbits were used at each time point. The ratio of lung total GAG to lung total collagen (Δ---Δ) was determined from data on lung hydroxyproline per unit dry weight previously determined in the same colony of New Zealand white rabbits (17).

TABLE I
Quantitation of the Specific Activity of GAG Precursors ($[^{14}\text{C}]\text{UDP-N-Acetylhexosamine}$) in Lung Parenchyma from Different Age Rabbits

Age of rabbit	UDP-N-acetylhexosamine concentration	$[^{14}\text{C}]\text{UDP-N-acetylhexosamine}$ concentration	$[^{14}\text{C}]\text{UDP-N-acetylhexosamine}$ specific activity
	$\mu\text{mol}/\text{mg DNA}$	$\text{cpm} \times 10^3/\text{mg DNA}$	$\text{cpm}/\mu\text{mol}$
Fetal (-6 days)	1,720	251	146
Neonate (7 days)	1,530	221	145
Weanling (45 days)	2,450	320	131
Adult (180 days)	950	368	388

Values are the average of 4- and 6-h incubation samples.

more marked in the adult lung while the chondroitin 4-sulfate fraction has continued to decrease.

Synthesis of GAG by lung parenchyma. The incorporation of D- $[1-^{14}\text{C}]\text{glucosamine}$ into total GAG in the parenchyma explants is low for the first 2 h of incubation but then rapidly increases linearly for at least 10 h (Fig. 3). During the linear period the specific activity of $[^{14}\text{C}]\text{UDP-N-acetylhexosamine}$ was unchanged. When incubations were carried out in 5% CO_2 -95% air rather than the usual 5% CO_2 -95% O_2 , the incorporation was identical to the 0-6-h portion of Fig. 3 but, then leveled off (data not shown).

The importance of measurement of the specific activity of labeled GAG precursors in the tissue is pointed

out by the data in Table I. In the growing rabbit lung, the UDP-N-acetylhexosamine concentration increases approximately 40% between the fetal and weanling periods, but markedly decreases as the animal becomes adult. There are also changes in the relative amount of incorporation of D- $[1-^{14}\text{C}]\text{glucosamine}$ into UDP-N-acetylhexosamine, so that the resulting specific activity of GAG precursors remains relatively constant from the fetal to weanling period but increases threefold in the adult.

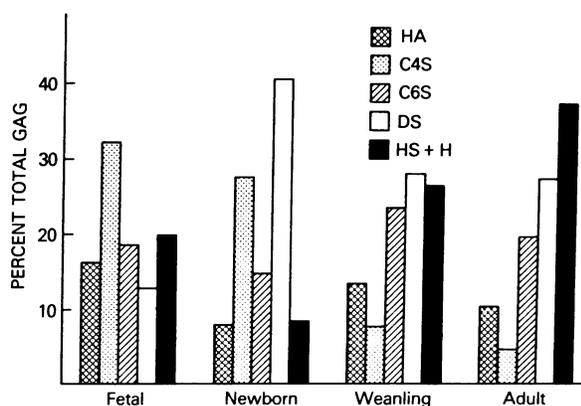


FIGURE 2 Relative amounts of types of GAG present in rabbit lung parenchyma at various ages. The amount of each type of GAG per unit dry weight was determined as described in Methods and expressed in micromoles of hexuronic acid per gram dry weight. At each age, the sum of the concentrations of each type equaled the total GAG per unit weight shown in Fig. 1. The relative percentages of each type of GAG are expressed by the height of the bar. HA, hyaluronic acid; C4S, chondroitin 4-sulfate; C6S, chondroitin 6-sulfate; DS, dermatan sulfate; HS + H, heparan sulfate + heparin. The four ages correspond to the same ages in Fig. 1.

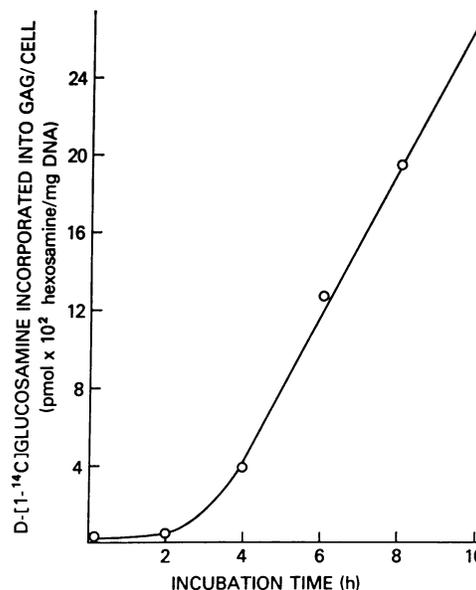


FIGURE 3 Incorporation of $[1-^{14}\text{C}]\text{glucosamine}$ into total GAG of lung parenchyma. For each time point, approximately 80 mg (wet wt) of minced lung parenchyma was incubated with 35.7 nmol ($2 \mu\text{Ci}$) of D- $[1-^{14}\text{C}]\text{glucosamine}$ in media under 95% O_2 -5% CO_2 , as described in Methods. The explants were homogenized at indicated times and measured aliquots were taken for determination of ^{14}C -total GAG, DNA, and specific activity of the GAG precursors in the explant ($[^{14}\text{C}]\text{UDP-N-acetylhexosamine}$).

The rate of synthesis of total lung GAG (averaged over all parenchymal cells and expressed on the basis of the specific activity of GAG precursors in the parenchyma) varies significantly in the growing lung (Fig. 4). The rate of synthesis of total GAG per cell increases from 125 to 400 pmol [^{14}C]hexosamine incorporated/mg DNA·h between fetal and weanling lung (Fig. 4A). This marked elevation in the rate of synthesis of total lung GAG precedes the increase in the concentration of lung GAG found in the adult (Fig. 1). As the concentration of GAG increases, the rate of synthesis of total GAG decreases.

The relative amounts of the specific types of GAG synthesized by parenchyma appears to parallel total GAG synthesis (Fig. 4B vs. 4A). The most marked change in rates of synthesis is in hyaluronic acid synthesis (weanling/fetal = 3) and chondroitin 4-sulfate plus chondroitin 6-sulfate synthesis (weanling/fetal = 5). Although less in absolute rates, the relative increases (in the same time period) in dermatan sulfate and heparin synthesis are actually greater (each seven- to eightfold). In contrast, there is very little change in heparan sulfate synthesis in this period.

All of the GAG synthesized by parenchyma was degraded by chondroitinase ABC followed by HNO_2 and, hence, contained no keratan sulfate (22).

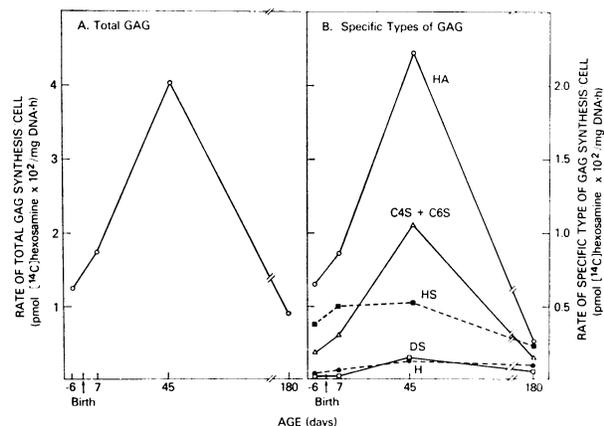


FIGURE 4 Rates of synthesis of GAG by rabbit lung parenchymal explants at various stages of maturity. (A) The rate of total GAG synthesis per cell at different ages. Rates of total GAG synthesis per milligram DNA per hour were calculated by determining the least square fits of the linear portion (4–10 h) of the plot of [^{14}C]hexosamine incorporated into GAG per milligram DNA against time (as shown in Fig. 3). In all cases the correlation coefficient of fit was over 0.95. (B) The rate of synthesis of specific type of GAG per cell at different ages. HA, hyaluronic acid (\circ — \circ); C4S + C6S, chondroitin 4-sulfate + chondroitin 6-sulfate (\triangle — \triangle); HS, heparan sulfate (\blacksquare — \blacksquare); DS, dermatan sulfate (\square — \square); H, heparin (\bullet — \bullet). The sum of the rates of synthesis of the various types (B) equaled the rate of synthesis of total GAG (A).

DISCUSSION

Approximately 86% of the total cells of the lung and 62% of the total connective tissue of the lung are in the parenchyma, with the remaining 14% of cells and 38% of connective tissue in the conductive blood vessels and airways (2, 23). When a wide excision is used to remove the hilar structures, the majority of the large blood vessels and airways are removed, so that any biochemical measurements in the remaining tissue reflect the cellular and connective tissue of the parenchyma (2). The cells of the parenchyma include primarily alveolar type I and II epithelial cells, endothelial cells, and interstitial mesenchymal cells, while the connective tissue of the parenchyma almost entirely reflects the connective tissue of the alveolar interstitium.

The concentration of parenchymal GAG (0.2–0.4% dry wt GAG/dry wt parenchyma) is low compared to collagen (15–20%, wt/wt) and elastin (5–10%, wt/wt). Thus, total parenchymal GAG is less than 2% of the total interstitial connective tissue. Even so, the demonstrated interaction of GAG with other connective tissue elements (5, 6), influence on connective tissue synthesis and cellular differentiation, and effect on the state of the ground substance “gel” (11) suggest that this relatively small amount of material could have a major impact on interstitial mechanical properties and on the rates of gas, solute, and fluid transfer through the interstitium.

The control of the amounts of GAG in the interstitium seems at least in part to be related to the rates of synthesis of GAG by the cells comprising the parenchyma, since maximum rate of GAG synthesis (Fig. 4) appears to precede the increase in GAG concentration found in the adult (Fig. 1). However, the relative changes in the concentration of each type of GAG in the parenchyma (Fig. 2) cannot be due only to changes in rates of synthesis of GAG (Fig. 4), since the rates of synthesis of specific types of GAG do not necessarily parallel the dramatic alterations in GAG types found with age. Presumably, interstitial GAG concentration is controlled by the balance of GAG synthesis and degradation by mechanisms as yet undefined in lung. Undoubtedly, these mechanisms include the changing populations of cell types in parenchyma with lung growth (23). Although it is known that epithelial, endothelial, and mesenchymal cells derived from organs other than lung synthesize and degrade GAG (24–27), there are no data available of GAG synthesis or degradation by isolated lung parenchymal cells. It is possible that each of the four major cell types in parenchyma contributes to the control of interstitial GAG accumulation. The increase in heparin plus heparan sulfate concentration found in adult lung may also be related to other cell types, such as mast cells.

Alterations in GAG concentration with age have been described in organs other than lung. Increases in GAG concentration with maturity have been found in brain (28), while decreases in GAG concentration with age have been found in skin (29), cartilage (30, 31), and the cardiovascular system (32). This latter finding may help to explain the observation by Bozovic and Bozovic (33) that total rat lung hexosamine and mucopolysaccharides (per unit wet weight of tissue) decreased with age, since when total lung tissue is analyzed, the increases in parenchymal GAG concentration in late maturity (Fig. 1) might be offset by decreases in GAG concentration in the large vasculature. Measurements of lung GAG must also take into account the state of the organ in regards to infection, since the infected rat lung shows marked increases in lung GAG (34). The lungs of the inbred strain of rabbits used in this study had no infection either grossly or microscopically.

The alteration in the ratio of interstitial GAG to collagen, particularly in the perinatal period (Fig. 1) may have significant implications for the function of the lung. Since the relative amounts of chondroitin 4-sulfate and dermatan sulfate are changing significantly at this time (Fig. 2), it is important to note that it has been shown in vitro that both GAG types will complex with tropocollagen and accelerate fiber formation, although to different degrees (5, 6, 10). Thus, not only may GAG influence mechanical properties through its interaction with interstitial collagen, it may also influence the quantity of newly synthesized interstitial collagen laid down as mature collagen fibrils.

Lung parenchyma contains all types of GAG except keratan sulfate (Fig. 2) (35). The age-related decrease in the ratio of parenchymal chondroitin 4-sulfate to chondroitin 6-sulfate is in contrast to rabbit cartilage, in which this ratio increases with age (36). In human costal and tracheobronchial cartilage this ratio decreases with age (34, 36). Thus, the relative content of each type of GAG may vary with tissue type, animal, and age.

Although early studies demonstrated that in vivo injection of [^{35}S O $_4$] $^{2-}$ or [^{14}C]glucosamine results in the labeling of lung GAG (37), it is very difficult to quantitate rates of synthesis of GAG by in vivo methods because of the variability of distribution of isotope and difficulty in measuring organ precursor pool sizes. A number of these variables can be eliminated by explant methods, in which slices or minces of lung are incubated in vitro in defined media. Under these conditions, the explant maintains the capacity to synthesize protein linearly for at least 24 h.² Rabbit lung parenchymal explants are capable of incorporating [^{14}C]glucosamine

² M. Cowan. Unpublished observations.

into GAG for at least 10 h (Fig. 3). The lag of 2 h before synthesis presumably represents the incorporation of glucosamine into the tissue from the medium, intracellular transport, conversion into the GAG precursors, and the growth of the GAG chain on the protein moiety of the proteoglycan. It is crucially important to measure the specific activity of the precursor pool of the GAG as demonstrated by the differences in lungs of varying ages (Table I). This concept, although qualitatively different, has been of equal importance in the quantitation of protein synthesis in lung (17).

The linear portion of the plot of [^{14}C]GAG per milligram DNA (adjusted for the specific activity of the precursor pool) against time allows the quantitation of the rate of total GAG synthesis averaged over all cells of the parenchyma. Whether the changes in rates of total GAG synthesis and specific types of GAG synthesis noted with age represent relative intracellular control, changing cellular populations, or both, is not known at this time.

It has been suggested that lung GAG may be fundamental to the pathogenesis of several lung disorders, including emphysema (38, 39) and silicosis (40). The methods described here are applicable to the examination of the rates of synthesis of GAG in biopsy specimens from patients with lung disease. Approximately 15–20 mg (dry wt) of tissue are needed for each time point, well within the range of a portion of an average open biopsy specimen. Work is continuing in our laboratory to correlate the role of interstitial GAG content, synthesis, and degradation to lung mechanical properties and lung cell types in both health and disease.

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