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K Bradley, S McConnell-Breul, R G Crystal

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Research Article

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Collagen in the Human Lung

QUANTITATION OF RATES OF SYNTHESIS AND PARTIAL CHARACTERIZATION OF COMPOSITION

KATHRYN BRADLEY, SALLY MCCONNELL-BREUL, and RONALD G. CRYSTAL

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

ABSTRACT The presence of collagen in lung is fundamental in normal lung structure and function. Methods have been developed to examine human fetal and adult lung collagen with respect to its composition and synthesis. The second trimester fetal lung has a large number of cells per unit lung mass (36.6±2.7 μg DNA/mg dry wt) and relatively small amounts of collagen (17.0±5.3 μg collagen/mg dry wt). The number of cells per unit lung mass in the adult lung (11.1±3.4 μg DNA/mg dry wt) is 30% of the number of cells in the fetal lung, but the adult has 11 times more collagen (196±25 μg collagen/mg dry wt).

The composition of fetal lung collagen can be partially characterized by extraction with salt at neutral pH, acetic acid, or guanidine. The extracted chains, representing 10% of the total lung collagen, chromatograph as α1 and α2 chains, each with a mol wt of 100,000 and an animo acid composition characteristic for collagen but not specific for lung.

Short-term explant cultures of fetal and adult lung synthesize α chains which can be isolated by ion-exchange chromatography. These chains, representing 30–40% of the total collagen synthesized by the explants, coelectrophorese with extracted collagen chains on acrylamide gels; they are destroyed by clostridial collagenase and they have a mol wt of 100,000.

Although the composition of the collagen synthesized by these explants can be only partially characterized, the rate of synthesis of both collagen and noncollagen protein can be quantitated. In fetal lung, 4.0±1.2% of the amino acids incorporated into protein per hour are incorporated into collagen. In normal adult lung, this percentage (4.2±0.9%) is remarkably similar. These values are almost identical to the relative rate of collagen synthesis in rabbit lung in the same age range. This technology should be applicable to answer specific questions regarding collagen synthesis and degradation in human lung disease.

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INTRODUCTION

Collagen is an integral component of lung. It maintains alveolar, airway, and vascular stability, limits lung expansion, and contributes significantly to lung recoil at all lung volumes (1, 2).

In the adult, the total amount of collagen and the concentration of collagen per unit lung mass remain constant (3). In the developing rabbit lung, there is a rapid accumulation of the amount of collagen per unit lung mass so that the adult rabbit lung has six times more collagen than the early third trimester rabbit fetal lung (4). This rapid accumulation of collagen is preceded by a shift in emphasis in the types of proteins synthesized by lung cells, manifested by an increase in the rate of incorporation of amino acids into collagen compared to noncollagen lung proteins. As the rabbit matures, the relative rate of collagen synthesis returns to a low level which continues throughout life (4). Analysis of the collagen synthesized by neonatal rabbit lung suggests there are probably four types of collagen present (5).

Two groups of human lung disorders may be associated with changes in the rate of collagen synthesis, the types of collagen synthesized, the rate of collagen proteolysis, and/or the types of collagen destroyed. In the emphysematous disorders, while the total amount of lung collagen appears to be stable (6), there may be "remodeling" so that the newly synthesized collagen appears in an abnormal array (7). In the fibrotic lung

disorders, histologic evidence suggests there is either an abnormal accumulation of collagen in total amount or in abnormal regions of the lung (8). The present study examines collagen composition and synthesis in the fetal and adult normal human lung.

METHODS

Human fetal lung was recovered intact from 12–17-wk-old fetuses obtained after therapeutic abortion by curettage under regional anesthesia. The investigators in this study had no knowledge of the patients or involvement in the surgical procedure. Human adult lung was recovered from surgical specimens removed at the time of lobectomy in patients with lung nodules. In all cases the anesthesia used was barbiturate, halothane, and nitrous oxide. None of the portion of lung used for these experiments contained tumor. Immediately after surgery, the lungs were placed in 0.9% saline at 4°C.

Structural analysis. 20 12–17-wk-old fetal lungs were trimmed to remove hilar structures and were subsequently rinsed in phosphate-buffered saline, pH 7.4 (PBS).[1] The combined lungs were homogenized in 1 liter of neutral salt (1 M NaCl-50 mM Tris-HCl, pH 7.4) in a Sorvall Omni-Mixer (16,000 rpm, 2 min) (Ivan Sorvall, Inc., Newtown, Conn.). Collagen was extracted sequentially with neutral salt (48 h), 0.5 M acetic acid (72 h), and 5 M guanidine, pH 7.5 (72 h) the methods used by Bornstein and Piez to extract human skin collagen (9). After isolation, the partially purified collagen components were dialyzed against 0.1 M acetic acid, lyophilized, and separated by carboxymethyl (CM)-cellulose chromatography (4). Fractions were pooled, dialyzed against 0.1 M acetic acid, and lyophilized. Aliquots were electrophoresed on 5% sodium dodecyl sulfate (SDS)-acrylamide gels to verify purity and to estimate mol wts (4). Purified collagen $\alpha 1$, $\alpha 2$, $\beta 11$, and $\beta 12$ preparations were hydrolyzed in constant boiling HCl at 110°C and chromatographed on a Beckman 120B amino acid analyzer (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) with a continuous gradient elution system (4).

Collagen synthesis by short-term lung explant cultures. Intact lungs (each 2.8–3.3 g wet wt) of fetuses, 17 wk of gestation, were obtained after curettage and adult lung (1–2 g wet wt) was obtained at surgery. The lungs were rinsed in PBS, sliced (0.5 mm) with a Stadie-Riggs tissue slicer or minced with scissors and rinsed in incubation medium (1 vol of Dulbecco's modified Eagles medium to 1 vol of PBS, 0.5 mM ascorbic acid, 0.6 mM β-aminopropionitrile) previously gassed with 95% O_2-5% CO_2. The β-aminopropionitrile is used to inhibit cross-linking between collagen chains, thus enhancing the ability to solubilize the newly synthesized collagen. Its presence does not affect protein synthesis in lung explant cultures (4). No differences were found in protein synthetic activity of lungs removed from rabbits killed by decapitation or barbiturate or halothane overdose. The slices from each of the lungs were placed in 5 ml of incubation medium and incubated at 37°C and 70 oscillations/min under 95% O_2-5% CO_2 in a Dubnoff shaker. After 45 min, the medium was decanted and replaced with 2 ml of medium containing 0.33 μM (final concentration) [5-^{3}H]proline (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.; 21 Ci/mmol). After 2–4 h of incubation, the tissue was rinsed three times with 10-ml portions of PBS at 4°C and then subsequently homogenized in 25 ml of 0.5 M acetic acid with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, N. Y.; 10,000 rpm, 30 s). The homogenate was subsequently handled as previously described for rabbit lung (4). Collagen chains labeled in vitro were separated on CM-cellulose either without carrier or (on separate columns) with acid-extractable collagen from human fetal lung or rabbit lung as carrier. Fractions (10 ml) were collected, and 1-ml aliquots were counted in 10 ml of Aquasol (New England Nuclear, Boston, Mass.; 30% efficiency). Fractions were pooled as indicated, lyophilized, dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid, lyophilized, and dissolved in H_2O for further analysis on SDS- and acidic acrylamide gels with human fetal lung, rabbit lung, or rabbit skin collagen as carrier. After electrophoresis, gels were stained (Coomassie blue for SDS gels, amido Schwarz for acidic gels), destained by diffusion, and scanned at 570 nm with a Gilford gel scanner (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Alternatively, they were frozen and sliced (1 mm) with a Mickle gel slicer (Brinkmann Instruments, Inc.) or fractionated with Gilson gel fractionator (Gilson Medical Electronics, Inc., Middleton, Wis.) and then counted in Aquasol with 30% efficiency (4).

Sensitivity of the in vitro product to purified bacterial collagenase (Form 3, Advanced Biofactures, Lynbrook, N. Y.) was tested by incubation (20 mM Tris-HCl, pH 7.4, 1 mM $CaCl_2$, 2.5 mM *N*-ethylmaleimide, 10 U of collagenase; 100 μl total volume, 1 h, 37°C) with the enzyme before chromatography (4).

Sensitivity of the in vitro product to trichloroacetic acid at 90°C was tested by exposing [^{3}H]$\alpha 1$ or [^{3}H]$\alpha 2$ chains isolated from the CM-cellulose column to 10% trichloroacetic acid at 90°C for 30 min before electrophoresis on SDS-acrylamide gels (4).

Quantitation of the amounts of collagen and DNA in human lung. Lungs were rinsed in PBS, finely minced with scissors, and homogenized in 0.5 M acetic acid. The homogenate was brought to a known volume with 0.5 M acetic acid, and aliquots were taken for determinations of dry wt, hydroxyproline, and DNA as previously described (4).

Quantitation of the rates of in vitro collagen and noncollagen protein synthesis. Fetal and adult lungs were rinsed in PBS (4°C) and finely minced in the incubation medium described above. For each lung, the lung mince was divided into three (fetal) or four (adult) equal portions which were incubated in 2 ml incubation medium as described above. After 45 min, the medium was removed, and fresh medium with [^{14}C]proline (final concentration 50 μM; Schwarz/Mann, 260 mCi/mmol) was added. Incubation of the aliquots was continued for 1, 2, or 3 h (fetal) or 1, 2, 3, or 4 h (adult). The subsequent handling of each incubation was identical to that described for rabbit lung incubations (4). At each hour of incubation, measurements were made of: dry wt, total hydroxyproline, [^{14}C]hydroxyproline, DNA, [^{14}C]proline incorporated into noncollagen protein, total free [^{14}C]proline in the tissue, and the total free proline in the tissue. The calculations of the rates of collagen synthesis, noncollagen protein synthesis, and total protein synthesis were as previously described (4). The percentage of amino acids incorporated per hour that is incorporated into collagen was calculated as: rate of collagen synthesis per cell × 100/(rate of collagen synthesis per cell + [2.06 × rate of noncollagen protein synthesis per

[1] *Abbreviations used in this paper:* CM, carboxymethyl; PBS, phosphate-buffered saline, pH 7.4; SDS, sodium dodecyl sulfate.

TABLE I

Quantitation of Amounts of Collagen and Rates of Collagen and Noncollagen Protein Synthesis in Human Fetal and Adult Lung

Source of lung	DNA/dry wt	Collagen/dry wt	Rate of collagen synthesis	Rate of noncollagen protein synthesis	Rate of total protein synthesis	Collagen synthesis
	μg/mg	*μg OHpro/mg*	*nmol[^{14}C]OHpro/mg DNA·h*	*nmol[^{14}C]pro/mg DNA·h*	*nmol[^{14}C]pro + [^{14}C]OHpro/mg DNA·h*	%
Fetal (17 wk)						
	36.6±9.3	1.93±1.16	0.708	10.0	21.3	3.32
	32.9±6.0	3.08±1.33	0.709	10.0	21.3	3.33
	39.0±11.0	2.57±0.33	0.756	6.45	14.0	5.40
	37.9±5.9	1.46±0.41	—	—	—	—
Mean	36.6±2.7	2.26±0.71	0.724±0.026	8.8±2.0	18.9±4.2	4.02±1.20
Adult (age, sex)						
R. E. (70, F)	10.2±1.9	30.3±3.2	0.570	4.75	10.4	5.48
T. J. (49, F)	6.8±1.5	22.2±1.9	0.504	6.74	14.4	3.50
B. T. (42, M)	12.8±2.5	26.1±3.9	0.697	8.85	18.9	3.69
G. S. (59, M)	14.6±2.6	25.9±5.7	0.494	5.57	12.0	4.12
Mean	11.1±3.4	26.1±3.3	0.566±0.092	6.48±1.78	13.9±3.7	4.20±0.89

From each lung, at least three determinations were made of DNA/dry wt and hydroxyproline/dry wt. The rate of collagen synthesis/cell and the rate of noncollagen protein synthesis/cell were determined as described in Methods and in the legend to Fig. 5. The rate of total protein synthesis per cell per hour was calculated from the formula: (rate of noncollagen protein synthesis per cell per hour × 2.06) + rate of collagen synthesis per cell per hour. The factor 2.06 corrects the differences between the (proline + hydroxyproline) content of lung collagen compared to the proline content of lung noncollagen protein. The percentage of the total amino acids incorporated into protein per hour that are incorporated into collagen was calculated from the formula: rate of collagen synthesis per cell per hour × 100/rate of total protein synthesis per cell per hour. Error estimates are 1 SD. In all cases, the correlation coefficient of the rate determinations was >0.9600. In the adult lungs, only nontumor areas of lung were used. The pathologic diagnoses of the tumors were: R. E., adenocarcinoma; T. J., adenocarcinoma; B. T., adenocarcinoma; G. S., squamous cell carcinoma.

cell]). The factor 2.06 is the relative difference in the proline content of lung noncollagen protein compared to the hydroxyproline content of lung collagen (4).

RESULTS

As in the fetal rabbit, the lung in the fetal human has relatively large numbers of cells but little collagen per unit lung mass (Table I). In the adult lung, there are 0.17×10^6 cells/μg DNA (10). Dispersal of human fetal lung (15th–18th wk) with trypsin into individual viable cells yields 0.168×10^6 cells/μg DNA (data not shown). Assuming 7.5 μg collagen/μg hydroxyproline (4), the human fetal lung has 6.1×10^6 cells/mg dry wt and 17 μg collagen/mg dry wt. The adult human lung has, on the average, 11 times more collagen per unit lung mass (196 μg collagen/mg dry wt) than the human fetal lung and almost twice as much collagen per unit lung mass as the adult rabbit lung (3, 4). Interestingly, there are 30% as many cells per unit lung mass in the adult human lung (1.8×10^6 cells/mg dry wt) as in the fetal human lung. The adult rabbit lung has one-half as many cells per unit lung mass as does the fetal rabbit lung (4).

Although an average of 19% of the dry wt of the adult human lung is collagen, it was not possible to extract intact collagen chains from the amount of adult lung available, probably because of the covalent cross-links between collagen chains (3, 5). In this manner, adult human lung is similar to adult rabbit lung.

In other tissues, collagen cross-linking is significantly less in the fetus than in the adult (11). This is probably the reason sufficient amounts of intact collagen chains could be extracted from human fetal lung to allow subsequent analysis. Even so, the total extractable collagen was less than 10% of the total collagen present. The yields of the three consecutive extraction procedures (neutral salt, acetic acid, and guanidine, see Methods) are comparable to those from human skin, the distribution being 2, 24, and 74% (by dry wt) of the collagen extracted. Under the conditions used, CM-cellulose separates the material extracted by these proce-

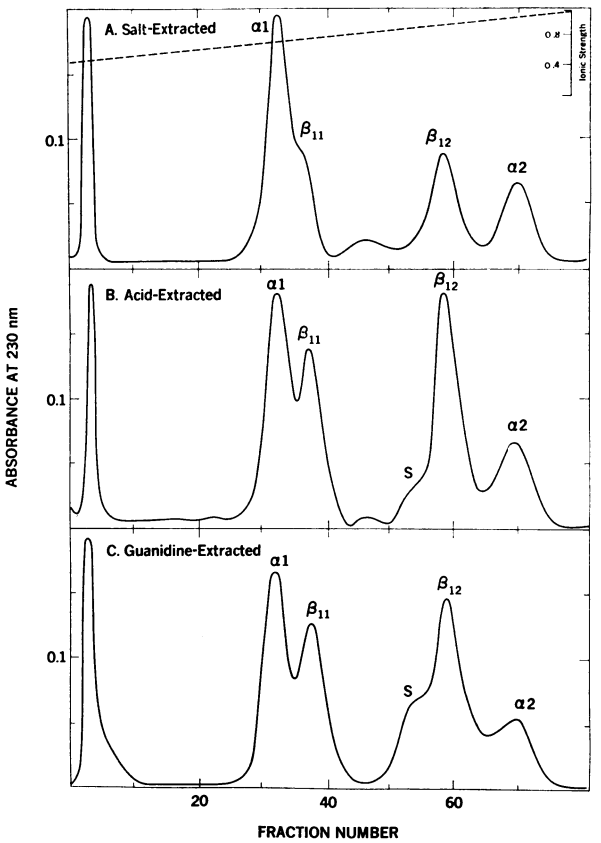

FIGURE 1 CM-cellulose chromatography of collagen extracted from human fetal lung. Collagen was eluted from a 1.5 × 10-cm CM-cellulose column (40°C, 270 ml/h, equilibrated with 0.04 M sodium acetate, pH 4.8) with a 400 × 400-ml linear NaCl gradient (0–0.1 M); 10-ml fractions were collected; columns were monitored at 230 nm (—). (A) Salt-extracted collagen (50 mg). (B) Acid-extracted collagen (50 mg). (C) Guanidine-extracted collagen (50 mg). Before chromatography, each sample was dialyzed against starting buffer, denatured at 50°C for 20 min and clarified.

dures into α and β components (Fig. 1A-C). The extracted α components are single chains of approximate mol wt 100,000 while the β components have a mol wt of 200,000 (Fig. 2A-C). Although the α2 chains electrophorese ahead of α1 chains on SDS-acrylamide gels, accurate determinations of skin α1 and α2 chains by other methods have demonstrated that they have similar mol wts (12).

The amino acid compositions of the α1 and α2 chains (Table II) conform to the values for α1 and α2 chains from most species and organs and are almost identical with acid-extractable collagen from newborn human skin (12). Of interest, however, is the relatively high proportion of hydroxylysine compared to human skin. The hydroxyproline/proline ratios are 0.79 and 0.74, respectively. These values are slightly lower than those from rabbit lung α chains (4). Comparison of the amino acid composition of human fetal lung β11 and α1 chains shows they are almost identical, compatible with the hypothesis that β11 chains are dimers of α1 chains. In a similar fashion, the amino acid composition of β12 chains is similar to the average composition of α1 and α2 chains, compatible with the concept that β12 chains are dimers of α1 and α2 chains.

The α1 chains extracted by 0.5 M acetic acid from rabbit lung are primarily α1(I) chains (Type I collagen) (5). This is probably true in human lung, although in the present study there was not sufficient material to do cyanogen bromide peptide mapping for comparison. Interestingly, the acid-extracted collagen has a small amount of material eluting before the β12 chains (Fig. 1B, labeled "S"). This peak becomes higher with the harsher extraction by guanidine (Fig. 1C, labeled "S"). This is the general region in which α1(III) collagen chains (Type III collagen) elute from CM-cellulose (13). As a possible correlate of this hypothesis, the SDS-acrylamide gel of the salt-extracted collagen has no material electrophoresing in the γ (300,000 mol wt) region, but the acid and guanidine extracted collagens have increasing amounts of γ components (Fig.

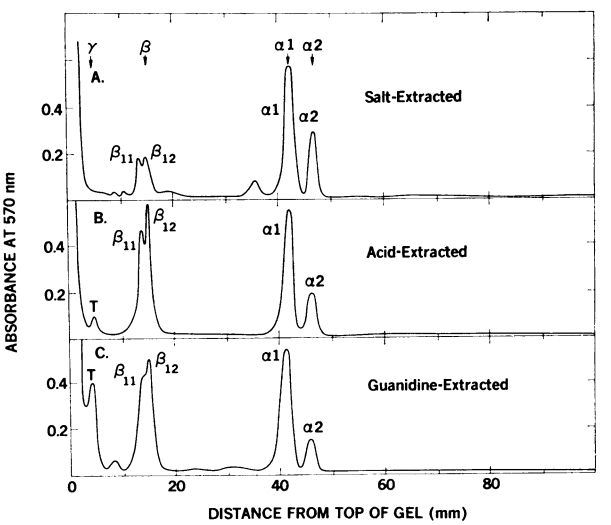

FIGURE 2 SDS-acrylamide gel electrophoresis of extracted human fetal lung collagen. After a 2-h incubation in 1% SDS, 1% β-mercaptoethanol in the gel buffer, samples were electrophoresed on 10-cm, 5% acrylamide gels for 10 h at 2 mA/gel. Stained gels were scanned at 570 nm, 0.5 cm/min with a 0.1 mm slit width. (A) Salt-extracted collagen (50 μg). (B) acid-extracted collagen (50 μg). (C) guanidine-extracted collagen (50 μg). The positions of purified rabbit skin and lung γ (mol wt 300,000), β (mol wt 200,000), and α1 and α2 (mol wt each 100,000) collagen components electrophoresed on parallel gels are shown in (A).

TABLE II

Amino Acid Composition of Human Fetal Lung Collagen

Amino acid	α1	α2	β11	β12	$\frac{\alpha 1 + \alpha 2}{2}$
Hydroxylysine	7.4	12.1	7.7	10.3	9.8
Lysine	30.0	20.2	27.8	24.1	25.1
Histidine	2.1	10.9	2.9	6.5	6.5
Arginine	56.8	52.7	53.2	56.1	54.8
4-Hydroxyproline	93.6	80.3	91.4	87.6	87.0
3-Hydroxyproline	<1	<1	<1	<1	<1
Aspartic acid	42.0	45.5	41.8	43.5	43.7
Threonine	16.5	18.4	16.5	16.9	17.4
Serine	34.8	34.2	34.0	31.8	34.5
Glutamic acid	75.7	70.4	76.1	73.7	73.1
Proline	119	109	118	110	114
Glycine	347	349	351	347	348
Alanine	113	101	112	108	107
Half cystine	0	0	0	0	0
Valine	19.1	33.1	19.1	26.2	26.1
Methionine	6.3	3.9	7.0	6.2	5.1
Isoleucine	6.4	16.2	7.2	11.3	11.3
Leucine	17.8	29.1	18.8	24.4	23.5
Tyrosine	1.6	2.9	1.6	2.5	2.3
Phenylalanine	10.0	9.7	10.3	10.4	9.8

The data are given as residues/1,000 total residues. The serine values were corrected by 4% for hydrolytic losses. There were no other significant differences in the 24-, 48-, and 72-h hydrolysates. All analyses were done on proteins that appeared to be more than 90% pure on SDS-gels. The α1, α2, β11, and β12 chains were purified from acetic acid-extracted collagen by CM-cellulose chromatography (Fig. 1B): α1 chains from fractions 27–31; α2 chains from fractions 67–73; β11 from fractions 37–41; β12 from fractions 57–64). α2 Chains analyzed from salt-extracted collagen (Fig. 1A, fractions 67–73) gave identical results. The similarity and the composition of β11 and α1 and their relative mol wts (Fig. 2) suggest β11 is composed of two α1 chains. The similarity of the average composition of α1 and α2 chains with β12 and their relative mol wts (Fig. 2) suggest β12 is a dimer of α1 and α2.

2B and C, labeled "T"). From what is known of Type III collagen, it would be expected that it would be extracted primarily in a γ form (13).

Explants of human lung incubated in tissue culture medium will synthesize intact collagen chains as demonstrated by the in vitro incorporation of [^{3}H]proline into α chains. Fetal lung explants synthesize α1 and α2 chains that cochromatograph on CM-cellulose with extracted unlabeled fetal lung α1 and α2 chains (Fig. 3A). Although in vitro synthesized human fetal lung α1 chains cochromatograph with in vitro synthesized rabbit newborn lung α1 chains on CM-cellulose, the human lung α2 chains elute after rabbit lung α2 chains (Fig. 3B). The same is true for human and rabbit skin α2 chains (4, 12). The in vitro synthesized human lung α1 and α2 chains coelectrophorese with purified human fetal lung α chains on SDS-acrylamide gels (Fig. 4A, B) and on acid-acrylamide gels (data not shown). These newly synthesized human fetal lung α chains are hydrolyzed by clostridial collagenase (Fig. 3A) and by trichloroacetic acid at 90°C (Fig. 4A and B).

CM-cellulose chromatographs of collagen synthesized by adult human lung explants also have α1 and α2 chains (data not shown). Interestingly, they also synthesize a collagen chain that elutes just before the α2 chains. This in vitro synthesized collagen is sensitive to clostridial collagenase and has a [^{3}H]hydroxyproline to [^{3}H]proline ratio of 0.9–1.1. When this peak from CM-cellulose is subsequently electrophoresed on a SDS-acrylamide gel, it displays multiple peaks, one eluting with α1 (mol wt 100,000), one just before α1 (mol wt 100–115,000), and one between α1 and β (mol wt 130–140,000) (Fig. 4 C). The two larger mol wt species may be precursor forms of α chains as described in rabbit lung (4) and other tissues (14).

The most convenient measure of lung collagen synthesis is the incorporation of [^{14}C]proline into [^{14}C]hydroxyproline. There is also hydroxyproline in elastin in lung, but the absolute amounts of lung elastin hydroxyproline are negligible compared with the absolute amounts of lung collagen hydroxyproline (4). The in vitro incor-

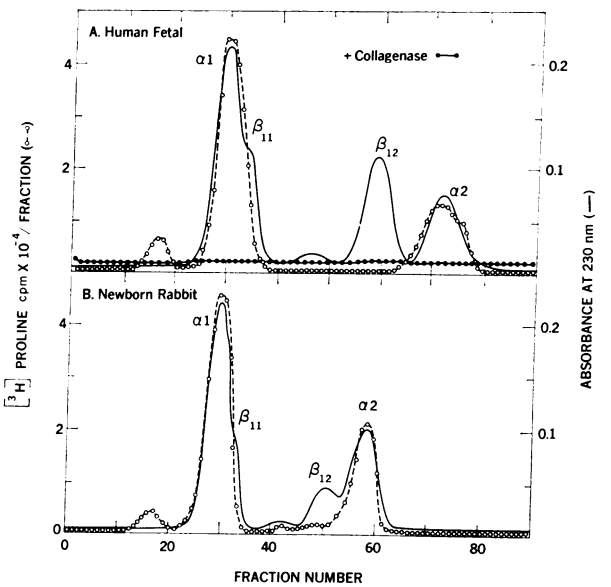

FIGURE 3 CM-cellulose chromatography of collagen chains synthesized in vitro by human fetal lung explants compared with the collagen chains synthesized in vitro by rabbit newborn lung explants. The conditions for the chromatography were identical to those in Fig. 1. (A) [^{3}H]-Proline incorporated into α1 and α2 chains by lung from a 17-wk-old human fetus (○- - -○). The in vitro synthesized α chains were extracted with 0.5 M acetic acid and partially purified with salt precipitation before chromatography. A chromatograph of the same quantity of [^{3}H]proline-labeled fetal lung collagen synthesized in vitro and then incubated with clostridial collagenase before chromatography is also shown (●- - -●). The carrier (—) was salt-extracted human fetal lung collagen. (B) [^{3}H]Proline incorporated into α1 and α2 chains by lung from a newborn rabbit (○- - -○). The conditions used for rabbit lung collagen synthesis were as previously described (4). The carrier (—) was 0.5 M acetic acid-extracted rabbit lung collagen.

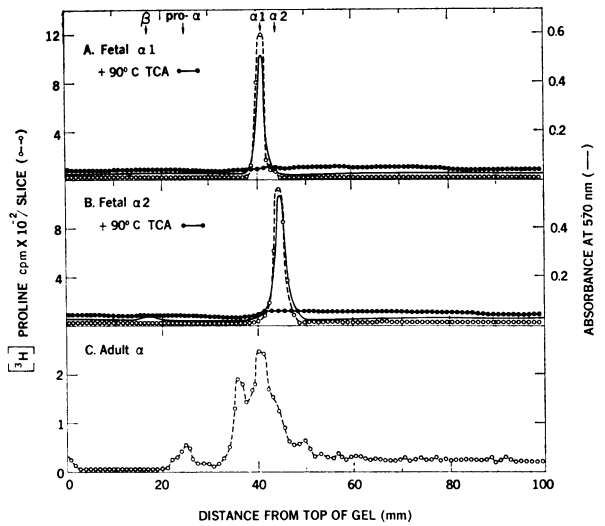

FIGURE 4 SDS-acrylamide gel electrophoresis of human fetal and adult lung collagen synthesized in vitro. Gels were prepared, electrophoresed, and scanned as described for Fig. 2. (A) [^{3}H]Proline α1 chains synthesized by lung from a 17-wk-old fetus. The α1 chains were isolated from fractions 28–35 of Fig. 3A; 5,000 cpm of [^{3}H]α1 chains were applied to the gel (○- - -○). An identical quantity of [^{3}H]α1 chains were exposed to 10% trichloroacetic acid (TCA) at 90°C for 30 min before electrophoresis and run on a parallel gel (●—●). The carrier was purified human fetal lung α1 chains (—). (B) [^{3}H]Proline α2 chains (5,000 cpm) isolated from fractions 66–75 of Fig. 3A and electrophoresed (○- - -○) under identical conditions as (A). [^{3}H]α2 chains (5,000 cpm) incubated 30 min at 90°C in 10% TCA before electrophoresis are completely hydrolyzed (●—●). The carrier was purified human fetal lung α2 chains (—). (C) [^{3}H]Proline α chains (3,000 cpm) isolated from CM-cellulose chromatography of collagen chains synthesized in vitro by lung from a 50-yr-old female (○- - -○). The collagen chains shown here eluted as a single peak before the α2 chains on the column. These chains were degraded by clostridial collagenase and had a [^{3}H]-hydroxyproline/[^{3}H]proline ratio of 0.9–1.1. The positions of purified rabbit lung β, pro-α, α1, and α2 components electrophoresed on parallel gels are shown in (A).

poration of [^{14}C]proline into collagen and noncollagen protein (all proteins other than collagen) in fetal and adult human lung is linear for at least 3–4 h (Fig. 5). These data can be used to calculate the rate of collagen synthesis per cell and the rate of noncollagen protein synthesis per cell (Fig. 5). The rate of total protein synthesis per cell is calculated from the rate of collagen synthesis per cell plus the rate of noncollagen protein synthesis per cell after corrections are made for the relative amounts of proline and hydroxyproline in each (see legend to Table II).

The rate of collagen synthesis per cell, the rate of noncollagen protein synthesis per cell and rate of the total protein synthesis per cell for three 17-wk-old fetal lungs and four adult lungs are listed in Table I. The average absolute rate of collagen synthesis per cell for fetal lung (0.724±0.026 nmol [^{14}C]hydroxyproline/mg DNA·h) is 30% higher than the average absolute rate

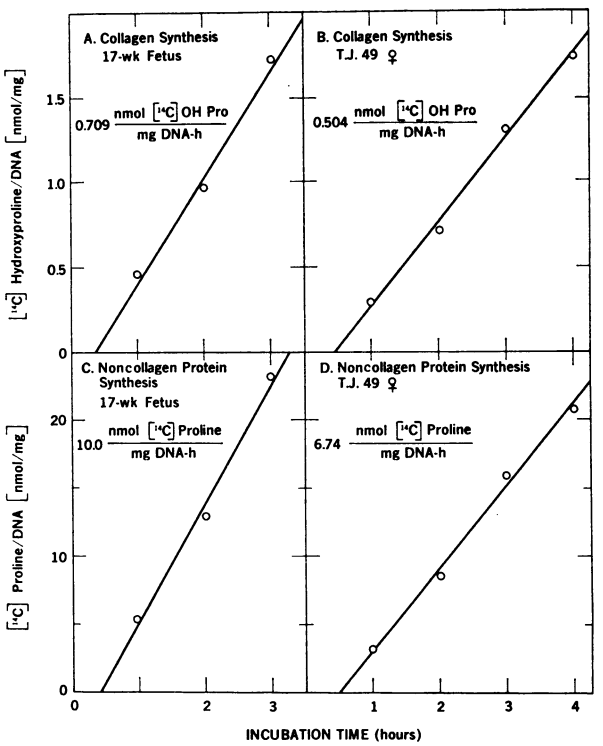

FIGURE 5 The synthesis of collagen and noncollagen protein by explants from fetal and adult human lung. Collagen synthesis was measured by the incorporation of [^{14}C]proline into [^{14}C]hydroxyproline in collagen expressed in relation to DNA content and the specific activity of the precursor isotope in each explant (nanomoles [^{14}C]hydroxyproline per milligram DNA). Noncollagen protein synthesis was measured as the incorporation of [^{14}C]proline into noncollagen protein (nanomoles [^{14}C]proline per milligram DNA) (4). These data were measured in the same explants as collagen synthesis. Collagen and noncollagen protein synthesis was measured at 1, 2, and 3 h in the fetal explants and at 1, 2, 3, and 4 h in the adult lung. Each point represents the mean of two determinations at each time. A least-squares fit regression analysis (Sony ICC-2550 W calculator, program SO310) was applied to the data giving the rate of collagen synthesis per milligram DNA per hour and the rate of noncollagen protein synthesis per milligram DNA per hour. (A) Collagen synthesis in lung explants from a 17-week-old human fetus. (B) Collagen synthesis in lung explants from a 49-yr-old female. (C) Noncollagen protein synthesis in the same explants as (A). (D) Noncollagen protein synthesis in the same explant as (B). Note that in all explants there is a lag of 20–30 min in the incorporation followed by a linear increase in protein synthesis for 3–4 h. The determinations of the rates of synthesis (slope) all had a correlation coefficient > 0.9400 and a coefficient of variation of less than 5%.

of collagen synthesis per cell for adult lung (0.566±0.092 nmol [^{14}C]hydroxyproline/mg DNA·h). Likewise, the average absolute rate of noncollagen protein synthesis per cell for fetal lung is 30% higher than that for adult lung (Table I). However, the percentage of total protein synthesis that is collagen (i.e., the relative number of amino acids incorporated into protein per hour that is incorporated into collagen) is remarkably similar (3.3–5.5%) for the fetal and adult lung (Table II).

DISCUSSION

As the lung matures from a relatively inactive fetal organ to a vital gas exchanging organ, there are significant changes in lung composition. In the rabbit, there is a decrease in the number of cells per unit lung mass and a significant increase in the amount of collagen per unit lung mass (3, 4). The same changes occur in the human, but they are more pronounced. Changes in the relative amount of collagen in other organs have been described but are not as significant as in the lung (11). The changes in the relative amounts of collagen per unit lung mass noted here may contribute to the pulmonary function changes found during development (15).

Although an average of 19% of the dry wt of the adult human lung is collagen, this macromolecule interacts with itself and other lung components to prevent the solubilization of intact collagen chains (16). The same is true in the rabbit, where it was necessary to treat growing animals with β-aminopropionitrile, an inhibitor of covalent cross-linking, so that intact collagen chains could be extracted (4). In other organs, it is known that intact collagen chains can be extracted from younger animals (11, 17). The same is true for human lung, as it is possible to extract 5–10% of the total collagen intact from the 12–17-wk-old fetal lung. The remainder, as in the adult, cannot be extracted intact.

The collagen chains extracted from the rabbit lung have been classified as α1 and α2 chains. It is known that these α1 chains are heterogeneous; the lung probably has four types of collagen α1 chains (3, 4). Because of the limited amount of normal human lung available, we have not had sufficient material to determine the types of collagen present with cyanogen bromide peptide mapping techniques. Hence, the extracted human lung collagen chains described here only represent a partial characterization of human lung collagen. It is probable, however, that the same type of heterogeneity noted in rabbit lung will be found in human lung.

Several studies by Massaro and his colleagues have demonstrated that lung explants will incorporate labeled amino acids into protein that remains within the cell as well as protein that is secreted (18, 19). At least some of this protein is collagen, since the adult and fetal human lung explants actively synthesize this macromolecule as identified by ion-exchange chromatography, mol wt, acid gel electrophoresis, sensitivity to clostridial collagenase, and sensitivity to trichloracetic acid at 90°C. Both adult and fetal human lung incorporate [^{3}H]proline into intact α chains; for fetal lung there are (at least) α1 and α2 chains. The adult lung also synthesizes these chains but, in addition, synthesizes collagen α chain(s) that elute at a different salt concentration on CM-cellulose than α1(I) and α2 chains. As with the extracted collagen chains, the characterization of the in vitro synthesized collagen chains represents only a partial characterization of the collagen present. Further identification of the types of collagen synthesized by explants of human lung will have to await the availability of additional normal human lung.

The rates of lung collagen synthesis can be quantitated in lung explants by measuring the conversion of [^{14}C]-proline to [^{14}C]hydroxyproline and relating this to the number of cells present. In the 17-wk-old human fetal lung, the rate of collagen synthesis per cell and the rate of noncollagen synthesis per cell are greater in absolute terms than similar measurements in adult lung. The percent collagen synthesis, however, is almost identical in both groups. Thus, at least in these age groups, approximately 4% of the total proteins being synthesized are collagen. Similar results have been obtained in the rabbit. In the rabbit, however, this percentage increases during times of rapid lung growth such as occurs in the neonatal period (4). As intact viable normal human lung of the equivalent age range becomes available, we will be able to examine whether this is true in humans as well.

These estimates of the rates of collagen and noncollagen protein synthesis are based on total DNA and hence average over all cells in the lung. It is not yet possible to quantitate the rate of collagen synthesis per specific cell type in the lung.

Since the amount of collagen per unit lung mass remains constant in the adult human, the continuous synthesis of collagen must be matched by a continuous destruction of collagen. In vivo studies by Pierce, Resnick, and Henry have suggested that in rats there is a continuous turnover of collagen in adult lung (20). Preliminary studies in our laboratory have demonstrated that of the collagen synthesized in vitro in these lung explants, 10–40% is destroyed within hours after synthesis. Undoubtedly, the control of the amounts and types of collagen in lung lies in a complex interaction of synthetic and proteolytic mechanisms.

The rate of collagen synthesis and the percentage of total protein synthesis that is collagen can be quantitated by using 100–300 mg (wet wt) of human fetal or adult lung. This is well within the amount of material obtained from lung biopsy for diagnosis in the interstitial

lung disorders. Hence, the technology is now available to answer specific questions about lung disease involving collagen. Studies in quantitation of the rates of collagen synthesis and destruction in the fibrotic lung disorders are currently in progress in our laboratory.

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