Protein synthesis in rat lung

Measurements *in vivo* based on leucyl-tRNA and rapidly turning-over procollagen I

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The relationships of the specific radioactivities of leucine in serum, leucine acylated to tRNA and leucine in procollagen I, procollagen III and total protein in lungs of unanaesthetized young male rats *in vivo* were assessed as a function of time during constant intravenous infusion of radiolabelled leucine. The specific radioactivity of free leucine in plasma reached a steady-state plateau value within 30 min of initiation of [³H]leucine infusion. Leucine acylated to tRNA isolated from lungs had the same specific radioactivity as free serum leucine. Leucine in procollagen I rapidly achieved a specific radioactivity equal to that of serum leucine and leucyl-tRNA, indicating that serum leucine and leucyl-tRNA isolated from total lung were in rapid equilibrium with the precursor leucine pool for procollagen I synthesis. On the basis of leucyl-tRNA or free serum leucine as the precursor, half-times of fractional conversion of procollagen I and III were calculated as 9 and 38 min respectively. The incorporation of leucine into mixed lung proteins calculated from the tracer studies was 6.8 μ mol/day for the first 30 min of the infusion, after which the calculated rate increased to 15.0 μ mol/day. This apparent increase correlated with the appearance of rapidly labelled plasma proteins trapped in the lungs. On the basis of short infusions lasting 30 min or less, followed by vascular perfusion of the lung, the average fractional synthesis rate of mixed pulmonary proteins in young male rats was 20%/day.

The application of radiolabelled-tracer methodology for accurate measurement of rates of protein synthesis in tissues *in vivo* requires knowledge of the specific radioactivity of precursor amino acids for the proteins of interest. Under conditions of constant intravenous infusion of radioactive amino acids in rats (Waterlow *et al.*, 1978), constant specific radioactivities of serum amino acids and cellular amino acid precursors for protein synthesis can be maintained for several hours (Waterlow *et al.*, 1978). When it is found that the measured specific radioactivities of tissue pools of free amino acids or amino acids acylated to tRNA are approximately equivalent to that of serum amino acids, the potential error of calculated rates of protein synthesis is small and the validity of the measurements relies only on the assumption that the measured precursor is homogeneous with respect to labelling and that the correct precursor

has been chosen. This appears reasonable for tissues such as muscle, which are composed predominantly of one cell type and where average synthesis rates of total protein are measured (Waterlow *et al.*, 1978). However, for measurements of the synthesis of specific proteins in tissues composed of multiple cell types, where a small proportion of cells may be responsible for the synthesis of a particular protein, as in lung, this assumption may not be correct, as it is therefore desirable to define the relationship between the measured precursor isolated from the whole tissue and the precursor of the specific proteins being synthesized. The rapid turnover of type I collagen precursors (Robins, 1979) provides an experimental approach for testing whether or not this is a problem in the measurement of collagen synthesis, since an amino acid in procollagen proteins will achieve, in a relatively short period of time, a constant specific radioactivity equal to that of the amino acid in the precursor pool for this protein.

Abbreviation used: SDS, sodium dodecyl sulphate.

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This proved to be the case in the present study for procollagen I in rat lung and permitted comparisons of the specific radioactivity of leucine in this protein with that of serum leucine and leucyl-tRNA isolated from the whole tissue. From this analysis, fractional rates of synthesis of total protein were determined in lungs of growing male rats.

Experimental

Animals

Male Fischer 344 rats weighing 175–225 g were obtained from Taconic Farms (Germantown, NY, U.S.A.). Housing for animals was remote from the central vivarium to avoid respiratory infection. Serological monitoring by the supplier as well as after prolonged housing within our barrier facility indicated that the animals remained free of common respiratory pathogens. Rats were given autoclaved Purina chow and water *ad libitum* and were illuminated on a 12h-light/12h-dark cycle.

Radioisotope infusion

We started all infusions during the morning between 08:00 and 10:00h. Animals had access to food and water during the night before, but not during, the infusion. Venous catheterization was carried out under brief anaesthesia with ketamine (60 mg/kg, intramuscularly) and methohexital sodium (3 mg, intraperitoneally). A Tygon catheter (0.76 mm outside diam., specially prepared by Norton Plastics, Akron, OH, U.S.A.) was inserted into the left jugular vein and advanced into the vena cava. The proximal end was brought out through the skin in the interscapular area, and the animal was allowed to wake up. Rats were weighed, placed in a clear plastic restrainer, and the venous catheter was flushed with approx. 0.4 ml of 0.9% NaCl containing 40 units of sodium heparin before connection to a sterile 10 ml glass syringe containing the labelled infusate.

Infusate contained 2.00 mCi of L-[4,5-³H]leucine (40–60 Ci/mmol; Amersham, Arlington Heights, IL, U.S.A.)/ml and a complete amino acid mixture in sterile 0.9% NaCl at the concentrations described by Morgan *et al.* (1971) as the normal plasma values in rats. The final leucine concentration infused was 0.166 mM. An infusion flow rate of 1.6 ml/h was maintained with an infusion pump (model 341A, Sage Instruments). Venous-blood samples (60 µl) were collected at frequent intervals from the tail vein in heparinized micro-capillary tubes and immediately centrifuged (500 g for 1 min) at room temperature for later determination of free plasma leucine specific radioactivity. Plasma proteins were precipitated with 5 vol of 95% (v/v) ethanol at –20°C, centrifuged in a Beckman Microfuge B at 7500 g for 30 s, and the supernatants

evaporated to dryness in a Speedvac apparatus (Savant Instruments, Hicksville, NY, U.S.A.) for determination of free leucine specific radioactivity. The steady-state (plateau) plasma leucine specific radioactivity was taken as the mean for two to four samples collected after the first 20–30 min of infusion.

Rats were killed by cervical dislocation while the infusion continued. The chest was opened and lungs were rapidly removed and placed in isopentane chilled in liquid N₂. The tissue was stored at –70°C until analysed. Before analyses, frozen tissue was pulverized with a stainless-steel mortar and pestle and cooled to the temperature of liquid N₂. In certain experiments, lungs were briefly perfused with 5 ml of 0.9% NaCl through the right ventricle in order to remove plasma proteins.

Isolation of leucyl-tRNA

Portions (approx. 0.5 g) of frozen tissue were added to 10 ml of 1% SDS/0.05 M-cacodylic acid buffer, pH 5.0, and homogenized with a Polytron (Brinkmann) for 2 min at setting 3. The Polytron probe was rinsed with 5 ml of SDS/cacodylic acid buffer for 15 s, the rinse was added to the original homogenate, and the combined volumes were centrifuged for 5 min at 10000 g. The sample was mixed with an equal volume of re-distilled phenol/SDS/cacodylic acid buffer (4:1, v/v) and stirred for 15 min at room temperature. The mixture was centrifuged for 15 min at 10000 g, and the supernatant was carefully removed and stirred for 10 min in the presence of 0.5 vol. of phenol and centrifuged as above. The resultant supernatant was transferred to a 50 ml capped plastic centrifuge tube, 0.1 vol. of 20% (w/v) potassium acetate buffer, pH 5.0, followed by 2.5 vol. of cold 95% (v/v) ethanol, were added, and the mixture was allowed to precipitate at –20°C overnight.

The precipitate was collected by centrifugation at 4°C for 15 min at 1300 g and washed twice with 95% ethanol. The precipitate was then flushed with N₂ until dry, dissolved at 0°C in 3.5 ml of acetate buffer (0.1 M-sodium acetate/0.01 M-magnesium acetate, pH 4.0) and the solution centrifuged at 100000 g for 1 h at 5°C. The supernatant was removed and tRNA precipitated by addition of 0.1 vol. of 20% potassium acetate, pH 5.0, followed by 2.5 vol. of 95% ethanol, and stored at –20°C overnight.

The tRNA was pelleted by centrifugation for 10 min at 1000 g. The pellet was washed once in 95% ethanol and dried. The tRNA was deacylated by the addition to the pellet of 100 µl of 0.05 M-NaHCO₃, pH 9.0. The tube was capped and incubated at 37°C for 90 min, after which 250 µl of 95% ethanol was added and the sample was left for 1 h at –20°C. The sample was centrifuged for

10 min at 1000g and the supernatant removed for dansylation (5-dimethylaminonaphthalene-1-sulphonylation) of released amino acid.

Amino acid specific radioactivity

The ^3H specific radioactivity of leucine was determined in samples of protein hydrolysates and plasma free amino acids by using [^{14}C]dansyl chloride binding and t.l.c., as previously described (Airhart *et al.*, 1979).

[^3H]Leucine incorporation and protein-synthesis rates

For determinations of protein synthesis, frozen lungs were homogenized in 5 ml of water in a Polytron (setting 4, 45 s), freeze-dried to dryness and weighed. Samples (5–10 mg) were placed in 6 ml of chilled 10% (w/v) trichloroacetic acid to precipitate protein overnight. The pellet was collected by centrifugation at 1000g for 15 min and washed with 3×3 ml of cold 5% trichloroacetic acid and once with 3 ml of 95% ethanol/ether (1:1, v/v), and transferred to a 20 ml glass scintillation vial with ether. The ether was evaporated under a stream of N_2 . Then 2 ml of NCS tissue solubilizer (Amersham, Arlington Heights, IL, U.S.A.) was added to each vial and the mixture was heated to 55–60°C for 1 h. The dissolved sample was cooled and 20 ml of Econofluor was added. The vials were counted for radioactivity in a liquid-scintillation spectrometer.

At least 98% of the total radioactivity in lung protein hydrolysates was in leucine, as determined by paper chromatography of the amino acid products of acid hydrolysis. Therefore total lung protein synthesis was calculated as follows:

$$\text{Synthesis rate } (\mu\text{mol of leucine/day}) = \frac{\text{leucine incorporation (d.p.m./min)} \cdot 1.44 \cdot 10^{-3}}{[S_{\text{max}}/t] \cdot \int_0^t (1 - e^{-kt}) dt} \quad (1)$$

where S_{max} is the plateau specific radioactivity of precursor leucine (d.p.m./pmol), t is the infusion time (min), and the integral in the denominator corrects for the rate of increase of plasma free leucine. For typical infusions lasting 20 min the integral was 0.71; for 60 min it was 0.90. The fractional synthesis rate (FSR) was calculated by dividing the synthesis rate by the mass of peptide-bound leucine in lungs of each rat (263 ± 30 nmol/mg of dried lung tissue; mean $\pm$ s.d., $n = 8$). The total peptide-bound leucine content of rat lungs was quantified by an isotope-dilution method (Airhart *et al.*, 1979).

Isolation of procollagens I and III

Procollagens I and III were isolated and sequentially purified by differential salt precipitation,

adherence to an anion-exchange resin, antibody affinity-column chromatography and polyacrylamide-gel electrophoresis (Byers *et al.*, 1974; Smith *et al.*, 1977). All procedures were performed at 0–4°C. Weighed samples of powdered lung (as little as 100 mg) stored at –70°C were thawed in 10 ml of procollagen-extracting buffer [150 mM-NaCl, 50 mM-Tris/HCl (pH 7.4)/20 mM-EDTA/1 mM-*p*-chloromercuribenzoate/1 mM-phenylmethanesulphonyl fluoride/0.5% Triton X-100]. The tissue was ground in a Waring blender for 1 min and homogenized with a Polytron for 60 s at setting 3. The homogenate was stirred at 4°C for 30 min, filtered through cheese-cloth, and centrifuged for 60 min at 35000g. The precipitate was discarded and lipid was removed by suction. Procollagens were precipitated by slowly adding solid $(\text{NH}_4)_2\text{SO}_4$ to 20% (w/v) and stirring overnight. The precipitate was collected by centrifugation for 1 h at 35000g and dissolved in 2 ml of the original extracting buffer without Triton X-100. NaCl was then added slowly to 20% (w/v) with stirring. The extract was allowed to precipitate for 5 h and then centrifuged at 50000g for 35 min. The precipitate was dissolved in 2 ml of buffer [200 mM-NaCl/50 mM-Tris/HCl (pH 7.4)/1 mM-*p*-chloromercuribenzoate/1 mM-phenylmethanesulphonyl fluoride] and dialysed against the same buffer overnight. To remove non-collagenous acidic macromolecules, this solution was mixed with a slurry of DEAE-cellulose (DE 52, Whatman, Clifton, NJ, U.S.A.) previously equilibrated in the same buffer. The mixture was stirred for 30 min and centrifuged at 7000g for 10 min. The supernatant was removed and pooled with the supernatant from a second wash of the resin. This supernatant was then dialysed for 8 h against distilled water containing proteinase inhibitors and finally in antibody-column buffer [0.05 M-Tris/HCl (pH 7.5)/0.5 M-NaCl] for 12 h.

Procollagen species were further purified by affinity chromatography and gel electrophoresis. Antibodies to procollagen I were prepared in rabbits by a multiple injection schedule. Procollagens I and III could then be separated by applying the sample to an immunoadsorbent affinity column of CNBr-activated Sepharose coupled with dialysed rabbit antiserum to procollagen I. The column was washed sequentially with 20 ml of column buffer [0.05 M-Tris/HCl (pH 7.5)/0.5 M-NaCl] to release procollagen III, its degradation products and other proteins, then with 20 ml of eluting buffer [0.05 M-Tris/HCl (pH 7.5)/3 mM-NaSCN] to release procollagen I and its immediate cleavage products P_N α I, and once again with 20 ml of column buffer. Pooled washes were dialysed overnight against distilled water and freeze-dried.

Type-I and type-III collagen precursors were further purified by separation by SDS/polyacrylamide-gel electrophoresis. Electrophoresis was performed on 1.5mm-thick slab gels of 7.5% acrylamide by the method of Laemmli (1970). The gel was stained with 0.25% Coomassie Brilliant Blue R, 50% (v/v) methanol/10% acetic acid for 90 min and destained overnight in fresh 10% methanol/7.5% acetic acid. The bands containing procollagen I and procollagen III were identified (Robins, 1979), cut from the gel, and washed briefly in three changes of distilled water for 20 min each. Proteins were extracted overnight with 1 ml of 70% (v/v) formic acid with gentle shaking at room temperature. The procollagen-I and -III samples were dried in the SpeedVac, and hydrolysed overnight at 110°C in 6M-HCl (redistilled). The hydrolysates were dried, washed twice with distilled water, dried again, dissolved in 20 µl of 0.1 M-NaHCO₃/Na₂CO₃ buffer (pH9) and made to react with [¹⁴C]dansyl chloride to determine the specific radioactivity of leucine (Airhart *et al.*, 1979).

Results

A typical curve for the rising specific radioactivity of free [³H]leucine in plasma is shown in Fig. 1. This curve can be described by

$$S_p = S_{\max} \cdot (1 - e^{-k_p t})$$

Fig. 1. Specific radioactivity of free leucine in plasma during continuous intravenous infusion of [³H]leucine. Multiple timed tail-vein blood samples were collected during [³H]leucine infusions in 16 rats. Data are plotted as S_p (specific radioactivity at time t) divided by $S_{\max}$ (plateau specific radioactivity achieved) versus time. The stippled area shows the rate of rise of plasma free leucine based on the mean half-time $\pm$ 1 s.d. in 16 rats. The eight closed circles represent the plasma free leucine in a single typical animal. The continuous line shows the best fit for the eight data points in the same animal. Plateau specific radioactivity was always achieved by 25 min.

where S_p represents the instantaneous free leucine specific radioactivity in plasma at time t , $S_{\max}$ is the plasma plateau value and k_p is the time constant. The half-time ($t_{1/2}$) for the rise in specific radioactivity of free plasma leucine was 4.0 ± 1.3 min (mean $\pm$ s.d., $n = 18$), with a corresponding mean time constant k_p of 0.172 min^{-1} . Thus a constant plateau plasma specific radioactivity was achieved within 15–25 min in all animals. After reaching a plateau value, the free leucine in plasma remained constant for as long as 2.5 h.

Fig. 2 shows the pattern of collagen precursors isolated from lung and separated on a SDS/7.5% polyacrylamide gel. Type III collagen precursors were not retained on the type I collagen affinity column and appear in the initial wash (track *a*). Type I collagen precursors were released from the column during elution with NaSCN (track *c*). Procollagens I and III were identified, cut from the gel, and eluted for determination of specific radioactivity of leucine.

To assess the relationship between leucine free in plasma and intracellular pools involved in protein synthesis, we examined leucine specific radioactivity in procollagens and tRNA. The rise in specific radioactivity of peptidyl-leucine of purified procollagen I was rapid (Fig. 3) and achieved a value equivalent to the plasma plateau value during infusion studies lasting 45 min or longer. Procollagen III turned over at a much slower rate and therefore its specific radioactivity failed to equilibrate with that of free leucine in plasma within 90 min.

The relationship between the specific radioactivity of lung tRNA and the plateau value for plasma free leucine was determined in rats infused for 40 min or longer (to allow accurate assessment of the plasma plateau). The specific radioactivity of leucine associated with tRNA of lung was 0.94 ± 0.06 (mean $\pm$ s.d., $n = 6$) of the free plasma leucine value. This agreement was achieved only when lungs were removed and immersed in liquid N₂ within 1 min of discontinuing the infusion. Delays of 1–2 min resulted in falls in specific radioactivity of leucyl-tRNA, to less than 40% of the plasma plateau value.

The agreement between specific-radioactivity determinations of leucyl-tRNA and procollagen I for infusions lasting 40 min or longer (Fig. 4) was extremely close ($r^2 = 0.91$, $P < 0.01$). We interpret such close agreement between these two biochemical species isolated by divergent analytical procedures to validate our measurements of leucyl-tRNA in freshly isolated lung tissue.

Whole-lung protein-synthesis rates were determined on trichloroacetic acid precipitates of weighed freeze-dried samples of lung tissue. Total

Fig. 2. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of collagen precursors

The Figure shows the patterns of collagen precursors isolated from lung and separated on a 7.5%-polyacrylamide slab gel. Individual fractions isolated by adherence to a procollagen I affinity column are shown. Track (a) shows type III collagen precursors which are not retained on the column. Track (b) demonstrates the absence of proteinaceous material after extensive washing of the column with neutral buffer. Track (c) contains the type I collagen precursor fraction, which is eluted from the column with NaSCN. Collagen precursors were identified with reference to previously published electrophoretic patterns (Robins, 1979). The locations of $\alpha 1(I)$, $\alpha 2(I)$, β dimers and bovine serum albumin (BSA) on the same gel are indicated. Pro, procollagen; $P\alpha 1(III)$, procollagen III intermediates; $P\alpha 1(I)m$ type-I-collagen precursor containing C-terminal extension peptides.

$[^3H]$ leucine incorporation calculated as d.p.m./lung was divided by the specific radioactivity of the precursor to yield a mass-based protein-synthesis rate. The precursor specific radioactivity used in this calculation was a time-weighted plasma value based on eqn. (1) (see the Experimental section) to reflect the mean precursor

Fig. 3. Time course of specific radioactivity of leucine in lung procollagens I and III

Data are plotted as specific radioactivity of procollagen divided by specific radioactivity of free leucine in plasma. Triangles represent specific radioactivity of leucine in procollagens (Pro) I (Δ) and III ($\blacktriangle$) in lungs of individual rats killed at different times during intravenous $[^3H]$ leucine infusions. The increase for procollagen I is rapid (half-time 9 min), whereas procollagen III equilibrates with the specific radioactivity of plasma free leucine only after 90 min (half-time 38 min).

Fig. 4. Relationship between leucyl-tRNA and procollagen I

The specific radioactivity of leucyl-tRNA is compared with the leucine specific radioactivity of purified procollagen I for infusions lasting 30 min or longer. Because soluble procollagen I has a small intracellular pool in lung, its leucine specific radioactivity quickly equilibrates with its precursors and validates the tRNA measurements. Each point represents data from a single animal; continuous line indicates linear regression [$S(tRNA) = 1.08 \cdot S(proI) - 2.27$; $r^2 = 0.91$, $P < 0.01$] where S represents specific radioactivity of leucine.

specific radioactivity through the duration of the infusion.

The lung protein-synthesis rate determined for infusions lasting 30 min or less was $6.8 \mu\text{mol}$ of leucine/day. When infusions lasting 30 min or longer are considered, leucine incorporation into lung proceeded at $15.0 \mu\text{mol}$ of leucine/day. However, this sudden increase in the protein-synthesis rate occurred at the same time as [^3H]peptidyl-leucine began to appear in serum (results not shown), suggesting that tritiated serum proteins trapped in the pulmonary vasculature and interstitium were falsely elevating the lung protein-synthesis rate. To calculate the fractional synthesis rate (FSR) of lung proteins, we divided the protein-synthesis rate (μmol of leucine incorporated/day) by the total peptidyl-leucine content of the lung. The FSR of total lung protein when long infusions were followed by vascular perfusion with 0.9% NaCl was $0.27 \pm 0.03 \text{ day}^{-1}$ (mean $\pm$ s.d.; $n = 6$). The FSR for short infusion followed by vascular perfusion was $0.20 \pm 0.01 \text{ day}^{-1}$ ($n = 7$).

Discussion

Although the technique of constant intravenous infusion has been applied successfully in the analysis of protein turnover in various tissues (Waterlow *et al.*, 1978), we are not aware of its extensive application in studying lung protein metabolism. This technique allows the study of intact awake animals and human subjects and thus differs from isolated lung perfusion systems (Watkins & Rannels, 1980). The infusion of relatively large amounts of [^3H]leucine used in the present study ensures amounts of radioactivity that can be easily measured in all compartments by the micro-methods that we have previously developed (Airhart *et al.*, 1979).

Our study demonstrates that lung pairs from individual rats infused intravenously with [^3H]leucine provide adequate amounts of tissue for analysis of tRNA, procollagens I and III, as well as [^3H]leucine incorporation into total protein. Clearly, the most limiting analysis in this study lies in the determination of leucyl-tRNA: approximately half of the total lung tissue available from individual animals is required to yield 50–70 pmol of leucine, or approx. 1500 d.p.m. under the conditions of labelling. In contrast, only 10–20% of the lung tissue is needed to yield adequate amounts of procollagens I and III for analysis of specific radioactivity, and less than 1% of the tissue yielded adequate counts for determination of total radio-tracer incorporation.

We chose leucine for these studies because it is an essential amino acid in the rat, gives a steep rise to plateau in plasma (Fig. 1), and has been shown to equilibrate with the tissue free pool and tRNA

in other tissues without the need to increase the concentration of leucine in plasma and tissue above normal values (Everett *et al.*, 1981). Although the leucine content of procollagen is low, we had no difficulty measuring the specific radioactivity of leucine in purified procollagens I and III from as little as 100 mg of wet lung tissue.

The kinetics of the rise of specific radioactivity of free leucine in plasma were adequately rapid to provide a constant plateau value within 15–25 min (Fig. 1), somewhat more rapid than reported by others (Everett *et al.*, 1981). The close agreement between the specific radioactivities of leucine free in plasma and associated with tRNA of lung indicates that these two compartments are in equilibrium. The correctness of the tRNA determinations is further corroborated by measurements of specific radioactivity of rapidly turning-over procollagen I of lung, which almost exactly parallel the tRNA measurements (Fig. 4).

Amino acids of extremely rapidly turning-over proteins such as procollagen I achieve specific radioactivities comparable with the plateau values for free amino acid in plasma (Robins, 1979). If the protein product can be observed to reach the same specific radioactivity as the extracellular precursor, then both of these values must be equal to the specific radioactivity of leucyl-tRNA. The finding that lung leucyl-tRNA and free leucine of plasma reached equivalent specific radioactivities suggests that the majority of the cells in lung derive their leucine for protein synthesis exclusively from the extracellular fluid or from a compartment which rapidly equilibrates with it. This observation contrasts with several other experimental observations in cell-culture systems and whole animals, where certain amino acids used for protein synthesis may be derived primarily from protein breakdown, from sequestered intracellular pools, or from newly synthesized amino acids (Hildebran *et al.*, 1981).

The half-time for procollagen I turnover was calculated by the method suggested by Robins (1979), based on the formula:

$$S_1 = S_p \cdot (1 + 0.05e^{-k_p t} - 1.05e^{-k_1 t})$$

where S_1 is the specific activity of procollagen I, S_p is the free plasma leucine specific radioactivity, and k_p and k_1 are the time constants ($\ln 0.5/t_1$) for the rise in specific radioactivity associated with leucine in plasma and procollagen I respectively. From studies in ten animals, k_1 was calculated to be 0.077 min^{-1} , with a corresponding half-time of 9 min. This half-time of procollagen I conversion into smaller collagen precursor forms is considerably more rapid than has been calculated in skin by using somewhat different approaches to radio-labelling techniques. From 2 h infusions of labelled proline, Robins (1979) calculated a half-time of

26 min in young rabbits, but pointed out that the actual time required for procollagen I conversion might be shorter than his estimate. Our estimate for the half-time of procollagen I conversion (9 min) is in close agreement with the known time for procollagen-chain elongation of 6 min as determined in cultured calvarium (Vuust & Piez, 1972) and estimates of secretion time of 10–20 min (Kao *et al.*, 1977). If chain elongation and secretion times are comparable in the rat lung, then procollagen I cleavage must occur close to the time of secretion.

The observation that procollagen III turnover is slower than that of procollagen I parallels similar observations in skin of growing rabbits (Robins, 1979). A comparably slower procollagen III turnover also occurs in lung in the rat (half-time 38 min). This observation suggests that the pool of procollagen III is large relative to its synthesis rate, with a proportionately decreased fractional conversion rate by procollagen peptidase (Robins, 1979). One might hypothesize alternatively that most of the procollagen III is produced in a small subpopulation of cells in which leucyl-tRNA is slower to equilibrate with plasma leucine. The exact cell population in lung responsible for type-III collagen synthesis has yet to be anatomically defined and isolated. Quantitative morphometric analyses have indicated that fibroblasts make up 52% of the interstitial cells of the rat lung (Crapo *et al.*, 1980). These cells are capable of simultaneous synthesis of both types I and III collagen in culture as well as *in vivo* (Gay *et al.*, 1976).

We found that measurements of protein turnover in lung by using prolonged exposure to radio-labelled precursor amino acids present some special problems not encountered in similar studies in other tissues. Both the high ratio of vascular volume to tissue in the lung and the rapid flux of vascular proteins (Wangenstein *et al.*, 1983) through the interstitial compartment and lymphatics confound measurements of the true lung protein-synthesis rate. Several experimental observations are consistent with the suggestion that rapidly labelled serum proteins, notably albumin, confound measurements of protein turnover in the lung. In this regard we noted that, when constant intravenous infusions are allowed to proceed for longer than 25–30 min, ^3H -labelled albumin and other proteins begin to appear in plasma and can be rapidly detected in crude lung homogenates (results not shown). Because intravascular albumin turnover exceeds 75%/day (Jeejeebhoy *et al.*, 1972), this source of exogenous labelled protein quickly begins to raise the apparent lung protein-synthesis rate.

Nevertheless, even when only brief infusions are considered, it is apparent that protein turnover as reflected by the fractional synthesis rate is far in

excess of growth requirements of the tissue. The protein content of lungs of 200 g animals, for instance, increases at 0.8%/day (results not shown). Thus most of the protein synthesis in the lungs of young male rats is directed toward turnover rather than tissue growth.

The FSR of lung protein calculated in these studies (0.20 day^{-1}) agrees with values determined in perfused isolated lungs. Watkins *et al.* (1983) found a FSR of 0.20 day^{-1} by perfusing [^3H]phenylalanine in rat lungs *in situ*. However, other measurements of lung protein-synthesis rates made in non-aesthetized small animals have indicated higher values, in excess of 0.3 day^{-1} (Garlick *et al.*, 1980; Laurent, 1982).

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