Postnatal Development of Rat Lung

CHANGES IN LUNG LECTIN, ELASTIN, ACETYLCHOLINESTERASE AND OTHER ENZYMES

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The development of rat lung from a primitive gas-exchange organ to the mature respiratory organ is in large part a postnatal phenomenon that has been well characterized by morphological and morphometric methods. The alveolarization of the lung is achieved during the first 3 weeks of life. Cholinergic innervation of rat lung also appears postnatally. We have monitored the presence or activity of several proteins during postnatal rat lung development. Newborn-rat lung contains negligible amounts of acetylcholinesterase, but the specific activity of acetylcholinesterase reaches adult values by postnatal day 10–11. Neonatal-rat lung does not contain significant amounts of β -galactoside-binding protein [Powell (1980) *Biochem. J.* 187, 123–129]. The activity of this endogenous lung lectin was apparent at about day 6, was maximal between days 10 and 13 before declining 8–10-fold to reach adult values. Elastin has been implicated from morphological evidence as critical to lung restructuring. We have quantified the amount of desmosine and isodesmosine per g wet wt. of lung. The concentration of elastin, by this criterion, was low and stationary until postnatal day 7; a dramatic increase in elastin concentration occurred between days 10 and 20, when adult values were reached. The peak of lung-lectin activity was coincident with the maturation of acetylcholinesterase and the beginning of rapid elastin cross-linking. The specific activities of angiotensin-converting enzyme, carbonic anhydrase, choline kinase and glucose 6-phosphate dehydrogenase were also monitored.

One of the most important criteria of lung maturity and consequent viability of the neonatal animal is the appearance of lung surfactant; the newborn lung must be ready to take over from the placenta the responsibility for gas exchange. Even though birth is such a critical event for the lung, considerable maturation of the lung occurs postnatally in humans (Boyden & Tompsett, 1965), dogs (Boyden & Tompsett, 1961), rats (Burri *et al.*, 1974) and probably most mammals. The postnatal anatomical development of the rat lung has received extensive study (Burri, 1974; Burri *et al.*, 1974; Hodson, 1977).

The newborn-rat lung has no alveoli; the rat breathes with smooth-walled air ducts and thick shallow saccules. Few morphological changes occur during the first 4 days of life. Between days 4 and 7, a sudden proliferation of thick-walled secondary alveolar septa occurs; these secondary septa appear

to contain the same double-capillary system as do the primary septa. This increase in surface area is continuous until day 13. Meanwhile, respiratory bronchioles are being fashioned from the purely conducting airways of the neonatal-rat lung; respiratory bronchioles can be found after day 10. Between days 13 and 21 the secondary septa elongate and become more slender as their double-capillary system is transformed into a single-capillary network. These latter changes are accompanied by an increase in lung volume, but a decrease in the volume density of the total tissue, including the interstitium. This terminates the dramatic phase of postnatal rat lung development.

We had become interested in a β -galactoside-binding protein, or lectin, endogenous to the lung (Powell, 1979, 1980), which is closely similar in its properties to the lectins in embryonic-chick tissues. The embryonic-chick lectins show striking changes of activity with development; the lectin activity of embryonic-chick muscle, liver and brain is low early

Abbreviation used: SDS, sodium dodecyl sulphate.

in development and rises 10–100-fold to maximum values at about 12 days of incubation and falls to about 10-fold within 1 week of hatching (Kobiler & Barondes, 1977). The activity of these and similar lectins has been correlated with synaptogenesis (Teichberg, 1978; Gremo *et al.*, 1978). Does the rat lung lectin show a similar developmental regulation? We had perhaps expected that the lectin activity in newborn-rat lung would be much higher than that of a juvenile or adult rat. Newborn-rat lung contained negligible lectin activity. Lectin activity was maximal between postnatal days 10 and 13 before declining slowly to adult values (Powell, 1979). Further, recent histochemical evidence suggests that the neonatal-rat lung has no cholinergic innervation, but acetylcholinesterase staining becomes apparent between postnatal days 5 and 9 (El-Bermani & Bloomquist, 1978). There is a paucity of biochemical data concerning the time period of postnatal lung development or alveolarization. Previous biochemical studies have emphasized the transition from foetus to neonatal animal. We have investigated the activities of lung lectin and acetylcholinesterase during postnatal lung development. We have also monitored the amounts of elastin during this period of alveolarization, since morphological studies have closely linked the appearance of elastin with the process of alveolar septation (Loosli & Potter, 1959; Burri & Weibel, 1977; Vaccaro & Brody, 1978). The activities of some other enzymes that might be used as index of rapid lung growth occurring during the first 21 days of rat life provide a background for this information.

Materials and Methods

Materials

Acetylthiocholine iodide, 5,5'-dithiobis-(2-nitrobenzoic acid), eserine sulphate, glucose 6-phosphate, NADPH, ATP, choline, hippuric acid, hippurylhistidyl-leucine, lactose, and thiodigalactoside [β -D-galactopyranosyl β -(1 $\rightarrow$ 4)-thiogalactopyranoside] were purchased from Sigma, St. Louis, MO, U.S.A. Bolton–Hunter reagent (^{125}I) and [^{14}C]methylcholine were obtained from New England Nuclear, North Billerica, MA, U.S.A. 2,4,6-Trichloro-*s*-triazine was purchased from Aldrich, Milwaukee, WI, U.S.A. Outdated human plasma was obtained from the John Elliot Blood Bank, Miami, FL, U.S.A. and Whatman GF/C filters from a local supplier. Other reagents were of the best commercially available grade. Elastin (from bovine ligament) was a gift from Dr. Noble of this University. Sprague–Dawley rats were bred by Dr. L. Frank in the Pulmonary Division.

Protein and enzyme assays

Protein was monitored by the dye-binding method

(Bradford, 1976). Lectin activity was determined by both haemagglutination and asialo-orosomucoid-fragment binding, as described by Powell (1980). Acetylcholinesterase (EC 3.1.1.7) was assayed at 30°C by the method of Ellman *et al.* (1961); eserine sulphate (100 μM) was used to inhibit acetylcholinesterase and monitor the non-specific esterase activity. Angiotensin-converting enzyme (EC 3.4.15.1) was assayed with the substrate hippuryl-histidyl-leucine (5 mM) (Hayakari *et al.*, 1978). Choline kinase (EC 2.7.1.32) was determined by the method of Weinhold *et al.* (1973). Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) was assayed at 30°C as previously described (Levy *et al.*, 1966). Carbonic anhydrase (EC 4.2.1.1) was assayed essentially as previously described (McKinley & Whitney, 1976). The rate of reaction, monitored as H^+ released/s, was determined at 5°C on addition of CO_2 -saturated 67 mM- Na_2SO_4 (1 ml) to the sample in 25 mM-triethanolamine sulphate containing 59 mM- Na_2SO_4 , pH 8.1 (4 ml). Soluble carbonic anhydrase was inactivated by incubation in the above buffer containing 5% sodium dodecyl sulphate (1 min, 37°C) to permit the assessment of membrane-bound carbonic anhydrase, which retained more than 90% of its activity under these conditions.

The elastin of lung was measured by determining the desmosine + isodesmosine content, essentially as described by Starcher (1977). The hydrolysed samples (in 6 M-HCl, 1% phenol, 110°C, 72 h *in vacuo*) were filtered, dried and then dissolved in 0.2 M-sodium citrate, pH 2.2. Analysis was performed on a JEOL 5 AH amino acid analyser by using a column (0.8 cm $\times$ 45 cm) of JEOL resin (10% cross-linking, $12 \pm 2 \mu\text{m}$ particle size). Four buffers were used to elute the amino acids; the temperature was 32°C for the first 0.5 h and 54°C for the remaining 6.5 h. The first buffer was 0.2 M-sodium citrate, pH 3.3, containing 1.5% (v/v) methanol to improve the resolution of serine and threonine. The second buffer (0.2 M-sodium citrate, pH 4.25) emerged after valine and continued into the phenylalanine peak. The third buffer was 0.51 M-sodium citrate, pH 6.1, and the fourth was 0.6 M-sodium citrate containing 0.75 M-NaCl, which emerged after NH_3 . Analysis of pure elastin showed that isodesmosine and desmosine eluted early in the third buffer. The high ionic strength required to separate desmosine and isodesmosine from contaminants, present in whole lung extracts, resulted in the desmosine being poorly resolved from the isodesmosine. Therefore isodesmosine and desmosine were quantified by manual integration of their absorbance.

Experimental procedures

Rats were given an intraperitoneal injection of

sodium pentobarbitol (30mg/kg) and killed by exsanguination from the abdominal aorta. The lungs were removed, trimmed, weighed and frozen in liquid N_2 . Frozen tissue was disrupted with a Polytron (Brinkman Instruments, Westbury, NY, U.S.A.) homogenizer with a ratio of 6ml of homogenizing medium to 1g of lung. The homogenizing medium was 0.25M-sucrose containing 10mM-lactose, 20mM-sodium phosphate, pH 7.5, and 10mM-2-mercaptoethanol. The fractionation scheme is outlined in Fig. 1. The homogenate was centrifuged at 3000g for 10min at 4°C and the precipitate was used for elastin determination (Starcher, 1977). The supernatant solution was then centrifuged at 100000g for 1h at 4°C. The residual supernatant solution was divided into two parts; after dialysis at 4°C against 0.14M-NaCl containing 20mM-sodium phosphate and 10mM-2-mercaptoethanol, one was used to determine lectin and choline kinase activity, the other half was dialysed against 0.14M-NaCl containing 20mM-sodium phosphate, pH 7.5, at 4°C, and angiotensin-converting enzyme, acetylcholinesterase and glucose 6-phosphate dehydrogenase activities were determined. (Lactose must be removed to determine lectin, and 2-mercaptoethanol must be removed to measure acetylcholinesterase and angiotensin-converting enzyme activities.) The activities of these latter two enzymes in this fraction provide an estimate of serum contamination of the preparation. The dialysis of many samples simultaneously was accomplished by using a disc-gel-electrophoresis apparatus to hold 18 samples (identified by number position), with buffer (3 litres) in the reservoir. The precipitate from the 100000g cen-

trifugation was dispersed by sonication (50W; 10s) in 5ml of potassium phosphate (0.02M, pH 8.3), and the washed pellet obtained by centrifugation at 100000g for 1h at 4°C. This pellet was again dispersed in 0.02M-potassium phosphate, pH 8.3 (2ml) and a portion removed to determine acetylcholinesterase activity and protein. Sodium deoxycholate was added to the remainder to a final concentration of 0.3%, and angiotensin-converting-enzyme activity was determined. After the determination of angiotensin-converting-enzyme activity, the remaining dispersion was centrifuged at 100000g for 1h at 4°C, the pellet taken up in 1% SDS in 25mM-triethanolamine sulphate, pH 8.1, containing 59mM-sodium sulphate for the determination of carbonic anhydrase activity. Rats were sampled at postnatal days 0, 2, 4–5, 6–7, 10, 12–13, 17 and 20–21. Each age group comprised 2–5 samples. The early-age-point samples contained pooled lungs from rats of the same litter to achieve the minimum weight, 0.3 g.

Results

The newborn-rat lung has a wet weight of about 0.1 g; this rises to 0.3 g at day 7; the lung wet weight in a 21-day-old rat is about 0.5 g, and that of an adult is 1–1.1 g. The weight gain is not even. During the first 10 days the increase in lung volume is proportional to body weight, after this time it is a function of (body weight)^{0.7} (Burri *et al.*, 1974). Therefore all results are presented as specific activities/mg of protein rather than as total activity. We used adult animals (body wt. 150–200 g).

Fig. 1. Lung-fractionation scheme

Abbreviations used: AChE, acetylcholinesterase; ACE, angiotensin-converting enzyme; G6-PDH, glucose 6-phosphate dehydrogenase.

Both soluble cytoplasmic enzymes (glucose 6-phosphate dehydrogenase and choline kinase) were recovered in good yield (65–80%) in the 100 000g supernatant fraction. The specific activity of choline kinase was not significantly lower in the adult lung (see Fig. 2).

Three enzymes were monitored in the 100 000g pellet. The yields of carbonic anhydrase varied widely, but the membrane-bound (or SDS-insensitive) carbonic anhydrase always had its highest specific activity in this fraction. This accounts for the wide scatter observed in Fig. 3; although carbonic anhydrase specific activity increased slowly between days 7 and 17, the most rapid increase appeared to be later. The most useful aspect of this assay was that it permitted an assessment of contamination of the 100 000g pellet by erythrocyte proteins. Erythrocyte carbonic anhydrase was inactivated by SDS (McKinley & Whitney, 1976). In each case >90% of the carbonic anhydrase activity of the 100 000g pellet was SDS-insensitive.

The specific activity of angiotensin-converting enzyme increased progressively during the first 3 postnatal weeks (Fig. 3). The specific activity observed at days 20–21 was only half that observed in adult rats (0.4–0.45 $\mu\text{mol}/\text{min}$ per mg). The recovery of angiotensin-converting enzyme in the 100 000g pellet varied from 45 to 60%. Some 2–8% of the total converting-enzyme activity was found in the 100 000g supernatant fraction and probably derives from serum enzyme. The remainder of the activity was found in the 3000g pellet.

The acetylcholinesterase specific activity of the

100 000g pellet was 15–25-fold that of the 100 000g supernatant fraction; 45–55% of the total acetylcholinesterase activity was recovered in the 100 000g pellet. Acetylcholinesterase activity in the neonatal lung was very low, but the specific activity had increased to adult values by days 10–11 (Fig. 4).

Lung lectin activity was assayed both by haemagglutination (inhibitable by 0.5 mM-thiodigalactoside or 2.5 mM-lactose) and by the binding assay (binding of the ^{125}I -labelled glycopeptide fragment of asialo-orosomucoid). These results are shown in Fig. 5, where minimal activity was observed before day 7 and the peak of activity was between days 10 and 13. The ratio of the two assay activities varies slightly, but in large part reflects the ratio observed for purified rat lung lectin: specific activity 480–600 haemagglutination units/mg, or 1 mg of rat lung lectin binds 0.8 mg of ^{125}I -asialo-orosomucoid glycopeptide. The efficiency of lectin extraction varied little from day 7 to day 13 and thence to adult lungs. Further, lectin purified from 13-day-old-rat lungs had the same specific activity as the lectin purified from adult rat lungs. This would suggest that the concentration of lung lectin increases 8–10-fold between days 10 and 13 when compared with the adult.

The elastin content of lung was determined by

Fig. 2. Specific activities of glucose 6-phosphate dehydrogenase (O) and choline kinase (●) in the developing rat lung

The specific activities of glucose 6-phosphate dehydrogenase and choline kinase were measured in the 100 000g-supernatant fraction. Values are means, with the range of results shown by the vertical bars. The numbers in parentheses indicate the sample size.

Fig. 3. Specific activities of angiotensin-converting enzyme (O) and carbonic anhydrase (●) in the developing rat lung

The specific activities of angiotensin-converting enzyme and carbonic anhydrase were determined on the washed 100 000g pellet. Values are means, with the range of results shown by the vertical bars. The numbers in parentheses indicate the sample size. The specific activity of angiotensin-converting enzyme in the same fraction of an adult rat lung was 0.43 ± 0.3 nmol/min per mg of protein (five samples).

Fig. 4. Specific activity of acetylcholinesterase in the developing rat lung

The specific activity of acetylcholinesterase per mg of protein was measured in the 100 000 g pellet. The pellet was washed to remove 2-mercaptoethanol and all these fractions were low in non-specific esterase activity (as determined in the presence of 100 μ M-serine sulphate). Values are means, with the range of results shown by the vertical bars. The numbers in parentheses indicate the sample size.

Fig. 5. Specific activity of β -galactoside-binding protein (lectin) in the developing rat lung

The specific activity of lung lectin was measured in the 100 000 g supernatant fraction after dialysis to remove the lactase present in the homogenization buffer. The units of the binding assay (O) were μ g of 125 I-glycopeptide of asialo-orosomucoid bound per mg of protein. One unit of lectin activity causes agglutination at 10^3 -fold dilution of a 100 μ l assay containing trypsin-treated rabbit erythrocytes at a final concentration of 0.6–0.8%. Identical samples were used for both sets of assays. Values are means, with the range of results shown by vertical bars. The numbers in parentheses indicate the sample size. The haemagglutination assays (●) were all performed in triplicate on the same day.

hydrolysis of the 3000 g pellet after repeated extraction for 48–72 h (Starcher, 1977) and quantification of isodesmosine and desmosine. The results are shown in Fig. 6 as (desmosine + isodesmosine)/g wet weight. Parallel profiles are obtained by plotting (desmosine + isodesmosine)/(phenylalanine or leucine); these latter two amino acids were always well resolved. Desmosine itself increased 25–30-fold during this time, the most rapid increase occurring between days 13 and 17. Rapid elastin cross-linking, as indexed by total desmosine, occurred between days 10 and 20, at which point an adult concentration of elastin cross-links had been achieved. Another distinct feature observed, despite incomplete resolution of desmosine from isodesmosine, was that the ratio isodesmosine/desmosine decreased rapidly during the first 3 postnatal weeks from about 3 at day 2 to 1.1 at day 20; this ratio is also shown in Fig. 6. The possibility that the changing isodesmosine/desmosine ratio results from the co-elution of contaminants remains, but seems unlikely, since none of the components resolved in the high-ionic-strength buffer showed more than a 2-fold change in concentration, whereas the total desmosine (isodesmosine + desmosine) increased 10–12-fold.

The pellet that we used for elastin determinations will also have contained collagen. Hydroxyproline

Fig. 6. Concentration of elastin in the developing rat lung

The concentration of isodesmosine + desmosine (nmol) was used as a criterion of elastin concentration per g wet weight of lung (O). The results between days 7 and 20 were from different lung samples; duplicate analyses always gave very closely similar values. Results are means, with the range of results shown by vertical bars. The numbers in parentheses indicate sample size. The ratio of isodesmosine to desmosine is also shown (□); the variation in ratio at each age point was trivial.

and hydroxylysine were also quantified; these two amino acids are used as biochemical markers for lung collagen (Hance & Crystal, 1976). The concentration of hydroxylysine increased only 4-fold between days 2 and 20, most of this increase occurring after day 13. The increase in hydroxyproline was similar, but elastin contains about 1% hydroxyproline, whereas hydroxylysine is unique to collagen.

Discussion

Lung development has been divided into four stages: glandular, canalicular, saccular and, finally, alveolar (Burri & Weibel, 1977). The development of alveoli from saccules is a postnatal phenomenon, which is accompanied by marked morphological changes, a changing ratio of cell types and rapid lung growth. Therefore little surprise should be expressed at changing amounts of various proteins and enzyme activities during this alveolarization of the lung. We should also caution that we measured enzyme activities at fixed substrate concentrations in crude extracts; the effects we observe may therefore result from changes in substrate specificity, molecular form, endogenous inhibitors as well as from enzyme concentrations themselves.

We have been particularly interested in the changes in lung lectin, acetylcholinesterase and elastin with postnatal rat lung development. In Fig. 7 these changes are correlated with a schematic representation of alveolarization. For the first 4 days the rat breathes with shallow thick-walled saccules. Between days 4 and 7 thick-walled secondary septa proliferate, still with the double-capillary network of the primitive saccules. The increase in surface area continues, but the slender single-capillary structures are not evident until about day 13. Within 3 weeks the alveoli are mature in form and composed entirely of a single-capillary network.

Lipid synthesis is important in any growing tissue; in the lung the synthesis of phosphatidylcholine is particularly noteworthy, since lung surfactant is composed mainly of disaturated phosphatidylcholine. We monitored two cytosol enzymes important to this lipid synthesis, namely glucose 6-phosphate dehydrogenase (which generates NADPH, the reducing power required for lipid synthesis), and, more specifically, choline kinase. The specific activities of both these enzymes remain constant during the postnatal lung growth. The results for choline kinase concur with those of Chida *et al.* (1973), who studied the enzymes of phosphatidylcholine synthesis in developing rat lung.

It is unfortunate that there is no specific protein or enzyme marker for the alveolar epithelial cells. Two probable markers are available for the alveolar endothelial cells; physiological evidence suggests

Fig. 7. Correlation of acetylcholinesterase, lectin and elastin profiles with postnatal-rat-lung development. The profiles of the changing content of acetylcholinesterase, lectin and elastin in the developing rat lung are taken directly from Figs. 4, 5 and 6 respectively; no axes are given but the scales are unchanged. We represent the development of the rat lung during this time period, from saccular to alveolar, in diagrammatic form.

that membrane-bound carbonic anhydrase is concentrated in endothelial cells (Klocke, 1978; Crandall & O'Brasky, 1978; Effros *et al.*, 1978) and angiotensin converting enzyme is situated along the luminal surface of pulmonary endothelial cells (Ryan *et al.*, 1975). Morphometric data on the developing rat lung have been published (Burri *et al.*, 1974). Intra-acinar pulmonary arteries and veins must develop in parallel with the alveoli. The ratio of alveolar capillary surface area to summed lung cell volume (endothelial + epithelial + fibroblast) increases rapidly during the first 3 weeks of lung growth (Burri *et al.*, 1974). The change of this ratio (capillary surface area/lung cell volume) with age approximately parallels the rise in the specific activity of angiotensin-converting enzyme. If we assume a constant membrane density for converting enzyme, our results might be explained on the basis of this relative increase in capillary surface area. Angiotensin-converting-enzyme activity in the developing rat lung has been the object of a previous study (Wallace *et al.*, 1978); our results are somewhat at variance with theirs, and this is probably related to methodology. Wallace *et al.* (1978) assayed a 20000g supernatant; we used activity in the 100000g supernatant as an indicator

of serum contamination and that of the 100 000g pellet as an index of the membrane-bound endothelial-cell enzyme.

Recent histochemical evidence has suggested that cholinergic nerves do not invade the intrapulmonary structure until 5–9 days after birth; acetylcholinesterase was absent at day 3, sparse peribronchiolar staining was observed at day 5 and an adult pattern observed by day 9 (El-Bermani & Bloomquist, 1978). Nerve endings are rarely observed at the alveolar level. The peribronchiolar parasympathetic cholinergic nerves probably regulate mucous secretion and airway diameter (Nadel, 1977). Rat smooth muscle contains at least two different molecular forms of acetylcholinesterase: 4S (loosely attached to the membrane) and 10S (firmly integrated into the membrane) (Vigny *et al.*, 1976). It is probable that, in the absence of detergents, only the 4S form would be solubilized from rat lung and that reaggregation of this 4S form could occur under our extraction conditions. Indeed we find acetylcholinesterase concentrated in a membrane fraction (3000–100 000g pellet). Further, this pellet showed little evidence of contamination by erythrocyte proteins; namely trivial SDS-sensitive carbonic anhydrase activity. Our results concur with the histochemical data; the specific activity of acetylcholinesterase was very low at days 0 and 2, had increased significantly by day 4 and reached adult values between days 10 and 13 (Fig. 4). Acetylcholinesterase activity has been used to indicate that cholinergic innervation of rat lung develops postnatally. It is probable that the increase in acetylcholinesterase specific activity that we observe between days 4 and 10 may result in part from a change in molecular form of acetylcholinesterase; developmental changes (4S → 10S form of acetylcholinesterase) have been observed in rat brain (Rieger & Vigny, 1976) and in chick brain (6S → 11S form) (Marchand *et al.*, 1977). Nevertheless, our biochemical data correlates well with the histochemical data of El-Bermani & Bloomquist (1978).

The junction of an axonal ending with a nerve cell, muscle cell or glandular cell is called a synapse. We deduce that the formation of cholinergic synapses in rat lung occurs between postnatal days 4 and 10. The peak in activity of β -galactoside-binding proteins (or lectins) has been correlated with synaptogenesis (Teichberg, 1978). The distribution of an endogenous β -galactoside-binding lectin has been studied in the developing chick optic tectum (Gremo *et al.*, 1978). Lectin activity in embryonic-chick brain is low early in development, rises to a maximum value at day 12 of incubation and then falls 5–10-fold within a week of hatching (Kobiler & Barondes, 1977). The peak of lectin activity in the chick embryo does not seem to be correlated with

extensive cellular proliferation or cellular migration; rather, lectin is prominent at a time when neurons mature and are sending out processes and receiving information (Gremo *et al.*, 1978). No specific relationship between the presence of lectin and innervation has been elaborated.

The similarity between the chick β -galactoside-binding proteins and lung lectins has already been mentioned (DeWaard *et al.*, 1976; Powell, 1980). The lectins are of similar size, subunit composition and saccharide specificity. The similarity extends further to the developmental regulation of lung lectin. In rat lung the lectin activity of the neonate is very low; lectin specific activity rises sharply to peak between postnatal days 10 and 13 and then declines 8–10-fold to adult values (Figs. 5 and 7). The sharp rise and fall of lectin specific activity is symptomatic of the lung lectin being important at a specific stage of lung development, although the peak occurs after the most rapid phase of cell proliferation. The profile of lectin activity parallels that of acetylcholinesterase quite closely until day 13. The biochemical properties of lung lectin suggest that it functions in a recognition process; circumstantial evidence would again suggest synaptogenesis, which within the rat lung would be confined to the peribronchiolar regions. This is an hypothesis that could be tested by immunofluorescence studies. In the interim we might put forward the working hypothesis that lectin activity is involved in the development and regulation of cholinergic innervation.

The peak of lectin activity also correlates with the peak of alveolarization of the lung, when single-capillary-network alveoli are first observed. Secondary septal outgrowth occurs between the tip and base; elastin frequently appears beneath the tip of newly formed alveolar septa and elastic fibres may play a prominent role in this restructuring of lung (Burri & Weibel, 1977). Two types of interstitial fibroblast have been described in the neonatal rat lung: one appeared at the tip of new septa and appeared to be engaged in the synthesis and secretion of elastin; the other type of fibroblast, which is lipid rich, occurred at the base of the septa (Vaccaro & Brody, 1978). The former type, or myofibroblast, was prominent between postnatal days 5 and 15. Vaccaro & Brody (1978) stated that 'at the ultrastructural level, elastin clearly predominates in the formation of new septa'; collagen plays a minor role. Burri & Weibel (1977) suggested that 'the elastic system might provide a kind of anchorage point for the formation of alveolar septa.' We have already remarked that the intracinar arteries and veins grow in parallel with the alveoli; therefore much of the gross increase in elastin concentration that we observe will be arterial. We have used the concentration of elastin cross-links as a criterion of elastin concentration (Starcher,

1977). The concentration of elastin was almost stationary for the first 7–10 postnatal days. After this time, particularly between days 13 and 17, we observed a dramatic increase in elastin cross-linking. The concentration of cross-links (isodesmosine + desmosine) increased 10–12-fold to reach adult values by day 20–21. The timing of this elastin proliferation correlates with the thinning of the alveolar septa (see Fig. 7) and the transformation from a double- to a single-capillary network. In this respect we would disagree with the ultrastructural observations (Vaccaro & Brody, 1978; Burri & Weibel, 1977) that link elastic-fibre formation with the earlier stage of septation. We would agree that collagen-fibril formation is less important, since the collagen concentration increases only 3–4-fold between days 2 and 20, some of which will be attributed to the growing arterial system. The other feature of these biochemical studies that cannot escape remark is the distinct change in the isodesmosine/desmosine ratio, from 3:1 in the neonatal-rat lung to 1:1 in the 20–21-day-old-rat lung. Elastin from most sources has an isodesmosine/desmosine ratio of near 1:1, which might be expected, since the final cross-linking step is a non-enzymic process. Preliminary observations on the isodesmosine/desmosine ratio in developing rat and turkey aorta appear to show a similar change, initially there is a high isodesmosine/desmosine ratio (R. Boucek, personal communication).

We have shown that, in the developing rat lung, the maximum activity of lung lectin appeared at the maturation point of cholinergic innervation and at the initiation of elastin proliferation. All these processes occur throughout the peak of alveolarization of the lung, but after the most extensive cellular proliferation. The role of lung lectin in this developmental process has not been clarified by the present study, although we have proposed a working hypothesis linking lectin activity with cholinergic innervation.

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