Shimane J. Med. Sci. Vol. 4, pp. 81–90, 1980

Histogenetic Study on Human Fetal Lungs

(lung/terminal bud/human fetuses)

OSAMU TANAKA[a], MITSURU OKI[a], and AKIRA SHIMATSU[b]

[a]*Department of Anatomy, Shimane Medical University, Izumo 693 and* [b]*Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto 606, Japan*

(Received July 7, 1980)

Using 27 externally normal human fetuses ranging from the four to the tenth lunar months gestation (M) (7.3 to 36.0cm in crown-rump length), the histogenesis of the lung was examined by the conventional methods. A part of the middle lobe of the lung was sectioned and stained with hematoxylin and eosin, respectively.

The epithelia of the terminal buds were the columnar or the low columnar at the fourth M, the low columnar to the cuboidal at the fifth M and the cuboidal to the flattened at the sixth M. The difference of the nuclei's stainability between the tubuli appeared at the end of the fourth M, and became evident at the fifth M, and was clear at the sixth M. The proliferation of the capillaries appeared in the mesenchyme near the epithelium between the end of the fourth M and the beginning of the fifth M. Thereafter, the capillaries began to invade at the proximal part of the terminal buds and were exposed directly to the air spaces at the seventh M. The mesenchymal condensation and irregularity of arrangement of the epithelium appeared at the proximal part of the terminal buds.

The prenatal developmental morphology and histology of the human lung have been described, illustrated, and bibliographically annoted in considerable detail by many authors (1–18). Clinically, on the other hand, the adaptability of premature infants to extrauterine conditions is an important problem in perinatal medicine, as well as the frequent occurrence of the respiratory disorders such as the respiratory distress syndrome. Therefore, it is helpful to give a close description on normal pulmonary maturation during the prenatal period. However, published articles of normal development in the lung have been mainly concerned with the connective tissue fibers and the alveolar epithelium and were based on the study of small number of the human fetuses.

Loosli and Potter (7) described three periods in prenatal lung : (a) the "glandular" period up to about the fourth month of gestation, during which the bronchial division is established ; (b) the "canalicular" period, fourth to sixth month of gestation, during which the respiratory portion of the lung is delineated ; (c) the "alveolar" period, sixth month of gestation to term, during which the definitive alveolar duct and alveoli are developing. Recently, Hislop and Reid (16), discussed the "pseudoglandular" (up to 17th gestational

week); the "canalicular" (14th−25th gestational week); the "terminal sac" (24th−38th gestational week). It is necessary to detect in greater detail the histogenesis of fetal lung during the middle stages of gestation as numerous changes in the cells and tissues occur from the fourth to seventh months of gestation.

The current investigation was undertaken to examine the development of terminal buds, the proliferation of the capillaries and the difference of nuclei's stainability between the tubuli in the human fetus, particularly from the fourth to seventh months of gestation.

MATERIALS AND METHODS

The materials for the present study were twenty-seven externally normal human fetuses ranging from 73 mm to 360 mm in crown-rump length, 30 gm to 3,780 gm in body weight. Their approximate gestation ages ranged from the fourth to the tenth months, as determined by clinical data and degree of development of the outer anatomical characteristics. The distribution of the fetuses by age, sex and size is shown in Table I.

TABLE I. *Specimens*

Number	Lunar month	Sex	Crown-rump length (mm)	Body weight (gm)
1	4	f	73	30
2	4	m	76	30
3	4	m	80	47
4	4	m	113	85
5	4	f	120	85
6	5	m	135	200
7	5	f	143	245
8	5	f	155	300
9	5	m	156	298
10	5	m	162	315
11	5	f	170	320
12	5	m	172	340
13	5	f	178	310
14	6	f	158	295
15	6	f	178	390
16	6	m	190	450
17	6	m	192	410
18	6	m	192	530
19	6	f	210	550
20	7	f	240	750
21	7	f	245	865
22	7	f	248	1,090
23	7	m	288	1,500
24	7	m	295	2,250
25	8	f	292	1,890
26	9	m	300	2,500
27	10	f	360	3,780

m : male ; f : female

The right lung was resected from the human fetus within three hours after death. Following enucleation, the small block was usually removed from the lateral region of the middle lobe in the lung, and fixed in Bouin's fluid,

and placed in 10% formalin. Serial 7μ paraffin sections were cut and stained with hematoxylin and eosin. Following the staining preparations, the pulmonary maturation of the human fetus was examined under a light microscope.

RESULTS

The development of the terminal buds of the lung is shown in Fig. 1. Characteristic findings by the gestational month are as follows :

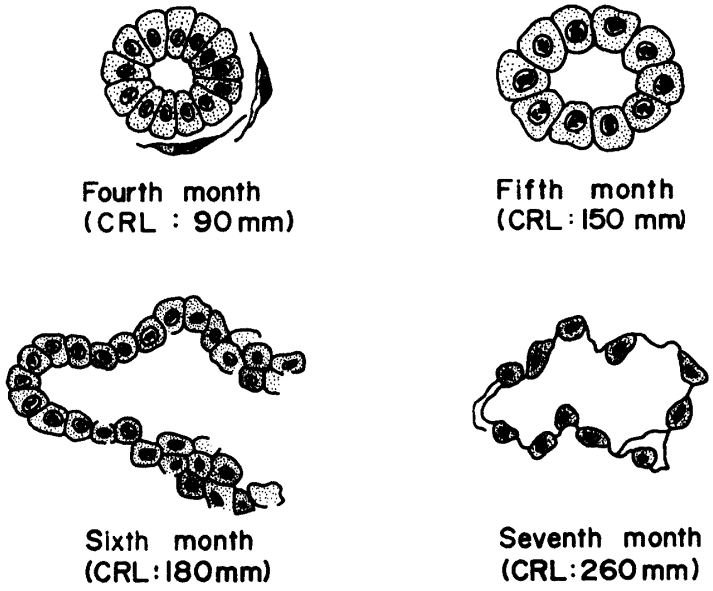


Fig. 1. Schema of the developing terminal buds.

First Half of the Fourth Month

The lung was recognized to be a tubular structure of the epithelial ducts in the mesenchyme. The bifurcation of the tubuli was observed to be scattered and three or four terminal buds had a tendency to group, but lobule formation did not appear. The terminal buds were compact. The tubuli near the terminal buds were covered with continuous simple columnar epithelium. The epithelial cells had eosinophilic cytoplasm at their apical parts and regularly arranged nuclei in the center. One layer of the mesenchymal cells was observed around the tubuli, but this layer did not condense.

The more proximal part of the bronchial tree could be distinguished with the terminal buds by the apical portion of nuclei and fold of its epithelium.

The lymphatics with endothelia and several capillaries were found in the mesenchyme. The mesenchymal cells near the bronchial tree were different from those observed in other areas. The former had spindle-shaped nuclei with a rich chromatin, the latter oval vesicular nuclei.

No precartilage or cartilage appeared, but smooth muscle fibers were seen around some of the tubuli.

The bronchial artery is present, in this stage.

Latter Half of Fourth Month

The lung still had a glandular appearance. The terminal buds increased in number. The epithelial lining consisted of low columnar cells with round nuclei in the middle portion and clear cytoplasm (Fig. 2-A). Some of the terminal buds had several mesenchymal cells in this stage, but no condensation. Most of the lumens of the terminal buds were round. However, the most proximal part of the bronchial tree had columnar epithelial lining with an irregular-shaped lumen. In the apical portion, the epithelial cells had chromatin-rich nuclei which were stained deeper than those of the terminal buds, and here there were two layers of mesenchymal cells condensed around the buds.

The ciliated cells at the bronchi had appeared. The epithelial cells were irregularly arranged, so that a fold developed. They had apical-portioned nuclei, and the basal cytoplasm was clear, though apical eosinophilic. Basement membrane became recognizable at this stage. Few capillaries were observed in the mesenchyme, except for specimen No. 1. Interlobular lymphatics were well developed.

Fifth Month

The lung began to loose its glandular appearance. Many tubuli were observed, but the lumens gradually became rough-surfaced and had an irregular shape. The epithelial linings of the terminal buds were still continuous with the low columnar or cuboidal cells which had round vesicular nuclei in the center and clear cytoplasm (Fig. 2-B). There was no mesenchymal cell condensation around them. The more proximal tubuli had irregularly arranged columnar to cuboidal epithelium with elongated or oval nuclei, deeper than in the terminal buds. The bronchi had columnar and ciliated epithelium with well developed folds. The basal cells appeared at some portions, and here the arrangement of the nuclei was irregular.

Lobular structure became evident at the interlobular lymphatics expanded, and mesenchymal cells were sparse. The capillaries developed gradually in the mesenchyme near the tubuli. In specimen No. 8, two types of lobule were observed, the first type was a lobule which had many expanded lumens, the second few expanded lumens. The epithelial cells of the second type were large and irregular arranged. In this case, the terminal buds seemed to be collapsed.

Sixth Month

In this stage, there were many expanded tubuli with a rough-surfaced lumen lined round-vesicular-nucleid cuboidal cells (Fig. 2-C). The expanded tubuli were invaded by capillaries at some places and contacted these capillaries. The tubuli were not continuous and had little cytoplasm.

There were also some not-expanded terminal buds with no invading capillaries and distinct basement membranes. Flattened nuclei were not observed.

The mesenchymal cells showed a tendency to condensate around bronchus and expand tubuli.

The bronchus had folded columnar epithelium with irregular-arranged elongated or oval nuclei which stained deeper than the nuclei of two distal tubuli. All epithelial cells were ciliated.

The lobular formation was not distinct because of the rapid growth of the respiratory part.

Seventh Month

The bronchial tree could be distinguished with the respiratory part of the lung by its deeper stained epithelial nuclei.

The mesenchymes reduced gradually proportion in the lung because of growth of the tubuli and they became more compact septa at the end of this month.

The capillaries exposed into the air space in some regions, and most capillaries were observed in the mesenchyme and had invaded the epithelium.

All of the expanded lumens were rough-surfaced and the epithelial cells had round vesicular nuclei or flattened dark nuclei (Fig. 2-D). The cytoplasm processes without nuclei were observed scattered throughout the lumens. The epithelial cells with flattened nuclei had little cytoplasm.

The lobular formation became evident as the interlobular connective tissue was more fibrous and had fewer cell components.

The well expanded lumens in the specimens had a grape-bunch-shape, which were lined by the flattened cells at some parts. In specimen No. 19, there were tubuli without a lumen, which had collapsed, but the epithelial structure could be traced.

Eighth, Ninth and Tenth Months

The nuclei of the epithelial cells became more flattened and the processes of the cytoplasm without nuclei increased in number.

The lobules were clearly recognized.

The ciliated cells were observed at the terminal bronchi in the ten month fetus.

DISCUSSION

Other investigators have described the general histogenesis of the fetal lung, as determined by light or electron microscopy and with or without histochemistry. The findings differed regarding epithelial growth and differentiation of the terminal buds, the difference of nuclei's stainability between tubuli, and the proliferation of capillaries in the routine histology. Therefore, the different views are discussed, in the present study.

Epithelial Growth and Differentiation of the Terminal Buds

As noted by previous investigators, the epithelial change from columnar to cuboidal usually appears between the fifth and the beginning of the sixth month. According to Shimada (12), Hozaka (19) and Arey (20), this change occurred at the fifth month, and Shimai (21) and Matsumoto (9) showed that this occurred at the end of the fifth month to the beginning of the sixth month, Akiyama (22) reported these findings at the 21th to the 22th week of gestation, however, Campische *et al.* (11) showed that the terminal buds were frequently cuboidal and slightly flattened at the fourth to the fifth months.

The present study indicates that the epithelia of the terminal buds are low columnar at the end of the fourth, low columnar to cuboidal at the fifth, and cuboidal to the flattened at the sixth month.

Difference in Stainability of the Nuclei between the Tubuli

According to Loosli and Potter (7), the spaces which were to become bronchi could be detected by the deeper staining of the epithelial cells, at the fourth month. Thereafter, the epithelial cells lining the bronchial tubular system stained deeply with hematoxylin-eosin, and the tubules which were to become the respiratory portion took the stain less deeply between the middle of the fifth and sixth months. Matsumoto (9) obtained similar findings in the sixth month specimens.

The present findings regarding the difference in stainability of the nuclei between the tubuli are as follows : no differences at the third month ; some differences at the fourth month ; slight difference at the fifth month ; clearly different at the sixth month, and clearly different from the seventh to term.

Proliferation of the Capillaries

Several authors have reported the development of the vascularization. At the fourth month, Shimada (12) found capillaries near the basement membrane of the epithelium, and Loosli and Potter (7) found no essential vascularization in the mesenchyme, however, Hozaka (19) found a distinct proliferation of the capillaries in the mesenchyme. At the fifth month, some investigations (7, 9, 23) indicated that the capillaries had invaded and were exposed at some places between the epithelia, and Akiyama (22) reported that the proliferation of the capillaries was evident in the mesenchyme between 19 and 20 weeks of the gestation. At the beginning of the sixth month, Loosli and Potter (7) and Short (24) found that the capillaries began to push against the epithelium and that there was vascularization between the cells. By the sixth month, the naked capillaries penetrated between the flattened cells and reached the surface of the bronchiolar wall (25—27). At the seventh month, the nature of the naked capillaries was more apparent and the capillaries seemed to be exposed directly to the air spaces (7, 9, 10, 23).

The present study reveals that the individual differences in proliferation of the capillaries is over a wide range during the fourth month. The proliferation

of the capillaries begins near the epithelium and the capillaries invade between the epithelia at the fifth month. At the sixth month, many capillaries are observed in the mesenchyme and invade progressively. The capillaries contact the tubuli, and penetrate wedge-like into the epithelia and are then exposed to the air space.

It is often difficult to ascertain the exact location of the observed epithelium in the fetal lung. If the term bronchi is used for tubules around which there is cartilage or precartilage and the term bronchioli for airways distal to those with cartilage, most tubules destinated to be bronchi could not be identified as such and were undistinguishable from bronchioli. Therefore, the present study was based on the terminology of Potter and Craig (25).

More precise terminology of the units of respiratory portion of the developing lung should be established.

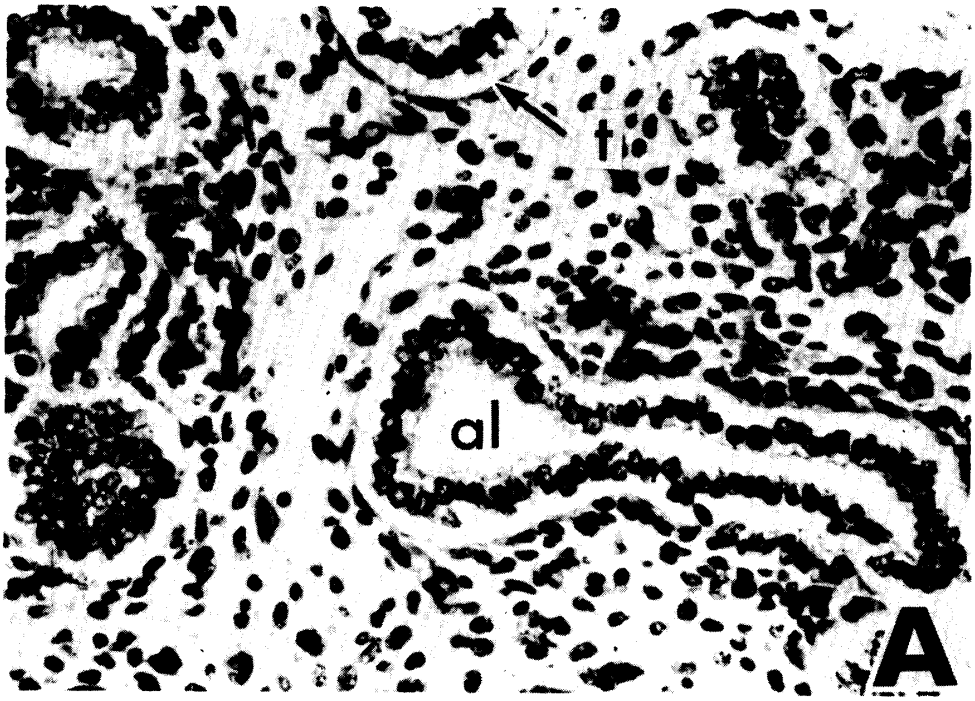
REFERENCES

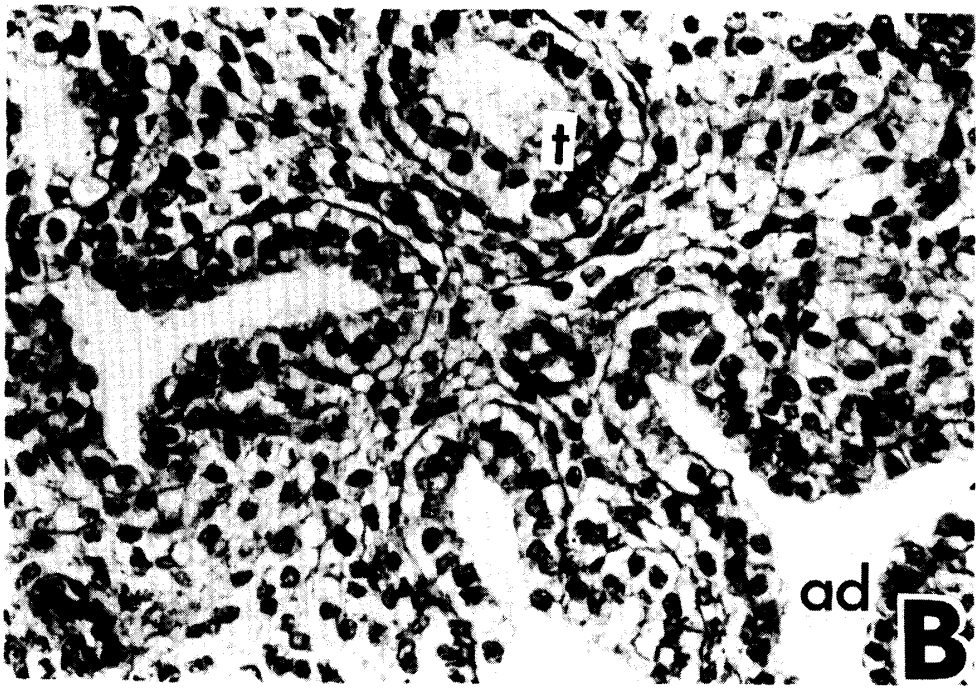
1) Flint, J. M. (1906) The development of the lungs. *Am. J. Anat.* **6**, 1–138
2) Addison, W. H. F. and How, H. W. (1913) On the prenatal lung. *Am. J. Anat.* **15**, 199–214
3) Heiss, R. (1919) Zur Entwicklung und Anatomie der menschlichen Lunge. *Arch. Anat. Physiol. Anat. Abt.* **1918–1919**, 1–129
4) Bender, K. W. (1925) Über die Entwicklung der Lungen. *Z. Anat. Entwicklungsgesch.* **75**, 638–704
5) Cooper, E. R. A. (1938) A histological investigation of the development and structure of the human lung. *J. Pathol. Bacteriol.* **47**, 105–114
6) Ham, A. W. and Baldwin, K. W. (1941) A histological study of the development of the lung with particular reference to the nature of alveoli. *Anat. Rec.* **81**, 363
7) Loosli, C. G. and Potter, E. L. (1959) Pre-and postnatal development of the respiratory portion of the human lung with special reference to the elastic fibers. *Am. Rev. Respir. Dis.* **80**, 5–25
8) Lelong, M. and Laumonier, R. (1954) Histological and histochemical evolution of the foetal lung. In : Anoxia of the Newborn Infant. (Delafresnage, J. F. and Oppé, T. E., eds.) pp. 61–84, C. C. Thomas, Springfield
9) Matsumoto, S. (1957) A histologic investigation on the lung of the human fiber. *Folia Anat. Jpn.* **30**, 275–298
10) Loosli, C. G. and Barker, R. F. (1962) The human lung : microscopic structure and diffusion. Ciba Foundation Symposium on Pulmonary Structure and Function, pp. 194–215
11) Campische, M., Gautier, A., Hernandes, E. I., and Reymond, A. (1963) An electronmicroscope study of the fetal development of human lung. *Pediatrics* **32**, 976–994
12) Shimada, M. (1964) Histological studies on the normal and the abnormal pulmonary development of fetuses and newborn infants. *J. Osaka City Med. Center* **13**, 457–488
13) Emery, J. (1969) The Anatomy of the Developing Lung. William Heinemann, London
14) Boyden, E. A. (1972) Developing of the human lung. In : Brenneeman's Practice of Pediatrics. Vol. 4, pp. 1–12, Harper and Row, Hagerstown
15) Hage, E. (1973) The morphological development of the pulmonary epithelium of human fetuses studied by light- and electron microscopy. *Z. Anat. Entwicklungsgesch.* **140**, 271–279
16) Hislop, A. and Reid, L. (1974) Scientific Foundations of Pediatrics. (Davis, J. A. and Dobbing, J., eds.) Heinemann, London
17) Stahlman, M. T. (1978) Anatomical development and maturation of the lungs. *Clin. Perinatol.* **5**, 181–185

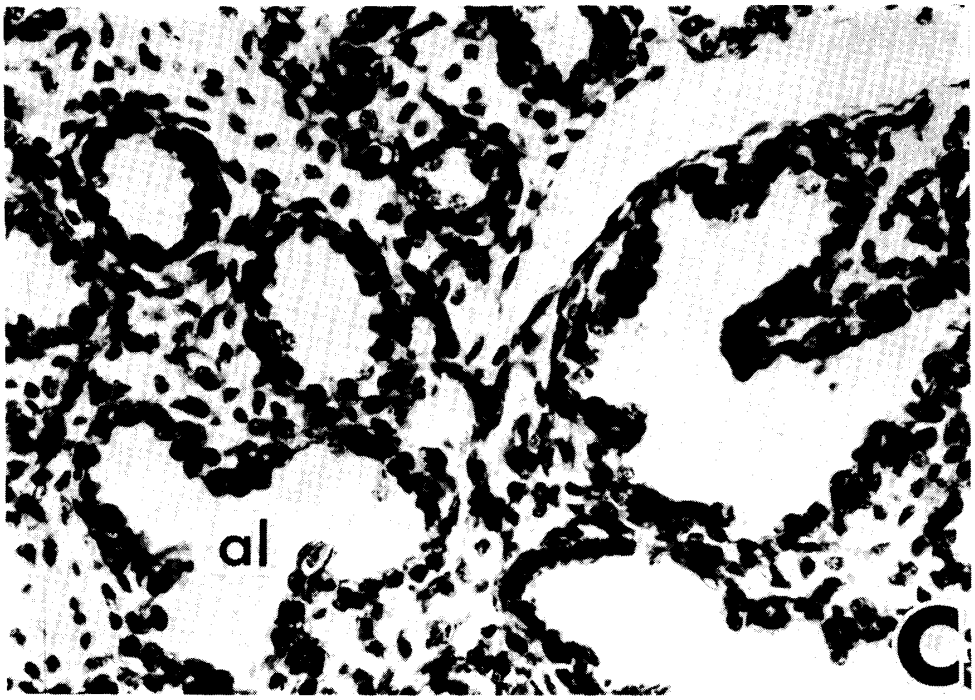
18) Valdés-Depena, M. A. (1979) The lower respiratory tract. In : Histology of the Fetus and Newborn. pp. 310–341, W. B. Saunders, Philadelphia
19) Hozaka, T. (1942) On the histogenetic study on the lung, with special reference to the alveolar epithelial cells. *Hokuetsu Igakkai Zasshi* **57**, 1112–1142 (Eng. Abstr.)
20) Arey, L. B. (1974) The lungs. In : Developmental Anatomy. pp. 266–268, W. B. Saunders, Philadelphia
21) Shimai, K. (1951) A histogenetic study of the human embryonic lung. II. The histogenetic study of the alveolar epithelium. *J. Keio Med. Soc.* **28**, 253–257
22) Akiyama, T. (1952) Histological studies on the development of the human lung. *Osaka Daigaku Igaku Zasshi* **4**, 309–322 (Eng. Abstr.)
23) Yanagihara, M. (1959) Histological and histopathological studies on the structure of the pleura. *Kyoto Daigaku Kekkaku Kenkyusho Kiyo* **7**(3), (Suppl. 1)148–172 (in Japanese)
24) Short, R. H. D. (1950) Alveolar epithelium in relation to growth of the lung. *Philos. Trans. R. Soc. Lond.* [*Biol.*] **235**, 35–48
25) Potter, E. L. and Craig, J. M. (1975) Lungs and trachea. In : Pathology of the Fetus and Infant. pp. 274–285, Year Book Medical Publishers, Chicago
26) Palmer, D. M. (1936) The lung of a human foetus of 170mm C. R. length. *Am. J. Anat.* **58**, 59–72
27) Norris, R. F., Kochenderfer, T. T., and Tyson, R. M. (1941) Development of the fetal lung with special reference to the lining of the alveoli and the effect of immaturity on respiration. *Am. J. Dis. Child.* **61**, 933–950

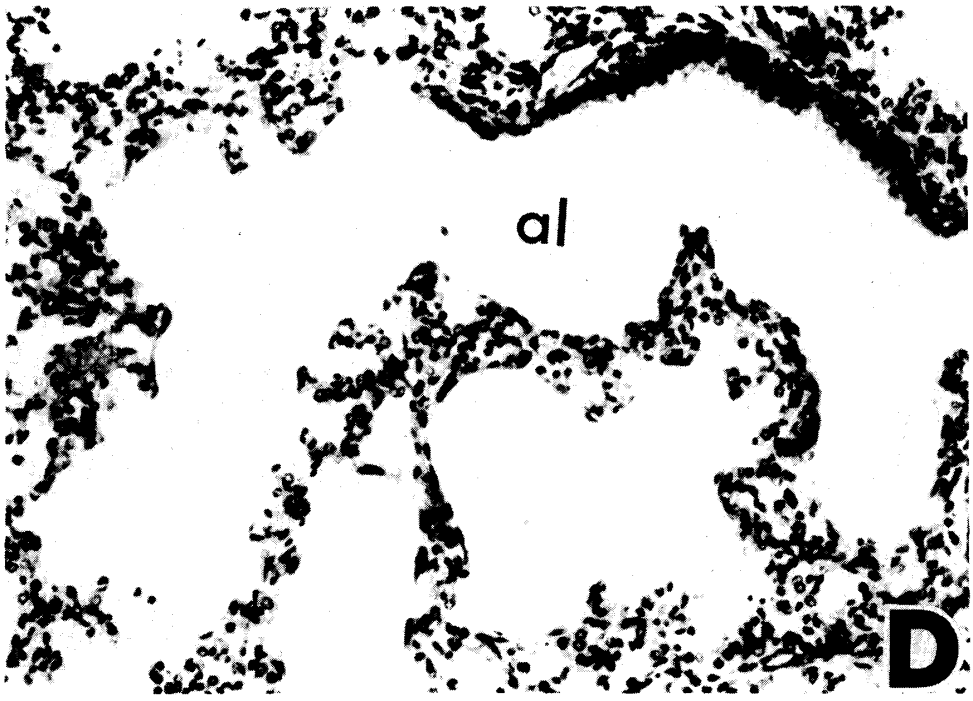
LEGENDS TO FIGURES

Fig. 2. Histological section of the lung of human fetus (H. E. staining).
t : terminal bud ; ad : alveolar duct ; al : alveolus
A. Fourth month, 113mm in CRL (×400)
B. Fifth month, 162mm in CRL (×400)
C. Sixth month, 190mm in CRL (×400)
D. Seventh month, 248mm in CRL (×200)


t
al
A


t
ad
B


al
C


al
D