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Use of DNA estimation for growth assessment in normal and hypoplastic fetal lungs

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SUMMARY Total DNA was estimated in the lungs of 80 fetuses and newborn infants varying in gestation from 14 weeks to term. In fetuses of appropriate weight for gestational age total lung DNA increased at a constant rate from about 35 mg at 17 weeks' gestation to 480 mg at term. The lungs of immature fetuses were heavier and contained more DNA relative to body weight than did those of mature infants. Small-for-dates infants had lower lung DNA levels for gestation than infants with weights appropriate for gestational age, but there was no difference when lung DNA was corrected for body weight. Lung hypoplasia defined in terms of lung/body weight ratio was associated with low lung DNA content for gestation, even when corrected for body weight. The total lung DNA at 34–40 weeks' gestation in infants with lung hypoplasia associated with fetal anuria or urinary outflow obstruction was equivalent to that seen in normal fetuses at 20–22 weeks' gestation. We conclude that the early second trimester is a critical period for human fetal lung growth.

The state of lung development at birth is one of the major factors determining death or survival in the perinatal period. Both qualitative and quantitative aspects of lung development are clearly of importance in this respect. Most research has been applied to the study of biochemical aspects of lung development related to surfactant metabolism and hyaline membrane disease.[1] Quantitative failure of lung growth has been less extensively studied although it appears to be the cause of perinatal death in a number of malformation syndromes affecting the renal tract, the central nervous system, or the thoracic cage,[2–4] it may follow prolonged amniotic fluid leakage,[5 6] and it has been reported as an isolated anomaly.[7]

One of the major problems in studying pulmonary hypoplasia is the difficulty of establishing criteria for the diagnosis of hypoplastic lungs at necropsy in babies who may vary widely both in gestational age and in weight for gestation. The ratio of lung weight to body weight has been suggested as the most practical means of overcoming the problem and it has been shown that lung:body weight ratios of 0·012 or less are generally associated with a reduction in alveolar number as indicated by the radial alveolar count.[4]

Wet weight may be rather a poor measure of tissue mass for the perinatal lung as it must be appreciably affected by the quantity of lung liquid retained within the airways or interstitial tissue and by superimposed pathological changes—such as inhaled amniotic fluid, oedema, or pulmonary haemorrhage.

DNA measurement as an index of cell population has been used extensively in the study of perinatal brain growth and has allowed useful deductions to be made about normal and abnormal brain development.[8 9]

We have now investigated the use of this method for study of human fetal lung growth and for assessment of severity and timing of fetal lung hypoplasia.

Materials and methods

A total of 80 fetuses or newborn infants was studied. Macerated stillbirths and anencephalics were excluded. Gestational age of the therapeutic termination cases was estimated from the crown to rump length, supplemented by menstrual dates if such data were available. Assessment of gestational age, both obstetric and paediatric, in the perinatal cases was confirmed by examination of brain convolutional pattern.[10] All necropsy examinations were performed or were closely supervised by one of us (JSW). After external inspection of the lungs the bronchi and pulmonary vessels were cut off flush with the parenchyma and the unopened

lungs were placed in a closed container until they could be weighed separately on a balance accurate to 0·01 g. Small portions of upper and lower lobes of each lung were taken for histology and the remainder of the right lung was reweighed and stored at −20°C for biochemical estimation. Lungs obtained from therapeutic terminations were examined for completeness. After removal of small portions of each lung for histology the whole of the remaining lung tissue from these specimens was retained for biochemical study. Lungs with histological evidence of pneumonia were excluded from the study.

Lung samples were homogenised in an equivalent weight of water using a Polytron PCU-2 tissue homogeniser at setting No 6 for at least 2 minutes. DNA was assayed fluorimetrically by the method of Le Pecq and Paoletti.[11] Calf thymus DNA (Koch-Light Laboratories Limited) was used as a standard and readings were measured in a Perkin-Elmer model 204a fluorescence spectrophotometer.

The total lung DNA was estimated from the equation,

total lung DNA = DNA of aliquot × total lung weight/weight of aliquot.

The total lung DNA was assumed to be directly proportional to lung cell population.[12]

Results

The total lung DNA in 41 fetuses and infants of normal weight for gestational age is shown in Fig. 1. The increase in cell population follows a straight line from about 17 weeks' gestation up to term. Total lung DNA increases from 35 mg at 17 weeks to 480 mg at term.

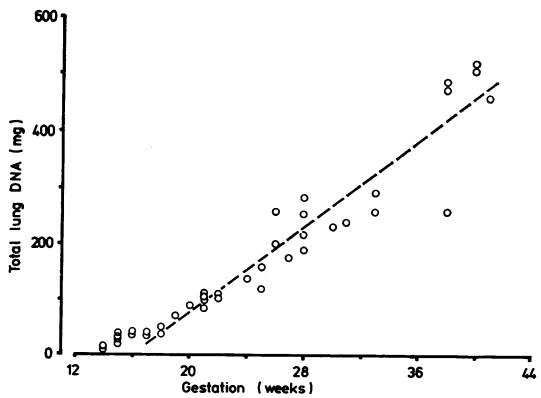

Fig. 1 *Total lung DNA in 41 fetuses and newborn infants of appropriate weight for gestation with regression line plotted for the data from 17 to 41 weeks* ($y = -308{\cdot}4 + 19{\cdot}04x$, $r = 0{\cdot}96$).

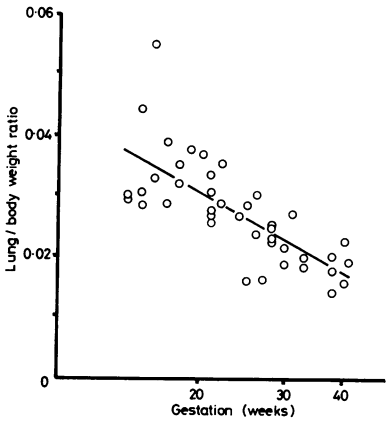

Fig. 2 *Lung/body weight ratio in 41 fetuses and newborn infants of appropriate weight for gestation, with regression line* ($y = 0{\cdot}088 - 0{\cdot}044x$ *where* $x = \log$ *gestation.* $r = -0{\cdot}74$).

The lung/body weight ratio of the same group of infants is shown in Fig. 2. It can be seen that the ratio falls with increasing gestational age. In view of this it seemed appropriate to define lung hypoplasia in infants or fetuses of <28 weeks' gestation as half the mean lung/body weight ratio of the 20–27 week group (that is ≤0·015) rather than taking the value of 0·012 used for infants of 28 weeks' gestation or more.

The lung DNA values in 14 small-for-dates infants (<10th centile) without malformation are shown in Fig. 3. Thirteen of the 14 values are below the regression line for the normally grown infants.

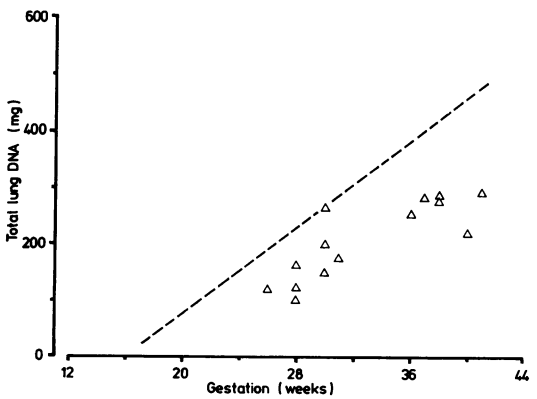

Fig. 3 *Total lung DNA in 14 small-for-gestational age infants. Thirteen of the values are below the regression line for controls (broken line).*

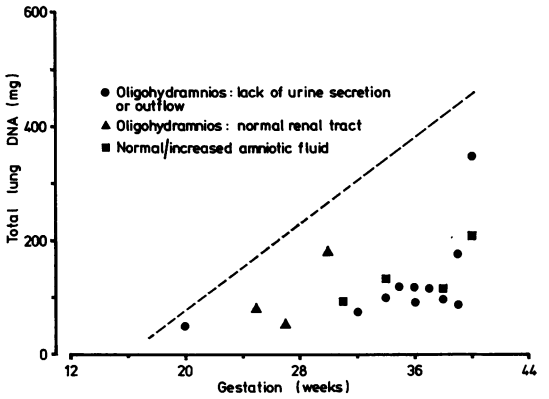

Fig. 4 *Total lung DNA for 18 infants with lung hypoplasia. Broken line is regression line for controls.*

The lung DNA values of 18 infants with lung hypoplasia defined according to lung/body weight ratio as mentioned above are shown in Fig. 4. Infants with renal agenesis, severe renal cystic dysplasia with thread-like ureters, or total urinary outflow obstruction had a mean lung DNA content at 34–40 weeks' gestation equivalent to that found in the normal fetal lung at 20–22 weeks' gestational age. The one fairly high value in this group was from a term infant of 4·1 kg birthweight (>90th centile) with urethral valve obstruction and hydronephrosis.

When the DNA figures for the three groups were replotted as total lung DNA/kg body weight (Fig. 5) there was no difference between the values for small-for-dates and normal weight for dates infants,

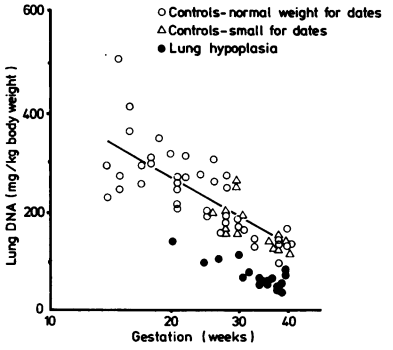

Fig. 5 *Total lung DNA/kg body weight for normal weight, small-for-dates, and lung hypoplasia groups. Regression line for normal and small-for-dates group ($y = 885·3 - 470·5x$ where x = log gestation. $r = -0·80$). Values for lung hypoplasia group differ significantly from other groups (analysis of variance with covariance $P<0·001$).*

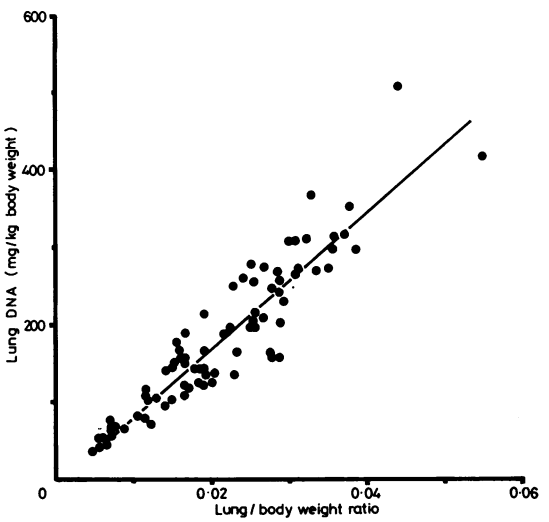

Fig. 6 *Relationship between lung DNA/kg body weight and lung/body weight ratio in 80 fetuses and newborn infants. Regression equation $y = -6·99 + 8751x$, $r = 0·93$.*

but the DNA levels of the hypoplastic lungs averaged only half the level expected on the basis of body weight for the particular gestational age.

If the values of lung/body weight ratio and lung DNA/kg body weight are plotted against each other using all the data we have available* (Fig. 6), it can be seen that there is in general a good correlation between the two methods of assessing lung hypoplasia, particularly for infants with low lung/body weight ratios. All infants with a lung/body weight ratio below 0·01 had a lung DNA level of less than 100 mg/kg body weight.

Discussion

Lung development has traditionally been assessed by the use of morphometric techniques. The method most often used is a modification of the radial alveolar count devised by Emery and Mithal,[13] whereby a line is drawn from a peripheral respiratory bronchiole at right-angles to the margin of the lobule, and a count made of the number of alveolar septa transected by the line. This method gives a measure of the degree of elaboration of the lobule into terminal sacs. It is not strictly quantitative and does not show whether there is a normal number of

*Additional cases included are those with malformations of renal tract or CNS or prolonged rupture of the membranes, but with lung/body weight ratios above 0·012.

lobules present. Hislop *et al.*[14] combined lung volume measurement with radial alveolar counts, estimation of alveolar size, measurement of arterial lumen, arterial wall thickness and branching pattern, and counts of airway generations. These studies were performed after barium injection of vessels and formalin inflation of airways to a standard pressure. These workers recorded a reduction in the number of generations of bronchial branching in the lungs of infants with renal agenesis or renal dysplasia, indicating that retardation of lung growth had occurred before 16 weeks.

Estimation of the cell population of an organ by measurement of the total DNA content is hardly more sophisticated than weighing it. The technique does however seem worthy of investigation for study of perinatal lung growth as wet weight can be influenced by so many physiological and pathological variables and the 'precise morphometric techniques' of Hislop *et al.*[14] are very time-consuming. The lung is also fairly homogeneous and, in the absence of an inflammatory reaction, most of the cells which comprise it belong to airways, interstitial tissue, or vessels, and may be regarded as contributing to respiratory function. Given qualitatively normal structure total lung DNA should therefore give some measure of the adequacy of lung development.

Our lung DNA measurements give new information on the time of origin of severe fetal lung hypoplasia in conditions associated with lack of amniotic fluid (renal agenesis or severe cystic dysplasia, urinary outflow obstruction, and severe prolonged rupture of membranes). Infants with hypoplastic lungs born at 34–39 weeks' gestation have a lung cell population appropriate to the normal fetus of 20–22 weeks. If one assumes that there has not been a net cell loss in the lungs of these infants the finding must indicate the latest time at which growth could have ceased. The trend of our fetal lung DNA measurements suggests that the hypoplastic lung continues to increase its cell population, but at a very slow rate, since the total lung DNA levels of the fetuses with hypoplastic lungs near term are consistently greater than those of similar preterm infants. However the lung hypoplasia recognised as the only organ abnormality in cases where oligohydramnios dated from 14 to 20 weeks' gestation was comparable in nature and severity with that seen in cases of renal agenesis (Fig. 4, and related paper page 606).[15] This focuses attention on the early second trimester as a critical period for human fetal lung growth. The graph of total lung DNA increase in normal fetuses shows that the lung cell population doubles between 17 and 20 weeks' gestation, a more rapid rate of proliferation than at any subsequent period. An influence which caused even a temporary impairment in lung growth at this stage might have a disproportionate effect on lung size at birth. Recent studies on lung growth in experimental animals have shown that both lung liquid secretion and neuromuscular function are important for normal fetal lung growth.[16–18] We have suggested that fetal respiratory activity may influence development of the acinus by controlling the volume of liquid retained within the lungs.[18] Since amniocentesis in experimental animals in the early fetal period is known to cause lung hypoplasia[19 20] and a wide variety of influences can inhibit fetal respiratory activity,[21] it seems probable that procedures—such as diagnostic amniocentesis and fetoscopy which are performed in the second trimester—could impair fetal lung development.

Although it would not be surprising to find unduly small lungs in growth-retarded fetuses, since they have often been subjected to hypoxia or ischaemia and may be associated with oligohydramnios,[22] lung cell population in our group of cases was reduced in proportion to the degree of body weight reduction.

The relationship between lung/body weight ratios and lung DNA per kg body weight seems to us of some practical help in deciding how to define lung hypoplasia. As was to be expected the relationship between lung weight and lung DNA shows considerable variation when the lungs are large relative to the body weight due to the wide range of fluid content. In fairly small lungs the correlation seems much closer. In all cases where the lung/body weight ratio was $<0{\cdot}01$ lung DNA was <100 mg/kg body weight. There are thus strict limits to the extent to which lung collapse or 'atelectasis' can account for undersized lungs in the perinatal period. Excessive fluid content in the form of haemorrhage or oedema does not often seem to conceal significant lung hypoplasia, presumably because the reduction in development of the peripheral part of the acinus limits the volume of fluid that can be accommodated.

It should be noted that the relationship between lung size (weight or DNA content) and body weight varies with gestational age; the lungs at 20 weeks' gestation are twice as large in proportion to body weight as they are at term. Collection of further data should allow us to establish improved definitions of lung hypoplasia based on the confidence limits for lung/body weight ratio and lung DNA/body weight ratio at varying gestational age.

Quantitative lung DNA measurements are in no way a substitute for careful morphometric studies on lung development. They do however provide useful

additional information both on normal and abnormal fetal lung growth and confirm the validity of low lung/body weight ratios as a simple means of assessing lung hypoplasia.

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References

1 Wigglesworth J S. Aetiology of hyaline membrane disease. *Arch Dis Child* 1979; **54**: 835–7.
2 Potter E L. Bilateral absence of ureters and kidneys: a report of 50 cases. *Obstet Gynecol* 1965; **25**: 3–12.
3 Finegold M J, Katzew H, Genieser N B, Becker M H. Lung structure in thoracic dystrophy. *Am J Dis Child* 1971; **122**: 153–9.
4 Askenazi S S, Perlman M. Pulmonary hypoplasia: lung weight and radial alveolar count as criteria of diagnosis. *Arch Dis Child* 1979; **54**: 614–8.
5 Bain A D, Smith I I, Gauld I K. Newborn born after prolonged leakage of liquor amnii. *Br Med J* 1964; **ii**: 598–9.
6 Perlman M, Williams J, Hirsch M. Neonatal pulmonary hypoplasia after prolonged leakage of amniotic fluid. *Arch Dis Child* 1976; **51**: 349–53.
7 Swischuk L E, Richardson C J, Nichols M M, Ingman M J. Primary pulmonary hypoplasia in the neonate. *J Pediatr* 1979; **95**: 573–7
8 Winick M, Rosso P, Waterlow J. Cellular growth of cerebrum, cerebellum, and brain stem in normal and marasmic children. *Exp Neurol* 1970; **26**: 393–400.
9 Dobbing J, Sands J. Quantitative growth and development of human brain. *Arch Dis Child* 1973; **48**: 757–67.
10 Dorovini-Zis K, Dolman C L. Gestational development of brain. *Arch Pathol Lab Med* 1977; **101**: 192–5.
11 Le Pecq J B, Paoletti C. A new fluorometric method for RNA and DNA determination. *Anal Biochem* 1966; **17**: 100–7.
12 Enesco M, Leblond C P. Increase in cell number as a factor in the growth of the organs and tissues of the young male rat. *J Embryol Exp Morphol* 1962; **10**: 530–62.
13 Emery J L, Mithal A. The number of alveoli in the terminal respiratory unit of man during late intra-uterine life and childhood. *Arch Dis Child* 1960; **35**: 544–7.
14 Hislop A, Hey E, Reid L. The lungs in congenital bilateral renal agenesis and dysplasia. *Arch Dis Child* 1979; **54**: 32–8.
15 Wigglesworth J S, Desai R, Guerrini P. Fetal lung hypoplasia: biochemical and structural variations and their possible significance. *Arch Dis Child* 1981; **56**: 606–15.
16 Alcorn D, Adamson T M, Lambert T F, Maloney J E, Ritchie B C, Robinson P M. Effects of chronic tracheal ligation and drainage in the fetal lamb lung. *J Anat* 1977; **123**: 649–60.
17 Wigglesworth J S, Winston R M L, Bartlett K. Influence of the central nervous system on fetal lung development. *Arch Dis Child* 1977; **52**: 965–7.
18 Wigglesworth J S, Desai R. Effects on lung growth of cervical cord section in the rabbit fetus. *Early Hum Dev* 1979; **3**: 51–65.
19 Blackburn W R, Logsdon P A, Delli-Bovi J. Fetal lung development after amniocentesis (abstract). *Pediatr Res* 1978; **12**: 515.
20 Moessinger A, Ballantyne G, James L S, Blanc W A. Lung hypoplasia and selective growth retardation in experimental oligohydramnios (abstract). *Fed Proc* 1978; **37**: 854.
21 Boddy K, Dawes G S. Fetal breathing. *Br Med Bull* 1975; **31**: 3–7.
22 Elliott P M, Inman W H W. Volume of liquor amnii in normal and abnormal pregnancy. *Lancet* 1961; **ii**: 835–40.

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