Fetal lung hypoplasia: biochemical and structural variations and their possible significance

J S WIGGLESWORTH, R DESAI, AND P GUERRINI

Department of Paediatrics and Neonatal Medicine, Institute of Child Health, Hammersmith Hospital, London

SUMMARY Quantitative biochemical criteria for lung growth and maturation were compared with the histological appearances in hypoplastic lungs from 20 fetuses and newborn infants. Cases associated with oligohydramnios showed a characteristic series of changes with narrow airways, retardation of epithelial and interstitial growth, delay in development of blood–air barriers, and low concentrations of phospholipid phosphorus, lecithin phosphorus, total palmitate, and lecithin palmitate. The growth and maturation arrest appeared to affect the peripheral part of the acinus. Examples of other types of lung hypoplasia showed different features. Hypoplastic lungs from infants with normal or increased amniotic fluid were of mature structure with phospholipid concentrations similar to those of infants with normally developed lungs at term. The hypoplastic left lung in 2 cases of congenital diaphragmatic hernia had an immature structure with low phospholipid concentrations, whereas the right lung was structurally and biochemically more mature. It is suggested that fetal lung growth may be impaired by any influence which reduces thoracic volume but that maturation arrest is due specifically to loss of the ability to retain lung liquid.

It is now recognised that hypoplasia of the fetal lungs is the immediate cause of neonatal death in a number of malformation syndromes. These include all conditions with failure of fetal urine production or obstruction of urine output,¹ aplasia or eventration of the diaphragm,² thoracic abnormalities—such as congenital thoracic dystrophy and thanatophoric dwarfism³—and abnormalities of the central nervous system—including anencephaly, iniencephaly, and Meckel-Gruber syndrome.^{4,5} In addition, pulmonary hypoplasia may be seen in conditions arising after the embryonic period including prolonged rupture of the membranes,^{6,7} or severe hydramnios,⁸ and as a primary condition in otherwise normal infants.⁹

Many of the structural and functional features of hypoplastic fetal lungs remain poorly defined despite an increasing interest in the condition. Morphometric studies suggest that an important component in several forms of pulmonary hypoplasia is reduction in the number of generations of bronchial branching.^{10–12} There is less agreement about other aspects of lung structure and development. It has been claimed that hypoplastic lungs occurring in conditions with reduced intrathoracic space show decrease in lung parenchyma and have closely packed normal bronchi, whereas those associated with renal agenesis may show generalised maturation

arrest.¹³ Other studies on infants with renal agenesis or dysplasia have found the lungs to be structurally mature with normal surface properties,^{11,14} or not to differ from those associated with other malformations.¹⁵ The left and right lungs of infants with unilateral congenital diaphragmatic hernia have been found not to differ,¹⁵ to differ structurally,² or to differ both structurally and biochemically.¹⁶ It has been claimed that all the differences between the hypoplastic lungs in different malformation syndromes are due to variation in the time at which lung growth became retarded.¹⁵

There is clearly a need to determine whether the different structural patterns of lung hypoplasia can indeed be explained on the basis of variation in timing of the growth arrest, or whether they reflect differences in the mechanism which interferes with lung growth in different circumstances. Resolution of this problem may well provide new information on the mechanisms controlling normal lung development.

We have attempted to answer this question by comparing quantitative biochemical criteria for lung growth and maturation with the histological appearances in a series of hypoplastic lungs associated with a variety of malformation syndromes.

Materials and methods

Lungs were examined from 18 fetuses or newborn infants with uniform bilateral lung hypoplasia defined as described previously¹⁷ (lung/body weight ratio of ≤ 0.015 at <28 weeks' gestation, and lung/body weight ratio ≤ 0.012 at 28 weeks' gestation or more). Two additional infants with left-sided congenital diaphragmatic hernia and hypoplasia predominantly of the left lung were also examined. Control material for phospholipid studies included lungs from 13 infants who died with hyaline membrane disease (HMD) within the first 36 hours of life at 27–36 weeks' gestation and the lungs of 10 infants without HMD who died during birth or within the first 4 days at 34–41 weeks' gestation. DNA measurements and histological material were available from all the lungs of normal and small-for-dates fetuses and infants reported in the preceding paper (page 601).¹⁷ Initial handling of the lungs and DNA estimation was as previously described. Lung lipids were extracted from the lung homogenates in chloroform/methanol and dried under nitrogen. Acidic and non-acidic phospholipids were separated by DEAE cellulose acetate column chromatography using the method of Gluck *et al.*¹⁸ Lecithin was isolated by thin-layer chromatography as an aliquot of the non-acidic phospholipid fraction on TLC-plastic sheet silica gel 60, No 5748 (Merck). The running solvent was chloroform:methanol:water (65:25:4 by volume). Dipalmitoyl lecithin (Sigma) was run as a standard.

The concentration of phosphorus in the lecithin fraction and in the total phospholipid extract of each lung was determined by the method of Bartlett.¹⁹ The palmitic acid content of the lung lecithin and of the total lung phospholipid was measured by gas-liquid chromatography. The fatty acids were esterified using 5% H_2SO_4 in methanol and extracted in petroleum ether with a boiling point of 60–80°C. An internal standard of margaric acid (Sigma) was added for quantitation. Separation was achieved in 10% (by mass) Silar 10c on Chromosorb W-HP 100–120 mesh (Field Instruments Ltd, Twickenham, Middlesex) at 180°C using a Pye Unicam Model 104a gas chromatograph.

Results

Clinical details and macroscopical findings. Details of the cases are summarised in Table 1. Cases 1–18 had uniform bilateral lung hypoplasia. In 14 this was associated with prolonged oligohydramnios due to failure of fetal urine production, obstruction to urinary output, or prolonged rupture of membranes,

but in the other 4 there was both a normal fetal urinary tract and intact amniotic sac. The external features of Potter's syndrome were recognised in all of the infants with renal agenesis or severe renal cystic dysplasia associated with failure of urine production (the latter evidenced by thread-like ureters and a tubular bladder). Similar external appearances were seen in Cases 2, 3, and 4 with prolonged oligohydramnios dating from before 20 weeks' gestation but normal renal tract. Of the 4 infants with normal renal tract and intact amniotic sac, 2 had exomphalos and 2 had conditions associated with polyhydramnios (intrauterine damage to brain stem nuclei and cytomegalovirus infection).

Cases 19 and 20 had congenital left diaphragmatic hernia with the asymmetrical lung hypoplasia characteristic of this condition. One of them had suffered amniotic fluid leakage for 5 weeks before birth at 30 weeks' gestation (lecithin/sphingomyelin ratio in the leaking amniotic fluid 2 weeks before delivery was 2.4), and the other was a term infant who died soon after corrective surgery.

The hypoplastic lungs in all instances had normal development of lobes and fissures. The 2 cases with prune-belly syndrome showed distortion of the rib cage due to pressure from the enlarged bladder. This was more pronounced at 35 weeks' gestation than at 20 weeks. The development and form of the thoracic cage, intercostal muscles, and diaphragm appeared normal in the rest of the oligohydramnios group. In most of the cases of renal agenesis or severe renal cystic dysplasia the lungs were deeply congested with obvious haemorrhage. Several showed pneumothorax and, despite periods of up to 22 hours' mechanical ventilation in life, all appeared totally airless and collapsed at necropsy. The lungs of the infants with exomphalos appeared oedematous and were associated with severe lordosis and rib cage distortion in one (Case 14), and thoracic narrowing in the other (Case 18). In one of the infants with congenital diaphragmatic hernia (Case 20) there was good aeration of the moderately undersized right lung, but the hypoplastic left lung was almost entirely airless in both cases.

Biochemical findings. The lung DNA content expressed in relation to body weight is shown in Table 1. All infants born at 31 weeks' gestation or more had values of less than 100 mg lung DNA per kg body weight. The DNA values for the left and right lungs of Cases 19 and 20 were assessed separately (Table 2). Both the total DNA and DNA concentration tended to be lower in the grossly hypoplastic left lung. The lung DNA findings in the cases of bilateral lung hypoplasia are illustrated and

Table 1 Details of 20 cases with lung hypoplasia

Case	Sex	Gestation (weeks)	Age at death (hours)	Body weight (g)	Lung weight (g)	Lung/body ratio	DNA (mg/kg)	Pathological summary
1	M	20	0	355	5.01	0.014	141	Prune-belly syndrome with total urine outflow obstruction
2	F	25	0	770	9.34	0.012	102	Prolonged rupture of membranes from 14 weeks. Normal renal tract
3	F	27	0	512	5.90	0.012	109	Repeated threatened abortion from 13 weeks. Prolonged oligohydramnios. Normal renal tract
4	M	30	6	1570	18.39	0.012	115	Prolonged rupture of membranes. Normal renal tract
5	F	31	0	1400	12.37	0.009	67	Fetal cytomegalovirus infection. Hydramnios
6	M	32	0	996	7.16	0.007	76	Renal cystic dysplasia with thread-like ureters. Hydrocephalus
7	M	34	2	1900	11.34	0.006	53	Renal agenesis
8	F	34	0	1980	15.34	0.008	68	Flexion deformities. Sclerosis of brain stem nuclei. Hydramnios
9	M	35	0	1900	13.35	0.007	63	Prune-belly syndrome with total urinary outflow obstruction and bilateral hydronephrosis. Oesophageal atresia with tracheo-oesophageal fistula. Anal atresia
10	M	36	3	1960	13.86	0.007	62	Renal cystic dysplasia with thread-like ureters and tubular bladder
11	F	36	<1	1615	9.54	0.006	56	Renal cystic dysplasia with thread-like ureters and tubular bladder
12	M	37	22	1651	20.64	0.012	70	Renal agenesis
13	M	38	3	2458	13.65	0.006	40	Renal cystic dysplasia with thread-like ureters and tubular bladder
14	F	38	0	2600	16.92	0.007	46	Exomphalos with distortion of thorax and diaphragm. Absent left kidney. Normal right kidney, ureter, and bladder. Anal atresia
15	M	39	6	3102	23.08	0.007	57	Renal cystic dysplasia with thread-like ureters and tubular bladder. Oesophageal atresia and tracheo-oesophageal fistula. Truncus arteriosus. Imperforate anus
16	M	39	5	2442	11.5	0.005	35	Renal agenesis
17	M	40	12	4190	43.41	0.010	83	Bilateral hydronephrosis associated with bladder hypertrophy due to valvular obstruction to posterior urethra
18	F	40	3 days	2680	31.45	0.012	78	Exomphalos
19	M	30	4	1500	12.42	0.008	120	Congenital left diaphragmatic hernia. Prolonged rupture of membranes
20	F	38	39	3005	9.15 right 3.27 left 23* 18 right 5 left	0.008	68	Congenital left diaphragmatic hernia. Death after operative repair

*Lungs of this case weighed to nearest 0.5 g.

Table 2 Lung DNA in congenital left diaphragmatic hernia

Case	Gestation (weeks)	Right lung DNA		Left lung DNA	
		Total (mg)	Concentration (mg/g wet weight)	Total (mg)	Concentration (mg/g wet weight)
19	30	90	9.8	31	9.4
20	38	171	9.6	33	5.5

discussed in greater detail in the previous paper (page 601).¹⁷

In Fig. 1 the lung phospholipid levels of the 9 infants with renal anomalies or urinary outflow obstruction of 34 weeks' gestation or more are compared with those of control infants without HMD of a similar gestational age range, and with those of control infants who died within the first 36 hours of life with HMD. As the phospholipid levels are expressed in proportion to lung DNA they

represent quantity of phospholipid per lung cell and are independent of lung size. In control infants there was no relationship between length of survival and lung phospholipids; both the highest and lowest values were seen in fresh stillbirths. All phospholipid parameters of the lung hypoplasia group were significantly lower than those of control lungs without HMD but similar to those of the lungs of less mature infants who died in the acute phase of HMD. The lung phospholipid levels in cases of lung

Fig. 1 Comparison of lung phospholipid levels between control lungs, cases of lung hypoplasia associated with oligohydramnios, and lungs of infants with hyaline membrane disease. All phospholipid parameters in the lung hypoplasia group are significantly lower than those of the controls ($P < 0.01$, Wilcoxon rank sum test) but do not differ from those of the group with hyaline membrane disease.

hypoplasia without oligohydramnios born after 34 weeks' gestation and the right and left lungs in diaphragmatic hernia are shown in Fig. 2. Values within the range of the control infants were seen in the lungs from infants without oligohydramnios and in the right lungs of the cases of diaphragmatic hernia, but the left lungs of the infants with diaphragmatic hernia had very low phospholipid levels.

Lung histology.

Hypoplastic lungs associated with oligohydramnios

The hypoplastic lungs of the babies with oligohydramnios showed changes which in most cases were

Fig. 2 Lung phospholipid levels in infants with hypoplastic lungs associated with normal or increased amniotic fluid and in right and left lungs of infants with diaphragmatic hernia.

readily recognisable as belonging to a similar pattern despite variations in gestational age.

At 20 weeks' gestation (Case 1) the lung had a pseudoglandular appearance, but with closely packed tubular epithelial elements with little intervening mesenchymal tissue (Fig. 3). Control lungs at the same gestation showed a canalicular structure with early formation of blood-air barriers and extensive mesenchymal tissue between the branching acini (Fig. 4).

At 25 weeks' gestation (Case 2) there was clear evidence of arrest both in growth and maturation (Fig. 5). By this stage the lung was in a canalicular phase and the peripheral parts of the acinus were represented by narrow tubules lined by cuboidal epithelium. Patchy haemorrhage had occurred into the sparse interstitial tissue. Blood-air barriers were seen only in the centre of the acinus near the termination of the respiratory bronchioles. In contrast the normal lung at this period was in an early sacular phase of development with well developed blood-air barriers but plentiful persistent interstitial tissue (Fig. 6).

Lungs of 8 infants, ranging from 27 to 38 weeks' gestational age, showed essentially similar features. There were well developed blood-air barriers in the central part of the acinus, but peripheral parts were represented by simple tubules lined by cuboidal epithelium (Figs 7 and 8). Interstitial tissue was in most instances disrupted by extensive haemorrhage, and in several by emphysema. Superimposed HMD was seen in lungs of 2 infants (Cases 4 and 12) who had survived for 6 and 22 hours. Lungs from 4

Visual comparison of abnormal and control at same gestation

Visual comparison of normal and abnormal at different gestations

Fig. 3 Lung of Case 1 (prune-belly syndrome with total urinary outflow obstruction), 20 weeks' gestation. Parenchyma consists largely of closely packed tubular epithelial structures. Compare with Fig. 4. H and E $\times 100$.

Fig. 4 Control lung at 20 weeks' gestation. Canicular phase of development. Plentiful interstitial tissue. H and E $\times 100$.

Fig. 5 Lung of Case 2 (prolonged rupture of membranes), 25 weeks' gestation. The lung is congested but shows little advance in maturation from that illustrated in Fig. 3. Compare with Fig. 6. H and E $\times 100$.

Fig. 6 Control lung at 25 weeks' gestation. The terminal airways show the irregular outline characteristic of the saccular phase of development and associated with the formation of blood-air barriers. H and E $\times 100$.

Fig. 7 Lung of Case 13 (severe renal cystic dysplasia), 38 weeks' gestation. The lung is distorted by interstitial haemorrhage but multiple tubular epithelial structures persist throughout the parenchyma. Compare with Figs 3 and 5. H and E $\times 100$.

infants (Cases 7, 15, 16, and 17) had less obviously abnormal structure with more advanced development of blood-air barriers throughout the acini. Careful examination showed persistent poorly developed

areas at the periphery of the acini in these cases also (Fig. 9). Small calibre of the bronchi and bronchioles and collapse of the acini was a feature of all cases.

Fig. 8 Part of section illustrated in Fig. 7. Epithelial structures 'ep' represent areas of growth and maturation arrest at the periphery of the acinus. Blood-air barriers 'bab' have developed in the central area near the end of the respiratory bronchiole 'RB'. H and E $\times$ 400.

Fig. 9 Lung of Case 16 (renal agenesis), 39 weeks' gestation. Retarded maturity is not obvious in this case but areas of cuboidal epithelium 'ep' persist at the periphery of some acini. H and E $\times$ 400.

Hypoplastic lungs from cases without oligohydramnios
The lungs of Case 5 appeared moderately immature, but with more interstitial tissue than seen in the oligohydramnios cases. Interpretation was complicated by the presence of the histological features of cytomegalovirus infection. The lungs of the other

3 infants appeared mature in structure. In particular, the severely hypoplastic lungs of Case 14 showed grossly dilated acini and thinned out saccules with plentiful blood-air barriers (Fig. 10). The appearance was in striking contrast to that of the cases associated with oligohydramnios.

Fig. 10 Lung of Case 14 (exomphalos with thoracic distortion), 38 weeks' gestation. Overdistended lung with thinned-out congested alveolar walls of mature structure. H and E $\times 150$.

Fig. 11 Right lung of Case 20 (left diaphragmatic hernia). Alveolar walls are collapsed and appear thick, but the acinar pattern beyond the respiratory bronchiole 'RB' appears normal. H and E $\times 150$.

Fig. 12 Left lung of Case 20 (left diaphragmatic hernia). Large, irregular, thick-walled air spaces. The appearance differs from that of the right lung and that of other forms of lung hypoplasia (Figs 7 and 10). The structure appears immature but is not normal for any stage of development. H and E $\times 150$.

Although neither lung of the diaphragmatic hernia cases appeared entirely normal the left lung in each instance was recognisably less mature in structure than the right (Figs 11 and 12). Case 19 showed HMD affecting the left lung only.

Discussion

This prospective study of hypoplastic lungs has shown a clear sequence of changes in those cases associated with oligohydramnios. Impairment in quantitative growth in terms of total DNA may be apparent by 20 weeks' gestation and is accompanied at histological level by retarded development of epithelial and interstitial tissues. There is poor saccular branching and retarded epithelial maturation at the periphery of the acinus. This is associated with poor development of interstitial tissue and delayed vascularisation of the saccules to form blood-air barriers. Lung collapse is a uniform feature, despite mechanical ventilation for varied periods in life. Low lung phospholipid levels confirm the impairment in lung maturation.

The few examples of lung hypoplasia from conditions not associated with oligohydramnios show a variety of different features. The mature saccular wall structure and normal phospholipid content of lungs of cases with exomphalos differ totally from the findings in the oligohydramnios group despite reduction of lung cell population to a similar extent.

The fluid-filled poorly developed saccules of the hypoplastic lung in diaphragmatic hernia at term are also unlike the lungs of the oligohydramnios group. The coincidence of diaphragmatic hernia and prolonged amniotic fluid leakage was associated with unilateral HMD despite a normal L/S ratio in the amniotic fluid which leaked before delivery.

The clarity of our findings may appear to conflict with previous studies on the subject. Reale and Esterly¹⁵ found no qualitative differences in the type of lung hypoplasia associated with renal anomalies, diaphragmatic hernia, and anencephaly. Their cases had been chosen according to the primary malformations rather than on the basis of lung hypoplasia, and lungs of a normal size were included in their study. Qualitative interpretation may have been influenced by the range of normal and hypoplastic lungs within each malformation group. Our studies have been limited to frankly hypoplastic lungs and we have been lucky to obtain specimens over a wide range of gestational ages. The low lung volume in hypoplastic lungs from cases of renal agenesis or severe renal cystic dysplasia recorded by Hislop *et al.*¹¹ is in agreement with our observation of impaired development of the periphery of the acinus in these cases. Hislop *et al.* considered that maturation of the acini was normal in their series although the terminal sacs were small. The process of preparing the lungs for morphometric measurements by injection of airways and vessels may well have

obscured some of the qualitative differences in the peripheral parts of the acinus and a larger proportion of the cases studied may have been at the more mature end of the histological spectrum. Pressure volume measurements by these authors suggested presence of surfactant in all cases and alveolar stability in some. Our quantitative phospholipid measurements do not give a precise measurement of lung stability and it is likely that the central parts of the acini, which appear structurally mature, do have a stable lining layer in some cases. Hyaline membrane formation was seen in only 2 instances in our series.

The pronounced difference in phospholipid level between the left and right lungs of the cases with diaphragmatic hernias is similar to the data for a larger series recorded by Blackburn *et al.*¹⁶ Such findings provide good evidence that endocrine factors stimulating surfactant production can only operate if the respiratory epithelium has reached an appropriate stage of differentiation.

We could detect no difference between the structural appearance of the lungs after rupture of the membranes dating from before 20 weeks' gestation in the presence of normal kidneys and that seen in cases of renal agenesis. The finding supports the view that the lung changes are due to oligohydramnios rather than to lack of some direct influence of the fetal kidney on the internal environment of the fetus.

It has been widely accepted that the mechanism of inhibition of fetal lung growth in oligohydramnios is by a process of fetal compression.^{1 20 21} As the thoracic cage is well developed and of normal form in such infants any lung compression *in utero* must be exerted by transmission of increased intra-abdominal pressure through a raised diaphragm. The total collapse, narrow airways, and small terminal sacs in these lungs indicates that they have contained very little lung liquid. Recent experimental evidence indicates that normal fetal lung development requires both secretion of lung liquid and its retention within the airways.²² We suggest that the impairment in lung growth and maturation of the fetal lung in oligohydramnios is due to loss of the ability to retain liquid within the fetal airways. Further investigation is needed to determine whether this lack of fluid retention is due purely to enhanced liquid outflow at the larynx by a simple pressure effect,²⁰ or whether it involves inhibition of neuromuscular activity or lung liquid secretion in addition.

It seems possible to explain the varied forms of lung hypoplasia if we assume that any reduction in intrathoracic volume will impair fetal lung growth

but only a total failure to retain lung liquid will retard maturation. On the basis of this hypothesis oligohydramnios of any cause results in lung hypoplasia with maturation arrest due to failure of lung liquid retention. Thoracic distortion associated with a fixed depressed diaphragm, as in exomphalos, allows liquid retention which enables the hypoplastic lungs to mature. Damage to the central nervous system bringing about impairment in fetal respiratory activity and fetal swallowing, but without positive lung deflationary pressures, will retard fetal lung growth but may have little effect on maturation. Congenital left diaphragmatic hernia causes total collapse of the periphery of the left lung, and will retard its maturation, but it will generally only cause partial reduction in space of the right pleural cavity. Indeed the distortion of trachea and main bronchi in this condition may allow very variable quantities of lung liquid to be retained in the right lung and the larger airways of the left lung. Such distortion would account for normal maturation of the right lung of our Case 19 despite prolonged oligohydramnios.

If this analysis is correct we may expect to encounter cases where (1) renal agenesis combined with obstruction or distortion to upper airways is associated with normally grown mature lungs despite oligohydramnios, and (2) intrauterine damage of the central nervous system causing severe atrophy of the diaphragm is associated with arrest of lung growth and maturation despite a normal amniotic fluid volume. Further study of structure and biochemical composition of lungs from the varied forms of human fetal lung hypoplasia may thus allow us to support or refute the hypothesis, quite apart from any tests of it we may make using experimental models.

Our studies indicate that growth and maturation of the human fetal lung can be profoundly retarded by influences such as amniotic fluid leakage arising after the period of organogenesis. Since overt hypoplasia is merely the end point of a spectrum of changes, this vulnerability of the developing fetal lung may well prove to be one of the major factors determining perinatal mortality and morbidity.

Addendum

Since submitting this paper we have studied 8 additional sets of lungs from cases of bilateral lung hypoplasia including 5 with normal or increased amniotic fluid. Further differences which we have established in this extended series are the presence of normal elastic tissue development and normal radial alveolar counts in cases with normal or increased amniotic fluid as compared with lack of

stainable elastic tissue and low radial alveolar counts in the oligohydramnios group. These findings will be the subject of a further publication.

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Correspondence to Dr J S Wigglesworth, Department of Paediatrics and Neonatal Medicine, Hammersmith Hospital, Du Cane Road, London W12 OHS.

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