

CONGENITAL POSTEROLATERAL
DIAPHRAGMATIC
HERNIA

*Pathophysiological studies
and
Clinical Picture*

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CONGENITAL POSTEROLATERAL
DIAPHRAGMATIC
HERNIA

Pathophysiological studies and Clinical Picture

CONGENITALE HERNIA DIAPHRAGMATICA

PROEFSCHRIFT

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aan de Erasmus Universiteit Rotterdam
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Prof. Dr. C.J. Rijnvos
en volgens het besluit van het College van Dekanen.
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This study was carried out at the Pediatric Surgical Intensive Care Unit, Department of Pediatric Surgery, Sophia Children's Hospital, and the Laboratory for Experimental Surgery, Erasmus University Medical School, Rotterdam, The Netherlands.



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aan Aukje en Elke

My heart leaps up when I behold
A rainbow in the sky:
So was it when my life began;
So is it now I am a man;
So be it when I shall grow old,
Or let me die!
The child is father of the man.

William Wordsworth -

When technology is master,
we shall reach disaster faster.

Piet Hein

VOORWOORD

Met veel plezier begin ik dit proefschrift met het uitspreken van mijn dank en waardering aan hen die aan mijn vorming tot kinderarts hebben bijgedragen. Met naam wil ik noemen mijn opleiders Prof. Dr. E.D.A.M. Schretlen en Prof. Dr. G.B.A. Stoelinga. Dr. A.J. van Vught heeft mij de kneepjes van de paediatrische intensive care bijgebracht en gewezen op de noodzaak om stoornissen in "vitale functies" te vertalen naar de onderliggende pathofysiologische processen.

Bij de voltooiing van dit manuscript hebben velen geholpen.

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Ik vind het jammer dat mijn vader en moeder deze dag niet meer kunnen meemaken.

Zonder Boudien en de kinderen zou mijn leven niet zijn wat het is.

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INTRODUCTION

Congenital diaphragmatic hernias are classified according to the location of the defect: posterolateral hernia with or without a sac (Bochdalek-type), parasternal hernia through the foramen of Morgagni, central hernia, and diaphragmatic eventration. The so-called hiatal hernia has a different pathophysiology and a different clinical presentation. Posterolateral defects account for 85% of congenital diaphragmatic hernias.^{1,2} Left-sided posterolateral defects occur eight times more frequently than right-sided defects in the neonatal period. Bilateral defects are rare and estimated at 3 to 5%.³

Children at the highest risk of dying are they who are symptomatic in the delivery room or within six hours after birth.⁴ The mortality rate reported in this group ranges from 40 to 50%.^{5,6} Conversely, those who do not develop respiratory failure in the first 24 hours of life have an almost 100% chance of survival.

In contrast to other major congenital anomalies the mortality due to congenital posterolateral diaphragmatic hernia remains high despite improvements in neonatal intensive care and pediatric surgery.^{7,8} The main reason is that pulmonary hypoplasia and pulmonary hypertension form the pathophysiological basis for the clinical picture of respiratory distress and right-to-left shunting that frustrates so many clinicians responsible for newborns with a diaphragmatic defect.

This thesis presents the research into clinical aspects of congenital posterolateral diaphragmatic hernia related to pulmonary hypoplasia and pulmonary hypertension; the pathophysiological background of these disorders was studied in an animal model. The clinical studies were all carried out in the Pediatric Surgical Intensive Care Unit of the Sophia Children's Hospital. The animal model was developed by TenBrinck and coworkers in the Laboratory of Experimental Surgery of the Erasmus University in Rotterdam.⁹ In this model congenital diaphragmatic hernia is induced in fetal rats by means of administering a single dose of Nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether) on the 10th day of gestation. The model interferes with lung development in an early stage, thus providing the opportunity to study several aspects of lung development in relation to both ventilatory capacity and pulmonary vascular reactivity.

The results of the conducted studies, as they have been reported and discussed in papers either published or accepted by international journals, form the core of this thesis. These papers are preceded by a review of the literature focussed on historical aspects, clinical picture and normal and abnormal lung development. They are followed by a concluding chapter in which changing concepts concerning pathogenesis and treatment are discussed.

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Chapter 1

REVIEW OF THE LITERATURE

- 1.1 *History*
- 1.2 *Epidemiology*
- 1.3 *Etiology and animal models*
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 - 1. Antenatal presentation
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REVIEW OF THE LITERATURE

1.1 History

Diaphragmatic defects caused by sword and knife were already known to Hippocrates of Cos, who thought that such wounds were inevitably "mortal". Congenital diaphragmatic hernia was first recorded in 1679 by Lazarus Riverius as an incidental observation during the autopsy of a 24-year-old man.¹ In 1701, Sir Charles Holt reported congenital diaphragmatic hernia in a two-month-old infant²,
...when we had opened the abdomen ...part of the duodenum passed thro a foramen in the diaphragm, placed on the left side.

In 1769, Morgagni, a pupil of Valsalva, described the congenital anterior diaphragmatic defect named after him.³ In 1824, Sir Astley Cooper published the case history of a 28 year-old woman who died after recurrent episodes of intestinal strangulation through a diaphragmatic defect. In the same treatise he mentioned two newborn infants who died within hours after birth due to congenital diaphragmatic hernia, and he already noted underdevelopment of the lung⁴,
...the left lobes of the lungs were not larger than a small nutmeg, and about two-thirds less than the right.

In 1848, Vincent Alexander Bochdalek, professor of Anatomy at Prague, described herniation of the bowel through the posterior part of the diaphragm, a defect that now bears his name.⁵ In this century, Bowditch reported the first series of congenital diaphragmatic hernia and gave a classic description of the clinical symptoms⁶,
...no respiration over whole of left chest. In its place a mixture of gurgling, whistling, and blowing sounds was heard, like those heard over the abdomen.

Among his patients were twenty-six with a congenital diaphragmatic defect. Eleven of them died within hours after birth, six within two years, one at the age of seven, and eight during adult life. In 1902, Heidenhain gave the first account of successful surgical repair; the patient was a nine-year-old boy.⁷ Greenwald and Steiner published a report on 43 cases of diaphragmatic hernia in infancy and childhood in 1929, and categorized the signs and symptoms by age groups. There were 13 stillborns and 24 of the other 30 neonates died within 36 hours of birth. They concluded⁸,
...for the patient in whom the hernia makes its appearance at birth, little or nothing can be done from a surgical standpoint.

Eleven years later Ladd and Gross reported 16 children who underwent operation and advocated early surgical repair in the first 48 hours of life⁹,

...the policy of waiting until the child gets older and stronger is apparently responsible for the loss of a great many lives that might have been saved by a timely operation.

The use of endotracheal intubation and assisted ventilation became routine procedure during early surgical repair since 1950. Gross reported 63 cases of Bochdalek hernia, and he optimistically stated in 1953¹⁰,

...surgical therapy is highly satisfactory regardless of the extent of the herniation and the apparent critical condition of the child.

In 1955, Campanale and Rowland described the presence of pulmonary hypoplasia in five newborn infants with a congenital diaphragmatic hernia, in whom they noted a marked reduction in number and differentiation of alveolar ducts and alveoli.¹¹ These observations were confirmed by Butler and Claireaux who drew the attention to the presence of hypoplasia in the contralateral lung as well. They also noted an association with other major congenital anomalies and their contribution to mortality.¹² Moore had already observed that the hypoplastic lungs are vulnerable to positive pressure ventilation¹³,

...the vigorous use of positive pressure through the endotracheal tube must be avoided. These lungs cannot be expanded by this type of effort and only harm can result.

In 1964, Gross looked back at experiences of the Boston Children's Hospital and found it disturbing that over the years the cure rates of congenital diaphragmatic hernia had dropped. This is possibly explained by a much higher proportion of babies less than 24 hours of age coming for treatment.¹⁴

Areechon and Reid drew attention to the long-term growth and development of the hypoplastic lung and stated¹⁵,

...no correction of the bronchial and bronchiolar deficiency will occur; but that growth within the acini will be great enough to provide the lung with its normal complement of alveoli.

They also pointed out that animal experiments were needed to study the normal growth of the lung as well as growth compensatory to disease.

The problem of neonatal hypoxemia due to pulmonary hypertension, and subsequent right-to-left shunting of undersaturated blood through fetal channels such as the foramen ovale and the ductus arteriosus, was gradually recognized during the 1960s. Naeye and Letts studied infants with neonatal hypoxemia and found thickened smooth muscle in pulmonary arterial vessel walls. They also noted that the usual postnatal decrease in musculature and thickness of the pulmonary vascular bed did not occur.¹⁶ Kitagawa made a detailed study of the lungs of one child with congenital diaphragmatic hernia and found not only a reduced number of

alveoli, but also a pathological, excessive muscularization of pre-acinar arteries.¹⁷ Both Murdock¹⁸ and Rowe¹⁹ reported large alveolar-to-arterial oxygen tension differences between radial artery and descending aorta during adequate alveolar ventilation. They both suggested venoarterial admixture in the lungs, or right-to-left shunting through the ductus arteriosus and foramen ovale.

At the time it was normal pediatric surgical practice to treat congenital diaphragmatic hernia in newborns as an emergency condition. Many afflicted newborns were in a poor clinical condition, due to a combination of hypothermia, asphyxia, and low systemic blood pressure. Postoperatively some patients improved and survived, but others improved only temporarily: the so-called "honeymoon period".

The observation that preoperative treatment aimed at correcting acidosis, hypoxia, and tissue perfusion improves survival, has led to the concept of delayed surgery.²⁰ In several centers around the world this "new" approach has been evaluated. In a prospective pilot study Hazebroek et al concluded that ventilation parameters that are satisfactory on admission, will remain good during the stabilization phase, and that unsatisfactory ventilation parameters may improve.²¹

Extracorporeal membrane oxygenation (ECMO) has been developed in the United States as a treatment modality for neonates with severe respiratory failure. Bartlett published a survey of the initial ECMO-experiences in 100 neonates and concluded that the best survival results were achieved in persistent fetal circulation, and the second best in congenital diaphragmatic hernia.²² Nakayama studied a group of patients treated according to a delayed-surgery protocol in combination with ECMO. He found improved survival but concluded that further studies are needed to determine the benefits of preoperative stabilization.²³ Compared with series from the 1970s and 1980s the mortality rate remained unchanged at 40 to 50% when ECMO was applied.^{24,25,26,27} Some patients may benefit from alternative ventilatory strategies such as high frequency oscillatory ventilation.²⁸ The application of surfactant in these patients or the development of newer drugs to modulate pulmonary vascular tone, however, are only described in anecdotal reports.^{29,30}

A completely different approach was made by the group of Harrison. They advocate repair before birth by means of fetal surgery. One of the disadvantages of this treatment modality is the impossibility to identify patients who really would benefit from such a risky surgical procedure.³¹

Despite all these new developments, congenital diaphragmatic hernia remains a serious congenital anomaly with a high mortality. There is growing awareness that it might be part of a more basic defect, the defect in the diaphragm probably being the least important.

1.2 Epidemiology

Congenital diaphragmatic hernia is a common neonatal disorder. Epidemiological studies from around the world, most of which include stillborns, are summarized in Table 1. They show an incidence of one in 2 to 3000 newborns. A higher incidence was found in Avon (UK) in 1980³⁶; Sarda mentioned a much higher prevalence in stillbirths (one in 74) than in livebirths (one in 2450).³⁷ In the northern part of Netherlands the prevalence is one in 5000 newborns³⁸, in the south-west of the country it is one in 3000.

Diaphragmatic defects are associated with a number of chromosomal, genetic, and nongenetic patterns of malformation. The types of abnormalities vary according to whether stillborns are included in the study or not. The original studies by Butler and Claireaux¹², and David and Illingworth³² included stillborns and showed anomalies other than congenital diaphragmatic hernia in about 50% of cases (Table 2). The central nervous system was affected most frequently (especially neural tube defects).

Table 1. Reported incidence of congenital diaphragmatic hernia

England, 1962 ¹²	1 : 2200
England, 1976 ³²	1 : 1870
England (Avon), 1980 ³⁶	1 : 1850
USA, 1981 ³³	1 : 2644
Hungary, 1985 ³⁴	1 : 2500
France, 1991 ³⁶	1 : 2500
France, 1991 ³⁷	1 : 2450
Netherlands ³⁸	1 : 5000

Table 2. Associated malformations

System	Ref. 12*	32	40*
Nervous	35 %	28 %	18 %
Cardiovascular	12 %	13 %	42 %
Gastrointestinal	5 %	19.6 %	13 %
Genitourinary	10 %	14.7 %	42 %
Skeletal	nr	11.7 %	16 %
Others	nr	13 %	nr
Incidence	47 %	50 %	48 %

*autopsy findings; nr: not reported

Most American studies concentrated on liveborn infants and excluded autopsy data. In 1985, Adzick reported data from a questionnaire on 94 cases of fetal congenital diaphragmatic hernia: 16% of the fetuses had associated lethal anomalies, and isolated nonlethal anomalies presented in an additional 8%.³⁹ A few years later Adzick published the experiences of the Boston Children's Hospital: 11 of the deaths were associated with other lethal anomalies, (almost 30%).⁴¹ Currif and coworkers reviewed the medical records of 102 liveborn children with congenital diaphragmatic hernia to establish patterns of malformation. They found associated anomalies in 40 of them (39%). Eleven patients had previously recognized patterns of malformation, such as chromosomal disorders or Fryns' syndrome. In addition, 13 patients had multiple congenital anomalies that did not represent a recognized pattern of malformation. Thirteen children had only one defect in addition to the diaphragmatic hernia. Cardiac defects were present in 18 newborns, and this group showed a much higher mortality rate (72 vs 38%). It was therefore suggested that preoperative cardiac evaluation should be part of the preoperative workup.⁴²

Congenital diaphragmatic hernia has been reported to present in association with chromosomal abnormalities. The abnormal fetal karyotypes most commonly observed are trisomies 18 and 21.^{39,43} As the fetal karyotype is abnormal in more than 10% of cases, karyotyping should be part of the assessment of children with congenital diaphragmatic hernia and other anomalies.⁴⁴

Diaphragmatic defects can also be part of other circumscribed syndromes such as Fryns' syndrome⁴⁵, Cornelia de Lange syndrome, Apert syndrome⁴², Goldenhar syndrome⁴⁶, Beckwith-Wiedemann syndrome⁴⁷, and Pierre Robin syndrome.³² Other phenotypes that may include diaphragmatic defects are, for instance, Cantrell's pentalogy (omphalocele, congenital diaphragmatic hernia, pericardial defect, intracardiac defect, and sternal cleft)⁴⁸, and rarely, the DiGeorge sequence.

Familial occurrence of congenital diaphragmatic hernia was first described in 1916 by Makela.⁴⁹ Since that time it is known that congenital diaphragmatic hernia may occur in identical twins, siblings, maternal uncles of affected siblings, and cousins.⁵⁰ There is no doubt that the incidence in familial cases is much lower than in sporadic cases. The mode of inheritance in familial cases of congenital diaphragmatic hernia is unknown. In 1979, Crane analyzed twelve afflicted families and argued that multifactorial inheritance is probably a more likely way of transmission than autosomal recessive inheritance; because of (1) the absence of consanguinity, and (2) the absence of equal distribution of affected males and females.⁵¹ In 1980, Wolff reviewed the literature on familial congenital diaphragmatic defects and also concluded that familial congenital diaphragmatic hernia follows a multifactorial inheritance pattern, because of the heterogeneity of the anatomy of the defect, the male predominance in the sex ratio, and the observation of associated anomalies.⁵² Norio also took this view, but added that multifactorial determination does not exclude other genetic causes in some familial cases.⁵³ Some years later Hitch described a family that had a second, third, and fourth infant with congenital

Table 3. Causes of malformations in man

Known genetic transmission	20 %
Chromosomal aberration	3-5 %
Environmental causes:	
- radiations	< 1 %
- infections	2-3 %
- maternal metabolic imbalance	1-2 %
- drugs and environmental chemicals	4-6 %
Unknown	65-70%

diaphragmatic hernia after a first normal male infant. The latter two children were fraternal twins. There was no known consanguinity in three generations of this family. Chromosomal studies yielded normal results. The question arises indeed whether autosomal recessive inheritance had been the way of transmission in this family.⁵⁴

1.3 Etiology and animal models

The causes of birth defects are widely ranging, but the etiology of the majority of malformations is a maze of unknowns. This has been emphasized by Wilson (Table 3).⁵⁵

The critical period of the diaphragm's organogenesis is estimated at day 40 of gestation, and the cause of absent closure of the diaphragm still is categorized as unknown.⁵⁶ Although a few drugs or groups of drugs have now been established as teratogenic agents in humans, none of these agents is associated with diaphragmatic hernia.

Experimental models

In animals, only a limited number of models has been developed to study pathogenic aspects of congenital diaphragmatic hernia. Harrison's group developed a model

mimicking diaphragmatic hernia by implanting and inflating a balloon in the chest of fetal lambs.⁵⁷ These lambs deteriorated soon after delivery, showing the clinical picture of respiratory distress. Deflation of the balloon in the neonatal period, mimicking correction of the defect, did not improve survival. However, deflation at day 120 of gestation (so-called simulated "correction"), resulted in catch-up lung

growth and development; after birth these lambs were easily resuscitated and all survived. They had significant increase in lung weight, air capacity, compliance, and area of the pulmonary vascular bed.^{58,59} In other words, this model established the pathophysiological efficacy of *in utero* repair.

Direct surgical repair in animals has been done by Harrison's group and by Soper and coworkers. It was shown that intrauterine repair indeed results in improved survival after delivery.^{60,61} Others induced diaphragmatic hernia in fetal rabbits by rupturing the left diaphragm *in utero* during the pseudoglandular period of pulmonary histological development.^{62,63}

In both animal models, studies into alveolar hypoplasia and characteristics of the pulmonary vascular bed showed a reduction in total lung weights in animals with a diaphragmatic defect. The ipsilateral lung was always more affected. The total size of the pulmonary vascular bed was decreased, the number of vessels per unit area lung was reduced, and there was increased muscularization of the arterial tree.^{58,62}

The defect of the diaphragm

Since 1971 Nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether) has been recognized as a potent teratogenic agent in rodents.⁶⁴ When administered to the pregnant mother during organogenesis, the heart, kidneys, diaphragm, and lung will develop abnormally. The exact mechanism of teratogenesis is not known, but evidence is accumulating that the teratogenic effects are mediated via alterations in thyroid hormone status.⁶⁵ The stereochemical configuration of Nitrofen greatly resembles that of triiodothyronine and thyroxine.

Iritani studied in mice whether hypoplasia of the lung bud might be responsible for the defect of the diaphragm.⁶⁶ He induced congenital diaphragmatic hernia in 80% of the offspring and concluded that pulmonary hypoplasia is a causal factor in the origin of congenital diaphragmatic hernia. This is in contrast to the generally accepted theory that lung hypoplasia is secondary to compression of the migrated viscera into the thoracic cavity. In other words, lung hypoplasia might not be the *result* but the *primary defect* in congenital diaphragmatic hernia.

The effect of Nitrofen on rat fetuses was further studied by Kluth and by Tenbrinck, who demonstrated the presence of lung hypoplasia in rats exposed to Nitrofen with or without a diaphragmatic defect.^{67,68,69} This finding is in accordance with the concept that Nitrofen primarily interferes with lung development and not with development of the diaphragm alone.^{71,72} In this model Nitrofen interferes with the closing mechanism of the diaphragm during organogenesis and not in fetal life as in the lamb model. Other drugs that are able to induce congenital diaphragmatic hernia in animals are quinine and thalidomide, but they are not currently used in experiments.⁷² Thus Nitrofen is probably the most promising drug to study pathophysiological and developmental aspects in congenital diaphragmatic hernia and pulmonary hypoplasia.

1.4 Clinical picture

1.4.1 Antenatal presentation

Congenital diaphragmatic hernia can be detected in utero by prenatal ultrasonography when herniated abdominal viscera in the fetal chest, abnormal upper abdominal anatomy, and mediastinal shift away from the side of herniation can be visualized.⁷³ Sometimes herniated viscera move in and out of the fetal chest, suggesting that fetal congenital diaphragmatic hernia is a dynamic process.⁷⁴ It has been suggested that the presence of the stomach in the chest, especially if it is dilated, is predictive of poor outcome.⁷⁵

In most cases, sonography is performed because the mother is large-for-date. Polyhydramnios usually develops after 24 weeks of gestation, suggesting that it is a relatively late manifestation of this disease.⁷⁵ The cause of polyhydramnios is unknown, but it may be functional upper gastrointestinal obstruction or impaired fetal swallowing.⁷⁶ Others have reported that congenital diaphragmatic hernia is associated with renal enlargement. They suggested that the kidneys produce a certain pulmonary growth factor and that due to compression the fetal lungs may be unable to respond. Pulmonary hypoplasia would maximally stimulate production of pulmonary-derived renotropin, resulting in continual stimulation of the kidneys, and renal enlargement. Subsequently, renal enlargement would lead to increased urine production by the fetus, and polyhydramnios.⁷⁷ In a large, retrospective study, polyhydramnios was present in 76% of cases, and was also a predictor of poor clinical outcome.⁵⁸ A recent report from England on fetal diaphragmatic hernia did not confirm this association; the incidence of hydramnios was only 29%.⁷⁸ The same study showed that a reduced left-to-right ventricular size and a lower aorta-to-pulmonary artery ratio is associated with poor prognosis. Sequential observations showed that changes occurred with advancing gestation. The cause of the underdevelopment of the left heart remains uncertain, although the evidence of right heart underdevelopment in right-sided hernia suggests that direct compression of cardiac structures may play a role.⁷⁹

Once a congenital diaphragmatic hernia is diagnosed, it is possible to perform chromosomal analysis by amniocentesis or percutaneous umbilical blood sampling. Screening for other anatomical abnormalities, including structural cardiac defects, should be carried out by an experienced obstetrical sonographer.

Prenatal diagnosis makes it possible to provide the best chances for survival for the yet unborn patient. It allows maternal transport to a specialized center, planned delivery, and immediate resuscitation. Ultrasonography may not detect small congenital diaphragmatic hernias *in utero*, but it apparently detects those fetuses that are most severely affected. Diagnostic errors may include cystic adenomatoid malformation of the lung, and cystic masses in the mediastinum.⁸⁰

1.4.2 Postnatal presentation

The classic symptom triad of the common left-sided congenital diaphragmatic hernia consists of cyanosis, dyspnea, and cardiac dextroposition. Physical examination reveals a scaphoid abdomen, decreased breath sounds, and distant heart sounds. Auscultation of bowel sounds in the chest is an inconsistent finding. A chest roentgenogram shows a mediastinal shift, air-filled bowel loops located in the chest, and a relatively gasless abdomen. Right-sided hernias may be asymptomatic because of tamponade of the defect by the liver. The children at highest risk are symptomatic in the delivery room or within six hours after birth.⁸¹ The mortality rate reported in this patient group ranges from 40 to 50%.^{82,83} The mortality rate has not decreased probably due to the fact that more patients with profound respiratory distress and circulatory collapse, who previously would have died, reach the specialized center earlier nowadays. It is this group of children that Harrison referred to as representing the "hidden" mortality.⁸⁴

Infants with congenital diaphragmatic hernia have abnormal pulmonary structure characterized by diverse degrees of hypoplasia of lung parenchyma and pulmonary blood vessels.^{15,17} As a consequence they present with the clinical symptoms of respiratory distress and persistent pulmonary hypertension.

Nguyen and coworkers conducted a large study of 40 autopsied congenital diaphragmatic hernia patients and found hypoplasia in all cases. They concluded that the mortality was caused by a combination of factors, one of which certainly bilateral lung hypoplasia.⁸⁵ A five-year study from the Boston Children's Hospital involved 50 neonates with a diaphragmatic defect, thirteen of whom underwent cardiac catheterization in the postoperative period.⁸⁶ Analysis of the data suggested the presence of two separate physiological groups, "responders" and "nonresponders" to conventional therapy. "Responders" often had left-to-right shunting. Episodes of right-to-left shunting in the postoperative period were due to vasoconstriction of the pulmonary vascular bed after the so-called honeymoon period. Pulmonary vasoconstriction can be triggered by many events such as suctioning of the endotracheal tube, pain, and loud noises. Continued administration of anesthetics reduced the incidence and frequency of this episodes.⁸⁷ "Nonresponders" had severe right-to-left shunting that was unresponsive to any attempts at pulmonary vasodilation. In a number of these children pulmonary arteriograms were made. Patients were grouped by the (in)ability to reach at least one postductal $\text{PaO}_2 > 100$ torr. Arteriograms in the group with PaO_2 values < 100 torr showed smaller ipsilateral and contralateral main pulmonary arteries, as well as smaller ipsilateral lungs with more severe peripheral vascular obstructive disease. The authors proposed the postductal PaO_2 as the clinical marker for identification of the degree of pulmonary perfusion.⁸⁸

As the degrees of pulmonary hypoplasia and pulmonary hypertension are by no means uniform in this disease, attempts have been made to identify the patients

who run the highest risk. Early reports predicted survival on the basis of acid-base balances and alveolar-arterial oxygen differences $\{(A-a)DO_2\}$.^{89,90} Later the site of the stomach was used as a prognostic sign: an abdominal site was associated with an excellent prognosis, an intrathoracic site with a high mortality.⁹¹ About the same time Touloukian described a scoring system based on the final preoperative thoracic X-ray. He scored the side of the diaphragmatic defect, the location of the stomach, presence of an ipsilateral or contralateral pneumothorax, the degree of mediastinal shift, amount of visceral distension, and relative volume of aerated ipsilateral and contralateral lung: high scores were associated with nonsurvival.⁹² Cloutier measured V/P values (expiratory tidal volume over inspiratory pressure) as an index of pulmonary expansion in newborns with congenital diaphragmatic hernia; he also recorded these values in lambs and correlated them with the amount of lung tissue found at post mortem. He found low V/P values in newborns and a significant correlation between V/P and fractional lung mass in lambs with a diaphragmatic defect, suggesting that this index of expansion could be an indicator of lung hypoplasia.⁹³ Bohn recently emphasized the importance of hypoplasia, and asserted that a better estimation of survival can be made in the relationship of the $PaCO_2$ and ventilation parameters.⁹⁴ He showed that the values measured 2 hours post surgery predicted outcome: if the $PaCO_2$ was less than or equal to 40 torr and the ventilation index was less than 1000, the chance of survival was excellent. The ventilation index is the product of the mean airway pressure and the respiratory rate. In another paper Bohn used these criteria both before and after surgical repair and confirmed his earlier report. He concluded that patients with elevated $PaCO_2$ levels, which cannot be reduced by hyperventilation, have bilateral lung hypoplasia associated with death.²⁸

Other diagnoses that should be considered in neonates presenting with the triad of cyanosis, dyspnea or dextrocardia are space-occupying developmental anomalies such as congenital lobar emphysema and cystic adenomatoid malformation of the lung. In congenital lobar emphysema there is even distribution of air in the affected lobe. In cystic adenomatoid malformation of the lung, however, multiple air and fluid-filled cystic spaces can be seen that resemble the bowel loops in congenital diaphragmatic hernia. Tachypnea will result in air swallowing with dilated bowel loops within the abdomen. In contrast, congenital diaphragmatic hernia is associated with a scaphoid abdomen with less abdominal gas.

In conclusion, *alveolar hypoplasia* and *persistent pulmonary hypertension* form the pathophysiologic background for the clinical picture in symptomatic newborns with congenital diaphragmatic hernia.

1.4.3 "Delayed" or "acquired" presentation

The late-presenting Bochdalek hernia often presents difficulties in diagnosis because its symptoms and signs are far from specific and resemble those of other disease

processes. Between 5 and 10% of congenital diaphragmatic hernia presents after the neonatal period.⁹⁵

Berman reported a 20-year review and suggested that there are two distinct groups. In one group the defect may be longstanding, but the viscera are confined by a hernial sac or obturated by the liver or spleen. The defect will present when the sac ruptures or the intra-abdominal pressure is raised, causing the viscera to herniate. This type of presentation is supported by previously normal chest X-rays and is called "acquired" hernia.⁹⁶ The other group probably has long-standing congenital diaphragmatic hernia which presents when a complication of the herniated bowel loops, such as strangulation, occurs. Berman found a high incidence of respiratory tract infections and acute respiratory distress. In contrast, other studies concerning late congenital diaphragmatic hernias and eventrations reported failure to thrive followed by abdominal pain and vomiting as the commonest symptom.⁹⁷ On the basis of clinical presentation, there are two types of patients: those presenting early in childhood with respiratory symptoms and those with gastrointestinal symptoms presenting later in childhood.⁹⁸ Mistaken diagnoses included pneumonia, congenital lung cysts, and tension pneumothorax.⁹⁹ Right-sided hernias may present as a pleural effusion, the obstructed hepatic venous outflow from the herniated liver causing vascular transudation from the liver surface.¹⁰⁰ Clinically, it may be difficult to distinguish between a hernia with a sac and an eventration, but an eventration is characterized by elevation of an attenuated, intact diaphragm.¹⁰¹ Group B streptococcal sepsis has been associated with congenital diaphragmatic hernia and has been thought to develop because of compression of the lung with resultant consolidation.¹⁰²

1.5 Normal developmental aspects

1.5.1 Diaphragm

The diaphragm has four developmental components: (1) anterior components from the septum transversum, (2) a posterior component from foregut mesentery, (3) a lateral component from body wall muscle, and (4) pleuroperitoneal canals. Two developmental phases can be identified: (1) development of the septum transversum and (2) closure of the pleuroperitoneal canals. The primary body cavity, or intraembryonic celom, is formed as early as the fourth week of gestation. The septum transversum is established between the pericardial cavity cranially and the peritoneal cavity caudally; it is a mesodermal structure. Dorsal to the septum transversum the pleuroperitoneal canals develop.

The septum transversum expands due to active growth of the developing heart and lungs. The pulmonary primordium appears at the caudal end of the laryngotracheal sulcus after two weeks.¹⁰³ The dorsal part of the future diaphragm is formed from mesenchymal cells around the aorta, the lateral parts from muscular parts of the body wall. There are no arguments that point to a primary defective

development of the septum transversum as the cause of the diaphragmatic hernia. The pleuroperitoneal canals close at approximately 52 days of gestation.¹⁰⁴ There is no agreement on the exact mechanism of closure. The closure of the diaphragm is completed by the ninth week of gestation.

1.5.2 Gas exchange area

Recently, several authors have reviewed the structural development of the fetal lung.^{105,106,107} It appears that the process of development can be subdivided into five stages.

The first is the *embryonic period* (0 to 7 weeks), when a small diverticulum of the foregut develops. Two bronchial buds appear and form the right and left main-stem bronchi. Respiratory epithelium already develops in the endodermal foregut bud and interacts with the bronchial mesoderm, growing invasively into the surrounding mesenchyme. The pattern of branching is determined by the associated mesenchyme.

The next stage is the *pseudoglandular phase* (8 to 16 weeks); in this phase, the primitive airways rapidly proliferate so that all airway divisions are completed by 16 weeks. The extreme peripheral structures are the terminal bronchioles which undergo further differentiation into respiratory bronchioles and alveolar ducts. Differentiation of epithelial cells begins as early as 10 weeks. Mucous glands appear at 10 to 12 weeks, and ciliated cells at 13 weeks of gestation.

The *canalicular phase* (17 to 27 weeks) shows further division and enlargement of distal airways. By 20 to 22 weeks the epithelium begins to differentiate into two cell types: type II cells containing lamellar bodies, and more flattened type I cells with large cytoplasmic extensions.

Another important event at this stage, is the development of the distal pulmonary circulation, where capillaries come in close contact with the epithelial layer. In other words, the first steps are made to form a thin air-blood barrier and sufficient quantities of surfactant material to enable future gas exchange.

The *saccular phase* (28 to 35 weeks) is characterized by large sack-like, peripheral airspaces. The sacculi are subdivided by ridges that protrude into the airspaces, taking part of the capillary bed with them, and forming a double capillary layer. In this way "primitive" alveoli are formed as early as 32 weeks of gestation.

This stage gradually proceeds into the *alveolar stage* (36 weeks to Term). It is still unsolved whether alveolar formation actually starts in this period. Boyden stated that there are only "primitive sacculi" before birth.¹⁰⁸ In 1984, Thurlbeck reported that alveoli started to appear as early as the 32nd or 34th week of gestation, and that there were 50 million alveoli at birth.¹⁰⁹ This is far more than was estimated previously.¹¹⁰ The volume of potential airspace, both within the airway lumen and within the future gas exchange area, increases with thinning of septal walls and attenuation of cuboidal cells. The postnatal growth of the lung results in a further

increase of lung volume (23-fold), number of alveoli (six-fold), alveolar surface area (21-fold), and lung weight (20-fold) into adulthood.

Lynne Reid summarized the process of lung development and introduced the three laws of lung development¹¹¹:

LAW I: the bronchial tree - bronchi and bronchioli - is developed by the 16th week of intrauterine life. Airway development is an intrauterine story.

*LAW II: alveoli develop after birth, and at birth the "alveolar" spaces are more primitive than in the adult. The alveolar story is mainly postnatal. The fastest multiplication takes place during the first 4 years of life. As thoracic growth exceeds alveolar multiplication, alveoli increase in size and their density decreases. The radial alveolar count (the number of alveoli between the last respiratory bronchiole and the outside margin of the acinus) is a convenient way to assess alveolar multiplication.*¹¹²

LAW III: the "vascular" law reflects the first two. The pre-acinar branches of the pulmonary artery appear at the same time as the accompanying airways. The intra-acinar vessels appear as alveoli grow.

Professor Reid concluded:

...that by using these laws as the timetable of development it is possible to decide at what stage in development a given anomaly has appeared.

Lung growth is influenced by various hormones, such as glucocorticoids, sex hormones, thyroid hormones, and growth hormone. Glucocorticoids enhance lung maturation and increase surfactant production.¹¹³ Thyroid hormones have been recognized as important agents in stimulating septation of alveoli and DNA synthesis in lung cells of newborn rats.^{114,115}

1.5.3 Pulmonary vascular bed

Recently, deMello and Reid emphasized the pre- and postnatal development of pulmonary circulation.¹¹⁶ The acinus is the unit of the lung supplied by a terminal bronchiolus. The respiratory bronchioli, the alveolar ducts, and the distal alveoli are part of it. The pulmonary artery supplies the intra-acinar structures; the capillary bed begins only at the level of the respiratory bronchiolus. The bronchial artery supplies the capillary bed within the bronchial wall and the structures of the perihilar region. The intrapulmonary structures drain to the pulmonary vein, whereas the hilar structures drain to the bronchial veins and to the azygos system.

The pulmonary arteries run together with the airways but give off more branches, so that "conventional" and "supernumerary" branches can be distinguished. The former are eventually distributed beyond the level of the terminal bronchiolus,

whereas the latter supply the capillary bed of alveoli immediately around the pulmonary artery. The process of pre-acinar branching follows branching of airways and is completed by 16 weeks of gestation. The intra-acinar arteries develop later in fetal life and continue to form after birth as the alveolar ducts and the alveoli develop.

During fetal life, the walls of muscular and partially muscular pulmonary arteries are thick. The wall structure is related to the diameter of an artery, and relatively few intra-acinar arteries contain muscle. As new arteries are formed, they acquire a muscle coat corresponding with their diameter. The number of muscularized arteries increases, but the muscle coat of each individual artery does not thicken. The structure of the fetal pulmonary vascular bed reflects the high pulmonary vascular resistance and the low pulmonary blood flow characteristic of fetal life.

The main features of adaptation to the postnatal period are concerned with maturation, adaptation to air breathing, and a major increase in pulmonary blood flow. After birth the pulmonary vascular bed is remodeled in order to effect an abrupt reduction in pulmonary vascular resistance. Postnatal adaptations can be classified into three different stages.¹¹⁷

The *first stage*, from birth to the first three or four days of life reflects the adaptation to extrauterine life. The wall-thickness of the peripheral pulmonary arteries will decrease during the first weeks of life¹¹⁸; more recent reports show that adaptation actually involves the entire arterial pathway, including the elastic and large muscular conducting arteries in addition to the arteries just proximal to the respiratory unit.¹¹⁹ Major roles seem to be reserved for the precapillary arteries. These vessels consist of endothelial cells surrounded by pericytes. They become thinner within minutes after birth, resulting in a thinner vessel wall and a larger lumen diameter.¹²⁰ Similar structural changes occur in the small muscular arteries where smooth muscle cells show a significant reduction in diameter during the first month of life.¹²¹

The *second stage* shows structural stabilization lasting until 3 to 4 weeks of age. Connective tissue is deposited, and the definitive elastic lamina is formed in the small muscular arteries.

The *third stage* is growth continuing into adulthood. This stage is characterized by an increase in arterial diameter, which reflects cell multiplication. The main burst of growth is seen within the acinus. Smooth muscle cells in the respiratory unit arteries differentiate from precursor cells, and muscle cells extend all along the arterial pathway with growth. The prominent intra-acinar arteries increase in size, and new alveolar wall vessels grow to supply the increasing gas-exchange area. The process of adaptation and growth during these stages is also known as growth by "fits and starts".¹¹

At birth almost the entire pulmonary vasculature bed is innervated. Nerve trunks run in the adventitia of the large arteries and veins. Both noradrenergic and cholinergic nerves are identified, containing several transmitters such as vasoactive

intestinal peptide and calcitonin gene-related peptide in the extrapulmonary arteries.^{122,123}

1.5.4 Surfactant

The existence of lung surfactant and its function were reported in 1950.¹²⁴ Surfactant has at least four important functions: (a) to stabilize the lung during deflation, (b) to prevent high surface-tension pulmonary edema, (c) to protect the lung against damage, and (d) to assist in the defense mechanisms of the lung. The alveolar surfaces are lined with extended, thin epithelial type I cells and with the more cuboidal type II cells. Autoradiography studies with various labeled precursors of surfactant have shown that surfactant is produced by the type II cells.¹²⁵ Surfactant consists of a number of lipids, primarily dipalmitoylphosphatidylcholine, and a number of surfactant-specific proteins (SP-A, SP-B, SP-C). These proteins have both a surfactant function and a non-surfactant function; they are probably involved in the homeostatic control of surfactant in the alveolus, and they have a potential defense function.¹²⁶

At 24 weeks of gestation type II cells are able to take up metabolic precursors and to produce the lipid and protein components of surfactant. They are also able to package these components in lamellar bodies: their storage and secretory organelle. Saturated phosphatidylcholine is present early in whole lung extracts and increases in level after 24 weeks.¹²⁷

The process of development and accelerating synthesis is under control of hormones like glucocorticoids and thyroid hormones.¹²⁸ Secretion of surfactant is regulated by several hormones and other agents.¹²⁹ The fetus releases large amounts of surfactant during labor and shortly after delivery.¹³⁰ During the last weeks of gestation there is an increase in adrenergic receptors in the lung, suggesting that the catecholamines may play an important role in the control of secretion.¹³¹

The development of surfactant replacement therapy started in the late 1950s after the original observation of Avery and Mead that neonatal respiratory distress syndrome was associated with the absence or delayed appearance of pulmonary surfactant.¹³² This study initiated further research into the ontogeny of the surfactant system and into the possibilities of surfactant replacement therapy. Highlights were the reports of Enhorning and Robertson, who showed improvement of pulmonary mechanics in prematurely born rabbits after the application of whole surfactant.¹³³ In 1980, Fujiwara published promising results of the treatment of newborns with an exogenous surfactant isolated from bovine lung.¹³⁴ Since that time several studies have been undertaken to define the optimal composition and mode of application of surfactant in prematurely born infants.^{135,136} Surfactant replacement therapy may have other indications in the neonatal or pediatric intensive care unit, such as shock lung syndrome, meconium aspiration syndrome, and pneumonia.¹³⁷

1.5.5 Antioxidant defense

Marx's statement that "breathing oxygen is, it seems, hazardous to your health", is certainly applicable to the newborn infant.¹³⁸ At birth the fetus leaves the quite hypoxic *in utero* environment for the relatively hyperoxic environment of the outside world. It therefore seems necessary that the fetal lung develops sufficient antioxidant defenses to prepare against potential oxygen-induced cytotoxicity when breathing begins.

An oxygen free radical is defined as any species capable of independent existence that possesses one or more unpaired electrons.¹³⁹ Induction of oxidant stress can occur due to raising ambient oxygen concentrations, toxins that augment intracellular oxidant formation, and ischemia followed by tissue reoxygenation.¹⁴⁰ Normally there is a balance between the toxicity of oxidants such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO), and the protective activities of antioxidant defense mechanisms. The cell can protect itself against free radicals by enzymatic conversion of free radicals to nontoxic products, nonenzymatic scavenging, and compartmentalization. The intracellular antioxidant enzyme system consists of superoxide dismutase (SOD), catalase, and the glutathione redox cycle (glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase). Superoxide dismutase handles superoxide, catalase is involved in the elimination process of hydrogen peroxide, and glutathione reduces hydrogen peroxide to H_2O . The nonenzymatic agents can be distinguished into water-soluble and lipid-soluble compounds. Vitamin E is the major lipid-soluble antioxidant, vitamin C the major water-soluble antioxidant. Compartmentalization is mainly related to the binding of iron and copper. Iron is bound to transferrin and lactoferrin outside the cell, and to ferritin within the cell. Copper is predominantly bound to ceruloplasmin.¹⁴¹

The developmental course of the antioxidant enzyme system is remarkably similar to that of the surfactant system. The levels of the three antioxidant enzymes, - SOD, catalase, and glutathione reductase - increase during the last part of gestation. Tanswell found an increase with advancing gestational age in rats and concluded that maturation of the antioxidant enzyme system is virtually complete before delivery.¹⁴² These observations have been confirmed by Gerdin and coworkers.¹⁴³

The first studies in fetal rats were followed by a demonstration of identical developmental patterns in other species. Frank found late gestational elevations of antioxidant enzyme levels in rats, hamsters, rabbits, and guinea pigs; he concluded that these changes represent a normal preparation for birth.¹⁴⁴ Frank also pointed out that investigation of the antioxidant enzyme system should not be aimed at baseline (normoxic) conditions only, but also at hyperoxic conditions. He found that term neonatal rats were able to induce antioxidant enzyme activity, whereas adult rats were not.¹⁴⁵ He also studied the ability of premature rabbits to induce antioxidant

enzyme activity in reaction to hyperoxia and found that unlike term rabbits, preterm rabbits were not able to increase the production of antioxidant enzymes.¹⁴⁶ Other studies revealed that intrauterine growth-retarded rats had decreased levels of glutathione peroxidase, and increased susceptibility to pulmonary oxygen toxicity.¹⁴⁷ The late gestational developmental pattern of antioxidant enzyme activity may be "switched on" by the same hormonal regulation mechanisms that account for the surfactant system. In vitro this could be demonstrated in rats.¹⁴⁸ The question remains how to translate this advancement in knowledge to the newborn infant.

1.6 Developmental aspects in congenital diaphragmatic hernia

1.6.1 Diaphragm

Several theories have been described to explain the diaphragmatic defect. Grosser supported the theory of improper development of the pleuroperitoneal membrane.¹⁴⁹ Holder suggested a failure of muscularization of the diaphragm resulting in a weak area.¹⁵⁰ A recent morphologic study, however, showed no pathological findings that favor these theories.¹⁵¹ Infiltration of the intestinal organs is a theory described in 1943.¹⁵² Gattone and coworkers suggested that lung tissue could be present in the pleuroperitoneal canal at the moment of closure resulting in a diaphragmatic defect.¹⁵³ Kluth and coworkers proved that closure of the diaphragm in the normal Sprague-Dawley rat takes place in two layers.¹⁵⁴ At the moment, the most exciting theory is the one proposed by Iritani; he suggested that lung hypoplasia may be merely the *cause* of the diaphragmatic defect rather than the *result*.⁶⁶

1.6.2 Gas exchange area

Potter described pulmonary hypoplasia as a circumscribed entity in anencephaly and renal anomalies.¹⁵⁵ Soon thereafter, morphometrical studies were carried out to define the degree of lung hypoplasia in congenital diaphragmatic hernia patients. Campanale found an abnormality variable in size and configuration, with the ipsilateral lung showing the most pronounced degree of retardation.¹¹ A classic, detailed study was reported by Areechon and Reid.¹⁵ They found a reduction in the number of bronchial branches, more marked on the affected side, and a reduction in the volume of alveoli. This means that the diaphragmatic defect occurs early in gestation during the pseudoglandular period, the period of bronchial development.

Because pulmonary hypoplasia is a poorly defined condition, several attempts have been made to establish reliable criteria for the diagnosis. Reale and coworkers reported a large study of 80 autopsy cases including 20 patients with congenital diaphragmatic hernia: they found decreased mean lung-body weight ratios and lower radial alveolar counts.¹⁵⁶ Askenazi reported the morphometrical lung analysis of 111 patients who had pulmonary hypoplasia and suggested that a lung-body

weight ratio of < 0.012 in combination with a radial alveolar count of < 4.1 (75% of mean normal count) are reliable diagnostic criteria of pulmonary hypoplasia.¹⁵⁷

Assessment of pulmonary hypoplasia by DNA estimation was carried out by Wigglesworth and coworkers in infants with pulmonary hypoplasia defined according to lung-body weight ratio. Lung hypoplasia was associated with lung DNA content low for gestation. In infants with hypoplastic lungs born at 34-39 weeks of gestation DNA values were equivalent to those seen in normal fetuses at 20-22 weeks of gestation.¹⁵⁸ In another study, including two infants with congenital diaphragmatic hernia, the quantitative biochemical criteria for lung growth and maturation were compared with the histological appearances. The hypoplastic ipsilateral lung had an immature structure with low phospholipid concentrations, whereas the contralateral lung was structurally and biochemically more mature.¹⁵⁹ The observation that the lungs of congenital diaphragmatic hernia survivors showed overdistension and emphysema, has drawn attention to the terminal lung unit in congenital diaphragmatic hernia.¹⁶⁰

George and coworkers assessed the maturity of the lungs in 10 infants with congenital diaphragmatic hernia who died in the immediate perinatal period. They showed that the lungs were all immature, the ipsilateral lung being most affected. The radial alveolar count was reduced in all cases, and they stated that hypoplasia is not only characterized by deficient airway generation but also by acinar hypoplasia. In several patients they also found hyaline membranes, despite term gestation.¹⁶¹ These observations were confirmed by others, who also found absence of elastic fibers in alveolar septa of the ipsilateral lung.¹⁶² This could be an important observation because elastic fibers might be involved in the process of septation of alveoli.¹⁶³

Mechanical factors that have been associated with late gestational lung growth include available space (fetal compression), amniotic and lung fluid volume, and fetal breathing movements. The classic theory of available space in congenital diaphragmatic hernia points at compression of the developing lung by the herniated viscera, beginning early in gestation. This situation has been mimicked in fetal lambs, after which pulmonary hypoplasia could be shown.⁵⁹ Fetal breathing movements are important for lung development, probably by the mechanism of stretch.¹⁶⁴

In conclusion: there is evidence that the hypoplastic congenital diaphragmatic hernia lung is immature, both in structure and in function.

1.6.3 Pulmonary vascular bed

One of the major clinical problems in congenital diaphragmatic hernia is persistent pulmonary hypertension. The structural basis of persistent pulmonary hypertension has been emphasized by Geggel and Reid.¹⁶⁵ They distinguish between

maladaptation, excessive muscularization, and underdevelopment of the pulmonary vascular bed.

In case of *maladaptation* there is a structurally normal vascular bed, but no normal, postnatal increase in compliance of the small muscular arteries. The neonatal pulmonary vascular bed is highly reactive, and acidosis, hypoxia, and hypercarbia may all contribute to pulmonary vasoconstriction and create a vicious circle of ongoing persistent pulmonary hypertension, which is thought to be caused by mediators such as the prostanoids, and is associated with acute perinatal stress.¹⁶⁶

Excessive muscularization is characterized by an increase of medial wall thickness of normally muscularized intra-acinar arteries and by extension of muscularization into nonmuscular arterioles. This form has been mentioned in chronic intrauterine hypoxia, and in *in utero* closure of the ductus arteriosus.¹⁶⁷

Underdevelopment of the pulmonary vascular bed may occur in several conditions associated with pulmonary hypoplasia, for instance congenital diaphragmatic hernia. Early reports on the pulmonary vascular bed in congenital diaphragmatic hernia mentioned increased mean wall thickness compared to normal newborns, but no extension of muscle into the intra-acinar arteries.¹⁸ In 1978, Levin reported a morphologic analysis of the pulmonary vascular bed in congenital diaphragmatic hernia and found: (a) a decrease in total size of the pulmonary vascular bed, (b) increased pulmonary smooth muscle, and (3) a decrease in the number of vessels per unit of lung.¹⁶⁸ In earlier studies he suggested the possibility that mechanical distortion of the fetal ductus arteriosus may cause partial constriction, resulting in fetal pulmonary hypertension due to excessive muscularization.¹⁶⁹ Geggel was able to demonstrate that abnormalities of the pulmonary vascular bed in patients who have persistent hypoxemia differ from those in patients who at first maintain normal blood gas values postoperatively, but after the so-called honeymoon period become hypoxemic. No-honeymoon patients have smaller lungs, increased muscularization of intra-acinar arteries, and decreased luminal area of pre-acinar and intra-acinar arteries due to reduced external diameter. Geggel concluded that clinical deterioration in the honeymoon group depends on a vasoconstrictive response of the hypoplastic vascular bed. Persistent hypoxemia in the no-honeymoon group is determined by the severity of pulmonary hypoplasia and by the structural remodeling of pulmonary arteries.¹⁷⁰ The consequences for the clinical course in these patients and the significance of these observations in terms of mortality risk has been emphasized by Shochat.¹⁷¹

Various animal models were developed to gain more insight in the developmental pathophysiology of abnormal pulmonary circulation associated with congenital diaphragmatic hernia. Changes of the pulmonary vascular bed have been demonstrated in fetal lambs. Fetal surgical repair of the hernia restored the pulmonary arterial bed, probably through compensatory lung growth.⁵⁸ A major disadvantage of the lamb model is that interference takes place in a relatively late stage of otherwise normal lung development. Others found similar results in a fetal

rabbit model.⁶² Notwithstanding all this, our knowledge of the morphology of the pulmonary vascular bed and the pathophysiology of persistent pulmonary hypertension is still limited, especially on the cellular level.

1.6.4 Surfactant and antioxidant defense

Reports on biochemical maturation of the lung in congenital diaphragmatic hernia are scarce. Hisanaga and coworkers observed low lecithin/sphingomyelin ratios (0.56 and 0.57 respectively) in two infants with a congenital diaphragmatic hernia. They argued that lung hypoplasia actually means a reduced number of type II pneumocytes and reduced production of surfactant.¹⁷² The lecithin-sphingomyelin ratio reflects to some extent the maturation of the fetal lung. The question remains, however, whether ratios are low because of the total reduction in lung tissue or because the type II cell in congenital diaphragmatic hernia functions at a lower level. The type II cell has been extensively studied, and reduced numbers of these cells as well as fewer lamellar bodies per cell have been associated with lung immaturity.¹⁷³ Hashimoto and Pringle investigated the morphologic characteristics of the type II cell in a fetal lamb model of congenital diaphragmatic hernia. Surprisingly, they found that type II cells were five to ten times more abundant in the lungs of animals with a diaphragmatic defect. No ultrastructural changes of immaturity were observed in type II cells.¹⁷⁴

Further studies into lung surfactant production in the fetal lamb with congenital diaphragmatic hernia showed: (1) a marked decrease in pulmonary compliance, (2) a reduction of the total amount of phospholipid in bronchoalveolar lavage fluid, and (3) a reduction in the synthesis rate of phosphatidylcholine by type II cells.¹⁷⁵ The authors concluded that congenital diaphragmatic hernia in the fetal lamb leads to profound lung hypoplasia and apparent immaturity of the surfactant system.

In neonatal rats congenital diaphragmatic hernia was induced by feeding of Nitrofen to the mothers during gestation. Their lungs showed hypoplasia and lower content of disaturated phosphatidylcholine per μg DNA and total disaturated phosphatidylcholine.¹⁷⁶

To our knowledge there are no reports on the maturation of antioxidant enzyme activity in the presence of congenital diaphragmatic hernia neither in humans nor in animal models.

1.7 Treatment

Management of the fetus with congenital diaphragmatic hernia has been recently emphasized by Caplan and MacGregor.⁷⁶ Antenatal management requires fetal karyotyping, assessing of the degree of herniation, locating of the stomach, and ultrasound evaluation for associated anomalies. The findings will determine antepartum and intrapartum management. In fetuses with a normal karyogram and

no additional lethal anomalies, follow-up sonograms are needed and maternal transport to a specialized tertiary center should be strongly considered. Some centers consider prenatal diagnosis of congenital diaphragmatic hernia before week 20 as an indication to advise the parents to terminate pregnancy, especially when other congenital anomalies have been detected as well. Only a multidisciplinary team approach can provide optimal information for the parents and adequate care for the yet unborn patient.

Delivery is generally planned after 37 weeks' gestation, and vaginal delivery is usually preferred.¹⁷⁷ In patients symptomatic at birth, initial resuscitation in the delivery room should be aimed at stabilization while preparing for transport to the intensive care unit. A nasogastric tube is inserted to minimize gaseous distension of the bowel. Assisted ventilation is preferably administered via an endotracheal tube, because handbagging the patient with a mask will aggravate respiratory distress due to increasing abdominal distension.¹⁷⁸

In the past, emergency surgical repair was the cornerstone of therapy. In the past few decades, however, one has gradually become aware that congenital diaphragmatic hernia is not an acute event, but rather an anomaly developing early in fetal life. The major cause of death is related to abnormal lung development.

The clinical picture shows respiratory distress as a consequence of lung hypoplasia, and persistent pulmonary hypertension with right-to-left shunting as a consequence of the abnormal vascular bed.

After Cartlidge observed that preoperative stabilization might improve the outlook, several groups have adopted the delayed surgical approach as a better treatment option.²⁰ This concept has led to ventilation of patients under muscular paralysis and sedation. The stomach and the bowel are decompressed by continuous suction using a nasogastric tube with a double lumen. Adjustment of ventilatory settings including high respiratory rates (60-150/min) is meant to avoid risk factors for persistent pulmonary hypertension. Therefore the objectives are: arterial pH values of 7.50 or higher, a PaCO₂ between 25 and 30 mmHg, and a PaO₂ between 100 and 150 mmHg.¹⁷⁹ Metabolic acidosis is treated vigorously with sodium bicarbonate, and further alkalization is aimed at pH values of 7.50-7.55. Circulatory stability may be achieved by administration of vasoactive drugs such as dobutamine and dopamine. Monitoring of pre- and postductal arterial blood gas values with indwelling catheters or transcutaneous PO₂ monitors is essential to establish persistent fetal circulation. In spite of all these efforts, delayed surgery has not brought the expected significant decrease in mortality.^{82,180}

The recognition of persistent pulmonary hypertension as a major factor contributing to death in these patients coincided with the application of ECMO in neonatal respiratory failure. In most cases of persistent pulmonary hypertension, the infant can be successfully treated by aggressive ventilation with high inspiratory pressures, high percentages of oxygen, and induced alkalosis by means of hyperventilation.¹⁸¹ For those in whom conventional therapies fail, ECMO has

provided new chances of survival. ECMO is the temporary use of an artificial lung and provides "lung rest" together with the possibility to overcome episodes of persistent pulmonary hypertension in the honeymoon period.

Many authors have proposed special techniques for postoperative management, such as balanced thoracic drainage and reconstruction of the dome of the diaphragm with a prosthetic patch to prevent enlargement of the hemithorax, with overexpansion of the hypoplastic lung. They all report higher survival rates.^{182,183}

Others have tried to define accurate predictors of mortality based on pH, PCO₂, (A-a)DO₂, or best preductal or postductal PO₂.^{89,184} Bohn used ventilatory parameters by plotting PCO₂ versus ventilation index (respiratory rate x mean airway pressure).⁹⁴ This method was reliable in the pre-ECMO period when patients who deteriorated after a stable interval or who never stabilized at all, usually died. The introduction of ECMO complicated this predictive model.

Wilson and coworkers examined the severity-of-illness parameters in 59 patients. They confirmed earlier observations that the best postductal PO₂ is an accurate predictor of mortality with values < 100 mmHg associated with death: depending, however, on the degree of ventilation and alkalosis. They introduced the oxygenation/ventilation index [(PO₂/mean airway pressure x respiratory rate) x 100] and found this a reliable, objective predictor of mortality.¹⁸⁵

1.8 Follow-up

There is increasing awareness that long-term morbidity in congenital diaphragmatic hernia survivors is related to the aggressive mode of treatment. The interaction between positive pressure ventilation with high percentages of oxygen and the hypoplastic and dysplastic lung may cause iatrogenic lung injury. Lung development may consequently be disturbed and its function may remain compromised in adulthood. Several observations support this hypothesis. The vulnerability of the hypoplastic lung in the direct postoperative period was already noticed in an animal model developed by DeLuca and coworkers.¹⁸⁶ Thurlbeck described a boy, who, when a newborn, had undergone an operation for left-sided congenital diaphragmatic hernia, but died because of an accident at the age of five. The study showed normal histology in the right lung, but parts of destructive emphysema in the left lung.¹⁶⁰

Long-term function studies show that although these patients are doing well and are functioning normally, the majority show a 20 to 30% reduction of normal lung function.¹⁸⁷ Falconer reported on 19 survivors operated on during the late 1970s, and found an increasing incidence of chest symptoms, subjective exercise intolerance and expiratory wheeze. Median values for maximum expiratory flow-volume curves were lower compared to controls. Radiographic left lung volumes suggested overdistension and nuclide scans revealed perfusion of the ipsilateral lung. Highly abnormal results were found in the patients who had been ventilated for four days

or more.¹⁸⁸ Although a morphometrical analysis of a large number of congenital diaphragmatic hernia lungs is lacking, several authors report the existence of hyaline membranes and thickened alveolar and intra-alveolar walls.

In other words, the usually *term* congenital diaphragmatic hernia lung reacts similar to artificial ventilation as the lung of the *premature* infant.^{29,161} Nowadays infants with even more severe hypoplastic lungs reach the specialized center, where they are subjected to aggressive treatment. From this point of view, it is noteworthy that Northway observed that most adolescents who had bronchopulmonary dysplasia in infancy have some degree of pulmonary dysfunction, consisting of airway obstruction, airway hyperreactivity, and hyperinflation.¹⁸⁹ What this observation means for the congenital diaphragmatic hernia survivor with hypoplastic lungs, having survived either with or without ECMO, is not clear at the moment.

Another aspect of long term morbidity in congenital diaphragmatic hernia survivors is the presence of gastro-esophageal reflux. It has already been noted that some fetuses with congenital diaphragmatic hernia develop polyhydramnios and that this is associated with poor prognosis.³⁸ Stolar reported a massive esophageal dilatation in congenital diaphragmatic hernia survivors and related this to the possibility of a kinked gastro-esophageal junction; this could explain fetal swallowing dysfunction leading to polyhydramnios.¹⁹⁰ In another study Stolar demonstrated symptomatic gastro-esophageal reflux in 69% of tested congenital diaphragmatic hernia survivors; all but one patients were manageable with conservative therapy without needing anti-reflux surgery.¹⁹¹

1.9 References

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Chapter 2

ETIOLOGICAL ASPECTS OF
CONGENITAL DIAPHRAGMATIC HERNIA:
RESULTS OF A CASE COMPARISON STUDY

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ETIOLOGICAL ASPECTS OF CONGENITAL DIAPHRAGMATIC HERNIA: RESULTS OF A CASE COMPARISON STUDY

2.1 Summary

Congenital diaphragmatic hernia is a serious malformation with an unknown etiology. The present study was aimed at the identification of possible risk factors such as occupational or environmental exposure to chemicals and herbicides as well as the use of medications during pregnancy. One of such herbicides is Nitrofen, a compound with a strong teratogenic potential that interferes with lung development in rats and greatly resembles thyroid hormone. A parental questionnaire about teratogenic risk factors and maternal thyroid dysfunction during pregnancy was completed by 33 parents whose baby had congenital diaphragmatic hernia, and by 43 couples whose baby had esophageal atresia. No association with the studied teratogens nor with maternal thyroid dysfunction was found in either group. The resemblance of Nitrofen with thyroid hormone, a well-known growth factor for the developing lung, is of particular interest from a pathogenetic point of view in the development of CDH. Whether lung hypoplasia is the result of the diaphragmatic defect or primarily the cause of failure of the diaphragm to close, remains to be elucidated. The inability to identify teratogenic factors causing CDH still is of value in counseling the parents.

2.2 Introduction

Congenital diaphragmatic hernia (CDH) is a serious malformation characterized by a diaphragmatic defect and bilateral lung hypoplasia.¹ In the Netherlands its incidence is estimated one in 3000 liveborns, which implies that yearly circa 70 newborns will present with this defect. Its mortality rate amounts to 40%. Recent changes in therapeutic approach, such as delayed postnatal surgery after stabilization, and extracorporeal membrane oxygenation, have not improved the outlook.^{2,3}

The etiology of CDH in the human newborn remains largely unknown⁴, but in animal models several teratologic processes including exposure to chemicals have been found to induce the induction of CDH.⁵ The present study was undertaken

because within a few months an unusual high number of five newborns from a circumscribed area with heavy industry and intensive agriculture and market-gardening were admitted with CDH. In these areas fertilizers and herbicides are extensively used. Nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether) is a herbicide with a strong teratogenic potential that in rats interferes with lung development resulting in bilateral lung hypoplasia and induces anatomical malformations including diaphragmatic hernias.⁶

The stereo-chemical configuration of Nitrofen greatly resembles that of triiodothyronine (T_3) and thyroxine (T_4) (Figure). Application of Nitrofen leads to a decrease in thyroid stimulating hormone (TSH) and T_4 of the pregnant rat as well as a decrease in T_4 and increase in T_3 in the rat fetus.⁷ Thyroid hormone is a well-known trophic factor for normal lung development and alveolar cell differentiation. Moreover, maternal hypothyroidism has been associated with an increased risk for a variety of birth defects in offspring⁸, but information on its role in CDH is lacking.

In this study we report the results of a parental questionnaire concerning possible etiological and teratologic factors in the development of CDH. The questions inquired after the use of medications during pregnancy, exposure to occupational and environmental chemicals (Addendum I and II) and the history of maternal thyroid disease. In addition we studied the presence of antibodies against thyroid microsomes and thyroglobulin in mothers that had given birth to babies with CDH.

2.3 Patients and Methods

The Sophia Children's Hospital is a tertiary referral hospital with a 13-bed Pediatric Surgical Intensive Care Unit (PSICU). The hospital serves a region with a population of ± 3 million inhabitants, and an annual birth rate of approximately 35,000 newborns. All live-born neonates with structural life-threatening congenital anomalies are admitted to this PSICU. We approached all parents of children with a posterolateral CDH admitted from 1984 to 1990; stillborns were excluded. We decided to take patients with an esophageal atresia (EA) admitted in the same period as a control group, since EA has a similar distribution of referral in the area. In the study period 48 patients with a diaphragmatic defect and 66 patients with EA were admitted.

All parents were approached by phone to ask their participation in the study and after their consent they received a questionnaire, consisting of 72 items. Questions were asked related to potential teratologic data: marital status, family planning, contraception, treatment for delayed fertility, complications and illnesses during

gestation, way of delivery, occupation and social class, drug abuse, alcohol abuse, smoking, medications, X-rays, and contact with hazardous compounds in home environment and occupational environment. Reference lists were included with the names of 40 chemicals and 42 commonly prescribed drugs (Addendum I and II).

All questionnaires were evaluated after completion, by one of us (APB), and additional information was obtained by phone when answers were not clear. Statistical analysis was based on chi-square analysis of contingency tables using the StataPC program (Computing Resource Center, Los Angeles, California, USA). Statistical significance was accepted at $p < 0.05$ level.

Blood was drawn immediately postpartum from nine mothers, chosen at random from each group, and maternal antibodies were determined against thyroglobulin and thyroid microsomes (Thymune test, Wellcome, United Kingdom), and TSH receptors (TRAK assay, Henning, Berlin).

2.4 Results

Questionnaire

The final study group consisted of 33 couples (response rate 68%) whose baby had CDH, and 43 couples whose baby had EA (response rate 65%). The results are given in table 1 and 2. The data show that mothers with a newborn with EA usually underwent prenatal sonography, probably because of polyhydramnios, and were given specialized obstetrical care in a hospital. The incidence of ovulation induction procedures and spontaneous abortions in these mothers was higher than in CDH mothers. The number of prescribed medications during pregnancy was higher in fathers and mothers (in the first month of pregnancy only) of CDH newborns. The type of drugs taken ranged widely and no specific association was found to be significant.

None of the mothers from either group, suffered clinically from chronic thyroid dysfunction.

Thyroid antibodies

Antibodies against thyroglobulin and TSH receptors were negative in all tested mothers of both groups. Antibodies against microsomes were also negative in all but one mother of a patient with a diaphragmatic defect.

Table 1. Differences between potential risk factors in CDH and EA patients

<i>Before pregnancy</i>		<i>p</i>
Marital status		0.5
Consanguinity		0.3
Incidence of illness		0.6
Induction of ovulation		0.07
Contraception, type of		0.8
Delayed conception		0.1
<i>Pregnancy</i>		
Obstetrical care	EA > CDH	0.03•
Polyhydramnios	EA > CDH	0.001•
Ultrasound	EA > CDH	0.003•
Admission to hospital	EA > CDH	0.05•
Hospital delivery	EA > CDH	0.09
Dietary prescriptions		0.6
<i>Illnesses</i>		
Flu, febrile disease		0.7
Cystitis		0.1
Fainting	EA > CDH	0.04
Diabetes gravidarum		0.8
Emesis		0.5
Fluxus		0.4
Fever	EA > CDH	0.04
Hypertension		0.7
Excessive weight gain		0.6

• $p < 0.05$

2.5 Discussion

CDH is associated with a number of chromosomal, genetic, and nongenetic patterns of malformation and it seems likely that CDH is a non-specific consequence of several teratological processes.⁹

Table 2. Differences between teratologic data
in CDH and EA patients

<i>Mother</i>		<i>p</i>
Alcohol		0.4
Smoking		0.7
Prescribed medications (first 4 wks of pregnancy)	CDH > EA	0.05
<i>Father</i>		
Alcohol		0.2
Smoking		0.1
Prescribed medications	CDH > EA	0.015*
<i>Residential area</i>		
Polluted area		0.7
Degree of industrialization		0.2
Contact with herbicides		0.7
Isolation of housing		0.5
<i>Occupational area</i>		
Polluted area		0.4
Industrialization		0.6
Contact with herbicides		0.6
Working with monitor-screens		0.8

* $p < 0.05$

Chemically-induced birth defects account for only 5 % of birth defects observed in the population.¹⁰ It remains worthwhile to identify potentially hazardous chemicals in the environment in order to minimize the risk of congenital malformations. In this era of heavy industrialization and pollution the number of environmental factors interfering with the developing fetus in early gestation, may have increased. A short report on the incidence of CDH in Avon (UK) suggests an increasing frequency of this defect.¹¹

The herbicide Nitrofen is a potent teratogenic agent in rodent species. Although Nitrofen has been withdrawn from the Dutch market in 1984 because of its teratogenic properties, it is still used in other parts of the world. Exposure to Nitrofen leads to cardiac and kidney malformations as well as diaphragmatic defects

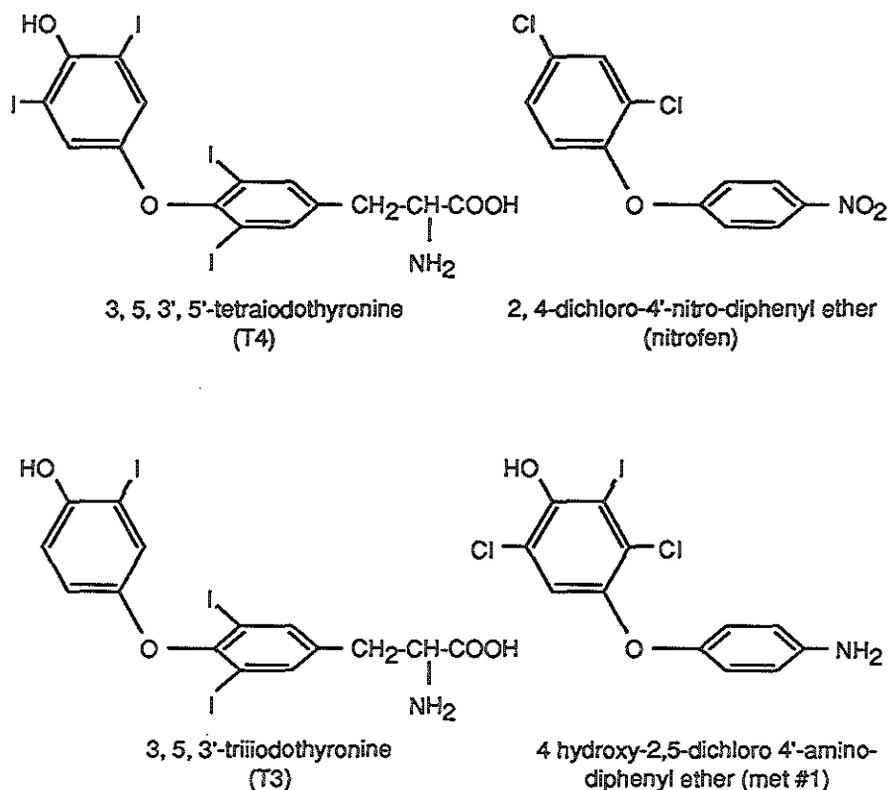


Figure. Stereochemical structures of thyroid hormones, Nitrofen, and metabolite #1. From Manson (1984), with permission.

in the offspring of pregnant rats.⁶ Nitrofen affects the cellular and structural development of the fetal lung resulting in bilateral lung hypoplasia.^{12,13} The exact mechanism of teratogenesis is not known but evidence is accumulating that the teratogenic effects are mediated via alterations in thyroid hormone status. In vitro binding studies show that Nitrofen competes with and replaces T₃ from its nuclear receptor (Brandsma et al, unpublished observations). After in utero exposure Nitrofen may interact with the embryonic nuclear receptors for thyroid hormone because it resembles thyroid hormone in structure (Figure). The interaction could interfere with lung differentiation and lung growth, and cause pulmonary hypoplasia in the fetus.⁶

This hypothesis links pulmonary hypoplasia and CDH with thyroid hormone status, and consequently, congenital defects with maternal thyroid disease. Maternal thyroid disease has been associated with an increased risk of birth defects, but reports on the subject are rather controversial.¹⁴ A large population-based, case-control study over a 12 year period showed that there was no relationship between the risk of birth defects and history of maternal hypothyroidism. In babies with multiple congenital anomalies a two-fold increased risk with hypothyroidism was found, but no discernible pattern of defects.⁸ It is also known that an intact thyroid gland is essential for normal lung development during gestation.¹⁵ Increased levels of thyroglobulin antibodies and thyroid microsomal antibodies were associated with pregnancies affected with Down's syndrome.¹⁶ In this study, neither an increased incidence of thyroid dysfunction was found, nor were antibodies detected against thyroglobulin, microsomes, and TSH-receptors in mothers of CDH patients. These findings do not support the hypothesis that maternal hypothyroidism is a risk factor for the development of lung hypoplasia or CDH.

During pregnancy, the EA group showed a higher incidence of polyhydramnios and subsequently more ultrasound examinations, hospital admissions, hospital deliveries, and care by a gynecologist. This is especially relevant to the current study because, in the Netherlands, 40% of deliveries takes place at home supervised by a midwife or a general practitioner, unless gestation is complicated by e.g. polyhydramnios. No teratologic differences between the groups were shown other than the incidence of prescribed medications to CDH parents during the first 4 weeks of gestation. Etiological studies in Hungary on isolated EA compared to normal controls also failed to show an association with studied teratogens.¹⁷

To enable further research into the etiology of CDH, the actual moment of maldevelopment should be defined first: absent closure of the diaphragm and subsequent pulmonary hypoplasia, or an alternate pathogenetic mechanism with primary poor development of the lung bud and subsequent delay of closure of the pleuroperitoneal membrane, as has been suggested by Iritani.¹⁸ The role of thyroid hormone status and that of thyroid hormone receptors in lung tissue and their relation with the development of lung hypoplasia in fetuses with CDH remains to be elucidated.

In conclusion, no etiological factors could be identified that are associated with either the development of CDH or EA. The birth of an infant with a congenital malformation will present the parents with complex emotional reactions¹⁹, such as reproach and lack of understanding. The fact that etiological relationships were not identified still can be of value in counseling the parents.

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*Addendum I**List of drugs*

Agiolax ^k	Semen Plantaginis Ovatae, Fructus Cassiae
Agré-gola ^k	Cetylpyridinium Chloride,
Algesal ^k	Diethylaminesalicylate
Antrenyl ^k	Oxyphenonium Bromide
APC ^k	Acetylsalicylic Acid, Caffeine, Paracetamol
Ascal ^k	Carbasalate Calcium
Augmentin ^k	Amoxycillin, Clavulanic Acid
Bactrimel ^k	Trimethoprim, Co-trimoxazole
Broxil ^k	Phenetillin
Buscopan ^k	Hyoscine Butylbromide
Canesten ^k	Clotrimazole
Clamoxyl ^k	Amoxycillin
Coldrex ^k	Acetylsalicylic acid, Phenylephrine , Ascorbic Acid
Daktarin ^k	Miconazole
Davitamon ^k	Vitamin A-B-E
Dolviran ^k	Acetylsalicylic Acid, Caffeine, Codeine
Dulcolax ^k	Bisacodyl
Duphalac ^k	Lactulose
Eryfer ^k	Ferrous sulfate
Fero-Gradumet ^k	Ferrous sulfate
Ferumat ^k	Ferrous fumarate
Finimal ^k	Paracetamol, Caffeine
Flagyl ^k	Metronidazole
Fortral ^k	Pentazocine
Gaviscon ^k	Sodium alginate, Sodium hydrogenocarbonate
Glifanan ^k	Glaferine
Gynodaktarin ^k	Miconazole
Hedex ^k	Paracetamol
Metamucil ^k	Plantago ovata
Mogadon ^k	Nitrazepam
Nizoral ^k	Ketoconazole
Noctamid ^k	Lormetazepam
Normison ^k	Temazepam
Seresta ^k	Oxazepam
Valdispert ^k	Valerian
Valium ^k	Diazepam

*Addendum II**List of herbicides*

Actor ^k	Paraquat-diquat
Actril ^k	Ioxynil
A.U.MCPA ^k	MCPA
Baycor anti-yeast agent	Bitertanol
Birlane ^k	Chlorfenvinphos
Bladafum ^k	Sulfotep
Curator ^k	Carbofuran
Dedevap ^k	Dichlorvos
Folimat ^k	Omethoate
Gusadeen ^k	Azinphos-methyl/propoxur
Lannate ^k	Methomyl
Mesuroil ^k	Methiocarb
Nitrofen ^k	Dinitrophenyl ether
Peropal ^k	Azocyclotin
Pirimor ^k	Pirimicarb
Ridomil ^k	Metalaxyl
Safrotin ^k	Propetamphos
Tamaron ^k	Methamidophos
Telone ^k	1,3-dichlorpropene
Thiovit ^k	Sulphur
TMTD ^k	Thiram
Topsin ^k	Thiophanate-methyl
Torque ^k	Fenbutatinoxide
Trimaton ^k	Metam-sodium
Vitigran ^k	Copperoxychloride
	Dinoseb
	Formothion
	Endosulfan
	Phosphamidon
	Zineb

Chapter 3

ASPECTS OF PERSISTENT PULMONARY HYPERTENSION

Part 3.1

ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IS
INCREASED IN LUNGS OF RATS WITH PULMONARY
HYPOPLASIA AND CONGENITAL DIAPHRAGMATIC HERNIA

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ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IS INCREASED IN LUNGS OF RATS WITH PULMONARY HYPOPLASIA AND CONGENITAL DIAPHRAGMATIC HERNIA

3.1.1 Summary

Lung hypoplasia and pulmonary hypertension are responsible for the high mortality rate in congenital diaphragmatic hernia patients. Angiotensin-converting enzyme plays a role in the regulation of pulmonary vascular resistance in the postnatal period and might be involved in the development of pulmonary hypertension of the newborn. We studied the development of angiotensin-converting enzyme activity spectrophotometrically in a rat model of congenital diaphragmatic hernia. In this model, it was previously shown that the lungs are hypoplastic and the muscularization of the pulmonary vascular bed is increased. Congenital diaphragmatic hernia was induced in fetal rats by oral administration of 115 mg/kg Nitrofen to the mother on day 10.5 of pregnancy. Fetuses were delivered by hysterotomy on day 19, 20, 21 and 22. We found a 1.5 fold higher activity in Nitrofen-exposed rats compared to controls. Angiotensin-converting enzyme converts angiotensin I to the vasoconstrictor angiotensin II, and it inactivates the vasodilator bradykinin. Increased angiotensin-converting enzyme activity may therefore contribute to pulmonary hypertension. Whether angiotensin-converting enzyme and angiotensin II levels are increased in human newborns with a diaphragmatic defect and whether they contribute to the development of persistent pulmonary hypertension, has not been studied up till now.

3.1.2 Introduction

Pulmonary hypoplasia has been described in various disease syndromes of newborn infants, such as Potter's syndrome (kidney agenesis or dysplasia), obstructive anomalies of the lower urinary tract or premature rupture of membranes.¹ Congenital diaphragmatic hernia (CDH) is a congenital anomaly that is often associated with hypoplasia of one or both lungs. CDH has a high mortality rate of over 40 %. The outcome depends either on the severity of pulmonary hypoplasia or on the combination of pulmonary hypoplasia with persistent pulmonary hypertension (PPH).^{2,3} Aggressive artificial ventilation with high percentages of

oxygen and high inspiratory peak pressures is often needed. Not only the lung parenchyma but also the pulmonary vascular system shows well-defined abnormalities consisting of decreased size of the pulmonary vascular bed, increased thickness of the pulmonary arterial muscle coat, and a decrease in the number of vessels per unit of lung.⁴ These abnormalities may interfere with the normal drop in pulmonary vascular resistance postnatally, leading to PPH and persistent fetal circulation with right-to-left shunting.

Angiotensin-converting enzyme and many other vasoactive compounds have been associated with the regulation of pulmonary vascular tone in the postnatal period.⁵ Angiotensin-converting enzyme is an enzyme located in the endothelial cells of the pulmonary vascular bed. It has two effects: (a) it converts angiotensin I to the potent vasoconstrictor angiotensin II and (b) it inactivates bradykinin, a vasodilator.⁶

In this study we investigated the activity of angiotensin-converting enzyme in an animal model of pulmonary hypoplasia and CDH.⁷

3.1.3 *Materials and methods*

Animal model

CDH was induced in fetal Sprague-Dawley rats by oral administration of 115 mg Nitrofen/kg body wt to the mother at day 10.5 of pregnancy as described previously.^{7,8} In this model animals with a diaphragmatic defect show bilateral lung hypoplasia (LH) and smaller lung/body weight ratios compared to controls.⁹ There are no significant intragroup differences in degree of LH. About 40% of the fetuses do not develop CDH, but solely LH. The distinction between animals with CDH (group:LH-CDH) and without CDH (group:LH) can only be made after autopsy. Fetuses of pregnant rats who were not given Nitrofen served as normal controls (group:control).

Fetuses were delivered by hysterotomy at day 19, 20, 21 or 22 (full term) of gestation. After determination of body weight the fetuses were killed by intraperitoneal injection of an overdose of pentobarbital (Nembutal; Sanofi, Tindenberg, The Netherlands). The presence of a diaphragmatic defect was evaluated by autopsy, after which the heart and lungs were removed en bloc, and the lungs were perfused with ice-cold 1/15 mol/L phosphate buffer (PBS; pH 7.8) via the pulmonary artery under constant pressure to remove the blood from the circulation. During perfusion the colour of the lungs changed to pale white. If part of the lungs remained pink, which indicated incomplete perfusion, the organs were discarded. The perfused lungs were then dissected, stripped of nonpulmonary tissue, weighed,

frozen with liquid nitrogen and then stored at -70°C until angiotensin-converting enzyme activity was determined.

Biochemical analyses

All biochemical analyses were performed on separate fetal lungs. After thawing, the lungs were diluted 1:15 (w/v) in ice-cold PBS, and homogenized with a Brinkmann Polytron (Brinkmann Instruments, Westbury, NY) for 15 sec at maximum speed. Next, the lung suspension was sonicated for 10 sec on ice. In this crude suspension concentrations of protein were estimated by Lowry's method using bovine serum albumin as a reference.¹⁰ To determine angiotensin-converting enzyme activity the crude suspensions were centrifuged at $20\,000 \times g$ for 30 min, and the pellets discarded.¹¹

Activity of angiotensin-converting enzyme in the lung supernatants was determined by the assay described by Neels et al.¹² In short, 11-fold diluted supernatant in buffered substrate solution consisting of 50 mmol/L HEPES, 300 mmol/L NaCl, 400 mmol/L Na_2SO_4 , 30 mmol/L hippuryl-glycyl-glycine and 2.5 ml/L saturated NaOH, pH 8.15, was incubated for 30 min at 37°C in the presence (control) or absence of 0.1 mM EDTA. After a Folin-Wu deproteinization, the liberated glycyl-glycine was derivatized with trinitrobenzenesulfonate (1.67 mmol/L) in borate buffer (55.6 mmol/L, pH 9.3) to form trinitrophenyl-glycylglycine. The absorbance was read at 420 nm vs the control. The resulting enzymic activity amounted to about ninefold that of the spectrophotometric assay of Cushman & Cheung¹³ as modified by Lieberman.¹⁴ One unit (U) of angiotensin-converting

Table 1. Developmental changes in lung weight of normal fetal rats and of fetal rats exposed to Nitrofen with and without a diaphragmatic defect*

GA (d)	n	Lung weight (mg) ^a		
		control	LH	LH-CDH
19	6	42.7 (7.8)	39.3 (3.5)	35.6 (6.2)
20	4	74.3 (8.5)	46.3 (15.5) ^b	47.4 (11.1) ^b
21	4	126.3 (11.9)	13.5 (9.3)	95.0 (10.1) ^b
22	6	151.8 (8.1)	114.5 (8.3) ^b	52.5 (7.7) ^b

* Data are means \pm SD; GA, gestational age

^a) Total lung weight (mg) of normal rats (control), and of rats exposed to Nitrofen without (LH) or with diaphragmatic defect (LH-CDH)

^b) Significantly different from the control group, $p < 0.01$

enzyme activity was defined as the amount of enzyme required to release 1 μmol of hippuric acid per min at 37°C. To facilitate comparison and exclude differences merely based upon differences in lung weight, the angiotensin-converting enzyme activities were all expressed as units per mg lung protein.

Statistical analysis

In this study lungs of 5-6 rat fetuses in each group were analyzed at day 19,20,21 and 22. The data are presented as the mean with one standard deviation (SD). Treatment effects were evaluated by analysis of variances or regression analysis.¹⁵ If a significant F-value in the analysis of variances was found, Bonferroni's correction method for multiple comparisons was used to identify differences among the groups. A difference was considered statistically significant when the P-value was 0.05 or less.

3.1.4 Results

Lung development during gestation

Total lung weight of normal fetal rats and of rats exposed to Nitrofen with or without a diaphragmatic defect (group LH-CDH and LH, respectively) increased during gestation in a similar fashion as found previously^{7,8}, (Table 1). The time-dependent increase in lung weight of LH-CDH and LH rats was significantly less than that of control rats ($P < 0.001$ for group-dependent effect of time).

Table 2. Developmental changes in lung/body weight ratio of normal fetal rats of fetal rats exposed to Nitrofen with and without a diaphragmatic defect*

GA (d)	n	Lung/body weight (mg/g) ^a		
		control	LH	LH-CDH
19	6	29.8 (4.2)	25.0 (0.8)	24.2 (2.0) ^b
20	4	32.6 (1.3)	22.8 (2.9) ^b	24.5 (3.7) ^b
21	4	32.6 (1.2)	29.9 (2.3)	26.7 (2.8) ^b
22	6	28.1 (2.6)	23.7 (1.1) ^b	10.9 (1.6) ^b

* Data are means \pm SD; GA, gestational age

^a) Lung/body weight ratio (mg/g) of normal rats (control), and of rats exposed to Nitrofen without (LH) or with diaphragmatic defect (LH-CDH)

^b) Significantly different from the control group $p < 0.01$

Table 3. Developmental changes in lung protein of normal fetal rats and of fetal rats exposed to Nitrofen with and without a diaphragmatic defect*

GA (d)	n	Lung protein ($\mu\text{g}/\text{mg}$ lung weight) ^a		
		control	LH	LH-CDH
19	6	71.8 (4.7)	74.7 (4.9)	75.6 (6.4)
20	4	72.4 (6.2)	68.4 (8.1)	63.6 (9.3)
21	4	59.5 (0.7)	56.0 (3.4)	62.3 (0.5)
22	6	60.2 (5.0)	61.4 (3.1)	87.4 (4.2) ^b

* Data are means \pm SD; GA, gestational age

^a) Lung protein (μg) per mg lung weight of normal rats (control), and of rats exposed to Nitrofen without (LH) or with diaphragmatic defect (LH-CDH)

^b) Significantly different from the control group and LH group at the same gestational age, $P < 0.01$

Exposure of the mother led to severe lung hypoplasia in the offspring as reflected by lung/body weight ratios, which were 15% lower than the controls, irrespective of the presence or absence of a diaphragmatic defect (group LH-CDH and LH, respectively), (Table 2). Lung protein content did not change significantly during gestation, and did not differ between the three treatment groups, except for day 22 at which the protein content of LH-CDH rat lungs was significantly higher than of normal and LH rat lungs ($P < 0.001$), (Table 3).

Angiotensin-converting enzyme activities during gestation

The angiotensin-converting enzyme activities during gestation are presented in Fig.1. Except for day 21, the angiotensin-converting enzyme activity in the lungs of rats exposed to Nitrofen was about 1.5 fold higher than in the normal controls. The Nitrofen-treated groups did not differ significantly in angiotensin-converting enzyme activity during gestation. Regression analysis showed that between day 19 of gestation and birth the angiotensin-converting enzyme activity in the normal fetal lung steadily increased about 17 fold to 53.04 ± 7.5 mU/mg prot ($P < 0.001$ for time dependency). In the lungs of LH-CDH and LH rats, angiotensin-converting enzyme activity increased significantly during gestation as well ($P < 0.001$ for time-dependent effect in both groups). Furthermore, the increase in lung angiotensin-converting enzyme activity during gestation differed significantly between normal rats and rats exposed to Nitrofen ($P < 0.001$), but not between rats exposed to Nitrofen with or without a diaphragmatic defect ($P = 0.24$).

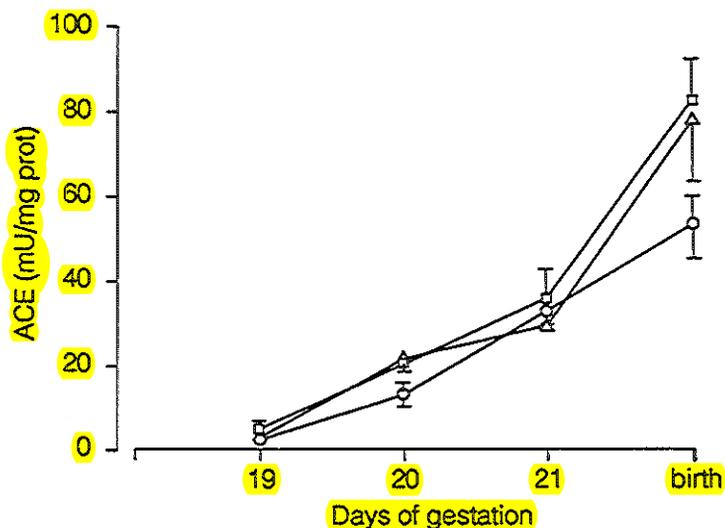


Fig. 1 Developmental changes in angiotensin-converting enzyme activity (mU/mg prot) in the lung of normal rats (○), and of rats with lung hypoplasia without a diaphragmatic defect (LH, △) or with a diaphragmatic defect (LH-CDH, □). Each point represents the mean ± 1 SD of 5-6 animals.

3.1.5 Discussion

Lung hypoplasia can be induced in fetal rats after maternal exposure to Nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether), a herbicide with a strong teratogenic potential. Nitrofen affects the cellular and structural development of the fetal lung which results in lower lung weight/body weight ratios and lower radial saccular counts.⁷ The lungs of treated rats also show increased muscularization of the pulmonary bed as in newborns with CDH.⁹ At day 11 of gestation, the process of diaphragmatic closure has proved to be highly sensitive to the teratogenic action of Nitrofen resulting in a concomitant diaphragmatic defect. In this study Nitrofen-treated rats showed a lower increase in lung weight during gestation but a similar protein content per unit of lung weight. The angiotensin-converting enzyme content in Nitrofen-treated rats was about 1.5-fold higher than in normal controls. The developmental pattern of angiotensin-converting enzyme activity in the normal fetal rat lung was similar to the pattern shown previously by Wallace et al.¹¹

Many factors are thought to regulate the pulmonary vascular tone in the postnatal period. The increase in pulmonary blood flow after birth could reflect a major shift from active vasoconstriction to active vasodilation. These changes are the result of a complex interaction between O_2 and vasoactive substances such as bradykinin, angiotensin II and the eicosanoids.⁵ The pulmonary arterial endothelial cells play a key role in this process. Endothelial cells release substances that affect adjacent smooth muscle cells or stimulate other endothelial cells downstream to paracrine activity.¹⁶ Angiotensin-converting enzyme is an enzyme that is located in the endothelial cells of the lung, near the luminal surface. It is responsible for the activation of angiotensin I to angiotensin II and the degradation of bradykinin in smaller inactive fragments.⁶ Chronic pulmonary hypertension in adult rats is associated with *decreased* levels of angiotensin-converting enzyme.^{17,18} Decreased angiotensin-converting enzyme activity would lead to a reduction of the vasoconstrictor angiotensin II and an increase in the amount of the vasodilator bradykinin that stimulates the production of the even more potent vasodilator prostacyclin and endothelium-derived relaxing factor.¹⁹ In this way decreased angiotensin-converting enzyme activity serves as a protective mechanism.

In contrast, *increased* angiotensin-converting enzyme levels would lead to an increased production of angiotensin II and an increased degradation of bradykinin. We speculate that the normal postnatal drop in pulmonary vascular resistance could be easily disturbed in the presence of increased angiotensin-converting enzyme activity together with an abnormally muscularized pulmonary vascular bed.

Another intriguing observation against the background of abnormal muscularization of the pulmonary vascular bed is the identification of angiotensin II itself as an endothelial growth factor for cell proliferation in the vessel wall.²⁰ In this concept of understanding systemic hypertension, vascular hypertrophy may be an adaptive process that normalizes vessel-wall stress in response to elevated arterial pressure, according to Laplace's law.²¹

In CDH patients the number of vessels per cm^2 lung tissue amounts to only one third of normal controls.²² Pulmonary vascular resistance can be evaluated basically from the Poiseuille's equation modified to take into account the numbers of lung vessels: $R_p = (8/\pi)(l/kr^4)\eta$, where l is the length of the vessels, k is the number of vessels, r is the radius, and η is the viscosity of the blood. As a consequence, a reduction in the number of vessels to one third would mean a three-fold increase in vascular resistance. This phenomenon could play a key role in the development of PPH and in this concept increased angiotensin-converting enzyme activity is rather a *consequence* than a *cause* of PPH.

The observation that maternal exposure to Nitrofen leads to lung hypoplasia, a reduction of the number of blood vessels in the lung and the development of a

diaphragmatic defect in the offspring, needs further clarification.^{7,9,23} A possible clue could be that Nitrofen resembles thyroid hormone in chemical structure²⁴ and competes for binding to the nuclear T₃ receptor (Brandsma et al, in press).

This is the first report of angiotensin-converting enzyme activity in a reproducible animal model for CDH characterized by bilateral lung hypoplasia. We found an increase in angiotensin-converting enzyme activity in fetal rats exposed to Nitrofen. Whether angiotensin-converting enzyme levels are increased in the lungs of human newborns with CDH and contribute to the development of PPH remains to be elucidated.

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Part 3.2

CONGENITAL DIAPHRAGMATIC HERNIA:
IMPACT OF PROSTANOIDS IN THE PERIOPERATIVE PERIOD

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CONGENITAL DIAPHRAGMATIC HERNIA: IMPACT OF PROSTANOIDS IN THE PERIOPERATIVE PERIOD

3.2.1 Summary

A prospective study of 10 neonates with congenital diaphragmatic hernia and five controls to determine the importance of prostanoid concentrations perioperatively and the relation with persistent pulmonary hypertension (PPH) is reported. In neonates with congenital diaphragmatic hernia postoperative concentrations of the vasoconstrictor thromboxane B₂ rose significantly and were higher during episodes of PPH; this rise may provoke PPH and subsequent right to left shunting.

3.2.2 Introduction

The outcome in patients with congenital diaphragmatic hernia depends either on the gravity of pulmonary hypoplasia or on the combination of pulmonary hypoplasia with persistent pulmonary hypertension (PPH).¹

Degradation products of arachidonic acid such as the leukotrienes² and the prostaglandins and thromboxanes (the so called prostanoids) have been associated with the generation of PPH in neonates.³

The purpose of this prospective study was to determine the importance of prostanoid concentrations and ventilatory variables in patients with congenital diaphragmatic hernia with and without PPH in the perioperative period.

3.2.3 Patients and Methods

During the study period, December 1987 to February 1989, 10 consecutive neonates with congenital diaphragmatic hernia (mean gestational age 37.6 weeks, birth weight 3050 g) were admitted to the pediatric surgical intensive care unit, because of severe respiratory insufficiency within six hours after birth. During the same study period five patients with oesophageal atresia (mean gestational age 36 weeks, birth weight 2645 g) served as a control group and they followed the same protocol.

Registration of ventilatory variables was carried out on admission, one hour before surgery, and one and six hours after surgery. These variables included the

alveolar-arterial oxygen differences $\{(A-a)DO_2\}$, mean airway pressure, oxygenation index, and ventilation index.⁴

Determination of thromboxane B_2 , a stable metabolite of the vasoconstrictor thromboxane A_2 , and 6-keto-prostaglandin $F_{1\alpha}$ (PgF), a stable metabolite of the vasodilator prostacyclin, were analyzed by radioimmunoassay.

The diagnosis of PPH was confirmed with the following diagnostic procedures: (i) hyperoxic hyperventilation test in which the arterial oxygen pressure (PaO_2) increased to >13.3 kPa after hyperventilation with 100% oxygen; (ii) preductal and postductal PaO_2 differential of >2.7 kPa considered evidence of a transductal shunt; and (iii) positive contrast echocardiography showing right to left shunting.⁵ As the distributions of most variables were highly skewed the median and range were used as descriptive statistics in the statistical analysis.

3.2.4 Results

Ten patients with congenital diaphragmatic hernia and five controls were examined. Two of the patients had a right sided defect and eight had a left sided defect, two had never had surgery because their condition deteriorated rapidly. These two patients died at 15 and 25 hours after admission and necropsy confirmed severe lung hypoplasia. A third patient did not undergo surgery because he had an isoelectric electroencephalogram after a prolonged period of hypoxia immediately after birth. One of the seven patients who underwent surgery had trisomy 18 and died. All five control patients were operated on. One prematurely born infant died after severe intracranial hemorrhage.

In patients with congenital diaphragmatic hernia median concentration of thromboxane B_2 rose significantly from 250 (range 81-703) pmol/ml preoperatively to 740 (range 443-1030) pmol/ml postoperatively (signed rank test, $p=0.018$). In the control group concentrations of thromboxane B_2 were 376 (range 250-497) pmol/ml preoperatively and 185 (range 50-1010) pmol/ml postoperatively ($p=0.7$). Plasma concentrations of PgF, increased from 260 (range 140-8835) to 2460 (range 1709-4150) pmol/ml in patients with congenital diaphragmatic hernia and from 455 (range 30-986) to 1145 (range 180-3682) pmol/ml in control patients; this was not significant. The increased concentrations of thromboxane B_2 at one hour postoperatively dropped to preoperative concentrations in all but two patients with congenital diaphragmatic hernia (Figure p.62).

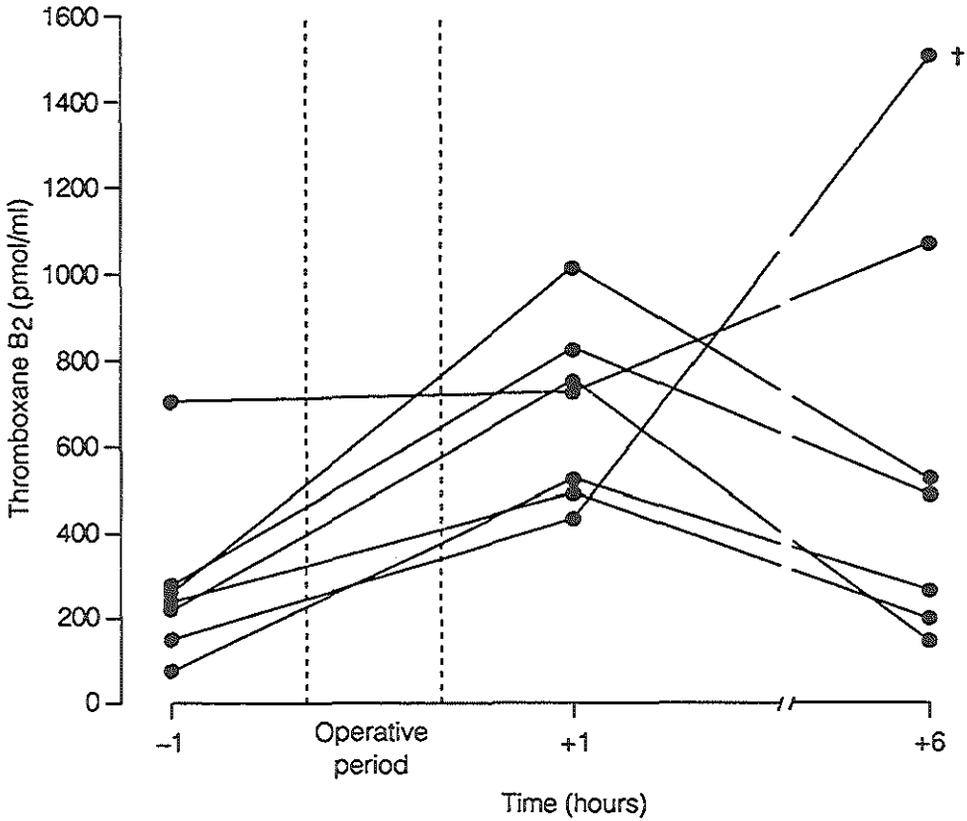


Figure. Concentrations of thromboxane B₂ before, immediately after, and six hours after surgery in seven patients with congenital diaphragmatic hernia. Patient (+) died. One of these patients died with high concentrations and the clinical picture of PPH.

In five patients with congenital diaphragmatic hernia at least one episode of PPH was diagnosed. The average values of the ventilatory variables and prostanoid concentrations are presented in the table, and compared with the values in patients without episodes of PPH. In patients with right to left shunting (A-a)DO₂, oxygenation index, and thromboxane B₂ concentrations were significantly higher than in those without right to left shunting.

Table 1. Median (range) ventilatory variables and prostanoid concentrations in patients with CDH with and without PPH

	CDH without PPH	CDH with PPH	p value
(A-a)DO ₂	201 (127-385)	559 (318-623)	0.016
Mean airway pressure	11 (8-15)	9 (8-15)	0.9
Oxygenation index	4 (2-10)	21 (8-30)	0.016
Ventilation index	630 (330-1296)	542 (497-1138)	0.8
Thromboxane B ₂ (pmol/ml)	312 (262-656)	774 (477-3699)	0.03
PgF (pmol/ml)	726 (562-1056)	938 (55-2713)	0.3

3.2.5 Discussion

Oxygenation of neonates with congenital diaphragmatic hernia in the perioperative period remains a challenging problem because of the combination of pulmonary hypoplasia and PPH.

Recently Hammerman and coworkers correlated the presence of PPH in neonates to increased concentrations of thromboxane B₂.³ The pulmonary vascular system of the patient with congenital diaphragmatic hernia differs from that of normal newborns and may overreact to different stimuli.⁶ In patients with congenital diaphragmatic hernia plasma concentrations of the vasoconstrictive metabolite thromboxane B₂ increased significantly during the surgical and anaesthetic procedure. If the pulmonary vascular system is still susceptible to vasoconstriction, it is obvious that in these cases pulmonary artery pressure may rise and right to left shunting may occur. In five out of seven patients thromboxane B₂ concentrations turned to baseline values at six hours postoperatively, showing that the rise of thromboxane B₂ is related to the operation.

In other words, the surgical procedure may provoke PPH in a 'susceptible' pulmonary vascular system. Ventilatory variables have proved their use in prediction of survival in patients with congenital diaphragmatic hernia.¹ The (A-a)DO₂ and the oxygenation index values are significantly higher in patients who have PPH. In these patients, concentrations of thromboxane B₂ have increased and might reflect an imbalance between vasodilation and vasoconstriction, dipping to vasoconstriction.

In conclusion: we have shown that during surgery there is activation of the arachidonic prostanoid cascade leading to a rise in thromboxane B₂ concentrations in patients with congenital diaphragmatic hernia. This may be an additional risk factor for right to left shunting postoperatively and it may be an argument for delayed surgery till the moment when the pulmonary vascular system has become 'stable'.

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Part 3.3

PERSISTENT PULMONARY HYPERTENSION IN HIGH-RISK
CONGENITAL DIAPHRAGMATIC HERNIA PATIENTS:
INCIDENCE AND VASODILATOR THERAPY

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J Pediatr Surg, in press

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PERSISTENT PULMONARY HYPERTENSION IN HIGH-RISK CONGENITAL DIAPHRAGMATIC HERNIA PATIENTS: INCIDENCE AND VASODILATOR THERAPY

3.3.1 Summary

Survival of congenital diaphragmatic hernia patients depends on the gravity of pulmonary hypoplasia and persistent pulmonary hypertension (PPH). Many vasoactive drugs have been used in the treatment of PPH, but often they also lower peripheral resistance, leading to a significant drop in arterial blood pressure. The incidence of PPH in 52 high-risk diaphragmatic hernia patients and the results of treatment with tolazoline and prostacyclin were evaluated in a study lasting 52 months and involving 52 patients. High risk patients require ventilatory support within six hours after birth. Study parameters were alveolar-arterial oxygenation difference $\{(A-a)DO_2\}$, oxygenation index (OI), and mean arterial blood pressure (MABP), measured at set times before and after administration of tolazoline or prostacyclin. Twenty-one patients had documented episodes of PPH (46%), and 18 of them died. Tolazoline did not lower $(A-a)DO_2$ and OI values, but MABP dropped significantly. Prostacyclin caused a significant decrease of $(A-a)DO_2$ and OI values without an effect on MABP. We concluded: (1) PPH presented in 46 % of our patients, associated with a high mortality rate; (2) tolazoline is not an effective dilator of the pulmonary vascular bed and lowers MABP; (3) prostacyclin is an effective pulmonary vasodilator as reflected by ventilation parameters without systemic side effects; it does not affect overall outcome but can be used as a "bridge" to ECMO.

3.3.2 Introduction

Newborn infants with congenital diaphragmatic hernia (CDH) presenting with respiratory distress within six hours after birth, still have a mortality rate of approximately 40 %.^{1,2}

The diaphragmatic defect is already present very early in gestation and leads to impaired branching of the bronchial tree and subsequent uni- or bilateral lung

hypoplasia.³ In addition, the muscularization of intra-acinar arteries is increased, and the external diameter of arteries is decreased.⁴ These structural abnormalities may result in increased pulmonary vascular resistance and the clinical picture of persistent fetal circulation with right-to-left shunting through the ductus arteriosus and the foramen ovale.⁵ A vicious circle of hypoxia, hypercarbia and acidosis, -well known risk factors for increased pulmonary vascular tone-, often leads to the death of the newborn with CDH due to therapy resistant pulmonary hypertension. Pharmacologic intervention to reduce pulmonary vascular resistance is often complicated by a concomitant decrease in peripheral resistance and a subsequent decrease in systemic blood pressure.⁶

In this study we report the incidence of persistent pulmonary hypertension (PPH) in newborns with CDH and the results of treatment with tolazoline and prostacyclin.

3.3.3 Patients and methods

The study was conducted as a retrospective study from January 1986 until May 1989 and continued prospectively until May 1991. In the whole study period 52 newborns (29 boys and 23 girls) with CDH were admitted to the Pediatric Surgical Intensive Care Unit of the Sophia Children's Hospital in Rotterdam. All were so-called high risk patients requiring ventilatory support within six hours after birth and all were so-called primary referrals. Six patients were excluded because of missing data, so the study group consisted of 46 patients. Eleven patients had a right-sided defect and 35 had a left-sided defect.

All patients were stabilized preoperatively and treated according to the "delayed surgery" protocol, as described previously⁷. In short the infants were paralyzed, sedated, and mechanically ventilated with frequencies of 60 to 100/min. Ventilatory support was aimed at pH values of 7.45 or higher, PaO₂ > 100 mmHg, and PCO₂ between 25 and 30 mmHg. The stomach and bowel were decompressed by continuous suction using a nasogastric tube with a double lumen. Circulatory failure was treated with plasma, dobutamine (7 to 15 µg/kg/min) together with low dose dopamine (3 µg/kg/min). Oxygenation was monitored using either preductal and postductal indwelling catheters, or two transcutaneous saturation monitors.

The diagnosis of PPH was confirmed with the following diagnostic procedures: (1) hyperoxic hyperventilation test in which the arterial PaO₂ increased to > 100 mmHg after hyperventilation with 100% oxygen; (2) preductal and postductal PaO₂ differential of > 20 mm Hg considered evidence of a transductal shunt; (3) positive contrast echocardiography showing right to left shunting⁸.

Table 1. Characteristics of patients with (+) and without (-) PPH*

	PPH +	PPH -	total
Number of patients	21	25	46
Gestational age.(wk)	38 ± 2.5	38.5 ± 2.5	
Birth weight (g)	2923 ± 647	2847 ± 886	
Side of defect			
- R	10	1*	11
number of deaths	9	1*	10
- L	11	24	35
number of deaths	9	4	13
Total number of deaths	18	5*	23

* Data are means ± SD; *p < 0.01

In the first part of the study period tolazoline (1 to 2 mg/kg/min) was the drug of choice to lower pulmonary vascular resistance, in the prospective part prostacyclin (Flolan[®], Wellcome) was the drug of first choice (5-20 nanog/kg/min). Registration of ventilatory and circulatory parameters was carried out shortly before, one hour after, and at 3-hour intervals after administration of vasoactive drugs. The variables were the alveolar-arterial oxygen difference $\{(A-a)DO_2\}$, oxygenation index (OI) and mean arterial blood pressure (MABP). The unpaired Student's *t* test was used to compare data between treatment groups. Statistical significance was accepted at $p < 0.05$ level.

3.3.4 Results

Twenty-one patients had documented episodes of PPH and 25 did not. Patients are divided according to the presence or absence of PPH; patient presenting characteristics are shown in Table 1. There were no significant differences between groups in terms of gestational age or birth weight. All patients without PPH were operated, except one with trisomy 18, one severely asphyxiated patient with an iso-electric EEG and a third with extreme lung hypoplasia. The incidence of PPH and the mortality rate was significantly higher in patients with a right-sided defect

Table 2. (A-a)DO₂, OI, and MABP before and after treatment in 21 patients with PPH*

	Tolazoline (n=12)		Prostacyclin (n=9)	
	pre	post	pre	post
(A-a)DO ₂	605	587	598	487*
SEM	±7	±16	±16	±48
OI	27	34	29	21
SEM	±3	±6	±5	±6
MABP	41	29*	41	42
SEM	±3	±3	±2	±3

*Data are means ± SEM; *p < 0.05

(p=0.002 and p=0.009 respectively). Ten of 21 patients had severe PPH preoperatively and were allowed to die without surgery, in the period before ECMO was available. Seven of them also had severe pulmonary hypoplasia as reflected by a highest pO₂ < 80 mmHg. In five of these seven patients autopsy was obtained, which confirmed lung hypoplasia showing lung body weight indices less than 0.012⁹. Table 2 contains the (A-a)DO₂, OI, and MABP values before and after treatment with tolazoline or prostacyclin. Tolazoline did not lower (A-a)DO₂ and OI values, but MABP dropped significantly (p=0.009). After administration of prostacyclin a significant decrease of mean (A-a)DO₂ values was seen (p=0.04), the decrease of OI values did not reach significance (p=0.3). There was no effect on MABP. The (A-a)DO₂ values in the individual patients after administration of prostacyclin are shown in Figure 1. All non-responders died. Patient 1 showed an initial drop of (A-a)DO₂ and OI but deteriorated later despite maximal treatment and died without the availability of ECMO. Patient 2 and 3 could be transferred for ECMO treatment and both could be weaned from ECMO. Patient 2 survived, but patient 3 died two weeks later with relapsing therapy-resistant episodes of PPH. Patient 4 remained stable under continuous administration of prostacyclin during 6 days and survived.

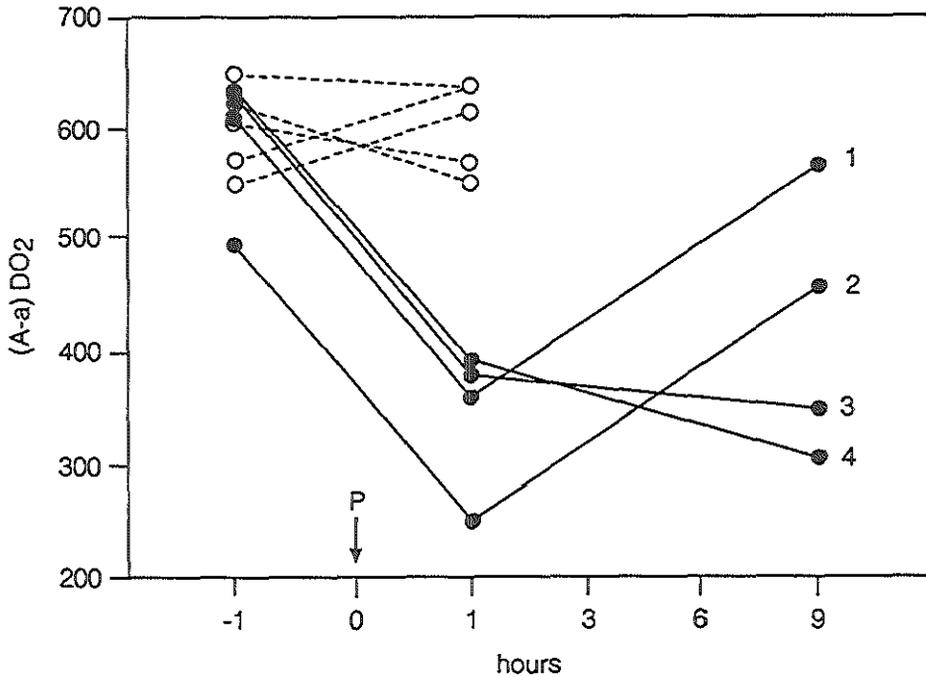


Fig. 1 (A-a)DO₂ values in nine patients after administration of prostacyclin (P).

3.3.5 Discussion

Newborns with CDH leading to respiratory insufficiency within 6 hours after birth have a poor outlook. Lung hypoplasia and (PPH) are the main causes of death. In a prospective study in 66 CDH patients Bohn et al. assessed the predictive value of preoperative PaCO₂, ventilatory measurements, in combination with morphometric analysis of the lungs of non-survivors. Their findings suggest that the degree of pulmonary hypoplasia rather than the pulmonary vascular abnormality determines survival.¹⁰ Our data show an incidence of PPH of 46 % associated with the death of 18 of 23 non- survivors.

The pulmonary vascular bed is abnormal in CDH patients and is characterized by decreased total size of the pulmonary vascular bed, increased pulmonary arterial muscle and decreased number of vessels per unit of lung.^{4,11,12} The normal resetting of pulmonary vascular tone in the postnatal period is a complex interaction between O₂ and several vasoactive compounds such as bradykinin, prostaglandins, and

thromboxanes.¹³ This process is disturbed in many CDH patients and the pulmonary vessels are excessively reactive to physiological stimuli, for example hypoxia and acidosis, resulting in vasoconstriction and subsequent right-to-left shunting.¹⁴ Mediators for this process of vasoconstriction probably are the prostanoids, because high levels of the vasoconstrictor thromboxane B₂ have been identified during right-to-left shunting in newborns with a diaphragmatic defect.¹⁵ During surgery the arachidonic cascade is activated, leading to a rise in thromboxane B₂ levels postoperatively, at a time when the pulmonary vascular bed has not "stabilized" yet.¹⁶

The ideal treatment for PPH would be selective pulmonary vasodilation without side effects on the systemic circulation.¹⁷ For many years Tolazoline was the drug of choice in newborns with PPH.¹⁸ But this drug has a long half-life, lowers systemic blood pressure in human newborns¹⁹, decreases cerebral blood flow in newborn dogs²⁰ and does not improve survival. In this study we found no positive effect on oxygenation and a significant decrease in MABP leading to a deterioration in almost all patients.

Prostacyclin is an arachidonic acid metabolite with strong vasodilative properties and a short half-life (1 to 2 min) that reduces resting pulmonary vascular tone and hypoxic pulmonary vasoconstriction.²¹ In children with PPH secondary to congenital heart disease, prostacyclin turned out to be a powerful but not selective pulmonary vasodilator.²² Animal studies showed that unwanted side effects on the systemic circulation occurred at doses above 20 nanog/kg/min.²³ Clinical experiences in neonates are scarce and not consistent, but showed a beneficial effect on oxygenation in a number of cases.^{24,25} We found a significant decrease of (A-a)DO₂ values after treatment with prostacyclin, without effects on MABP. Overall outcome did not improve but one patient benefitted from prolonged prostacyclin administration, and in three other patients time was gained to institute other forms of treatment such as ECMO. No side effects were noted.

In conclusion, PPH is a major problem in CDH which occurs in almost half of the patients and contributes to the high mortality rate. Vasodilator therapy with Tolazoline was not successful and led to systemic hypotension in almost all patients. Administration of prostacyclin did not influence overall outcome but, in responders, time was gained to make other forms of treatment possible. In other words prostacyclin is a valuable alternative in the treatment of PPH in newborns with a diaphragmatic defect. Prostacyclin can be of use as a "bridge" to ECMO.

3.3.6 References

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Chapter 4

ASPECTS OF HYPOPLASIA AND IMMATURITY

Part 4.1

NITROFEN INDUCED DIAPHRAGMATIC HERNIAS IN RATS:
PULMONARY ANTIOXIDANT ENZYME ACTIVITIES

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NITROFEN INDUCED DIAPHRAGMATIC HERNIAS IN RATS: PULMONARY ANTIOXIDANT ENZYME ACTIVITIES

4.1.1 Summary

We developed an experimental rat model of congenital diaphragmatic hernia (CDH) to elucidate the etiology and pathogenesis of this serious congenital anomaly in humans, and in particular to study the effects of a short period of artificial ventilation on the CDH lung in relation to antioxidant defense mechanisms. CDH was induced in about 60% of the offspring by maternal exposure to 2,4-dichlorophenyl-p-nitrophenylether (Nitrofen) during pregnancy. This herbicide resembles thyroid hormone in chemical structure. The lungs of fetal rats (day 19, 20, 21, and 22) were examined for protein and DNA content, and activity of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX). The same parameters were assessed in tracheotomized newborn rats after pressure-controlled artificial ventilation with either room air or pure oxygen during a short period of 5 hours.

Both in CDH-rats and controls wet lung weight increased during gestation. At term CDH-rats had significantly lower mean lung weights than controls. Both groups did not differ in protein and DNA content per mg lung, and SOD, catalase and GPX activity before and at birth.

After artificial ventilation of neonates with air and pure oxygen SOD activity tended to decrease, whereas catalase activity remained virtually unchanged in the CDH lung. However, GPX activity in the CDH lung was reduced to 80% of initial activity at term after ventilation with air and to 70% with pure oxygen. Literature supports that tissues can tolerate loss of catalase activity during oxidative stress, but no loss of GPX activity. In fact, this enzyme is one of the last remedies a cell has to repair free-radical induced lipid peroxidation of its membranes and prevent lethal damage. The present finding of a decline in GPX activity in this animal model after a short period of artificial ventilation may indicate that the CDH rat neonate is at risk to develop oxygen-related lung damage.

4.1.2 Introduction

Congenital diaphragmatic hernia (CDH) is a serious congenital anomaly with unknown etiology which occurs in approximately one in 2000 to 5000 newborns. In

contrast to other congenital abnormalities the mortality rate of CDH has not dropped since the introduction of neonatal intensive care and artificial ventilation.¹ Mortality still remains 50% due to pulmonary hypoplasia with or without persistent pulmonary hypertension.² Those infants with severe respiratory compromise need maximal ventilatory support, and are often subjected to high inflation pressures and high percentages of oxygen. The side-effects of respiratory treatment are well known³ and the clinical picture of bronchopulmonary dysplasia (BPD) has become one of the major sequelae of neonatal intensive care.⁴ Only few reports describe defense mechanisms against oxidative stress in the human neonatal lung.⁵ Most experimental studies on the development of antioxidant enzyme activity (AOA) have been done in small rodents.^{6,7} The effect of artificial ventilation on the lung has been studied only in larger animals such as the baboon.⁸

We described a model in which CDH was induced in fetal Sprague-Dawley rats by maternal exposure to a single dose of 2,4-dichlorophenyl-p-nitrophenyl ether (Nitrofen) during pregnancy.^{9,10} The incidence of CDH in the treatment group was up to 60%. The diaphragmatic defect was mainly right-sided with severe hypoplasia of the lung as reflected by decreased lung weight/body weight ratios. No correlation was found between the size of the defect and the ipsilateral and contralateral lung weights.⁹ Without artificial ventilation more than 80% of the CDH animals died within 2 hr after birth due to severe respiratory insufficiency.

In the present study we report (a) the activities of superoxide dismutase (CuZnSOD) (EC.1.15.1.1), catalase (EC 1.11.1.6) and glutathione peroxidase (GPX) (EC.1.11.1.9) just before and at birth in the diaphragmatic hernia lung, and (b) the effect of a short period of artificial ventilation on the activity of these antioxidant enzymes.

4.1.3 *Materials and Methods*

Animal model

CDH was induced in fetal Sprague-Dawley rats by oral administration of 115 mg Nitrofen/kg body wt to the mother at day 10.5 of pregnancy as described previously.^{9,10} Animals with a diaphragmatic defect showed a bilateral lung hypoplasia and smaller lung/body weight ratios compared to controls. There were no significant intragroup differences in degree of lung hypoplasia. About 40% of the fetuses did not develop CDH. The distinction between animals with CDH (group:CDH) and without CDH (group:NDH) could only be made after autopsy. Fetuses of pregnant rats who were not given Nitrofen served as normal controls (group:control).

Fetuses were delivered by hysterotomy at day 19, 20, 21 and 22 (full term) of gestation. After determination of body weight the fetuses were killed by intraperitoneal injection of an overdose of pentobarbital (Nembutal; Sanofi, Tindenberg, The Netherlands). The presence of a diaphragmatic defect was evaluated by autopsy, after which the heart and lungs were removed en bloc, and the lungs were perfused with ice-cold 1/15 mol/L phosphate buffer (PBS; pH 7.8) via the pulmonary artery under constant pressure to remove the blood from the circulation. During perfusion the color of the lungs changed to pale white. If part of the lungs remained pink, which indicated incomplete perfusion, the organs were discarded. The perfused lungs were then dissected, stripped of nonpulmonary tissue, weighed, frozen with liquid nitrogen and then stored at -70°C until antioxidant activity was determined.

The effect of a short period (for practical reasons) of artificial ventilation with air or pure oxygen on pulmonary AOA was investigated in spontaneously delivered newborn rats. The presence of CDH could be suspected in Nitrofen-treated rats by observing a high ventilatory rate and persistent cyanosis with the inability to suck spontaneously.⁹ Artificial ventilation was performed using the technique described by Lachmann.¹¹ In short, the newborn rats underwent tracheostomy under pentobarbital anaesthesia (35 mg/kg body wt/4 hr) to allow insertion of a small canula into the trachea. After muscle relaxation by administration of pancuroniumbromide (0.1 mg/kg body wt/hr) the animals were transferred to a multichambered heated body plethysmograph, which was connected to a modified Servo 900 B ventilator system (Siemens, Elema, Sweden) designed for parallel ventilation. Pressure-controlled ventilation was performed with inspiratory peak pressures of 17 cm H₂O and 2 cm H₂O of PEEP, respiratory rate 40/min, I:E ratio 1:2 according to the original reports by Lachmann et al.¹¹ on artificial ventilation in premature delivered rabbit pups. We did not increase the respiratory rate further, because the rat pups showed normal thoracic excursions and remained pink. Artificial ventilation was limited to five hours, since CDH-rats did not survive artificial ventilation with room air for longer periods. After this period the animals were killed by an overdose of pentobarbital, after which the diaphragmatic defect in the treatment group was evaluated by autopsy. Then, the lungs were processed for AOA assay as described above. After ventilation, the lungs of control rats were processed in the same way as of rats exposed to Nitrofen.

Biochemical analyses

All biochemical analyses were performed on separate fetal lungs. After thawing, the lungs were diluted 1:15 (w/v) in ice-cold PBS, and homogenized with a Brinkmann Polytron (Brinkmann Instruments, Westbury, NY) for 15 sec on maximum speed

according to Yam et al.¹² Next, the lung suspension was sonicated for 10 sec on ice. In this crude suspension concentrations of protein and DNA were estimated respectively by Lowry's method¹³, using bovine serum albumin as a reference, and by a sensitive fluorimetric assay.¹⁴ To determine AOA the crude suspensions were centrifuged at 20 000 x g for 30 min, and the pellets discarded. The activity of the most prevailing superoxide dismutase isoenzyme in the lung, i.e., the copper-zinc SOD (CuZnSOD)¹⁵, was assayed in the supernatants by the inhibition of the xanthine (0.1 mM) xanthine-oxidase(0.025 U/ml)-catalyzed reduction of ferricytochrome c at pH 10.2 in the presence of 0.1 mM EDTA to chelate free copper.^{16,17} Under those conditions no spontaneous reduction of ferricytochrome c occurred in the presence of the supernatants under investigation. After initiating the reaction by adding xanthine oxidase, the rate of increase in absorbance at 550 nm was recorded. Various volumes of each sample were used to determine the 50% inhibition point. Only linear reaction rates were taken into account to eliminate interference of cytochrome oxidase or peroxidase with the assay. In lysates of rat erythrocytes we found the CuZnSOD to be 9.7 times more active at pH 10.2 than at pH 7.8, which is in accordance with earlier reports.¹⁵ Since the manganese SOD has the same apparent activity at pH 10.2 and pH 7.8¹⁸, and the results of a pilot study indicated that this isoenzyme contributes to maximally 20% of total SOD activity (data not shown) confirming earlier observations¹⁵, the SOD assay at pH 10.2 is useful to determine changes in CuZnSOD activity. One unit of SOD activity was defined according to Fridovich¹⁶ as the amount of enzyme needed for a 50% inhibition of the reduction of ferricytochrome c in the xanthine-xanthine oxidase system at pH 7.8 using the aforementioned conversion factor of 9.7. Catalase was measured according to Bergmeyer^{17,19}, one enzyme unit (IU) was defined as the amount of enzyme that will decompose 1 μmol of H_2O_2 in 1 min at 25°C at pH 7.0, when the initial concentration of peroxide, which has a molar extinction coefficient of 41/cm at 240 nm, is 10.5 mmol/L. Glutathione peroxidase (GPX) activity was determined as previously described by Paglia and Valentine^{17,20}, who defined one unit of GPX as the amount of enzyme that convert one μmol of NADPH/min using a molar extinction coefficient of $6.22 \times 10^3/\text{cm}$ at 340 nm.²¹ To facilitate comparison and exclude differences merely based upon differences in lung weight, the activities of SOD, catalase and GPX were all expressed as units per mg lung DNA.

Statistical analysis

In this study lungs of 4-10 rat fetuses in each group were analyzed. The data are presented as the mean with one standard deviation (SD). After rank transformation treatment effects were evaluated by analysis of variances.²² If a significant F-value was found, Bonferroni's correction method for multiple comparisons was used to

identify differences among the groups.²³ A difference was considered statistically significant when the P-value was 0.05 or less.

4.1.4 Results

Lung development during gestation

Wet lung weight of normal controls increased with gestational age between day 19 up to birth to 136.4 ± 21.5 mg (for time dependency, $P < 0.001$); that of CDH-rats increased during gestation as well ($P = 0.007$), but the mean lung weight amounting to 62.9 ± 10.3 mg at birth was significantly ($0.01 < P < 0.05$) lower compared to the control and the NDH group (Table 1). The mean lung weight in the CDH and NDH rats was lower at birth than at day 21 of gestation and also the large SD found in the control rat neonates at birth is obvious. No statistical significant differences in body weight were found between the groups during gestation (Table 1). As expressed by the significantly lower lung/body weight ratios the CDH-rats apparently developed severe lung hypoplasia in utero (Table 1), which affected both lungs as shown by Tenbrinck et al.⁹ During gestation lung protein content and DNA

Table 1. Developmental changes in lung weight, body weight; and lung/body weight ratios of control rats and rats exposed to Nitrofen with and without diaphragmatic defect*

	Group [ⓐ]	n	Gestational age (d)			
			19	20	21	Birth
Lung weight (mg)	C	10	43.0 ± 7.1	89.6 ± 5.2	121.6 ± 7.8	136.4 ± 21.5
	NDH	7	40.8 ± 3.7	65.8 ± 30.1	137.0 ± 12.4 [ⓑ]	109.7 ± 8.9 [ⓑ]
	CDH	6	35.2 ± 5.6 [ⓑ]	47.6 ± 18.2 [ⓑ]	99.5 ± 17.1 ^{ⓑⓓ}	62.9 ± 10.3 ^{ⓑⓔ}
Body weight (g)	C	10	1.5 ± 0.2	2.7 ± 0.3	3.9 ± 0.1	5.4 ± 0.3
	NDH	7	1.6 ± 0.1	2.6 ± 1.0	4.5 ± 0.4	4.8 ± 0.2
	CDH	6	1.5 ± 0.1	2.0 ± 0.8	4.2 ± 0.7	5.0 ± 0.4
Lung wt/body wt ratio (mg/g)	C	10	28.2 ± 2.4	33.8 ± 1.7	33.5 ± 0.9	28.1 ± 2.6
	NDH	7	25.8 ± 1.6	27.1 ± 2.7	30.9 ± 1.1	22.6 ± 1.2 [ⓑ]
	CDH	6	24.1 ± 1.8 [ⓑ]	26.6 ± 2.7 [ⓑ]	24.8 ± 5.1 ^{ⓑⓔ}	12.2 ± 2.1 ^{ⓑⓔ}

*Data are means \pm SD.

[ⓐ] C, control group; NDH, rats exposed to Nitrofen without diaphragmatic defect; CDH, rats exposed to Nitrofen with diaphragmatic defect.

[ⓑ] Significantly different from control, $0.01 < p < 0.05$.

[ⓓ] Significantly different from control, $p < 0.01$.

[ⓔ] Significantly different from NDH group, $p < 0.01$.

[ⓕ] Significantly different from NDH group, $< 0.01 < p < 0.05$.

content expressed per mg lung weight remained virtually unchanged in all groups (Table 2); this was reflected in the protein/DNA ratio which did not differ with time and amounted to a mean value of about 7 (Table 2). The apparent increase in lung DNA and protein within the three groups between day 21 of gestation and at birth indicates that a loss of amniotic fluid from the lungs could explain the decrease in lung weight at birth.

Antioxidant enzyme activities during gestation

Between day 19 of gestation up to birth the level of SOD activity remained fairly constant within the three groups (Fig.1, p. 80). Catalase activity showed a significant increase in activity between day 21 of gestation and birth in each group (control: $P = 0.007$; NDH: $P < 0.001$; CDH: $0.05 > P > 0.01$, respectively); however, there was no significant difference between the groups (Fig.2, p. 80). GPX activity steadily increased in each group with age amounting at birth to 174%, 162% and 184%, respectively for the control, NDH and CDH groups, of the initial GPX activity at day 19 of gestation (for time dependency in each group, $P < 0.001$; Fig.3, p. 81).

Table 2. Developmental changes in lung protein and protein/DNA ratio of control rats and rats exposed to Nitrofen with and without diaphragmatic defect*

	Group [ⓐ]	n	Gestational age (d)			
			19	20	21	Birth
Protein (µg/mg lung wt)	C	10	71.5 ± 4.3	71.1 ± 4.7	62.8 ± 3.9	64.1 ± 7.8
	NDH	6	72.7 ± 4.3	61.7 ± 6.3	59.8 ± 6.3	65.0 ± 6.8
	CDH	6	76.9 ± 3.7	65.2 ± 6.7	63.5 ± 3.5	86.9 ± 7.1
DNA (µg/mg lung wt)	C	10	10.4 ± 0.7	8.2 ± 1.5	9.6 ± 0.7	10.3 ± 1.1
	NDH	6	11.0 ± 0.5	8.9 ± 1.4	7.5 ± 1.2	8.8 ± 0.5
	CDH	6	11.2 ± 1.0	9.7 ± 1.1	9.2 ± 0.8	12.1 ± 1.6
Protein/DNA ratio	C	10	6.9 ± 0.1	9.0 ± 1.8	6.6 ± 0.7	6.3 ± 0.5
	NDH	6	6.6 ± 0.1	7.2 ± 0.8	8.3 ± 2.0	7.4 ± 0.8
	CDH	6	6.7 ± 0.1	6.7 ± 0.1	7.0 ± 0.9	6.6 ± 0.7

*Data are means ± SD.

[ⓐ]C, control group; NDH, rats exposed to Nitrofen without diaphragmatic defect; CDH, rats exposed to Nitrofen with diaphragmatic defect.

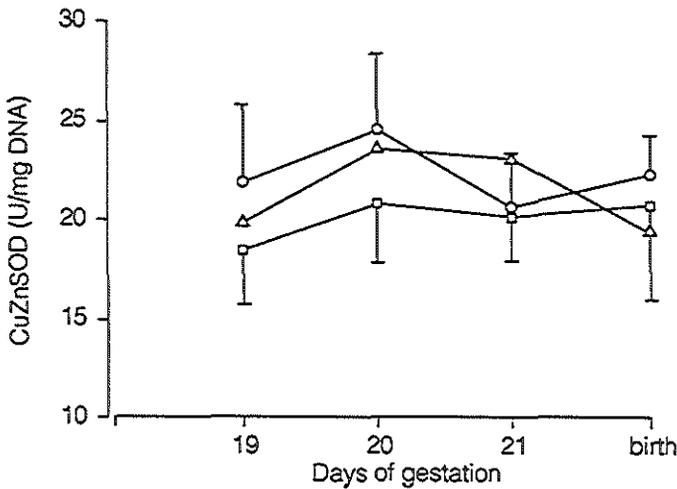


Fig.1. CuZn-superoxide dismutase activity before and at birth expressed per mg lung DNA. Each point represents the mean + 1 standard deviation of four to 10 lungs of normal control fetuses (●), and of fetuses exposed to Nitrofen without a diaphragmatic defect (▲), and fetuses with CDH (◻).

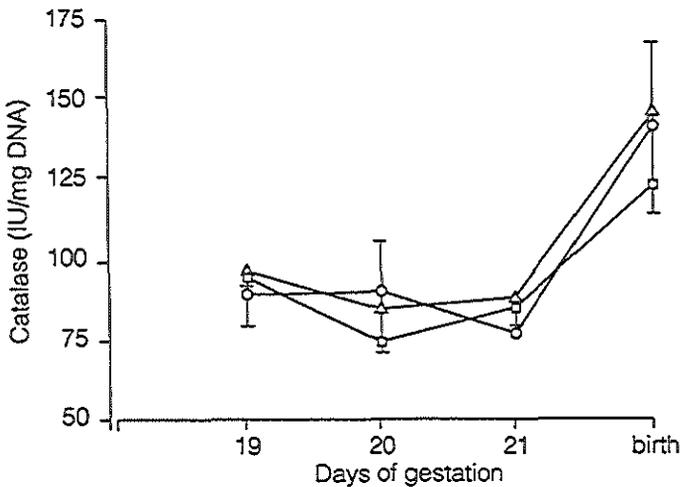


Fig.2. Catalase activity before and at birth expressed per mg lung DNA. Each point represents the mean + 1 standard deviation of four to 10 lungs of control fetuses (●), and of fetuses exposed to Nitrofen without a diaphragmatic defect (▲), and fetuses with CDH (◻).

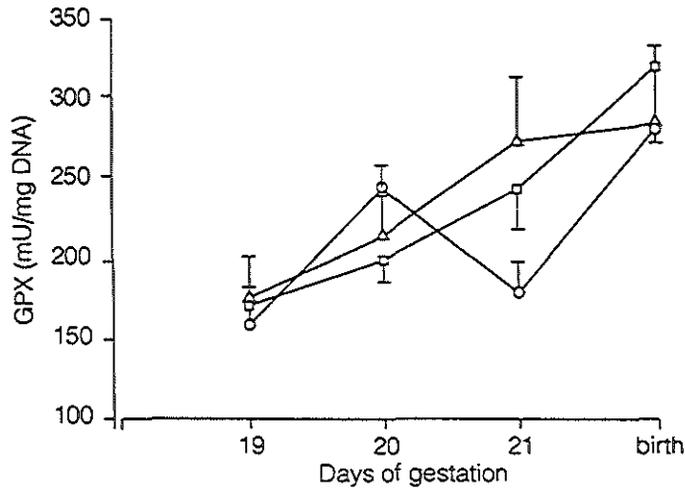


Fig.3. Glutathione peroxidase activity before and at birth expressed per mg lung DNA. Each point represents the mean + 1 standard deviation of four to 10 lungs of normal control fetuses (○), and of fetuses exposed to Nitrofen without a diaphragmatic defect (Δ), and fetuses with CDH (◻).

Effect of a short period of artificial ventilation on AOA

In the control group and the NDH group subjected to a 5-hr period of ventilation with room air or pure oxygen immediately after birth SOD, catalase and GPX activity remained at about the initial level (Table 3, p. 82). Without ventilation none of the CDH animals survived the 5 hr. Under both ventilatory conditions SOD in CDH-rats showed a tendency to decrease to about 85% of the initial activity at birth (Table 3), which did not reach statistical significance ($P = 0.12$, and $P = 0.18$, for the difference between initial activity and activity after ventilation with room air and with pure oxygen, respectively). Catalase activity remained under both ventilatory conditions at the level reached at birth and resembled normal controls and NDH rats (Table 3). After ventilation with room air GPX activity in the CDH lung decreased to about 80% and after ventilation with pure oxygen to almost 70% of initial activity at birth ($P = 0.05$, and $P = 0.01$, respectively) (Table 3); compared to the normal neonatal rat lung the CDH lung shows 20% less activity under the latter condition ($P = 0.05$; Table 3).

Table 3. Effect of short period of artificial ventilation on pulmonary SOD, Catalase, and GPX activities of control rats and rats exposed to Nitrofen with and without diaphragmatic defect*

Group ^①	Ventilation ^②	n	SOD (U/mg DNA)	Catalase (IU/mg DNA)	GPX (mU/mg DNA)
C	Birth	10	22.1 ± 2.2	142.1 ± 27.6	276.9 ± 40.5
	Air	7	20.9 ± 3.5	136.5 ± 22.7	275.8 ± 18.7
	Oxygen	8	22.8 ± 4.5	125.5 ± 13.7	271.9 ± 41.2
NDH	Birth	5	19.4 ± 4.4	146.5 ± 30.5	283.9 ± 43.6
	Air	7	20.9 ± 3.5	116.9 ± 12.4	264.1 ± 13.8
	Oxygen	9	24.6 ± 5.1	144.8 ± 31.1	255.4 ± 40.7
CDH	Birth	7	20.7 ± 4.7	124.3 ± 30.9	315.4 ± 48.4
	Air	4	17.5 ± 0.2	119.8 ± 10.1	247.9 ± 14.9 ^③
	Oxygen	6	17.9 ± 1.9	134.0 ± 40.1	216.3 ± 54.2 ^{③④}

*Data are means ± SD.

① C, control group; NDH, rats exposed to Nitrofen without diaphragmatic defect; CDH, rats exposed to Nitrofen with diaphragmatic defect.

② Neonatal rats were artificially ventilated for 5 h with air or pure oxygen.

For comparison, birth values are given, because unventilated CDH neonates did not survive the first hour of life.

③ Significantly different from the same group at birth, $p = 0.05$.

④ Significantly different from the same group at birth, $p = 0.01$.

⑤ Significantly different from the control group under the same ventilatory condition, $p = 0.05$.

4.1.5 Discussion

The present study showed that before and at birth the level of SOD, catalase and GPX activities in the lungs of CDH and normal rats were similar. Since lung weight was significantly lower in the CDH group, total AOA, however, was reduced. This indicates that the CDH lung does not differ qualitatively from the normally developed rat lung, but only quantitatively. Furthermore, upon a short period of artificial ventilation with pure oxygen only the CDH lung showed a small but significant decline in GPX activity.

Several studies focussed on the prenatal development of pulmonary antioxidant enzymes in the rat showed that the activities of total SOD, catalase, and GPX were low early in gestation and increased during fetal life.⁶⁷ The activity of at least one of those antioxidant enzymes is also affected by the amount of trace metals present

in the food.²⁴ Gerdin et al.²⁵ were the first to establish that the three antioxidant enzymes in the lung, i.e., SOD, catalase and GPX, developed in different ways. GPX in the lung shows a remarkable increase during the last 3 days prior to birth, and catalase is doubled in 2 days after birth. The SOD activity, on the other hand, increases slowly with time, with less age-related variation than catalase and GPX. In the present study we focussed on the late gestational profiles (from day 19 onward) to establish whether CDH fetuses differ in antioxidant enzyme activities from normal rats. The results show that this was not the case. Within our observation period the developmental profiles of SOD, catalase and GPX were fairly comparable to those reported by Gerdin et al.²⁵

In normal newborn rats the effect of normobaric hyperoxia on the pulmonary antioxidant enzyme system has been extensively investigated.^{12,26-28} We are, however, not aware of any study on the effect of artificial ventilation on the neonatal rat antioxidant enzyme system. To explore this aspect, we subjected spontaneously delivered newborn rats immediately after birth to a short period (for practical reasons) of artificial ventilation with room air or pure oxygen. The results of this study showed that under both ventilatory conditions GPX activity decreased significantly in the CDH lung, but not in the controls. SOD activity tended to decrease in these CDH-rats as well, whereas catalase activity remained at about the initial level. We do not know what caused the decrease in SOD and GPX activity of the CDH lung. At birth the pO_2 of room air to which the neonate is exposed, is about five-fold higher than the pO_2 in utero.⁶ Under such conditions of reoxygenation incompletely reduced forms of oxygen, i.e., oxygen-derived free radicals, become abundant. It is well known that SOD can be inactivated by hydrogen peroxide^{29,30} and GPX is highly susceptible to inactivation by superoxide, hydroxyl radicals, and hypochlorous acid.³⁰⁻³² If the rate of degradation of these enzymes during artificial ventilation is not compensated by an increased rate of synthesis, a decline in enzyme activity can be expected.

The maturation of the pulmonary antioxidant enzyme system is under hormonal control.³³⁻³⁵ Thyroid hormone stimulates metabolism generally causing an increased oxygen consumption, stimulates tissue maturation, but is shown to depress the development of antioxidant enzymes if administered at pharmacological doses leading to a markedly poorer survival of preterm rats during the first hr of life.³⁵ It is not known whether decreased enzyme synthesis, increased enzyme inactivation, or increased enzyme turnover rate is underlying the depressed increase in antioxidant enzyme activity during gestation. Furthermore, it has been shown that in rats rendered hyperthyroid the heart showed decreased GPX activity and increased lipid peroxidation.³⁶ Earlier studies have revealed that Nitrofen, which resembles thyroid hormone in chemical structure, is able to cross the placenta³⁷ and appears to have

thyromimetic activity affecting the heart, the diaphragm^{38,39}, and the lungs^{9,40-42}. From the low lung/body weight ratio and the reduced alveolar space as found in an earlier study⁶ it can be concluded that the CDH lung in our animal model is indeed hypoplastic. At the dose of Nitrofen administered apparently subtle differences in the exposure to this drug may exist among individual fetuses in utero, since about 40% of them did not develop diaphragmatic hernia. However, with respect to lung weight and lung/body weight ratio (Table 1) and maybe to the GPX response under artificial ventilation as well (Table 3), those rats could be considered as intermediate between control and CDH. This led us to propose that Nitrofen due to its thyromimetic activity has stimulated the differentiation of the CDH neonatal lung resembling the normal adult lung in its inability to respond to oxidative stress by an increase in antioxidant enzyme activity. Further investigations are necessary to assess the validity of this hypothesis.

Under normal steady-state conditions antioxidant enzymes afford effective protection to tissue damage by reducing the concentration of reactive oxygen species in the lung. Superoxide is converted by SOD to hydrogen peroxide. Both catalase and GPX can effectively detoxify hydrogen peroxide, but only GPX can reduce toxic lipid hydroperoxides formed by free radical attack in the cell membrane to the less toxic hydroxy fatty acids, and is therefore directly involved in the repair of the cell membrane.⁴³ If, however, the oxygen concentration is suddenly increased, e.g., during birth or artificial ventilation, the formation of reactive oxygen species may overwhelm the pulmonary antioxidant enzyme system. A pivotal role of GPX in protection against oxidant injury is supported by the finding that tissues can tolerate loss of catalase activity during oxidative stress, but no loss of GPX activity.^{44,45} In that respect the present finding of a decrease in pulmonary GPX activity after artificial ventilation may indicate that the CDH rat neonate is at risk to develop oxygen-related lung damage.

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Part 4.2

RADIOGRAPHIC EVIDENCE OF BRONCHOPULMONARY
DYSPLASIA IN HIGH-RISK CONGENITAL DIAPHRAGMATIC
HERNIA SURVIVORS

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RADIOGRAPHIC EVIDENCE OF BRONCHOPULMONARY DYSPLASIA IN HIGH-RISK CONGENITAL DIAPHRAGMATIC HERNIA SURVIVORS

4.2.1 Summary

Congenital diaphragmatic hernia is a congenital malformation associated with pulmonary hypoplasia which often leads to respiratory failure requiring artificial ventilation with high inflation pressures and high percentages of oxygen. Radiographic evidence of bronchopulmonary dysplasia in survivors, who presented with respiratory distress within 6 hours after birth so-called high-risk patients was evaluated by a radiographic scoring system measuring the severity of bronchopulmonary dysplasia (Toce score) and the degree of pulmonary hypoplasia (Touloukian score). Fifteen of 45 survivors (33%) had clinical and radiological lung disease resembling bronchopulmonary dysplasia. As a group they had significantly higher Touloukian and Toce scores than survivors without bronchopulmonary dysplasia. Morbidity expressed as duration of artificial ventilation, supplemental oxygen, and hospital stay was much higher in the bronchopulmonary dysplasia group. The hypoplastic lung in infants with congenital diaphragmatic hernia appears to be as susceptible to barotrauma and pulmonary oxygen toxicity as the lungs of prematurely born infants. To what extent bronchopulmonary dysplasia in congenital diaphragmatic hernia survivors might influence future development of lung function is not known yet.

4.2.2 Introduction

Congenital diaphragmatic hernia (CDH) is a congenital malformation with an unchanging high mortality rate from respiratory failure caused by pulmonary hypoplasia and persistent pulmonary hypertension.¹ Its incidence is approximately 1:3000 live births and this exceeds the incidence of cystic fibrosis (1:3500), the most common genetic disorder in The Netherlands. Recent developments in obstetric care, such as prenatal ultrasound study, may result in early detection of the fetus with a diaphragmatic defect leading to increased maternal transport and planned delivery.² As a consequence, many more patients, including those with severe pulmonary hypoplasia reach the specialized center.

These patients often require artificial ventilation with high inflation pressures and high percentages of oxygen to provide adequate oxygenation. Bronchopulmonary dysplasia (BPD) is a chronic pulmonary disease mainly presenting in premature infants who have been mechanically ventilated with high concentrations of inspired oxygen for respiratory distress syndrome.³ The pathogenesis of BPD is believed to be multifactorial with pulmonary oxygen toxicity and barotrauma having important roles.⁴

Few reports describe the effect of artificial ventilation on the hypoplastic CDH-lung, indicating an increased sensitivity to barotrauma.⁵ To test the hypothesis that the incidence of chronic lung disease, resembling BPD in these patients might be increased, we reviewed all high-risk CDH-survivors. After repair of the diaphragmatic defect we scored radiographic and clinical evidence of BPD and related morbidity expressed as duration of artificial ventilation, supplemental oxygen, and hospital stay. The preoperative chest X-ray was evaluated in order to determine whether it might predict pulmonary hypoplasia and subsequent development of BPD. Furthermore, we assessed the severity of the BPD and we tried to correlate BPD with the degree of lung hypoplasia as measured by the Touloukian score.

4.2.3 Patients and Methods

A total number of 74 high-risk CDH patients were treated at the Sophia Children's Hospital from January 1981 through December 1990. Infants were included in the high-risk group if they presented with respiratory distress within the first 6 hours of life. All patients had been treated according to a protocol described previously.⁶ In brief, patients were ventilated under sedation and muscle paralysis using time-cycled pressure-controlled ventilators (Amsterdam Infant Ventilator Mk IIIS or Servo Siemens 900 C) with a respiratory rate of 60-120 breaths per minute. The goals of ventilatory support were $\text{PaO}_2 > 100$ mmHg, $\text{PaCO}_2 < 30$ mmHg, and $\text{pH} > 7.40$. Forty-six patients (62 %) survived. Twenty-eight patients died pre-operatively or in the direct postoperative period, either due to lung hypoplasia (maximal $\text{PaO}_2 < 80$ mmHg, $n=12$) or due to therapy resistant persistent pulmonary hypertension ($n=16$). One of the survivors was not enrolled in the study group because of missing X-rays and thus the study group consisted of 45 patients. The medical records of these patients were reviewed for gestational age, birth weight, gender, side of defect, duration of artificial ventilation, duration of supplemental oxygen, and duration of hospital stay. In addition ventilation indices, and preoperative and postoperative (A-a)DO₂ and P_aCO₂ values were scored. Clinically, BPD was defined according to Bancalari as a dependency on oxygen for more than 28 days following mechanical

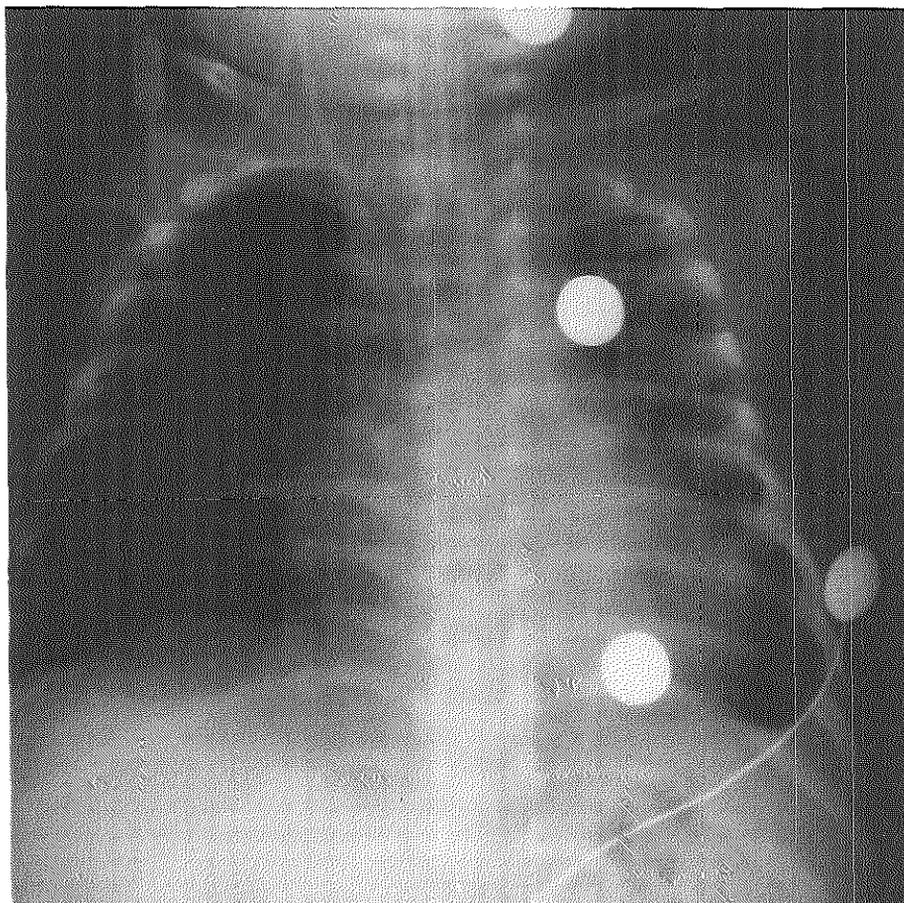


Fig. 1 Typical chest X-ray of a patient after surgical repair of a left-sided diaphragmatic defect, compatible with BPD. There is slight cardiomegaly (score, 1 point). Right hemidiaphragm projects at ninth posterior and ninth anterior rib (rib count of 18; score, 2 points). Multiple tiny bubbles of emphysema are apparent especially on the left side (score, 1 point). Interstitial markings are abnormally prominent (score, 1 point). Patient appears moderately diseased (score, 1 point). Overall score is thereby 6 points.

ventilation during the first week of life.⁷ The chest X-rays were scored by two experienced pediatric radiologists in a blinded fashion as follows: the first X-ray for risk assessment of pulmonary hypoplasia according to Touloukian⁸, and the X-ray on day 28 for BPD-assessment according to Toce.⁹ The Touloukian score included: (1)

side of the diaphragmatic defect, (2) location of the stomach, (3) presence of an ipsilateral or contralateral pneumothorax, (4) degree of the mediastinal shift, (5) amount of visceral distension, (6) relative volume of aerated ipsilateral, and (7) contralateral lung. Each roentgen finding was graded on a sliding scale from 0 to 2 with the higher grades assigned to the less favorable findings. The maximal score was 14 points. The Toce score is largely based on four of the most prominent roentgenographic abnormalities in BPD: overall lung expansion, interstitial densities, focal emphysema, and cardiovascular abnormalities (Fig.1). These factors were scored on a scale from 0 to 2 to indicate increasing severity. A fifth factor, scored likewise, is the roentgenographic impression of the overall severity of disease. The range of scores is thus 0 to 10, with 10 being the most severe. Statistical analysis for differences of means was done with the unpaired t-test. Results are given as mean \pm standard error. The relationship between Touloukian and Toce scores was evaluated by linear regression analysis. Significance was accepted at the $p < 0.05$ level.

4.2.4 Results

Twelve patients had already been discharged or were without supplemental oxygen on day 28, and consequently did not have BPD.⁷ Radiologic evaluation of chest X-rays taken on day 28 or later was possible in 33 patients. Fifteen of them had chest X-rays showing BPD at day 28 of life, and 18 did not. The 15 BPD-patients were compared with the 30 patients that did not have clinical BPD (Table I, p. 92). The group with BPD had a shorter gestational age and a lower birth weight, but the difference did not reach a significant level. Side of defect and male:female ratio did not differ between groups. The Touloukian scores and the Toce scores were significantly higher in the BPD group. The Touloukian score was not correlated with the Toce score in the individual patient ($r=0.15$, $p=0.62$). Ventilation indices, and preoperative and postoperative (A-a)DO₂ and P_aCO₂ values were higher in BPD-patients, however, without reaching statistical significance. As expected, patients with BPD required prolonged artificial ventilation, prolonged administration of supplemental oxygen, and had a much longer hospital stay.

4.2.5 Discussion

The radiologic score of BPD developed by Toce confirmed the clinical diagnosis in 15 out of 45 surviving high-risk patients. Preoperative risk assessment aimed at establishing the degree of pulmonary hypoplasia showed higher mean Touloukian scores in the BPD group; so this scoring system may help identify patients at risk

Table 1. Profiles of survivors of CDH with and without BPD*

	BPD (n = 15)	non-BPD (n = 30)
<i>Clinical data</i>		
gestational age (wk)	37.7 ± 0.5	39.1 ± 0.5
birthweight (kg)	2.93 ± 0.14	3.1 ± 0.13
Touloukian-score	10.3 ± 0.2	9.0 ± 0.3*
Toce-score	3.9 ± 0.6	0.7 ± 0.2*
hospital stay (days)	126 ± 20	46 ± 8*
artificial ventilation (days)	38 ± 10	12 ± 2*
supplemental oxygen (days)	45 ± 11	14 ± 2*
<i>Ventilation parameters</i>		
Ventilation index	793 ± 421	569 ± 51
(A-a) DO ₂		
preop	176 ± 103	150 ± 78
postop	237 ± 186	162 ± 83
PaCO ₂		
preop	37.3 ± 6	33.6 ± 7
postop	36.5 ± 4.8	35.9 ± 6.6

* Data are means ± SEM; * p < 0.05

Ventilation index: Mean Airway Pressure x Respiratory Rate

for BPD. No correlation with the Toce score was found in the individual patient. Morbidity is very high in these patients, as is reflected by long-term artificial ventilation, administration of supplemental oxygen, and hospital stay.

One of the characteristics of CDH is a variable degree of hypoplasia of the ipsi- and contralateral lung. The maturation of alveolar structures is delayed. The number of alveoli and the number of generations of the bronchial tree is reduced.^{10,11} Consequently respiratory failure immediately after birth is common in these patients and may be due primarily to lung hypoplasia. Oxygen toxicity and barotrauma may then lead to further damage of the lung and further need for ventilatory support. There is evidence that the CDH-lung is anatomically and biochemically immature. Hisanaga et al. have shown that the amniotic fluid of infants with CDH has lower lecithin/sphingomyelin ratios.¹² Animal experiments showed that antioxidant enzyme activity in the lungs of neonatal rats with a diaphragmatic defect and lung hypoplasia is decreased when exposed to artificial ventilation with 100% oxygen. Normal controls were able to produce normal lung antioxidant enzyme activity.^{13,14} If this finding is applicable to the human infant with a diaphragmatic defect, the

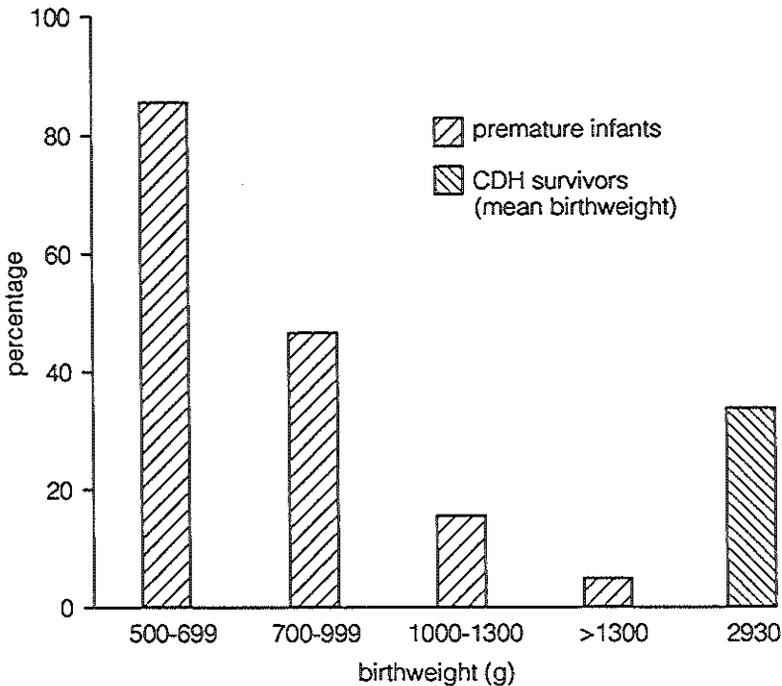


Fig. 2 Incidence of BPD related to birth weight in premature infants (data from Bancalari⁷ (with permission), and CDH survivors).

vulnerability of the CDH-lung to hyperoxia might be explained. Artificial ventilation with high percentages of oxygen and high inflation pressures may then lead to lung damage, even at full term.

The pathogenesis of BPD has been thought to be multifactorial and is related to tissue damage by hyperoxia and barotrauma.⁴ BPD is one of the major sequelae of mechanical ventilation in the neonatal period.³ It affects in particular prematurely born infants with a low birth weight.⁷

Patients with CDH have hypoplastic lungs that are often immature and functionally resemble the lungs of the premature infant.¹⁵ The risk for barotrauma in CDH patients might be even increased, because the asymmetry of the lungs results in areas of differential compliance and subsequent risk for hyperinflation¹⁶ and overexpansion of alveoli.¹⁷ Sakai and co-workers found that respiratory system compliance values dropped after surgical repair, due to the tight closure of the

diaphragm, the relocated abdominal viscera, and particularly hyperinflation, resulting in barotrauma of the contralateral lung.¹⁸ This probably explains the relatively high incidence of BPD despite normal birthweight (fig 2, p.93).

There are few reports in which pulmonary function after repair of CDH is evaluated. Pulmonary function tests within 30 days after surgical repair of CDH revealed a decrease in forced vital capacity and respiratory system compliance to half the values in normal newborn infants. In addition, airway reactivity was a prominent feature in CDH survivors, with the site of the reactivity probably located in the periphery of the lungs.¹⁹ In long-term survivors reduced perfusion and ventilation of the ipsilateral lung in combination with airway obstruction has been reported.²⁰ In one report destructive emphysema of the hernia-side lung was evident.²¹ Since the introduction of prenatal ultrasound study and maternal transport, many more neonates with severe pulmonary hypoplasia will be able to reach the specialized center and will receive artificial ventilation. On the one hand, preoperative stabilization before surgical repair has been proven beneficial in CDH patients resulting in a nearly twofold increase in respiratory system compliance and subsequently in better chances of survival.²² The application of extracorporeal membrane oxygenation (ECMO) also contributes to initial survival of the so-called high-risk patients in the neonatal period. On the other hand, a recent analysis of ECMO treatment showed a considerable number of deaths in these patients within a few months. Most of them still needed artificial ventilation after coming off ECMO and died of BPD. This is why the mortality rate remains 40%.²³

In conclusion: CDH is a serious congenital malformation with a high mortality rate and a high morbidity. This is the first documented report on the incidence of chronic lung disease, resembling BPD in high-risk CDH survivors, showing an increased incidence despite term gestation and mean birth weights around 3000 g. These patients require long-term artificial ventilation and hospital stay following surgical correction.

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Chapter 5

CLINICAL ASPECTS: TREATMENT

Part 5.1

"DELAYED" AND "ACQUIRED" PRESENTATION

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Eur J Pediatr 1987; 146:440-1

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"DELAYED" AND "ACQUIRED" PRESENTATION

5.1.1 Summary

A case of late presentation of left congenital diaphragmatic hernia (CDH) in a boy of 9 months is reported. A chest X-ray taken after (premature) birth gave normal results; hence this type of CDH is called "acquired". Not being associated with pulmonary hypoplasia, this condition is difficult to diagnose. The patient presented as an emergency and the initial diagnosis was tension pneumothorax. This resulted in the insertion of a chest tube, fortunately without damage to the herniated stomach and spleen. Doubling upward of the tip of the nasogastric tube led to the correct diagnosis. After successful repair of the diaphragm the child made a full recovery.

5.1.2 Case History

A boy of 9 months was found early one morning in his crib with respiratory arrest. Mouth-to-mouth respiration was applied successfully by the father and the child was rushed to the hospital. It appeared that he had been suffering from an upper respiratory tract infection for several days with increasing dyspnoea. He had been born prematurely at 35 weeks gestation with hydrops fetalis due to rhesus antagonism, which necessitated exchange transfusions. Mild respiratory distress symptoms in the neonatal period were diagnosed as "wet lung", and treated with continuous positive airway pressure (CPAP) and additional oxygen. A chest X-ray taken at the time gave normal results.

On admission, the boy was found to be dyspnoeic and cyanotic. His body temperature was 38.5°C, pulse rate 160/min, respirations 40/min. No breath sounds were heard on auscultation of the left chest. A chest X-ray (Fig.1) demonstrated air in the left pleural cavity with a mediastinal shift to the right, which was interpreted initially as left-sided tension pneumothorax. Laboratory findings were: White blood count $18.7 \times 10^9/l$; Capillary blood gas analysis: Ph 7.03; pCO_2 8.3 kPa; Base excess -14.4; O_2 -saturation 46%. A drain was inserted in the left pleural cavity but the left lung did not expand. It was then noted that the nasogastric tube curled upward, suggesting that the stomach was in the left pleural cavity. Contrast administration through the nasogastric tube confirmed this, leading to a provisional diagnosis of left diaphragmatic hernia (Fig.2, p. 100).

Subsequent laparotomy demonstrated a Bochdalek congenital diaphragmatic hernia on the left side, through which stomach and spleen were herniated. There

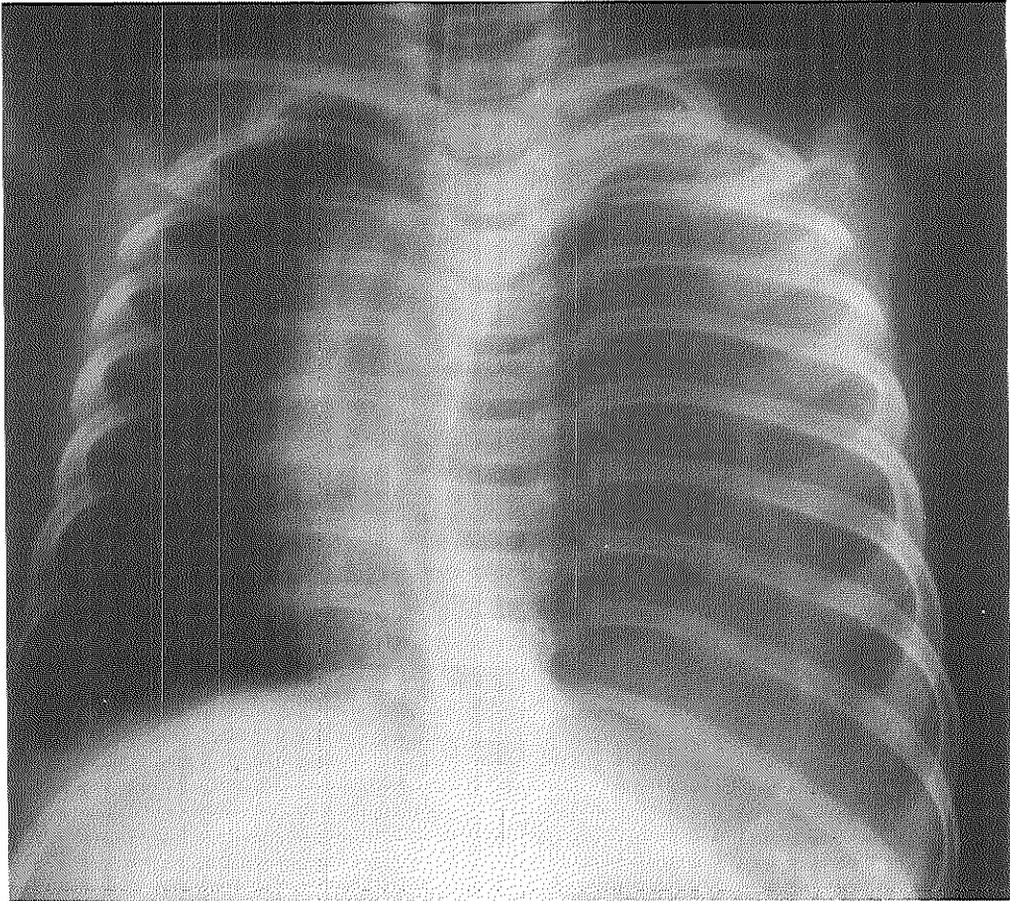


Fig. 1 Chest X-ray made on admission at 9 months

was no hernial sac. The organs were intact and could be reduced without difficulty. A normal left lung was seen to expand. The diaphragm was then repaired.

The post-operative course was complicated by recurrent atelectasis of the right upper lobe, which necessitated prolonged artificial ventilation and repeated bronchoscopy. After successful weaning from the ventilator on the seventh post-operative day, the child developed an intestinal obstruction. Laparotomy revealed ileo-ileal intussusception, which was reduced without resection. One month after the hernia repair the infant left the hospital in good condition.

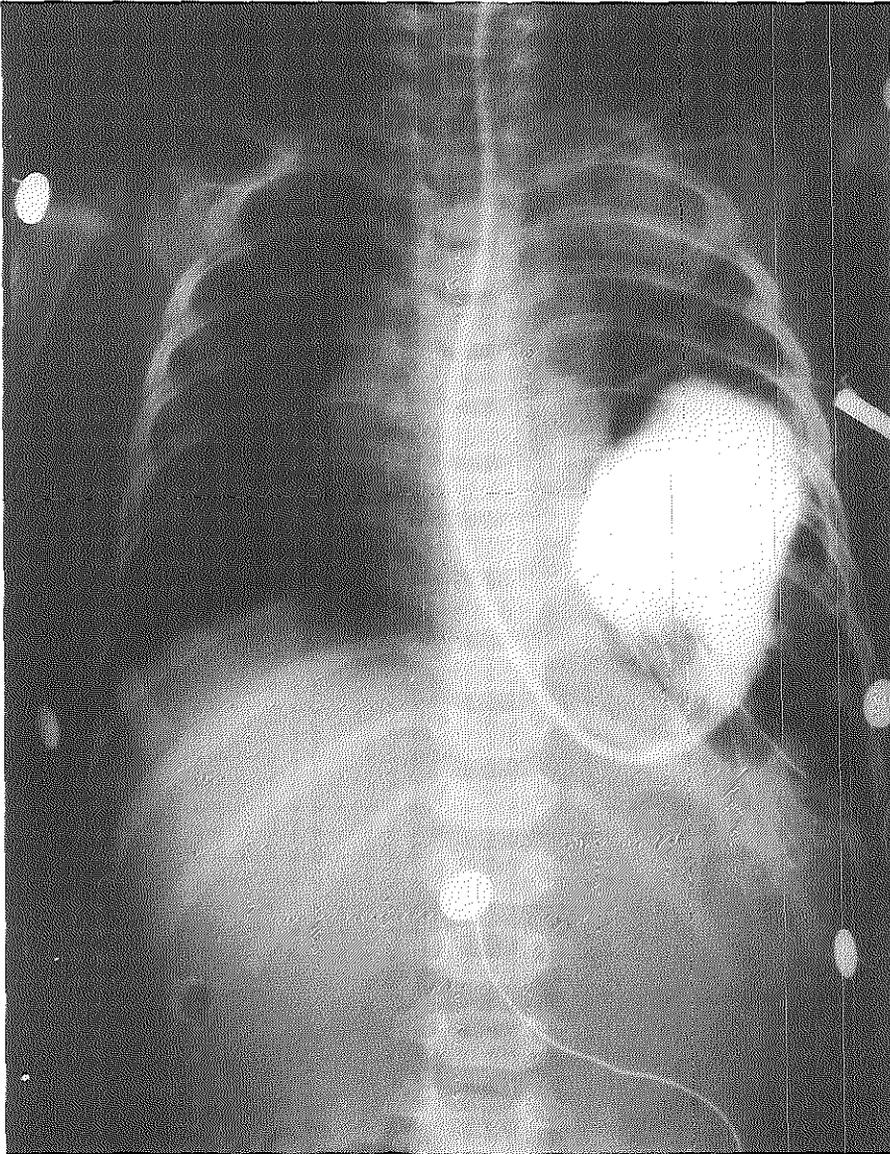


Fig. 2 Radiograph after administration of gastrografin via nasogastric tube, revealing the stomach in the left pleural cavity

5.1.3 Discussion

Wiseman and McPherson¹ differentiated between four types of congenital diaphragmatic hernia (CDH). In type 1 herniation occurs early in the course of bronchial branching, resulting in fatal bilateral pulmonary hypoplasia. In type 2 there is herniation at the stage of distal bronchial branching, resulting in unilateral hypoplasia with some chances of survival. In type 3 there is herniation at a late stage of gestation with little or no lung changes. Type 4 CDH presents some time after birth in children who are free from symptoms initially while this type is not associated with pulmonary hypoplasia. Therefore, the prognosis is very good.

In all 17 type 4 cases reported by Wiseman and McPherson¹, results of the initial chest X-ray had been normal. Consequently, they speak of acquired CDH. In their series herniation symptoms occurred between 12 h and 5 years after birth, but in several cases it took days to months to reach the proper diagnosis. Left- or right-sided CDH occurred with equal frequency and there was a male predominance.

Presenting symptoms of type 4 CDH are either pulmonary (pneumonia, dyspnoea, cyanosis) or gastrointestinal (obstruction).² Bowel sounds may be heard on auscultation of the chest. According to Wiseman and McPherson herniation in type 4 CDH takes place most often in the neonatal period because physiological events at that time favour a visceral shift from abdomen to chest.

In our patient normal results of a chest X-ray in the neonatal period demonstrated no CDH, therefore he can be classified as acquired or type 4 CDH. It is uncertain when and how herniation occurred, but it appears plausible that his upper respiratory tract infection with dyspnoea may have led to aerophagy with increasing distention of the stomach. This resulted in a mediastinal shift and circulatory problems. Mouth-to-mouth respiration forced more air into the stomach, leading to the clinical and radiological picture of tension pneumothorax. In retrospect, insertion of a chest tube was a risky procedure in view of the presence of stomach and spleen in the chest. Fortunately, the correct diagnosis was reached soon afterwards and surgical correction posed no problems.

Awareness of the possibility of late presentation of CDH will lead to a proper diagnosis and prevent hazardous insertion of a chest tube under the mistaken diagnosis of pneumothorax.

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Part 5.2

PRE-OPERATIVE STABILIZATION
WITH DELAYED REPAIR
IN CONGENITAL DIAPHRAGMATIC HERNIA

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Z Kinderchir 1989; 44:139-43

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PRE-OPERATIVE STABILIZATION WITH DELAYED REPAIR IN CONGENITAL DIAPHRAGMATIC HERNIA

5.2.1 Summary

Congenital diaphragmatic hernia (CDH) remains one of the major challenges for pediatric surgeons and pediatric intensive-care specialists. Death in patients with CDH is 30-60% worldwide due to severe pulmonary hypoplasia or pulmonary hypoplasia associated with persistent pulmonary hypertension, secondary to hypoxia, metabolic acidosis or myocard insufficiency. Pre-operative stabilization of CDH patients might reduce the risks of these complications. In a two-year period 16 high-risk patients with CDH (respiratory insufficiency < 6 hrs after birth) underwent delayed surgery following a stabilization period (mean 14 hrs). Continuous suctioning on a nasogastric tube resulted in total resolution of the mediastinal shift on repeat x-rays. The use of the ventilation parameters arterial alveolar oxygen gradient (A-A)DO₂, oxygenation index (OI) and mean airway pressure (MAP) revealed three different groups of patients: I consisting of 6 survivors, II two preventable deaths and III eight non-survivors.

In this way selection of patients with CDH is possible. In patients who do not improve during the stabilization period alternative ways of treatment have to be evaluated such as extracorporeal membrane oxygenation (ECMO), high frequency oscillation (HFO) or high frequency jet ventilation (HFJV). Application of the ventilation parameters in prospective trials of patients with CDH enables comparison between different ways of treatment in the future.

5.2.2 Introduction

The mortality rate for patients with congenital diaphragmatic hernia (CDH) varies from 30-60%.^{1,2,3} Patients admitted within 6 hours of birth generally constitute a high risk group. Death in patients with CDH is due to severe pulmonary hypoplasia⁴ or to less severe pulmonary hypoplasia associated with persistent pulmonary hypertension. Attempts to decrease the high pulmonary arterial pressure by means

of vasoactive drugs is not always successful.⁵ In many cases high pulmonary arterial pressure is secondary to hypoxia, metabolic acidosis or electrolyte disturbances.^{6,7,8,9}

Pre-operative stabilization of CDH patients might reduce the risk of these complications. In patients treated in this way new questions arise about the indication and timing of the operation. Ventilation parameters collected during the stabilization period can be evaluated as predictors for survival.

5.2.3 Patients and Methods

In a two year period from January 1986 to December 1987 16 patients (8 males and 8 females) with CDH were admitted within 6 hours after birth to the pediatric surgical intensive-care unit of the Sophia Children's Hospital in Rotterdam. The mean birth weight was 2900 grams (1885-3700 g), mean gestational age 38 weeks (32-42 wk). Two children were born preterm.

Overall mortality was 10 out of 16 (61%). The diaphragmatic defect was predominantly left-sided involving 10 patients versus 6 with a right-sided defect.

Four patients had concomitant congenital anomalies, consisting of hypoplastic left ventricle, aortic coarctation, trisomy 21, and cheiloschisis. In the patient with aortic coarctation hypertension of the upper part of the body was diagnosed only two months after birth and successfully repaired by resection of the coarctation. All patients suffered from respiratory insufficiency within 0-3 hours after birth, requiring endotracheal intubation and artificial ventilation.

In one patient bilateral pneumothorax was drained successfully in the referral hospital, while in another patient pneumothorax was diagnosed on arrival at the surgical intensive-care unit and drained. All patients were ventilated by a time-cycled volume-controlled ventilator (Siemens Servo 900C) with frequencies between 60-80 per minute under general muscular paralysis (Pavulon[®]) and sedation (Diazepam[®]).

Adjustment of ventilatory settings was always aimed at arterial pH values between 7.45 and 7.50, PaCO₂ between 25 and 30 mmHg and PaO₂ between 100 and 150 mmHg. The ventilatory requirement was based on arterial blood gas analysis using pre- and postductal indwelling catheters that enabled continuous monitoring of the systemic blood pressure as well. In case one of the arterial catheters could not be placed, pre- and postductal transcutaneous PO₂ measurements were done in addition.

All patients underwent cardiac evaluation at least once preoperatively to exclude structural cardiac anomalies as well as evaluation of myocardial contractility. Hypoxic cardiomyopathy was suspected in many patients secondary to postnatal

asphyxia. If required, the systemic blood pressure was supported by dopamine or dobutamine (2-10 µg/kg/min), in a few cases in combination with isoprenaline (3-10 µg/kg/hr). In case of hypoxic cardiomyopathy sodium-nitroprusside or nitroglycerin was administered for afterload reduction in a dosage ranging from 0.5-10 µg/kg/min.

Metabolic acidosis was treated by vigorously by sodium bicarbonate or TRIS-buffer until the arterial pH had risen above 7.40. A nasogastric tube was inserted in all patients enabling continuous suctioning of 5 cm of water to reduce the shift of the mediastinum which was always present. Repeat thoracic X-rays 6-12 hrs after admission served to assess any decrease in mediastinal shift as well as the amount of air in the digestive tract.

Twelve of our 16 patients were operated upon through a subcostal abdominal incision enabling reduction of the abdominal organs from the thoracic cavity and closure of the defect in the diaphragm. The mean duration of the stabilization period was 14 hours (range 2-33 hrs). Primary repair was possible in three patients, all others required a durapatch. Prior to the diaphragmatic closure, a chest tube was placed in the pleural space without suctioning and kept in place for at least 3-5 days.

Autopsy was performed in 8 of the 10 patients who died. The diagnosis of pulmonary hypoplasia was based on a lung-bodyweight index less than 0.012 as described by Askenazi et al (1979).¹⁰ In all patients the ventilation parameters were determined at six-hour intervals using the following formulas:

Arterial alveolar oxygen-gradient ¹¹

$$(A-a)DO_2 = FiO_2 (Pb-47) - PaO_2 - PaCO_2 (FiO_2 + 1 - FiO_2/R)$$

Mean airway pressure ²

$$MAP = 2/\pi(It \times TRC) \times (PIP-PEEP) + PEEP$$

Oxygenation index ¹²

$$OI = \frac{FiO_2 \times 100 \times MAP}{PaO_2}$$

FiO₂ = inspired oxygen concentration

Pb = atmospheric pressure

PaO₂ = arterial pO₂

pACO₂ = alveolar pCO₂,
assumed is paCO₂ (arterial pCO₂)

R = respiratory quotient, assumed to be 0.8

It = inspiratory time
 TRC = total respiratory cycle
 PIP = peak inspiratory pressure
 PEEP = positive end-expiratory pressure

5.2.4 Results

Ten of our 16 patients died, amounting to an overall mortality of 61%. This included all six patients with a right-sided defect but only four of ten patients with a left-sided defect, a comparative mortality of 100% versus 40%.

For two patients death was not directly related to the diaphragmatic defect. One of them contracted a pulmonary infection following successful surgical repair requiring increased oxygenation. The occurring persistent foetal circulation did not respond to vasoactive drugs and *Pseudomonas aeruginosa* were isolated from tracheal cultures. At the age of seven days this patient died.

Table 1. (A-a)DO₂, OI and MAP values measured at admission, after 12 and 24 hours*

	n	0 hrs	12 hrs	24 hrs
(A-a)DO₂				
Survivors	6	321 ± 269	147 ± 105	189 ± 105
Prevent. death	2	198 - 289	192 - 172	562 - 198
Non-survivors	8	607 ± 12	606 ± 39 [°] (n = 7) ⁺	565 - 176 (n = 2) ⁺
OI				
Survivors	6	16 ± 20	5 ± 1	5 ± 2
Prevent. death	2	6.7 - 5.2	3.1 - 7.3	3.8 - 2.1
Non-survivors	8	27 ± 24	95 ± 78 [°] (n = 7) ⁺	67.5 - 186 (n = 2) ⁺
MAP				
Survivors	6	13 ± 5	3 ± 1	13 ± 5
Prevent. death	2	9 - 9.4	9.7 - 9.9	9.4 - 9.9
Non-survivors	8	14 ± 5	23 ± 5 [°] (n = 7) ⁺	19.6 - 33.6 (n = 2) ⁺

* Data are means ± SD; ⁺ Number decreased by deaths; [°] p<0.05

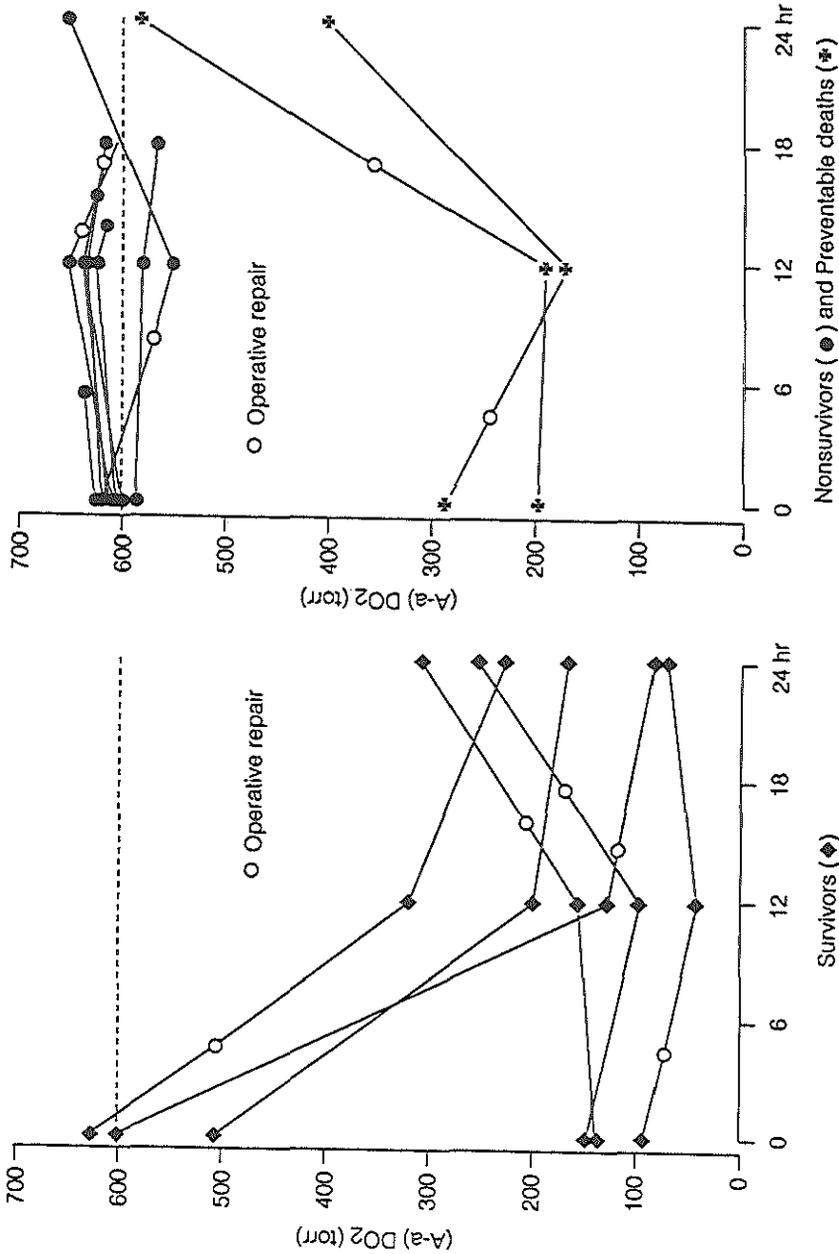


Fig. 1 (A-a)DO₂ in survivors (a) and nonsurvivors (b) including preventable deaths. Broken line indicates more than 80% mortality rate. Hr: hours after arrival at ICU.

The other patient returned to the ICU with persistent foetal circulation which did not respond to ventilatory assistance or vasoactive drugs and was subsequently found to have developed pneumothorax at surgery that had remained undetected. It was felt that these two deaths might have been prevented and these two non-survivors were therefore classified separately. Consequently, the assessment of results concerned three groups of patients: I consisting of six survivors, II two "preventable deaths", and III eight non-survivors. For all patients, regardless of survival or mortality, pre-operative stabilization successfully reduced the mediastinal shift as shown by the repeat thoracic x-rays taken before surgery.

Table 1 (p. 106) gives the values determined for the ventilation parameters at different intervals per group of patients, showing a decrease in numbers for the non-survivors. Figure 1 (p. 108) gives the values of the arterial alveolar oxygen gradient in relation to time as well as the moment of operative repair. For eight patients these values amounted to ≥ 600 with minor changes in time; all these patients died. Two other patients with an $(A-a)DO_2 > 600$ on arrival had a rapid decrease of this gradient and both patients survived. In contrast, two of the remaining six patients with low $(A-a)DO_2$ values on arrival showed a significant increase within the first 24 hours after admission and died (preventable deaths).

Figures 2 and 3 shows that both the OI and MAP at 12 hours after arrival in survivors and non-survivors differ significantly with exception of the two patients whose deaths were considered preventable (p. 110 & 111) Figure 4 (p. 112) gives the relationship between FiO_2 and preductal arterial PaO_2 at 12 hours after arrival. A preductal PaO_2 above 100-150 was achieved in all surviving patients, while in all non-survivors the preductal paO_2 was below 60 mm Hg even with a FiO_2 of 1.0. The two patients with preventable death clearly showed an intermediate pattern.

Four patients were never operated upon because of persistent arterial hypoxemia ($PaO_2 < 60$ mm Hg). Lung hypoplasia (lung-bodyweight index < 0.012) was diagnosed postmortally in all eight non-survivors for whom permission for autopsy was granted, including two patients who never underwent surgery.

5.2.5 Discussion

Congenital diaphragmatic hernia is generally considered to be a surgical emergency. The bowel loops that protrude into the thoracic cavity during the foetal period have to be removed as soon as possible.

In our series of 16 CDH patients primary non-operative treatment revealed two divergent patterns. In the first group of six patients no pathological values of the

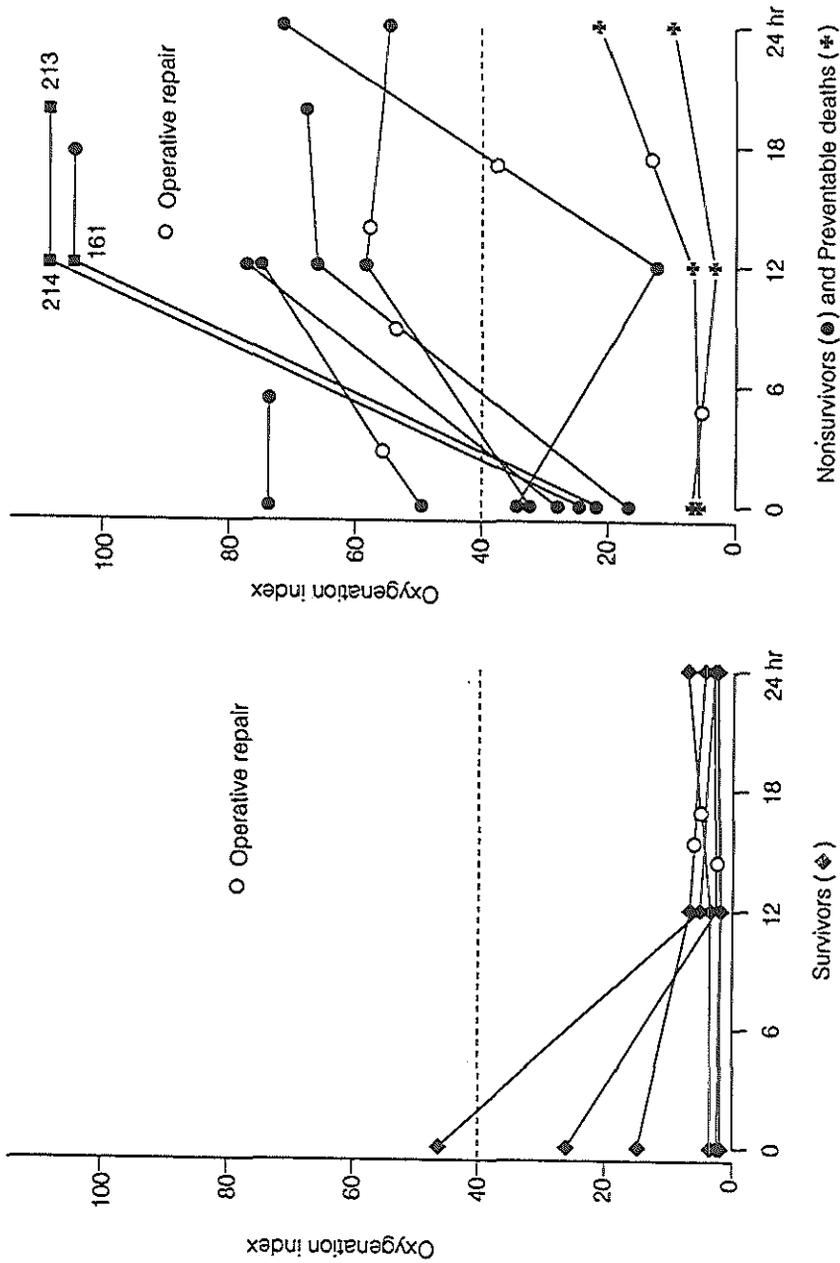


Fig. 2 OI in survivors (a) and nonsurvivors (b) including preventable deaths. Broken line indicates more than 80% mortality rate. Hr: hours after arrival at ICU.

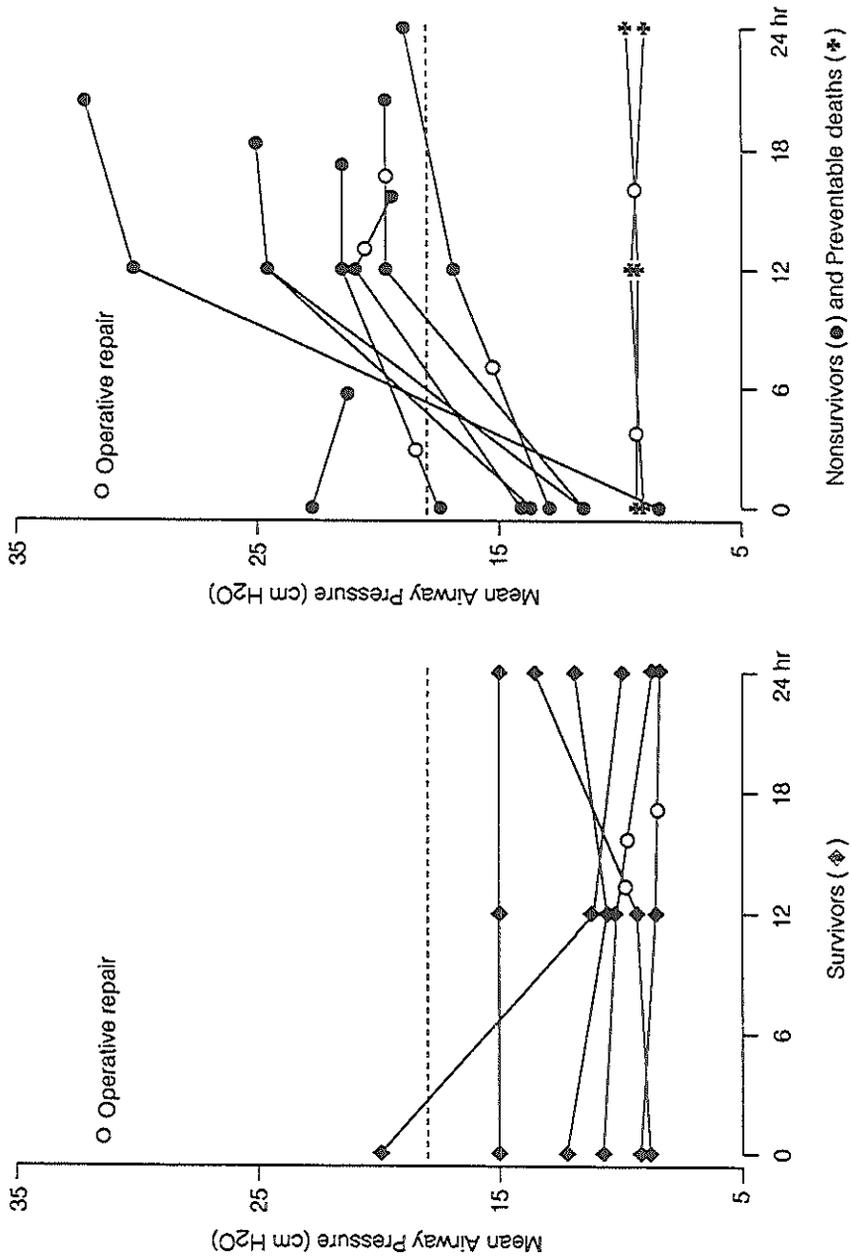


Fig. 3 MAP in survivors (a) and nonsurvivors (b) including preventable deaths. Broken line indicates more than 80% mortality rate. Hr: hours after arrival at ICU.

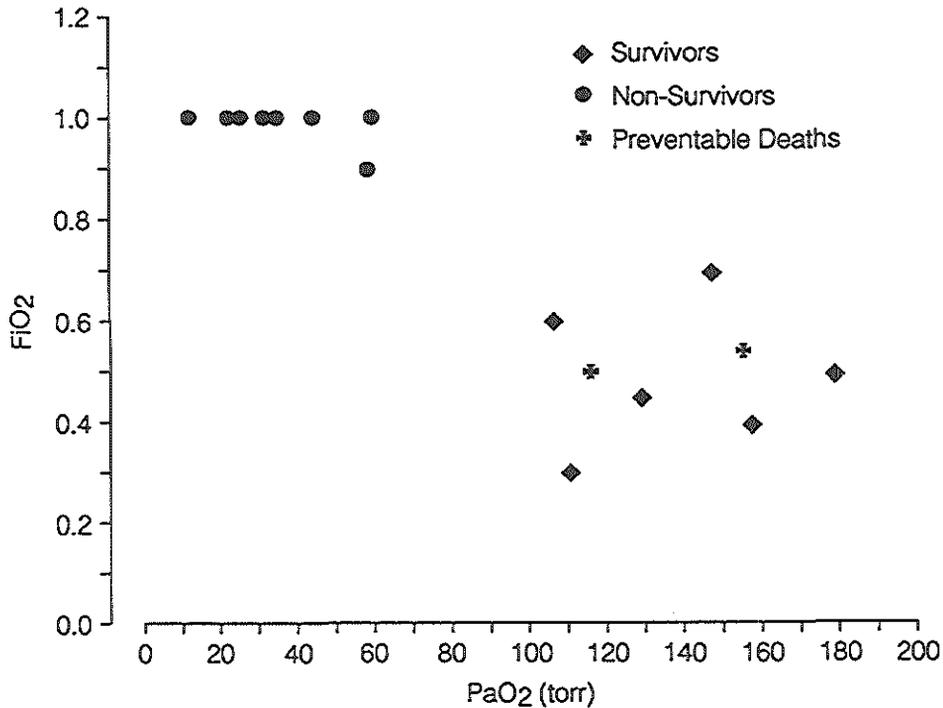


Fig. 4 Relationship between FiO_2 and the preductal PaO_2 at 12 hours after arrival at the ICU.

arterial alveolar oxygen gradient, oxygenation index and low mean airway pressures were observed. These infants had an excellent prognosis and survival should have been 100%. Unfortunately two patients did die, but their death was clearly not directly related to the CDH and could have been prevented.

The second group of patients, 10 out of 16, had pathological values of (A-a)DO₂ on arrival. In two cases in which pathological values were noted (>80% predicted mortality)¹² on arrival at the ICU pre-operative stabilization resulted in a rapid normalization of the (A-a)DO₂, and to a lesser extent of the OI and MAP and these patients survived as well. In the remaining eight patients adjustment of ventilatory settings in combination with medication to improve cardiac output did not lead to improvement of arterial blood gas values. These patients showed no response to treatment and died within 4-32 hours after admission.

Many patients with CDH pass a period of asphyxia following birth leading to hypoxic cardiomyopathy and persistent R-L shunt due to persistent pulmonary hypertension. For this reason other authors^{4,13} have advocated delayed repair of

CDH. Carlidge et al described 17 patients with CDH who developed severe respiratory insufficiency within one hour after birth.¹³ Their survival rate increased from 12% in patients, who underwent emergency repair to 53% following delayed repair. Bohn reported the use of the ventilation index as predictor of survival in 36 patients with CDH who underwent delayed repair.⁴ The same group demonstrated that lung compliance may decrease significantly following surgical repair.¹⁴

Other ways of ventilation have been suggested as a way to improve survival of patients with congenital diaphragmatic hernia such as high frequency oscillation or ECMO. ECMO may be considered for patients who survive the operation by at least 8 hours and meet the criteria of Bartlett.¹² A prospective randomized trial using ECMO and other forms of ventilatory support (high frequency jet ventilation or oscillation) has not yet been performed. In contrast to the investigations of Raphaely and Downes¹⁵ who attributed a significant decrease of the (A-a)DO₂ in surviving patients with CDH to surgical correction we have shown that this value decrease regardless whether the patients are operated upon or not.

In other words, the improvement of the (A-a)DO₂ cannot be considered as a result of the surgical correction. It can be achieved by optimal artificial ventilation and the use of vasoactive drugs alone. In all our patients nearly complete resolution of the mediastinal shift took place by continuous suction of the stomach. Repeat x-rays revealed that the deviation of the mediastinum had disappeared. In our opinion this phenomenon plays a significant role in the improvement of the arterial blood gas values observed in a number of patients during the preoperative period. This dismisses a persistent shift of the mediastinum and compression of the contralateral side as an argument for emergency operation. Although our approach did not lead to a significant decrease of the mortality rate it does enable selection of patients. In patients who do not improve alternative ways of treatment have to be evaluated. The great number of right-sided diaphragmatic hernias in our group might have contributed significantly to the total mortality.¹⁶

In conclusion: (A-a)DO₂, OI and MAP values obtained during the preoperative stabilization period in CDH patients have a predictive value for survival. In case no significant improvement of the values under study is observed during the stabilization period, surgical repair does not change the ultimate prognosis for these patients.

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Part 5.3

SURFACTANT REPLACEMENT THERAPY IN
HIGH-RISK CONGENITAL DIAPHRAGMATIC HERNIA

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Lancet 1991; 338:1279

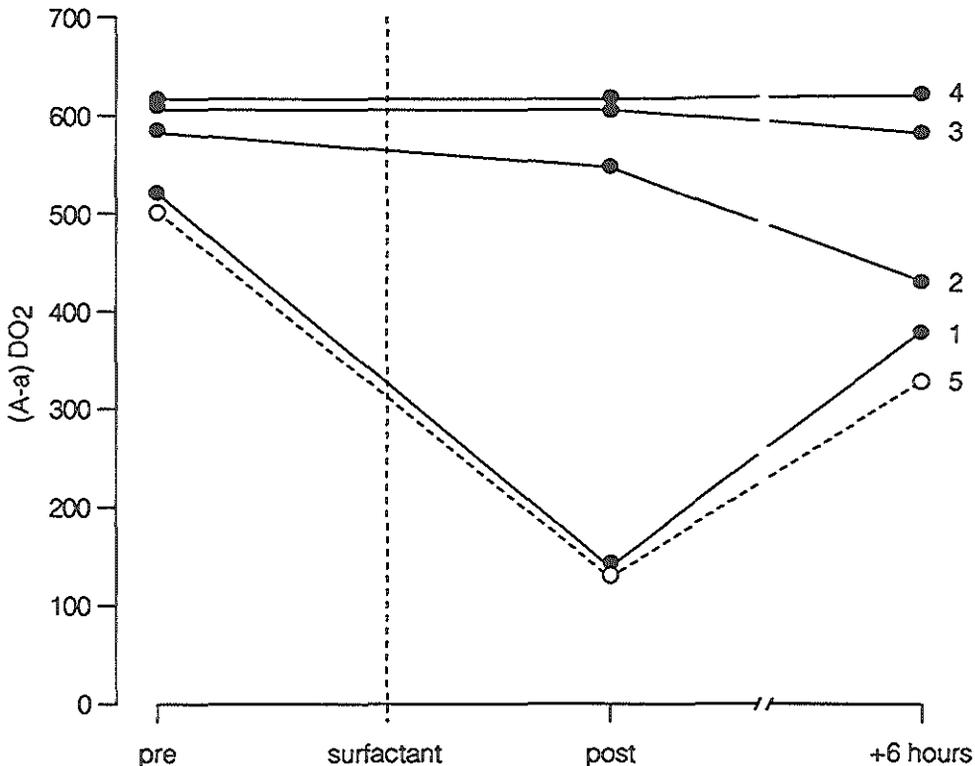
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SURFACTANT REPLACEMENT THERAPY IN HIGH-RISK CONGENITAL DIAPHRAGMATIC HERNIA

5.3.1 Letter

SIR,- Clinical trials of surfactant replacement therapy have shown a reduction in the frequency and severity of respiratory distress syndrome (RDS) in premature newborn babies,¹ and first clinical trials in adults have also shown the beneficial effects of this therapy.^{2,3}

Does surfactant have a role in the treatment of more mature infants with pneumonia and meconium aspiration syndrome, or in infants with hypoplastic lungs, as seen in congenital diaphragmatic hernia? Congenital diaphragmatic hernia present in 1 in 3000 liveborn babies. Although new treatments, such as delayed surgery and



extracorporeal membrane oxygenation (ECMO), have been advocated, the case fatality rate for this malformation still remains 30-50% worldwide because of pulmonary hypoplasia and persistent pulmonary hypertension.⁴ The hypoplastic lung in these patients is probably biochemically immature, and low lecithin/sphingomyelin ratios in amniotic fluid have been recorded.⁵ We investigated the effect of surfactant replacement therapy in 4 newborn babies with a congenital diaphragmatic defect. Their mean gestational age was 37 (range 36-40) weeks and their mean birthweight 2880 g (range 2560-3400). All 4 patients had respiratory insufficiency within 6 h after birth. They were ventilated with a time-cycled pressure controlled ventilator ("Servo 900C", Siemens Elema, Sweden) with high inflation pressures and 100 % O₂. We did not use surfactant prophylactically, but as rescue therapy within 24h of birth to improve oxygenation and to diminish further damage to the hypoplastic lung. The surfactant used was a natural substance isolated from minced bovine lungs. It consists of about 83% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), and other lipids such as cholesterol, glyceride, and free fatty acids, but does not contain SP-A. The freeze-dried preparation was resuspended in a 0.9% NaCl solution resulting in a total concentration of 40 mg phospholipids per ml. We gave 150 mg/kg of the surfactant extract endotracheally, and recorded the alveolar-arterial oxygen tension difference (A-a)DO₂, which indicates gas exchange by the lungs, immediately before and after administration and after 6 h. In patients 1 and 2 (figure), (A-a)DO₂ fell strikingly and led to a decrease in FiO₂ and inspiratory peak pressures. The effect was most pronounced immediately after administration. Repeated doses had only a short-term effect. The diaphragmatic defect was closed afterwards and both patients survived. In the other two patients no effect was noted; both had had ECMO treatment. Patient 3 died on ECMO with uncorrectable coagulation disorders and a massive intracranial hemorrhage.

After being weaned from ECMO successfully at age 12 days, patient 4 died at six weeks because of relapsing episodes of persistent pulmonary hypertension. Our preliminary conclusion is that in some newborn infants with congenital diaphragmatic hernia, surfactant therapy may induce a transient but important improvement of oxygenation. This improvement may lead, especially when used as a prophylactic measure, to a reduction of barotrauma to the hypoplastic lung and a reduction of risk factors for persistent pulmonary hypertension (ie, hypoxia) as well as to the possibility of gaining time to introduce other treatments such as ECMO. The best dosing schedule and the optimum preparation of surfactant for these patients needs to be established. We have lately treated a fifth baby born at term (patient 5 in figure) with congenital cystic adenomatoid malformation of the lung who responded well to treatment and who survived without chronic lung disease.

5.3.2 References

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Chapter 6

CHANGING CONCEPTS

CHANGING CONCEPTS

During the past few decades we have seen a changing approach to the treatment of newborns with congenital diaphragmatic hernia presenting with symptoms soon after birth. The original report by Ladd and Gross published in 1940, advocated emergency surgery.¹ The significance of the degree of pulmonary hypoplasia and the pathophysiological role of the abnormal pulmonary vascular bed have been recognized since then: they contribute to patterns of mortality and morbidity and limit the extent of successful treatment. Despite highly-skilled care by neonatologists and pediatric surgeons, and despite antenatal diagnosis by means of sonography, the mortality rate has remained unchanged at approximately 40 to 50%.

It was common experience that aggressive artificial ventilation, using high inspiratory peak pressures and high percentages of oxygen, was needed to achieve adequate gas exchange and reduction of risk factors for persistent pulmonary hypertension. In other words, a regimen destructive to the hypoplastic lung, which often resulted in early onset of bronchopulmonary dysplasia. Therefore, several attempts have been made to improve the outlooks.

1). The effect of *DELAYED SURGERY* on survival was first reported by Cartlidge². Different groups around the world have reported their experiences with this approach using a standardized protocol.^{3,4} The concept of delayed surgery is based on several premises: (a) surgical repair of the diaphragmatic defect is not an emergency, except in case of bowel strangulation; (b) risk factors for persistent pulmonary hypertension such as hypoxia, acidosis, hypothermia, and low systemic blood pressure can be treated⁵; (c) lung compliance decreases significantly following surgical repair⁶; and (d) patients with pulmonary hypoplasia incompatible with life can be recognized.⁷ Prospective clinical studies have shown that the operative procedure itself has a major impact on circulatory vasoconstrictive agents.⁸

The delayed surgery approach has become standard procedure around the world; however, evaluation of the patients treated this way, did not show a reduction of mortality. Nevertheless it allowed for a far better pathophysiological understanding of the adaptation of the pulmonary vascular bed after birth and of the intrathoracic pressure relationships before and after closure of the diaphragm. An unsolved

question is the duration of the stabilization period: what criteria should be used to determine the optimal moment of surgical intervention? If the long-term outcome in larger series should still be disappointing, the use of this treatment modality will have to be re-evaluated. However, a certain period of stabilization provides the possibility to institute other forms of treatment that may contribute to better chances of survival.

2). If conventional artificial ventilation should not result in adequate gas exchange, with acceptable oxygenation and elimination of CO₂, thereby reducing the risk factors for persistent pulmonary hypertension, some children will benefit from new ventilatory strategies using high-frequency jet ventilators and high-frequency oscillators. *HIGH FREQUENCY OSCILLATION VENTILATION* has been successfully used in a number of neonates.^{3,9,10,11,12} One of its advantages is that it enables CO₂ removal with lower mean airway pressures, minimizing the risk for persistent pulmonary hypertension and barotrauma and subsequent development of bronchopulmonary dysplasia in case the patient survives.

3). In some centers patients are given a trial of high frequency ventilation before *EXTRACORPOREAL MEMBRANE OXYGENATION* (ECMO) is instituted as rescue therapy for persistent pulmonary hypertension.¹³ ECMO provides lung rest together with the opportunity to overcome episodes of persistent pulmonary hypertension.

Historically, at least a single measurement of PaO₂ > 80 mmHg was needed to prove the presence of the absolute minimum number of alveoli to guarantee survival after coming off ECMO. The first reports on survival rates in congenital diaphragmatic hernia patients treated with ECMO were encouraging.

Heaton reported a survival rate of 73% in 30 patients.¹⁴

Stolar treated only patients with a best preductal PO₂ > 100 mmHg and PCO₂ < 50 mmHg to avoid treatment of overwhelming pulmonary hypoplasia, and found a survival of 68% in 27 patients.¹⁵

Langham reported 41 survivors in 93 patients (42%) in a study from the ECMO Central Registry, and suggested that these children would not have survived without ECMO.¹⁶

Radmond found a survival rate of 73% in a 3-year period (30 patients) and a strong indication that the maximum postoperative mean-airway-pressure value accurately predicts survival.¹⁷

Heiss even reported a survival rate as high as 87% and stated "the reversal of mortality with ECMO" has started.¹⁸

The initial optimism waned when it was observed that results of ECMO in congenital diaphragmatic hernia patients did not parallel the results in other patient

groups, for instance in those suffering from the meconium aspiration syndrome.^{19,20} According to O'Rourke and coworkers, the mortality rate did not decrease after the introduction of ECMO. They distinguished different hospital courses: (1) survival with conventional therapy; (2) survival with ECMO; (3) early ECMO death (direct ECMO complications and absence of improvement in lung function); and (4) late ECMO deaths: patients who were weaned from ECMO, but died secondarily to complications from chronic lung disease.^{21,22} The early ECMO death group may have such severe lung hypoplasia that ECMO only postponed death. Recognition of this group is difficult in the immediate postnatal period, and conventional ECMO entry criteria may not be applicable for the congenital diaphragmatic hernia patient. The late ECMO death patients deteriorated on conventional mechanical ventilation and probably represent a group of patients with a combination of moderate lung hypoplasia and iatrogenic lung injury. O'Rourke's group also compared the results of immediate repair with those of delayed repair using ECMO when necessary. They reported survival rates of 43% versus 45%. The best postductal PO_2 proved to be an accurate predictor of outcome in these patients.

ECMO certainly supports some congenital diaphragmatic hernia patients and prevents progressive pulmonary destruction and early onset of bronchopulmonary dysplasia. Therefore, others advocated ECMO for all congenital diaphragmatic hernia patients regardless of entry criteria based on gas exchange values and regardless of predictors of mortality.²³

4). In our institution we observed that some congenital diaphragmatic hernia patients benefit from early application of *SURFACTANT*.²⁴ The hypoplastic lung is anatomically immature, but there is direct and indirect proof that production of surfactant is also diminished.^{25,26} If the hypoplastic lung really is unable to produce sufficient amounts of surfactant, replacement therapy can be of value to reduce ventilator settings and subsequent development of bronchopulmonary dysplasia. The patients could be selected when predetermined ventilator settings are reached, and analysis of bronchoalveolar fluid lavage, as described by Stenmark, has been obtained.²⁷

5). Persistent pulmonary hypertension is one of the determining factors of the outcome in patients with congenital diaphragmatic hernia. Numerous *VASO-ACTIVE AGENTS* have been advocated as the ultimate solution to modulate pulmonary vascular tone, but a selective vasodilator of the pulmonary vascular bed is still not available. Tolazoline has been advocated, but it may have deleterious side-effects on the systemic circulation.^{28,29} Elevated levels of the vasoconstrictor thromboxane have been reported in congenital diaphragmatic hernia patients during persistent pulmonary

hypertension.^{30,31} Promising results have been reported concerning the application of prostacyclin after cardiac surgery in children and in persistent pulmonary hypertension in neonates.^{32,33} Ford and coworkers reported elegant studies in a lamb model of congenital diaphragmatic hernia and investigated the effectiveness of several vasodilators. They concluded that prostaglandin D2 came closest to the ideal vasodilator, with isoprenaline as second best.³⁴ More recent reports suggested magnesium sulfate as a promising alternative.³⁵ New data on the cell-biological aspects of vascular tone regulation mediated by endothelium-derived relaxing factor, stimulated the use of nitric oxide as a local intratracheal drug.³⁶

6). *FETAL SURGERY* is an approach developed by Harrison and coworkers after having defined the pros and cons of prenatal diagnosis by ultrasound.^{37,38} They found mortality rates exceeding 75% in antenatally diagnosed patients, and identified polyhydramnios and timing of the herniation as bad prognostic signs.³⁹ In the concept of fetal surgery catch-up growth of the lung can be achieved by antenatal repair of the diaphragm. This concept has been documented in the fetal lamb model. Finally they developed techniques for maternal anesthesia, fetal monitoring and tocolytic control of preterm labor in a monkey model.⁴⁰ Clinical application remains more or less experimental although this treatment modality is proposed to parents of a fetus with congenital diaphragmatic hernia. In 1991 Harrison reported the initial results of *in utero* repair: three survivors out of 16 treated cases.⁴¹ In other words, the risks of prenatal correction have not been weighed against the benefits and remain high to both the mother and the fetus.

7). The concept that lung hypoplasia, and not a closing defect of the diaphragm, is the primary event in congenital diaphragmatic hernia is gaining increasing popularity. If this hypothesis would prove to be valid, only *PRENATAL MODULATION OF LUNG GROWTH* or lung transplantation following birth, may provide ways of survival for the most severely affected patients. Fetal lung maturation has been enhanced by the combined application of corticosteroids and thyrotropin-releasing hormone, agents that both pass the placenta easily.⁴² These studies have been confirmed by de Zegher and coworkers, who showed a reduction of respiratory distress syndrome in prematurely born infants treated this way.⁴³ It seems worthwhile to establish whether this approach will be of benefit for the congenital diaphragmatic hernia patient. Fetal surgery combined with prenatal modulation of lung growth remains another option worthy of investigation.

8). The ultimate therapy for the congenital diaphragmatic hernia patient with extremely hypoplastic lungs might be the option of *LUNG TRANSPLANTATION* as early as

possible after birth to prevent damage due to barotrauma and hyperoxia. Harrison's group performed pulmonary lobar transplantation in pigs with a diaphragmatic defect, and found that the graft was well tolerated.⁴⁴ In humans, lung transplantation has been carried out on ECMO support.⁴⁵ Theoretically this would result in a congenital diaphragmatic hernia patient who is no longer a patient, but a baby with normal lungs, both anatomically and biochemically. At the moment the concept of using ECMO as a bridge to lung transplant in newborns with high risk congenital diaphragmatic hernia, might still be a bridge too far.⁴⁶

In effect, the degree of lung hypoplasia is the limiting factor in survival and should be considered the primary defect.

I hope that this concept is the key to new insights into pathogenesis and treatment of the disease. Our ultimate goal could then be prevention of the defect.

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Chapter 7

SUMMARY

This thesis was undertaken in order to investigate selected aspects of the clinical picture of congenital diaphragmatic hernia. The estimated incidence of congenital diaphragmatic hernia is one in 3000 newborns. It exceeds the incidence of cystic fibrosis, the commonest genetic disorder in the Netherlands. Patients presenting with symptoms within 6 hours after birth are called high-risk patients.

Chapter 1 is an outline of the literature on congenital diaphragmatic hernia. It describes historical and epidemiological aspects. The clinical picture of the high-risk patient is discussed, and it is shown that despite new therapeutic modalities, mortality has remained unchanged during the past decades. Developmental aspects of the lung in congenital diaphragmatic hernia are discussed against the background of normal lung development.

Two observations prompted us to undertake the study presented in **Chapter 2**: (1) the admittance, within a few months, of a cluster of congenital diaphragmatic hernia patients from an area with heavy industrialization and agriculture including extensive use of fertilizers and herbicides, and (2) the resemblance of the herbicide Nitrofen with thyroid hormone: a well known growth factor of the fetal lung. In this study we compared congenital diaphragmatic hernia and esophageal atresia patients. A questionnaire inquiring after possible etiological and teratologic factors during pregnancy was completed by 33 couples whose baby had congenital diaphragmatic hernia, and by 43 couples whose baby had esophageal atresia. Specific attention was directed at maternal thyroid dysfunction, and in a number of mothers antibodies against thyroglobulin, thyroid microsomes, and TSH receptors were determined. This study did not reveal any differences in exposure to well-known teratogenic agents in the home environment or the occupational environment. No specific drug could be identified that is associated with congenital diaphragmatic hernia. Nevertheless the fact that etiological relationships were not identified can still be of value in counseling the parents, especially against the background that over 95% of congenital anomalies have an unknown cause.

In the next chapter the problems of persistent pulmonary hypertension and persistent fetal circulation are discussed. Part 3.1 reports on the development of

angiotensin-converting enzyme in the rat model of congenital diaphragmatic hernia. The pulmonary vascular bed is abnormal in congenital diaphragmatic hernia patients and is characterized by a decreased size of the pulmonary vascular bed, increased thickness of the pulmonary arterial muscle coat, and a decrease in the number of vessels per unit of lung. Many compounds play a role in regulating pulmonary vascular tone in the postnatal period, and angiotensin-converting enzyme is one of them. It has two effects: it converts angiotensin I to the potent vasoconstrictor angiotensin II, and it inactivates bradykinin, a vasodilator. Angiotensin converting enzyme activity in the lungs of rats exposed to Nitrofen was about 1.5 fold higher than in normal fetal rat lungs. Increased angiotensin-converting enzyme levels would lead to increased production of angiotensin II and increased degradation of bradykinin. The normal postnatal drop in pulmonary vascular resistance could be easily disturbed in the presence of increased angiotensin-converting enzyme activity together with an abnormally muscularized pulmonary vascular bed. In the human newborn with congenital diaphragmatic hernia the number of pulmonary vessels per cm^2 lung tissue amounts to only one third of that in normal controls. According to Poiseuille's equation this would mean a three-fold increase in vascular resistance. Angiotensin II is a growth factor for cell proliferation in the vessel wall and vascular hypertrophy may be an adaptive process in normalizing vessel-wall stress. Increased angiotensin-converting enzyme activity, leading to increased angiotensin II levels, may be rather a consequence than a cause of persistent pulmonary hypertension.

Part 3.2 describes a prospective study of ten neonates with a diaphragmatic defect and five esophageal atresia patients. Ventilatory parameters were registered on admission, one hour before surgery, and one and six hours after surgery. Determination of thromboxane B_2 , a stable metabolite of the vasoconstrictor thromboxane A_2 , and 6-keto-prostaglandin $F_{1\alpha}$, a stable metabolite of the vasodilator prostacyclin was carried out at the same moments. In patients with congenital diaphragmatic hernia the median concentration of thromboxane B_2 rose significantly after surgery and dropped to preoperative values in all but two patients. In patients with right-to-left shunting the ventilatory parameters (alveolar-arterial oxygen difference, oxygenation index), and thromboxane B_2 concentrations were significantly higher than in those without right-to-left shunting. It is stated that during surgery for congenital diaphragmatic hernia, the arachidonic cascade is activated and leads to a rise in the vasoconstrictor thromboxane B_2 . This may be an additional risk factor for the development of persistent pulmonary hypertension at a time when the pulmonary vascular bed still is in the perinatal adaptation phase.

Part 3.3 reports the use of vasodilators to lower pulmonary vascular resistance in the clinical situation. In this study the effectiveness of the "historical" vasodilator

tolazoline is compared with the newer drug prostacyclin. Ventilatory parameters and mean arterial blood pressure were recorded during episodes of right-to-left shunting and at set times after the administration of vasoactive drugs. Tolazoline did not lower ventilatory parameters, but mean arterial blood pressure dropped significantly. After administration of prostacyclin (A-a)DO₂ values decreased significantly, without side effects on mean arterial blood pressure. Theoretically the ideal treatment for persistent pulmonary hypertension would be a selective pulmonary vasodilator. Indeed, prostacyclin turned out to be an effective pulmonary vasodilator in some patients, as is reflected by ventilatory parameters, and can be used as a "bridge" to ECMO.

The following chapter discusses functional properties of the hypoplastic lung related to tissue damage by oxygen. Part 4.1 is the report of research into antioxidative enzyme activity during gestation and under artificial ventilation with hyperoxia in the rat model. Between day 19 of gestation and birth the level of superoxide dismutase remained fairly constant in the rats with and without a diaphragmatic defect. Catalase showed a significant increase in activity between day 21 of gestation and birth. Glutathione peroxidase activity steadily increased with age in each group. After artificial ventilation with air and pure oxygen, superoxide dismutase activity tended to decrease, whereas catalase activity remained unchanged. Glutathione-peroxidase activity in the congenital diaphragmatic hernia lung was reduced to 70% of initial activity after artificial ventilation with pure oxygen. This enzyme is one of the last remedies of a cell to repair free-radical induced lipid peroxidation of its membranes and to prevent lethal damage. It is stated that this finding may indicate that the newborn rat with a diaphragmatic defect is at risk to develop oxygen-related lung damage.

It is known that the *term* lung of the congenital diaphragmatic hernia patient behaves as the lung of the *preterm* infant and that it reacts on artificial ventilation with the formation of hyaline membranes (Gaillard, unpublished observations). Part 4.2 confirms the suspicion that as a consequence the incidence of chronic lung disease resembling bronchopulmonary dysplasia should be high in congenital diaphragmatic hernia survivors despite their term delivery. In this retrospective study the preoperative chest X-ray was evaluated in order to determine whether it might predict pulmonary hypoplasia and subsequent development of bronchopulmonary dysplasia. Furthermore, we assessed the severity of the bronchopulmonary dysplasia and tried to correlate bronchopulmonary dysplasia with the degree of lung hypoplasia. Thirty-three percent of the survivors had clinical and radiological lung disease resembling bronchopulmonary dysplasia. Morbidity expressed as duration of artificial ventilation, application of supplemental oxygen,

and hospital stay was much higher in this group. After repair of congenital diaphragmatic hernia, pulmonary function tests show a decrease in forced vital capacity and respiratory system compliance. Reduced perfusion and ventilation of the ipsilateral lung and airway obstruction have been reported in long-term survivors. It is stated that a new population of congenital diaphragmatic hernia survivors might arise, those who will have compromised lung function in early adulthood.

Chapter 5, Part 5.1 briefly describes the clinical picture of the "delayed" or "acquired" presenting congenital diaphragmatic hernia. The main difference with the so-called high-risk patient is the lack of pulmonary hypoplasia. Presenting symptoms are either pulmonary (pneumonia, dyspnea, cyanosis) or gastrointestinal (obstruction). Still these patients may present as an emergency under the initial diagnosis tension pneumothorax. It is stated that intended drainage is a hazardous procedure in these patients. Part 5.2 and Part 5.3 discuss the experiences with the so-called delayed surgical approach and summarize the preoperative stabilization procedure. This concept was developed to minimize risk factors for persistent pulmonary hypertension and to select patients with inadequate lung mass who would not benefit from surgical repair. The first experiences with surfactant-replacement therapy are reported; they indicate that the hypoplastic lung is biochemically immature indeed and resembles the premature lung in function.

In conclusion, congenital diaphragmatic hernia remains a continuing challenge for pediatric surgeons and neonatologists. Basic questions remain to be answered concerning the functional properties of the hypoplastic lung, its reaction to treatment, and the consequences for future lung development and function.

SAMENVATTING

In dit proefschrift wordt verslag gedaan van een onderzoek naar de pathofysiologische mechanismen die een rol spelen bij de symptomatologie van de congenitale hernia diaphragmatica patiënt. Congenitale hernia diaphragmatica komt voor bij 1 : 3-5000 pasgeborenen. De incidentie is daarmee hoger dan die van cystic fibrosis, de meest voorkomende erfelijke aandoening in Nederland. Patiënten die symptomen van respiratoire insufficiëntie tonen binnen zes uur na de geboorte, worden high-risk patiënten genoemd. De mortaliteit in deze groep wordt geschat op 40 tot 50%.

Hoofdstuk 1 bevat een literatuuroverzicht. De geschiedenis van congenitale hernia diaphragmatica wordt behandeld en de veranderende inzichten in tijdstip van behandeling worden besproken. Epidemiologische en etiologische studies vermelden een vergelijkbare incidentie in verschillende landen. Congenitale hernia diaphragmatica patiënten worden geclassificeerd op grond van de plaats van het defect in het middenrif. De posterolaterale hernia met of zonder een breukzak draagt de naam van Bochdalek en is het onderwerp van dit proefschrift. Deze vorm komt het meest voor; bovendien zijn linkszijdige defecten acht maal zo frequent als rechtszijdige defecten. Bilaterale defecten zijn zeldzaam en hun incidentie wordt geschat op 3 tot 5%. Tot de overige gevallen van congenitale hernia diaphragmatica worden de hernia's door het foramen van Morgagni, de centrale hernia's en de eventratie van het middenrif gerekend. Congenitale hernia diaphragmatica kan ook voorkomen als onderdeel van chromosomale en genetisch bepaalde aandoeningen. Wanneer doodgeboren kinderen in de analyse worden betrokken, is er in 50% van de onderzochte gevallen sprake van andere aangeboren afwijkingen. Met name neurale buis defecten en hartafwijkingen komen vaak voor. Chromosomale afwijkingen die het meest worden gevonden in geval van congenitale hernia diaphragmatica, zijn trisomie 18 en trisomie 21. Defecten van het middenrif kunnen ook onderdeel zijn van omschreven syndromen zoals het Fryns' syndroom of het Cornelia de Lange syndroom. Het familiair voorkomen van congenitale hernia diaphragmatica is bekend sinds 1916. Multifactoriële overerving wordt het meest waarschijnlijk geacht.

Antenataal kan met behulp van echografie de diagnose worden gesteld, wanneer abdominale organen in de foetale borst- holte worden gezien of er een verplaatsing van het mediastinum zichtbaar kan worden gemaakt. Vaak is er sprake van een polyhydramnion, die zich na de 24e zwangerschapsweek heeft ontwikkeld. Het ontstaan van een polyhydramnion is niet geheel duidelijk, maar wordt in verband gebracht met functionele obstructie van het maag-darm kanaal. Wanneer de diagnose is gesteld, dient antenataal chromosomenonderzoek te worden verricht en dienen andere structurele afwijkingen te worden uitgesloten. Ook kan dan intra-uterien

transport en een geplande partus met adequate neonatologische opvang worden geadviseerd.

Het klassieke trias symptomen van de congenitale hernia diaphragmatica patiënt bestaat uit cyanose, dyspnoe en verplaatsing van het hart (meestal) naar rechts. Bij lichamelijk onderzoek valt een vlakke buik op, zacht ademgeruis, en soms de aanwezigheid van darmgeruisen bij auscultatie van de thorax. De mortaliteit is het hoogst bij patiënten die binnen 6 uur na de geboorte symptomen tonen. De prognose is goed, wanneer de symptomen zich voordoen meer dan 24 uur na de geboorte. Het klinisch beeld wordt gekenmerkt door respiratoire insufficiëntie en persistente foetale circulatie, op grond van longhypoplasie en het bestaan van abnormale muscularisatie van het pulmonale vaatbed.

De sluiting van het middenrif vindt plaats rond de negende zwangerschapsweek. De ontwikkeling van de long tijdens de zwangerschap kent vijf stadia. De eerste fase is de embryonale periode. Aan het eind van de tweede fase, de pseudoglandulaire fase, die duurt van de 8e tot de 16e zwangerschapsweek, zijn alle luchtwegvertakkingen voltooid. In de canaliculaire fase vindt differentiatie plaats van type II en type I cellen. Daarna vindt de ontwikkeling van sacculi en waarschijnlijk ook alveoli plaats tijdens de sacculaire en alveolaire fase. De ontwikkeling van alveoli gaat ook na de geboorte nog door tot de leeftijd van ongeveer 5 jaar. Het pulmonale vaatbed volgt de vertakkingen van de grote luchtwegen tot de acinus en is eveneens rond de 16^e zwangerschapsweek voltooid. Verdere uitbreiding binnen de acinus vindt daarna plaats. Voor de geboorte is er een sterke muscularisatie van het pulmonale vaatbed, leidend tot een hoge longvaatweerstand en een lage pulmonale bloedstroom. Na de geboorte dient de pulmonale bloedstroom toe te nemen en daarvoor is adaptatie van het vaatbed noodzakelijk. Deze aanpassing vindt grotendeels in de eerste uren en dagen na de geboorte plaats.

Functionele eigenschappen van de long zijn de productie van surfactant en van antioxydatieve enzymen. Surfactant bestaat uit een aantal lipiden en eiwitten. De type II cellen zijn vanaf de 24e zwangerschapsweek in staat om in toenemende mate surfactant te produceren. Dit proces staat onder invloed van glucocorticoïden en schildklierhormoon. De ontwikkeling van het antioxydatieve systeem vertoont grote gelijkenis met die van surfactant. De productie van superoxide dismutase, catalase, en glutathionreductase neemt sterk toe naarmate de zwangerschap vordert.

De theorieën over het uitblijven van de sluiting van het middenrif zijn goed samengevat door Kluth. De meest intrigerende theorie is die van Iritani, die suggereerde dat longhypoplasie eerder de *oorzaak* is dan het *gevolg* van het diaphragma defect. De long van de congenitale hernia diaphragmatica patiënt wordt gekenmerkt door hypoplasie, uitgedrukt als een verlaging van de long/lichaamsgewicht ratio, een afname in het aantal vertakkingen van de bronchiaalboom, een verminderde "radial

alveolar count", een afname van het DNA-gehalte per hoeveelheid longweefsel, lagere phospholipide-concentraties, en de afwezigheid van elastische vezels. Het pulmonale vaatbed is gekenmerkt door een afname van het totale oppervlak, een toename van de hoeveelheid spierweefsel in de vaatwand en een afname in het aantal vaten per hoeveelheid longweefsel. Functioneel is er sprake van een verminderde surfactantproductie door de congenitale hernia diaphragmatica longen. Over de productie van antioxydatieve enzymen door deze longen is niets bekend.

Van oorsprong bestond de behandeling van congenitale hernia diaphragmatica uit een zo spoedig mogelijke sluiting van het diaphragma defect. Met de vaststelling dat longhypoplasie en pulmonale hypertensie de oorzaken zijn van de klinische symptomen van congenitale hernia diaphragmatica is er een nieuwe behandelingswijze ontstaan. Deze is er op gericht om de patiënt te stabiliseren en risicomomenten voor pulmonale hypertensie zo veel mogelijk te vermijden. Operatieve correctie vindt dan in een latere fase plaats, wanneer het pulmonale vaatbed minder gevoelig is voor stimuli die pulmonale hypertensie kunnen uitlokken. De mortaliteit is door deze behandeling echter niet gedaald, wel zijn de inzichten in de pathofysiologie er door verdiept. De toepassing van nieuwe beademingstechnieken, de toepassing van extracorporele membraanoxygenatie, en de ontwikkeling van nieuwe medicamenten om de pulmonale vaatweerstand te beïnvloeden hebben baat gebracht voor een aantal patiënten, maar de mortaliteit binnen de groep als geheel is echter niet gedaald. Mogelijkerwijs houdt dit verband met het feit dat congenitale hernia diaphragmatica steeds vaker wordt gediagnostiseerd vóór de geboorte waardoor ook kinderen met ernstige longhypoplasie na intrauterien transport het ziekenhuis kunnen bereiken.

Twee waarnemingen vormden de reden om de studie te ondernemen waarvan in hoofdstuk 2 verslag wordt gedaan. Allereerst werd binnen enkele maanden een groep patiënten opgenomen, die allen afkomstig waren uit een streek met intensieve landbouw en zware industrie. Ten tweede viel de gelijkenis op tussen Nitrofen en schildklierhormoon -een bekende groeifactor voor de foetale long. Een vragenlijst gericht op mogelijke etiologische en teratologische factoren werd ingevuld door 33 ouderparen van een kind met congenitale hernia diaphragmatica en door 43 ouderparen van een kind met oesophagus atresie. Bovendien werd een aantal vragen gesteld, gericht op het vóórkomen van ziekten van de schildklier. Bij een aantal moeders werd bloed afgenomen om antistoffen tegen thyroglobuline, schildklier-microsomen en TSH receptoren te bepalen. Er werden geen verschillen gevonden tussen de twee groepen voor wat betreft teratologische invloeden uit de woon- of werkomgeving. Er was geen medicijngebruik waarvan bekend is dat het congenitale hernia diaphragmatica kan veroorzaken. Het ontbreken van een relatie tussen invloe-

den uit woon- en werkomgeving heeft zeker waarde in de begeleiding van de ouders van deze patiënten.

In de volgende hoofdstukken wordt het probleem van de persisterende pulmonale hypertensie besproken. **Hoofdstuk 3.1** doet verslag van een prospectieve studie bij 10 pasgeborenen met een congenitale hernia diaphragmatica en 5 controle patiënten met een oesophagus atresie. In deze studie werd een aantal beademingsparameters vastgelegd bij opname, één uur voor chirurgische correctie, en één en zes uur na de operatie. Op dezelfde tijdstippen werden spiegels bepaald van het circulerende thromboxane B_2 , een stabiele metaboliëet van de vasoconstrictieve stof thromboxane A_2 , en 6-keto-prostaglandin $F_{1\alpha}$, een stabiele metaboliëet van prostacycline. Bij patiënten met een congenitale hernia diaphragmatica steeg de concentratie van thromboxane B_2 onmiddellijk na de operatie, maar daalde later terug naar de pre-operatieve waarde. Bij patiënten met een rechts-links shunt werden hogere waarden gevonden van de beademingsparameters (A-a)DO₂ en oxygenation index. Bij deze patiënten waren de thromboxane B_2 spiegels significant hoger. Er is dus gedurende de operatieve ingreep activatering van de arachidonzuur cascade, waardoor een verhoogde uitstort van de vasoconstrictieve stof thromboxane A_2 plaatsvindt. Met name wanneer het pulmonale vaatbed nog in de aanpassingsfase is aan het extrauteriene leven en gevoelig is voor prikkels die pulmonale hypertensie kunnen uitlokken, vormt de verhoogde uitstort van thromboxane A_2 daarvoor een risico factor.

Hoofdstuk 3.2 beschrijft de ontwikkeling van angiotensin-converting enzyme activiteit in het diermodel dat door TenBrinck en collega's van het Instituut Kinderheeskunde werd ontwikkeld in het Laboratorium voor Chirurgie van de Erasmus Universiteit Rotterdam. Het pulmonale vaatbed is abnormaal bij congenitale hernia diaphragmatica patiënten en wordt onder andere gekenmerkt door een toename van de hoeveelheid spierweefsel in de kleinere vertakkingen van de arteria pulmonalis. Talrijke stoffen, waaronder angiotensin-converting enzyme, hebben een invloed op het dalen van de pulmonale vaatweerstand na de geboorte. Angiotensin-converting enzyme heeft twee effecten: het zet angiotensine I om tot de krachtige vasoconstrictor angiotensine II en het inactiveert bradykinine, een vasodilator. Angiotensin-converting enzyme activiteit in de longen van ratten die blootgesteld waren aan het herbicide Nitrofen was 1.5 maal hoger dan in controledieren. Een toename van de angiotensin-converting enzyme activiteit zal leiden tot een toename van de productie van angiotensine II en een toename van de afbraak van bradykinine. De normale postnatale daling van pulmonale vaatweerstand zou gemakkelijk kunnen worden verstoord in de aanwezigheid van een toegenomen hoeveelheid angiotensin-converting enzyme, zeker wanneer ook nog het pulmonale vaatbed een abnormale structuur heeft. De hoeveelheid bloedvaten in de longen van een pasgeborene met een congenitale hernia diaphragmatica bedraagt slechts een derde van de hoeveelheid

die normaal aanwezig is. Volgens de wet van Poiseuille zou dit een verdrievoudiging van de vaatweerstand betekenen. Angiotensine heeft naast vasoconstrictieve werking ook nog een stimulerend effect op celproliferatie in de vaatwand, en hypertrofie van de vaatwand kan een mechanisme zijn om de drukverhoudingen in het vat te normaliseren. De verhoging van de angiotensin-converting enzyme activiteit, die leidt tot verhoging van de angiotensine II spiegels, zou dan eerder een oorzaak dan een gevolg zijn van pulmonale hypertensie.

Hoofdstuk 3.3 vergelijkt het gebruik van twee vasodilatatoire stoffen in de klinische situatie. Beademingsparameters en gemiddelde arteriële bloeddruk werden vastgelegd tijdens episoden van een rechts-links shunt en op gezette tijden na toediening van hetzij tolazoline of prostacycline. Tolazoline had geen invloed op de beademingsparameters, maar de gemiddelde arteriële bloeddruk daalde significant. Na toediening van prostacycline werd een significante daling van de beademingsparameter (A-a)DO₂ gezien zonder effecten op de gemiddelde arteriële bloeddruk. Hoewel in de kleine studiegroep geen verbetering van de prognose werd aangetoond, kan prostacycline in ieder geval tijdelijk leiden tot een daling van de pulmonale vaatweerstand en daardoor de toepassing van andere behandelingen, zoals extracorporele membraan oxygenatie mogelijk maken.

In het volgende hoofdstuk worden de functionele eigenschappen van de congenitale hernia diaphragmatica longen en het gevolg daarvan voor de prognose besproken. **Hoofdstuk 4.1** is het verslag van studies in het diermodel die gericht waren op de ontwikkeling van de antioxydatieve activiteit tijdens de zwangerschap en onder kunstmatige beademing met 100% zuurstof. Tussen dag 19 en dag 22 (à terme) bleef de enzymactiviteit van superoxide dismutase constant binnen de groepen met of zonder een diaphragma defect. Catalase toonde een sterke stijging in activiteit tussen dag 21 en de geboorte. Glutathion-peroxidase toonde een gelijkmatige stijging naarmate de zwangerschap vorderde.

Beademing met zuivere zuurstof leidde tot een daling van de activiteit van superoxide dismutase, terwijl de catalase activiteit onveranderd bleef. De glutathion-peroxidase activiteit nam af tot 70% van de initiële activiteit na beademing met 100% zuurstof. Dit enzym is één van de laatste mogelijkheden die de cel heeft om beschadiging door vrije zuurstof radicalen te voorkomen. Deze waarneming zou kunnen betekenen dat de pasgeboren rat met een congenitale hernia diaphragmatica gevoeliger is voor zuurstofschade dan de normale contrôle rat. Wanneer deze bevinding zou worden vertaald naar de situatie bij de mens, zou dat betekenen dat de long van de pasgeborene met een congenitale hernia diaphragmatica zich gedraagt als de long van een premature pasgeborene. De waarneming dat de congenitale hernia diaphragmatica long reageert op kunstmatige beademing met de vorming van

hyaline membranen (Gaillard, niet gepubliceerde observaties) is daarmee in overeenstemming.

Hoofdstuk 4.2 bevestigt het vermoeden dat dienten-gevolge de incidentie van longbeschadiging gelijkend op bronchopulmonale dysplasie hoog moet zijn. In deze retrospectieve studie werd aangetoond dat bij 33% van de overlevenden sprake was van klinische en röntgenologische tekenen van bronchopulmonale dysplasie. De morbiditeit, uitgedrukt als duur van kunstmatige beademing, duur van extra zuurstofbehoefte, en hospitalisatie, was hoog in deze groep. In dit hoofdstuk komt ter sprake dat longfunctie-onderzoeken ongeveer een maand na operatie een afname van de vitale capaciteit en de compliantie laten zien. Longfunctie-onderzoeken na langere tijd tonen een verminderde perfusie en ventilatie, en verhoogde bronchiale prikkelbaarheid. Waarschijnlijk is de onderzochte populatie nog maar de voorbode van een groep die ondanks een nog ernstiger longhypoplasie kan overleven en mogelijk nog ernstiger longbeschadiging zal overhouden.

Hoofdstuk 5 behandelt een aantal klinische aspecten. **Hoofdstuk 5.1** beschrijft kort de klinische presentatie van de zogenaamde "verworven" congenitale hernia diaphragmatica. De prognose is bij late presentatie goed, maar er bestaat een groep patiënten die zich alsnog als "acute" patiënt kan presenteren óf met het beeld van een spanningspneumothorax, waarbij de uitgezette maag wordt aangezien voor de pneumothorax, óf ten gevolge van strangulatie van de darm door de breukpoort. Drainage van de "pneumothorax" is een gevaarlijke procedure in deze patiëntengroep. **Hoofdstuk 5.2** verslaat de ervaringen met de zogenaamde uitgestelde operatie na een stabilisatiefase. Dit concept is ontwikkeld om de risicofactoren voor pulmonale hypertensie tot een minimum te beperken en om patiënten te selecteren die een zodanig ernstige longhypoplasie hebben dat een chirurgische sluiting van het middenrif hun levenskansen niet zou verbeteren. In **hoofdstuk 5.3** worden de eerste gunstige ervaringen met de toediening van surfactant beschreven bij een aantal patiënten. Deze ervaringen vormen een indirect bewijs dat de hypoplastische long inderdaad biochemisch onrijp is.

De hypothese dat congenitale hernia diaphragmatica meer is dan alleen maar een defect van het middenrif en mogelijk onderdeel van een aandoening waarbij hypoplasie van de long centraal staat, krijgt meer en meer steun. Deze bewustwording heeft geleid tot een andere behandeling van de pasgeborene met een congenitale hernia diaphragmatica, en in de afgelopen jaren is een aantal verschillende behandelingswijzen geïntroduceerd. Antenatale diagnostiek heeft geleid tot intra-uteriene overplaatsing naar centra waar neonatologische opvang en kinderchirurgische behandeling na de geboorte mogelijk zijn. Het concept van de uitgestelde chirurgische correctie is in Europa ontwikkeld, welhaast tegelijk met de introductie van extracorporele membraan oxygenatie in de Verenigde Staten. Verbeterde beademings-

strategieën en de ontwikkeling van nieuwe medicamenten om de pulmonale vaatweerstand te beïnvloeden leken aanvankelijk veelbelovend, maar in grotere series bleef de mortaliteit onveranderd hoog. Ook foetale chirurgie lijkt niet de ideale behandelingsmethode te kunnen worden, omdat de bronchiaalboom zijn definitieve vorm al heeft gekregen rond de 16e zwangerschapsweek, lang voordat deze behandeling mogelijk is. In hoeverre prenatale modulatie van de longgroei de prognose kan verbeteren lijkt de moeite van verder onderzoek waard. De optie om te streven naar longtransplantatie waarbij extracorporele membraan oxygenatie als overbruggende therapie kan dienst doen, lijkt vooralsnog eerder science fiction dan realiteit.

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