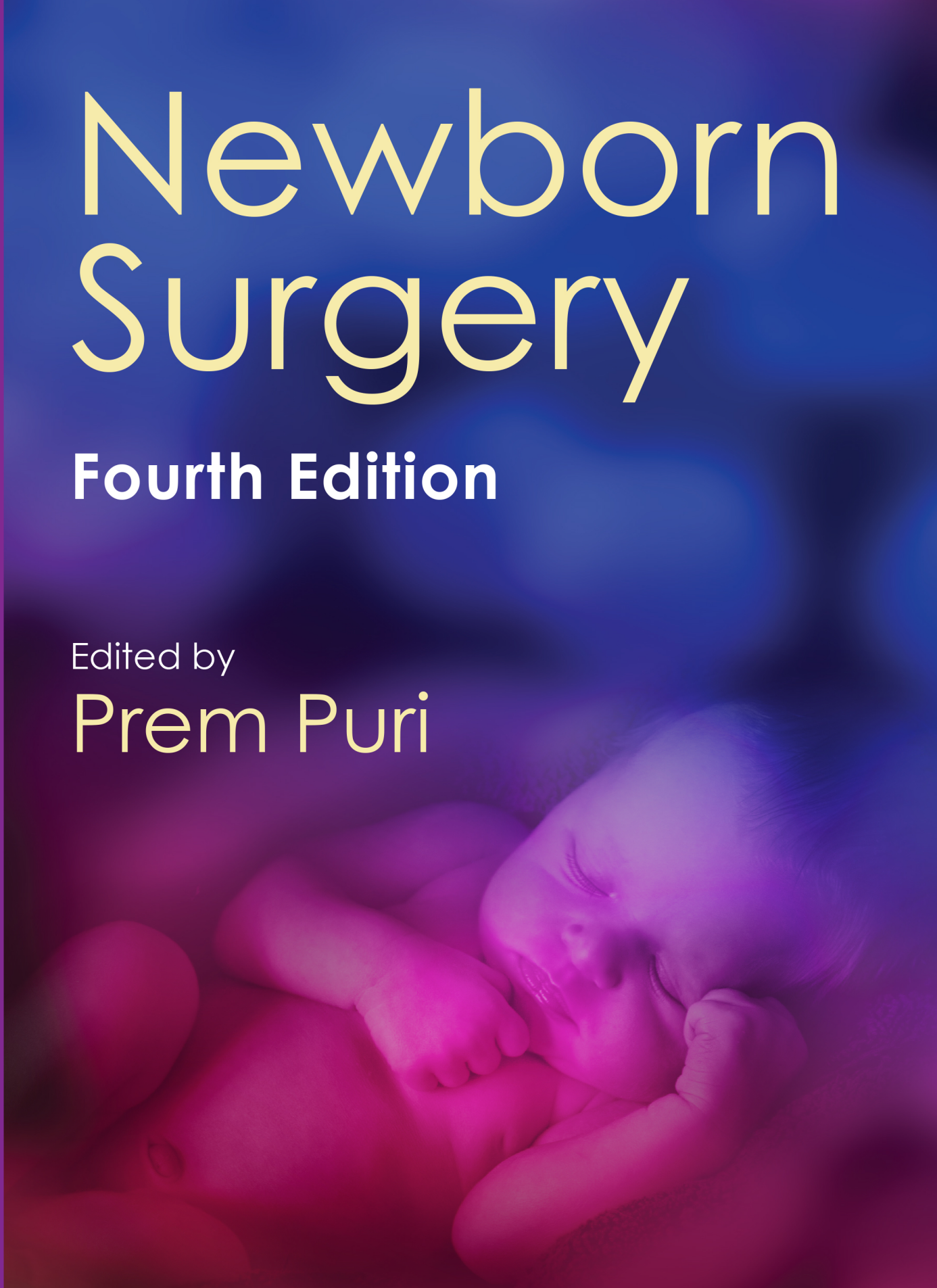
Newborn Surgery

Fourth Edition

Edited by

Prem Puri



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To Veena, Abir, Anita, and Niki, for their love and patience.

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Preface to the fourth edition

Newborn surgery has reached a high degree of sophistication and is now recognized as an independent discipline in pediatric surgery. The more comprehensive understanding of the pathophysiology of various neonatal disorders, advances in specialized neonatal anesthesia and intensive care, as well as the introduction of new surgical techniques including minimally invasive surgery have dramatically improved survival in neonatal surgical conditions. Recent advances in the fields of regenerative medicine and tissue engineering offer hope in the future to provide stem cell–based constructs for the reconstruction of some birth defects.

It has been six years since the third edition of the book (published in 2011). The fourth edition of *Newborn Surgery* has been thoroughly revised and updated, and contains 112 chapters by 194 contributors from five continents. This edition contains nine new chapters on key topics, including transition to extrauterine life, specific risks for the preterm infant, access for enteral nutrition, patient safety, tissue engineering and stem cell research, surgical aspects of HIV infection, stridor in infants, stomas of small and large intestine, and spontaneous intestinal perforation. Each chapter has been written by internationally renowned leaders in their respective fields. Several younger surgeons were selected as coauthors, who will become the next generation of leaders in pediatric surgery.

This book is intended for those who have a clinical responsibility for newborn babies. It provides an authoritative, comprehensive, and complete account of the pathophysiology and surgical management of neonatal disorders. The book is specifically designed for pediatric surgeons, trainees in pediatric surgery, pediatric urologists, as well as neonatologists and pediatricians seeking more detailed information on newborn surgical conditions. It is my sincere hope that the readers will find this textbook a useful reference in the management of surgical disorders in the newborn.

I wish to thank most sincerely all the contributors from around the world for their precious time and outstanding work in the preparation of this innovative textbook. I also wish to express my gratitude to Dr. Julia Zimmer and Dr. Hiroki Nakamura for their help with the galley proofs of the book. I wish to thank the editorial staff of CRC Press, particularly Ms. Miranda Bromage, for all their help during the preparation and publication of this book. I am thankful to the National Children's Research Centre, Our Lady's Children's Hospital, Dublin for their support.

Prem Puri
2017

Preface to the third edition

It has been eight years since the second edition of the book was published in 2003. Over the last decade, major advances have occurred in the understanding and treatment of neonatal surgical conditions. Advances in prenatal diagnosis, imaging, intensive care, and minimally invasive surgery have transformed the practice of surgery in the newborn. The third edition of *Newborn Surgery* has been extensively revised and contains 105 chapters by 160 contributors from five continents of the world. This edition contains many new chapters taking account of the recent advances in neonatal surgery. The new chapters include the following: Perinatal Physiology; Clinical Anatomy of the Newborn; Epidemiology of Birth Defects; Fetal Counselling for Surgical Malformations; Neonatal Sepsis; Liver Transplantation; Congenital Pouch Colon; Megacystis Microcolon Intestinal Hypoperistalsis Syndrome; and Urinary Tract Infections. Each chapter has been written by world-class experts in their respective fields, along with their coauthors.

This textbook provides an authoritative, comprehensive, and complete account of the pathophysiology and treatment of various surgical conditions in the newborn. This book should be of interest to all those who have a clinical responsibility for newborn babies. It is particularly intended for trainees in pediatric surgery, established pediatric surgeons, general surgeons with an interest in pediatric surgery, as well as neonatologists and pediatricians seeking more detailed information on newborn surgical conditions.

I wish to thank most sincerely all the contributors for their outstanding work in producing this innovative textbook. I also wish to express my gratitude to Ms. Vanessa Woods and Ms. Lisa Kelly for their skillful secretarial help. I am grateful to Dr. G.P. Seth for reading each and every word of the galley proofs of the entire book. I wish to thank the editorial staff of Hodder Arnold, particularly Mr. Stephen Clausard, for their help during the preparation and publication of this book. I am thankful to the Children's Medical & Research Foundation, Our Lady's Children's Hospital, Dublin for their support.

Prem Puri
2011

Preface to the second edition

The second edition of *Newborn Surgery* has been extensively revised. Many new chapters have been added to take account of the recent developments in the care of the newborn with congenital malformations. This edition, which comprises 97 chapters by 121 contributors from five continents of the world, provides an authoritative, comprehensive, and complete account of the various surgical conditions in the newborn. Each chapter is written by the current leading expert(s) in their respective fields.

Newborn surgery in the twenty-first century demands of its practitioners detailed knowledge and understanding of the complexities of congenital anomalies, as well as the highest standards of operative techniques. In this textbook, great emphasis continues to be placed on providing a comprehensive description of operative techniques of each individual congenital condition in the newborn. The book is intended for trainees in pediatric surgery, established pediatric surgeons, general surgeons with an interest in pediatric surgery, as well as neonatologists and pediatricians seeking more detailed information on newborn surgical conditions.

I wish to thank most sincerely all the contributors for the outstanding work they have done for the production of this innovative textbook. I also wish to express my gratitude to Mrs. Karen Alfred and Ms. Ann Brennan for their secretarial help and to the staff of Hodder Arnold for their help during the preparation and publication of this book. I am grateful to the Children's Medical & Research Foundation, Our Lady's Hospital for Sick Children, Dublin for their support.

Prem Puri
2003

Preface to the first edition

During the last three decades, newborn surgery has developed from an obscure subspecialty to an essential component of every major academic pediatric surgical department throughout both the developed and the developing world. Major advances in perinatal diagnosis, imaging, neonatal resuscitation, intensive care, and operative techniques have radically altered the management of newborns with congenital malformations. Embryological studies have provided new valuable insights into the development of malformations, while improvements in prenatal diagnosis are having a significant impact on approaches to management. Monitoring techniques for the sick neonate pre- and postoperatively have become more sophisticated, and there is now greater emphasis on physiological aspects of the surgical newborn, as well as their nutritional and immune status. This book provides a comprehensive compendium of all these aspects as a prelude to an extensive description of surgical conditions in the newborn. Modern-day newborn surgery demands detailed knowledge of the complexities of newborn problems. Research developments, laboratory diagnosis, imaging, and innovative surgical techniques are all part of the challenge facing surgeons dealing with congenital conditions in the newborn. In this book, a comprehensive description of operative techniques of each individual condition is presented. Each of the contributors was selected to provide an authoritative, comprehensive, and complete account of their respective topics. The book, comprising 90 chapters, is intended primarily for trainees in pediatric surgery, established pediatric surgeons, general surgeons with an interest in pediatric surgery, and neonatologists.

I am most grateful to all contributors for their willingness to contribute chapters at considerable cost of time and effort. I am indebted to Mr. Maurice De Cogan for artwork, Mr. Dave Cullen for photography, and Ms. Ann Brennan and Ms. Deirdre O'Driscoll for skillfull secretarial help. I am grateful to the Children's Research Centre, Our Lady's Hospital for Sick Children, for their support. Finally, I wish to thank the editorial staff, particularly Ms. Susan Devlin, of Butterworth-Heinemann for their help during the preparation and publication of this book.

Prem Puri
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PART 1

General

1

Embryology of malformations

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INTRODUCTION

Approximately 3% of human newborns present with congenital malformations.[1] Without surgical intervention, one-third of these infants would die since their malformations are not compatible with sustained life outside the uterus.[1,2] In figures, this means that in a country such as Germany, nearly 6000 children are born every year with a life-threatening malformation.

Due to the development of prenatal diagnostic procedures, advanced surgical techniques, and intensive postoperative care, most infants with otherwise fatal malformations can be rescued by an operation in the neonatal period. However, morbidity remains high in some of these children[2] with the necessity of repeated operations and hospitalizations despite a successful primary operation. This may also be the fate of many children with non-life-threatening malformations such as hypospadias or cleft palate.

Mortality is still high in newborns with certain malformations such as congenital diaphragmatic hernias (CDHs) or severe combined defects. As a consequence, congenital malformations today are the main cause of death in the neonatal period. In the United States, 21% of neonatal mortality can be related to congenital malformations.[3]

These figures probably do not reflect a real increase in the actual incidence of congenital malformations. The observed mortality shift might rather be due to improved intensive care medicine in today's Western world countries where neonates (even those with birth defects) have a better chance of survival. On the other hand, this statistical shift indicates that knowledge about congenital malformations lags behind the progress clinical research has made in the surrounding fields. Efforts are needed to close the gap and learn more about baby killer no. 1. Identification of teratogens will help to reduce the incidence of malformations when exposure can be avoided, and pathogenetic studies might aid in designing therapeutic measures. Both treatment and prevention critically depend on basic embryological research.

GENERAL REMARKS ON EMBRYOLOGY AND THE EMBRYOLOGY OF MALFORMATIONS

Despite many efforts, the embryology of numerous congenital anomalies in humans is still a matter of speculation. This is due to the following reasons:

1. A shortage of study material (both normal and abnormal embryos)
2. Various technical problems (difficulties in the interpretation of serial sections, shortage of explanatory three-dimensional reconstructions)
3. Misconceptions and/or outdated theories concerning normal and abnormal embryology

Fortunately, a number of animal models are known today, which allow advanced embryological studies in various embryological fields. Especially for the studies of anorectal malformations, a number of animal models are at hand. In addition, a scanning electron microscopic atlas of human embryos had been published recently, which provides detailed insights into normal human embryology.[4]

Appropriate and illustrative findings in various fields of embryology are still lacking. This explains why today many typical malformations are still not explained satisfactorily. Pediatric surgeons are still confused when they are confronted with the embryological background of normal and abnormal development.

For the described misconceptions and/or outdated theories, Haeckel's "biogenetic law"[5] is one example. According to this theory, a human embryo recapitulates in its individual development (ontogeny) the morphology observed in all life forms (phylogeny). This means that during its development, an advanced species is seen to pass through stages represented by adult organisms of more primitive species.[5]

This theory still has an impact on the nomenclature of embryonic organs and explains why human embryos have "cloacas" like adult birds and "branchial" clefts like adult fish.

Another very popular misconception is the theory that malformations actually represent "frozen" stages of normal embryology ("Hemmungsmißbildung").[6] As a result, our understanding of normal embryology stems more from pathological–anatomic interpretations of observed malformations than from proper embryological studies. The theory of the "rotation of the gut" as a step in normal development is a perfect example for this misconception.

DEFINITION OF THE TERM "MALFORMATION"

After birth, neonates can present with a broad spectrum of deviations from normal morphology. This extends from minor variations of normal morphology without any clinical significance to maximal organ defects with extreme functional deficits of the malformed organs or of the whole organism.

The degree of functional disorder is decisive when dealing with the question of whether a variation of normal morphology has to be viewed as a dangerous malformation requiring surgical correction. This means that functional disturbance is essential when using the term "malformation." Inborn deviations can be detrimental, neutral, or even beneficial; otherwise, evolutionary progress could not take place. An example of a beneficial deviation is the longevity syndrome of people with abnormally low serum cholesterol levels. Abnormalities with little or no functional disturbance might still require surgical correction when patients are in danger of social stigmatization. Coronal or glandular hypospadias might serve as an example for this condition.

ETIOLOGY OF CONGENITAL MALFORMATIONS

In most cases, the etiology of congenital malformations remains unclear. Possible etiological factors are listed in Table 1.1.

In about 20% of cases, genetic factors (gene mutation and chromosomal disorders) can be identified.[1,2,7] In 10%, an environmental origin can be demonstrated.[1,2] In 70%, the factors responsible remain obscure.

Environmental factors

A large number of agents are known that might interfere with the normal development of organ systems during embryogenesis.[1,7] The underlying mechanisms of this interference are poorly understood in most cases. Characteristically, during organogenesis, different organs of the embryo show distinct periods of greatest sensitivity to the action of the teratogen. These phases of greatest sensitivity are called the "teratogenetic period of determination."[8] The typical patterns of some syndromes can be explained by an overlap of these phases during embryological development.

Table 1.1 Etiology of congenital malformations

Genetic disorders	20%
Environmental factors	10%
Unknown etiology	70%

In 1983, Shepard[2] published a catalogue of suspected teratogenic agents. Over 900 agents are known to produce congenital anomalies in experimental animals. In 30 agents, evidence for teratogenic action in humans could be demonstrated. Teratogenic agents can be divided into four groups (Table 1.2).

The teratogenic potential of virus infections,[1] especially rubella and herpes, and that of radiation[1] has been clearly established. Maternal metabolic defects and lack of essential nutritives can be teratogenic. After a vitamin A–free diet[9] and riboflavin-free diet,[10] various congenital malformations were observed in rats and mice. Among these were diaphragmatic hernias, isolated esophageal atresias, and isolated tracheo-esophageal fistulas. Similarly, inappropriate administration of hormones can be associated with intrauterine dysplasias.[11]

Industrial and pharmaceutical chemicals such as tetrachlor-diphenyl-dioxin or thalidomide have inflicted tragedies by their teratogenic action. When thalidomide was prescribed to women in the early 1960s as a "safe" sleeping medication, numerous children were born with dysmelic deformities.[7,12,13] In addition, atresias of the esophagus, the duodenum, and the anus were observed in some children.[12] The data collected suggest that teratogenic agents do not cause new patterns of malformations but rather mimic sporadic birth defects. This had posed problems in identifying thalidomide as the responsible agent. It appears likely that among those 70% congenital malformations with unclear etiology, a considerable percentage might be precipitated by as yet unidentified environmental factors. In a rat model, the herbicide nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether) has been shown to induce CDHs, cardiac abnormalities, and hydronephrosis.[14–18] In 1978, Thompson et al.[19] described the teratogenicity of the anticancer drug Adriamycin in rats and rabbits. More recently, Diez-Pardo et al.[20] re-described

Table 1.2 Teratogenic agents in congenital malformations

Physical agents (radiation, heat, mechanical factors)
Infectious agents (viruses, treponemes, parasites)
Chemicals and drugs (thalidomide, nitrofen)
Environmental agents (hormones, vitamin deficiencies)
Maternal, genetic, and chromosomal disorders
Multifactorial inheritance

Source: Nadler HL. Teratology. In: Welch KJ, Randolph JG, Ravitch MM, O'Neill JA, Rowe MJ (editors). *Pediatric Surgery*. 4th Edition. Chicago: Year Book Medical Publishers, 1986:11–3.

this model with emphasis to its potentials as a model for foregut anomalies. Today, the Adriamycin model is generally described as a model for the VACTERL association (V = vertebral, A = anorectal, C = cardiac, T = tracheal, E = esophageal, R = renal, L = limb).[21,22] Thus, classic malformations such as atresias of the esophagus and the intestinal tract, intestinal duplications, and others can be mimicked by teratogens in animal models.

Genetic factors

Approximately 20% of congenital malformations are of genetic origin. Most surgically correctable malformations are associated with chromosomal disorders, e.g., trisomy 21, 13, or 18, or are of multifactorial inheritance[23] with a small risk of recurrence. The assumption of multifactorial inheritance results from the fact that with nearly all major anomalies familiar occurrences had been observed.[1] In animals, inheritance has also been found for some malformations.[24–27]

EMBRYOLOGY AND ANIMAL MODELS

Over the last two decades, a number of animal models were developed with the potential to gain a better understanding of the morphology of not only malformed but also normal embryos. These animal models can be divided into four subgroups.

Surgical models

In the past, the chicken was an important surgical model to study embryological processes. Due to the easy access to the embryo, its broad availability, and its cheapness, the chicken is an ideal model for experimental studies. It has been widely used by embryologists especially in the field of epithelial/mesenchymal interactions.[28–30] Pediatric surgeons used this model to study morphological processes involved in intestinal atresia formation,[31,32] gastroschisis,[33] and M. Hirschsprung.[34]

The Czech embryologist Lemez[35] used chicken embryos in order to induce tracheal agenesis with tracheo-esophageal fistula.

Apart from these purely embryonic models, a large number of fetal models exist. However, these models were mainly used in order to demonstrate the feasibility of fetal interventions.[36]

Chemical models

A large number of chemicals can have an impact on the normal development of humans and animals alike. The following are the most important chemicals today: (1) Adriamycin,[19,20] (2) etretinate,[37,38] (3) all-trans retinoic acid (ATRA),[39–41] (4) ethylenethiourea,[42–44] and (5) nitrofen.[15,16,18]

While models 1 to 4 are used to study the embryology of atresias of the esophagus, the gut, and the anorectum, model 5 was developed to study the malformations of the diaphragm, the lungs, the heart, and kidneys (hydronephrosis).

Genetic models

A number of genetic models had been developed that had been used for embryological studies in the past. These animals can be the product of spontaneous mutations or are the result of genetic manipulations, mainly in mice (transgenic mice).

1. Models of spontaneous origin: the SD mouse model[25,27]
2. Inheritance models: the pig model of anal atresia[24,26]
3. "Knockout" models[45–47]

The number of transgenic animal models is growing fast. For pediatric surgeons, those models are of major importance, which result in abnormalities of the fore- and hindgut. Here, interference with the sonic hedgehog pathway has proven to be very effective.[45–47] There are two ways to interfere with that pathway:

1. Targeted deletion of sonic hedgehog (Shh)[45,46]
2. Deletion of one of the three transcription factors Gli1, Gli2, and Gli3[46,47]

In the foregut, targeted deletion of Shh causes esophageal atresia/stenosis, tracheo-esophageal fistulas, and tracheal/lung anomalies in homozygous Shh null mutant mice.[45] In the hindgut, the deletion of Shh caused the formation of "cloacas,"[46] while Gli2 mutant mice demonstrated the "classic" form of anorectal malformations and Gli3 mutants showed minor forms like anal stenosis.[46,47] Interestingly, the morphology of Gli2 mutant mice embryos resembles that of heterozygous SD-mice embryos, while Shh null mutant mice embryos had morphological similarities with homozygous SD-mice embryos. Interestingly, after administration of Adriamycin, changes in the normal pattern of Shh distribution in the developing foregut were demonstrated.[48]

Viral models

Animal models that use virus infections to produce malformations important for pediatric surgeons are very rare. One exception is the murine model of extra hepatic biliary atresia (EHBA). In this model, newborn Balb/c mice are infected with rhesus rotavirus group A.[49] As a result, the full spectrum of EHBA develops as it is seen in newborns with this disease. However, this model is not a model to mimic failed embryology. But it highlights the possibility that malformations are not caused by embryonic disorders but by fetal or even postnatal catastrophes.

This part on embryology and animal models further highlights the importance of the study of normal animal embryos. Today, much information in current textbooks on human embryology stems from studies done in animals of varied species. Many of these are outdated. However, the

wide use of transgenic mice in order to mimic congenital malformations makes morphological studies of the various organ systems in normal mice mandatory. Otherwise, the interpretation of the effects of the deletion of genetic information can be very difficult.[50]

EMBRYOLOGY OF MALFORMATIONS

Disturbances of normal embryological processes will result in malformations of organs. This was first shown by Spemann[51] in 1901 by experimentally producing supernumerary organs in the triton embryo after establishing close contact between excised parts of triton eggs and other parts of the same egg. Spemann and Mangold[5] coined the term "induction" to describe this observation. They found that certain parts of the embryo obviously were able to control embryonic development of other parts. These controlling parts were called "organizers."[5] The process of influence itself was called "induction."

It was believed by many scientists in the field that "induction" could serve as the overall principle of hierarchical control of embryonic development. Ensuing investigations, however, made modifications necessary, which finally resulted in a very complex model of organizers and inductors. The nature of inductive substances remained obscure, and attempts to isolate inductive substances, currently called "morphogenes," were unsuccessful.[52] Interestingly, not only live cells could induce development in certain experiments but also dead and denatured materials.[5]

A process essential for the formation of early embryonic organs is the invagination of epithelial sheets. This invagination is preceded by a thickening of the epithelial sheet,[53] a process known as placode formation. The thickening itself is caused by elongation of individual cells of the placode. This process can be studied in detail in epithelial morphogenesis.[54] The same sequence of developmental events has been observed in the formation of the neural plate, in the formation of the optic and lens placode, and in the development of most epitheliomesenchymal organs including the lung, the thyroid gland, and the pancreas. From these observations, it can be concluded that most epithelial cells behave uniformly in the early phase of embryonic development.

Today it is generally accepted that early embryonic organs are especially sensitive for alterations. Therefore, researchers are more and more interested to understand the formation of early embryonic organs.

In 1985, Ettersohn[55] stated that most invaginations are the result of mechanical forces that are local in origin. He focused on three possible mechanisms that might lead to placode formation and subsequent invagination:

1. Change of cell shape by cell adhesion
2. Microfilament-mediated change of cell shape
3. Cell growth and division

In the following part, we will discuss some aspects of these mechanisms.

A teratological method used to determine the function of cell adhesion molecules (CAMs) in vivo during embryogenesis has been reported recently.[56] Mouse hybridoma cells producing monoclonal antibodies against the avian integrin complex were grafted into 2- or 3-day-old chick embryos. Depending on the site of engraftment, local muscle agenesis was observed. This is an example that the immunologic immaturity of the embryo can be exploited to study the contribution of cell attachment molecules to organ development in a functional fashion. A number of monoclonal antibodies directed against cell attachment molecules of various species have become available over the last years, and the structure of the binding molecules has been elucidated biochemically and by cDNA cloning. Functionally, adhesion molecules may be grouped into three families: (1) CAMs, which mediate specific and mostly transient cell recognition of other cells; (2) substrate adhesion molecules (SAMs), necessary for attachment to extracellular matrix proteins; and (3) cell-junctional molecules (CJMs), found in tight and gap junctions. Whereas CJMs apparently play an important role for metabolic signaling within established tissues, CAMs and SAMs are necessary for the formation of histologically distinct structures and directed migration of single cells. Among CAMs and SAMs, at least three families have been identified biochemically: integrins,[57] members of the immunoglobulin superfamily, and LEC-CAMS.[58] Integrins are heterodimeric molecules consisting of a larger α chain, which is associated with a smaller β chain in a calcium-dependent way. Usually, one given α chain might be found in association with various chains, but promiscuity of β chains has been described recently. Functionally, members of the integrin family present as SAMs (adhesion to vitronectin, collagen, fibronectin, complement components, or other intercellular matrix proteins) or CAMs (direct adhesion to other cells via corresponding cell surface target molecules). For example, cells bearing the integrin LFA-1 on their cell surface bind to cells expressing ICAM-1 or ICAM-2, both of which are members of the immunoglobulin superfamily.[59,60] Other members of the immunoglobulin superfamily that are known to be important during morphogenesis include liver CAM[61] (L-CAM) and neural CAM[62,63] (N-CAM). Both show homophilic aggregation; that is, N-CAM serves as a target structure for N-CAM, and L-CAM serves as a target structure for L-CAM, but there is no cross-reactivity. In developing feather placodes in avian embryos, L-CAM and N-CAM are mutually exclusive expressed on epidermal or mesodermal cells, respectively. When the placodes are incubated with antibodies to L-CAM, primarily only epidermal cell-to-cell contact is disturbed.[64] However, the structure of the surrounding mesoderm is altered subsequently, suggesting an inductive signal loop between epidermal and mesodermal cells. A third group of adhesion molecules has been termed LEC-CAMs to indicate that their extracellular part consists of a lectin domain, an epidermal growth factor (EGF)-like domain, and a complement regulatory protein repeat domain. The lectin domain is presumed to contain the active center; binding mediated by the murine homolog

to the leukocyte adhesion molecule 1 (LAM-1)[65] can be blocked by mannose-6-phosphate or its polymers.[66] Lectin-dependent organ formation should be accessible experimentally by administration of the respective carbohydrates, but few, if any, data have been reported so far.

Cell shape is mainly maintained by microtubules forming the cellular cytoskeleton. In addition, contractile elements exist such as actin, which are essential for cell movement, the so-called microfilaments. These structures are thought to be essential for the process of placode formation and invagination.[67] Microfilament-mediated change of cell shape is based on the idea that actin filaments could alter the shape of cells by contraction. Most of these filaments are found at the apex of epithelial cells. Contraction of these filaments in each individual cell of a cell layer would result in an increasing infolding of the whole cell layer,[67,68] finally resulting in invagination. It is a disadvantage of this model, however, that there is no apparent reason why apical constriction should proceed by cell elongation.[55]

Cell proliferation is probably an essential factor in the morphogenesis of epitheliomesenchymal organs. During morphogenesis of these organs, repeated invagination can be observed, which might be dependent upon cell proliferation.[69] The way in which epithelial cell growth and proliferation is controlled in the embryo is not clear. However, it is believed that the surrounding mesenchyme might regulate the timing and location of invagination of the epithelial layer. Goldin and Opperman[28] proposed that EGF might be excreted by mesenchymal cells, which would stimulate epithelial cell proliferation and repeated invagination. When agarose pellets impregnated with EGF were cultured alongside 5-day embryonic chick tracheal epithelium, supernumerary buds were induced to form at those sites. EGF and the related peptide transforming growth factor-β (TGFβ) have been shown to lead to precocious eyelid opening when injected into newborn mice.[70] Thus, complex changes of late-stage organ development can be induced by physiological stimuli in the laboratory. Interestingly, EGF is a mitogen for many epithelial cells in vitro without affecting most mesenchymal cells. A large variety of cells have been demonstrated to display the receptor for EGF/TGFβ on their cell surface, which is encoded by the cellular proto-oncogene c-erbB. Structural alterations of this receptor are known to result in uncontrolled proliferation and ultimately malignant transformation. When secreted locally, EGF might provide physically associated cells with appropriate on and off signals required for the formation of complex organs. Other polypeptides, such as platelet-derived growth factor or transforming growth factor-α (TGFα), appear to function in an antagonistic way in that they stimulate rather the proliferation of mesenchymal cells.[71,72] In defined experimental situations, TGFα has been shown to be a mitogen for osteoblasts while being a potent inhibitor of the proliferation of epithelial and endothelial cells at the same time. Embryonic fibroblasts, however, are also inhibited by TGFα.[73] TGFα is a powerful chemotactic agent for fibroblasts and enhances the production of both collagen and fibronectin by these cells. There are, however, little data available concerning the involvement of these factors during normal and pathologic development of the embryo. Future investigations using such powerful approaches as in situ hybridization with cloned genes, preparation of transgenic animals, and direct administration of the recombinant proteins to various parts of the embryo might shed some light on signaling pathways mediated by soluble cytokines.

The surrounding mesenchyme might limit the expansion of the epithelial bud[74] forcing the epithelial sheet to fold in characteristic patterns. If a growing cell layer is restricted from lateral expansion, "mitotic pressure" by dividing cells will result in elongation of cells and then invagination of the "crowded" cell sheet. This does not necessarily imply that cells divide more rapidly in the region of invagination than in the surrounding areas. The main effect is caused by restriction of lateral expansion.[29,30] In the early anlage of the thymus, cell proliferation counts are actually lower in the thymus anlage than in the surrounding epithelium.[75] Steding[29] and Jacob[30] have shown experimentally that restriction of lateral expansion might be responsible for thickening and subsequent invagination of epithelial sheets. In their experiments, restriction of lateral expansion was caused by a tiny silver ring placed on the epithelium of chick embryos.

EXAMPLES OF PATHOLOGICAL EMBRYOLOGY

The focus of our research has been the embryology of foregut, anorectal, and diaphragmatic malformations. We studied the normal development of all embryonic organs involved by scanning electron microscopy (SEM).[76–82] In addition, we employed two rodent animal models to study malformations of the anorectum and the diaphragm. Pathogenetic concepts concerning these malformations were controversial in the past due to lack of detailed data.

EMBRYOLOGY OF FOREGUT MALFORMATIONS

The differentiation of the primitive foregut into the ventral trachea and dorsal esophagus is thought to be the result of a process of septation.[83] It is guessed that lateral ridges appear in the lateral walls of the foregut, which fuse in midline in a caudocranial direction, thus forming the tracheo-esophageal septum. This theory of septation has been described in detail by Rosenthal[84] and Smith.[85] However, others[86,87] were not able to verify the importance of the tracheo-esophageal septum for the differentiation of the foregut. They instead proposed individually that the respiratory tract develops simply by further growth of the lung bud in a caudal direction.

Using SEM, we studied the development of the foregut in chick embryos.[76,77] In this study, we were unable to demonstrate the formation of a tracheo-esophageal septum (Figure 1.1). A sequence of SEM photographs of staged chick embryos suggests that differentiation of the primitive

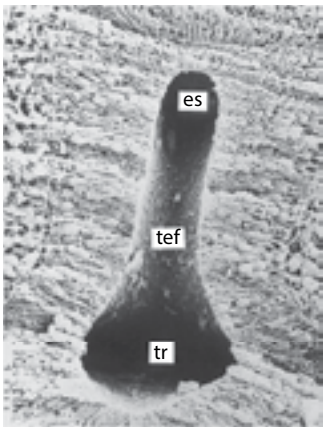

Figure 1.1 SEM photograph of the inner layer of foregut epithelium in a chick embryo (approx. 3.5 days old). View from cranial. Between trachea (tr) on bottom and esophagus (es) on top, the tip of the tracheo-esophageal fold (tef) is recognizable. Lateral ridges or signs of fusion are not found.

foregut is best explained by a process of "reduction of size" of a foregut region called the "tracheo-esophageal space" (Figure 1.2). This reduction is caused by a system of folds that develops in the primitive foregut. They approach each other but do not fuse (Figure 1.2).

Based on these observations, the development of the malformation can be explained by disorders either of the formation of the folds or of their developmental movements:

1. Atresia of the esophagus with fistula (Figure 1.3a): The dorsal fold of the foregut bends too far ventrally. As a result, the descent of the larynx is blocked. Therefore, the tracheo-esophageal space remains partly undivided and lies in a ventral position. Due to this ventral position, it differentiates into trachea.

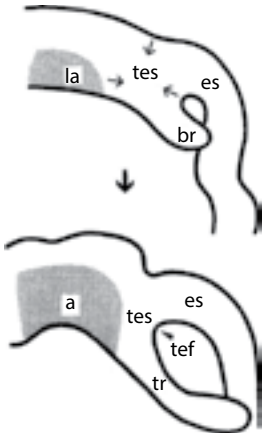

Figure 1.2 Summarizing sketch of foregut development. The tracheo-esophageal space (tes) is reduced in size by developmental movements of folds (indicated by arrows) (es, esophagus; la, anlage of larynx; br, bronchus; tr, trachea). Short arrow marks tip of tracheo-esophageal fold (tef) (compare Figure 1.1).

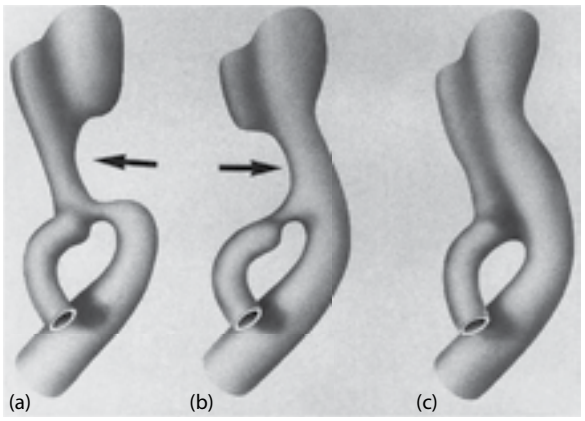

Figure 1.3 Sketch of formal pathogenesis of typical foregut malformations (see text for details): **(a)** atresia of esophagus with fistula; **(b)** atresia of trachea with fistula; **(c)** laryngotracheo-esophageal cleft. Arrows indicate sites of possible deformation of the developing foregut.

2. Atresia of the trachea with fistula (Figure 1.3b): The foregut is deformed on its ventral side. The developmental movements of the folds are disturbed, and the tracheo-esophageal space is dislocated in a dorsal direction. Therefore, it differentiates into esophagus.
3. Laryngotracheo-esophageal clefts (Figure 1.3c): Faulty growth of the folds results in the persistence of the primitive tracheo-esophageal space.

Recently, it has been shown that esophageal atresias and tracheo-esophageal fistulas can be induced by maternal application of Adriamycin into the peritoneal cavity of pregnant rats.[19,20] The dosage may vary between 1.5 and 2.0 mg/kg depending on the number of days it will be given. In most reports, the most promising dosage is 1.75 mg/kg given on days 6–9 of pregnancy. The Adriamycin model has been intensively studied over the last 20 years, resulting in more than 90 reports between 1997 and 2017.[88] It could be demonstrated that, in this model, not only foregut malformations but also atypical patterns of malformation can be observed, which are usually summarized under the term "VATER" or "VACTERL" association.[21,22] Therefore, this model is promising for the studies of not only foregut anomalies but also of anomalies of the hind- and midgut.

DEVELOPMENT OF THE DIAPHRAGM

In the past, several theories were proposed to explain the appearance of posterolateral diaphragmatic defects:

1. Defects caused by improper development of the pleuroperitoneal membrane[89,90]
2. Failure of muscularization of the lumbocostal trigone and pleuroperitoneal canal, resulting in a "weak" part of the diaphragm[89,91]
3. Pushing of intestine through the posterolateral part (foramen of Bochdalek) of the diaphragm[92]

4. Premature return of the intestines into the abdominal cavity with the canal still open[89,91]
5. Abnormal persistence of lung in the pleuroperitoneal canal, preventing proper closure of the canal[93]
6. Abnormal development of the early lung and posthepatic mesenchyme, causing nonclosure of pleuroperitoneal canals[18]

Of these theories, failure of the pleuroperitoneal membrane to meet the transverse septum is the most popular hypothesis to explain diaphragmatic herniation. However, using SEM techniques,[78] we could not demonstrate the importance of the pleuroperitoneal membrane for the closure of the so-called pleuroperitoneal canals (Figure 1.4).

As stated earlier, most authors assume that delayed or inhibited closure of the diaphragm will result in a diaphragmatic defect that is wide enough to allow herniation of gut into the fetal thoracic cavity. However, this assumption is not the result of appropriate embryological observations but rather the result of interpretations of anatomical/pathological findings. In a series of normal staged embryos, we measured the width of the pleuroperitoneal openings and the transverse diameter of gut loops.[82] On the basis of these measurements, we estimated that a single embryonic gut loop requires at least an opening of 450 μm in size to herniate into the fetal pleural cavity. However, in none of our embryos, the observed pleuroperitoneal openings were of appropriate dimensions. This means that delayed or inhibited closure of the pleuroperitoneal canal cannot result in a diaphragmatic defect of sufficient size. Herniation of gut through these openings is therefore impossible. Thus, the proposed theory about the pathogenetic mechanisms of CDH development lacks any embryological evidence. Furthermore, the proposed timing of this process is highly questionable.[79,80]

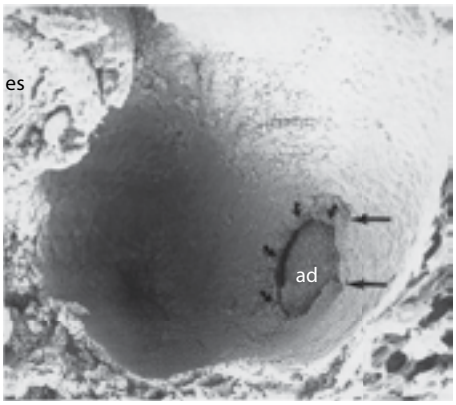

Figure 1.4 SEM photograph of right pleural sac in a rat embryo (approx. 16.5 days old). View from cranial. The so-called pleuroperitoneal canal (PPC) is nearly closed. Small arrows point at the margin of PPC. In the depth of the abdomen, the right adrenals (ad) are seen. Large arrows point at margins of the so-called pleuroperitoneal membrane. Its contribution to the closure of the canal is minimal (es, esophagus).

Recently, an animal model for diaphragmatic hernia has been developed[14–18] using nitrofen as the noxious substance. In these experiments, CDHs were produced in a reasonably high percentage of newborns.[15,16] Most diaphragmatic hernias were associated with lung hypoplasias. Using electron microscopy, our group[79–82] used this model to give a detailed description of the development of the diaphragmatic defect. Our results are as follows.

Timing of diaphragmatic defect appearance

Iritani[18] was the first to notice that nitrofen-induced diaphragmatic hernias in mice are not caused by an improper closure of the pleuroperitoneal openings but rather the result of a defective development of the so-called posthepatic mesenchymal plate (PHMP). In our study in rats, clear evidence of disturbed development of the diaphragmatic anlage was seen on day 13 (left side) and day 14 (right side, Figure 1.5).[79,82] In all embryos affected, the PHMP was too short. This age group is equivalent to 4–5 week old human embryos.[79]

Location of diaphragmatic defect

In our SEM study, the observed defects were localized in the PHMP (Figure 1.5). We identified two distinct types of defects: (1) large "dorsal" defects and (2) small "central" defects.[79] Large defects extended into the region of the pleuroperitoneal openings. In these cases, the closure of the pleuroperitoneal openings was usually impaired by the massive ingrowth of liver (Figures 1.6 and 1.7). If the defects were small, they were consistently isolated from the pleuroperitoneal openings closing normally at the 16th or 17th day of gestation. Thus, in our embryos with CDH, the region of the diaphragmatic defect

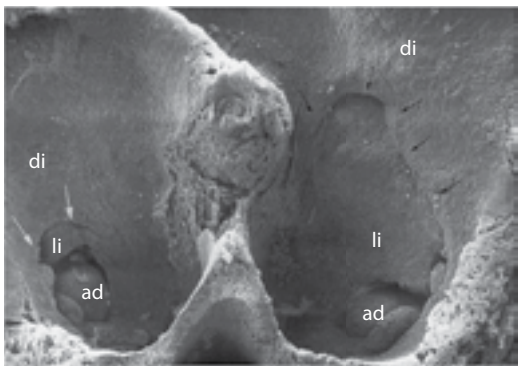

Figure 1.5 Cranial view of the pleural sacs in a rat embryo after exposition to nitrofen on day 11 of pregnancy. The embryo is approx. 15 days old. Note the big defect of the right diaphragmatic primordium. Small black arrows point at margins of the defect, which leaves parts of the liver (li) uncoated. On the left, the diaphragmatic anlage is normal. Note the low position of the cranial border of the pleuroperitoneal opening on this side (white arrows) (ad, adrenals; di, anlage of diaphragm).

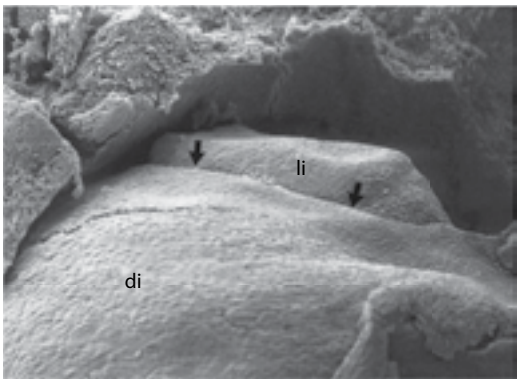

Figure 1.6 Liver (li) protrudes through diaphragmatic defect. Arrows point to the margin of the defect (di, diaphragmatic anlage). Rat embryo (approx. 16 days old), nitrofen exposition on day 11 of pregnancy.

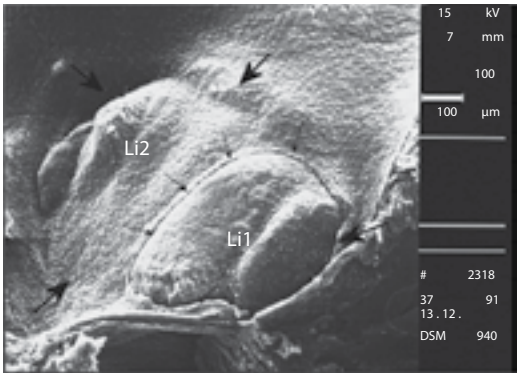

Figure 1.7 SEM photograph of a right pleural sac in a rat embryo after nitrofen exposure on day 11 of pregnancy. The embryo is approximately 15.5 days old. Note the big defect of the right dorsal diaphragm (large arrows). The closure of the pleuroperitoneal canal (PPC) is impaired by the ingrowths of liver (small arrows). Li1 = liver growing through PPC. Li1 + Li2 = liver growing through the defect of the diaphragm.

was a distinct entity and was separated from that part of the diaphragm where the pleuroperitoneal "canals" are localized. We conclude therefore that the pleuroperitoneal openings are not the precursors of the diaphragmatic defect.

Why lungs are hypoplastic

Soon after the onset of the defect in the 14-day-old embryo, liver grows through the diaphragmatic defect into the thoracic cavity (Figure 1.6). This indicates that, from this time on, the available thoracic space is reduced for the lung and further lung growth is hampered. In the following stages, up to two-thirds of the thoracic cavity can be occupied by liver (Figure 1.7). Herniated guts were found in our embryos and fetuses only in late stages of development (21 days and newborns). In all of these, the lungs were already hypoplastic when the bowel entered the thoracic cavity.[79]

Based on these observations, we conclude that the early ingrowth of the liver through the diaphragmatic defect is the crucial step in the pathogenesis of lung hypoplasia in CDH. This indicates that growth impairment is not the result of lung compression in the fetus but rather the result of growth competition in the embryo: the liver that grows faster than the lung reduces the available thoracic space. If the remaining space is too small, pulmonary hypoplasia will result.

DEVELOPMENT OF THE EMBRYONIC CLOACA (EC)

General remarks on the development of the EC

The terminology of malformations is sometimes confusing. As already mentioned, there is a strong belief that human embryos go through a phase in their development where they have a "cloaca." This belief is based on Haeckel's "biogenetic law."[5] According to this theory, a human embryo recapitulates in its individual development (ontogeny) the morphology observed in all life forms (phylogeny). This means that the development of advanced species passes through stages represented by adult organisms of more primitive species. This theory still has an impact on the nomenclature of embryonic organs.

Literature on "normal cloacal development"

In the literature, several theories have been put forward to explain the differentiation of the "cloaca" into the dorsal anorectum and the ventral sinus urogenitalis. To many authors, this differentiation is caused by a septum that develops cranially to caudally and thus divides the cloaca in a frontal plane. Disorders in this process of differentiation are thought to be the cause of "cloacal" anomalies such as "persistent cloaca" and anorectal malformations.

However, there is no agreement on the mechanisms of septation. While some authors[94,95] believe that the descent of a single fold separates the urogenital part from the rectal part by ingrowth of mesenchyme from cranial, others[96] think that lateral ridges appear in the lumen of the cloaca, which progressively fuse along the midline and thus form the septum. In a recent paper,[97] the process of septation had been questioned altogether.

Own observations

Using SEM techniques, our group studied cloacal development in rat and SD-mice embryos. The SD-mouse is a spontaneous mutation of the house mouse characterized by having a short tail (Figure 1.8). Homozygous or heterozygous offspring of these mice show skeletal, urogenital, and anorectal malformations.[25,28] Therefore, these animals are ideal for the study of the development of anorectal malformations.

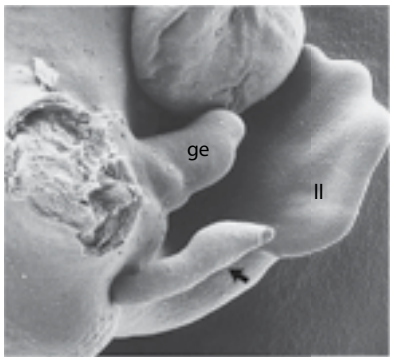

Figure 1.8 Characteristic short tail (arrow) of SD-mouse embryo (approx. 13 days old) (ll, left lower limb; ge, genital tuberculum, abnormal).

Normal "cloacal" embryology (rat)

As in the foregut of chick embryos, signs of median fusion of lateral cloacal parts could not be demonstrated during normal cloacal development in the rat. However, in contradiction to van der Putte,[97] we think that downgrowth of the urorectal fold takes place, although it is probably not responsible for the formation of "cloacal" malformations.

Abnormal "cloacal" embryology (SD-mouse)

"Cloacal" malformations are caused by improper development of the early anlage of the cloacal membrane as demonstrated in SD-mice embryos.[98,99] Our studies of abnormal "cloacal" development in SD-mice had the following results:

1. The basis of the pathogenesis of anorectal malformations is too short a cloacal membrane.
2. The anlage of the cloacal membrane is too short and results in a maldeveloped anlage of the "cloaca," which is missing in its dorsal part (Figure 1.9).
3. The caudal movement of the urorectal fold is impaired in the malformed "cloaca." Thus, the hindgut remains in abnormal contact with the urethral part. This opening is true ectopic and will develop into the recto-urogenital fistula (Figure 1.10).

It is interesting to note that the morphology of the anorectal malformations observed is very similar in all animal models used irrespective of the source of the malformed embryo (spontaneous mutation vs. chemically induced vs. transgenic models).

Critical remarks on the terminology of the "cloaca"

It must be kept in mind that the term "cloaca" is used to describe not only a transitional organ system in human

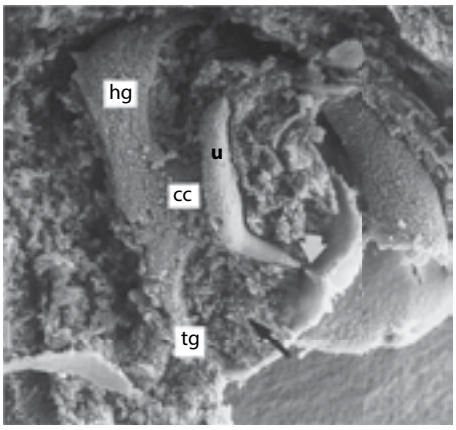

Figure 1.9 Malformed cloaca of SD-mouse embryo (approx. 11 days old). The surrounding mesenchyme is removed by microdissection. View on the basal layer of the cloacal entoderm. The cloaca has lost its contact to the ectoderm of the genitals (white arrow). The dorsal part of the cloaca is missing (black arrow). Tailgut (tg) and hindgut (hg) are hypoplastic. This malformed cloaca developed because the anlage of the cloacal membrane was too short in early embryogenesis (see text for details) (cc, rest of cloaca; u, urachus, rudimentary).

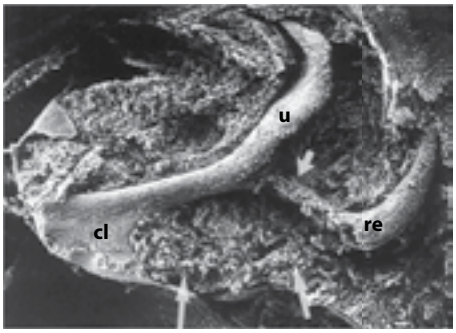

Figure 1.10 Malformed cloaca of SD-mouse embryo (approx. 13 days old). Urachus (u) and rectum (re) nearly normal (cl, ventral part of cloaca with short cloacal membrane). The dorsal part of the cloaca is missing (long white arrows). Short white arrow points to the region of the future fistula.

embryos but also a congenital anomaly in human female newborns and a normal organ in birds.[99]

This may lead to the false conclusion that the morphology of these three entities is similar. This is not the case. Despite the same name, embryonic "cloacas" are morphologically completely different from "cloacas" in female newborns with ARM. And these female "cloacas" are morphologically completely different from cloacas in birds.[100]

The main difference is the presence/absence and position of the anus:

1. In normal embryonic "cloacas," the future anal region is always present.
2. In human "cloacas," the anus is always missing.
3. In birds, the "cloaca" is part of the rectum.

Thus, the anus is always present in birds.

Furthermore, it is obvious that a true definition of the term embryonic "cloaca" is missing. In many papers and textbooks, it is replaced by observations made in female newborns born with the malformation called "cloaca." These are defined as defects "in which the rectum, one or two vaginas and the urinary tract converge into a common channel."[101]

It is believed that they represent malformations that occur in a very early stage of development.[101] Therefore, many authors believe that these malformations are "persistent cloacas."[101]

However, recent observations on the development of the vagina in mice[102] show that the development of the vagina and its downgrowth takes place after the complete separation of urethra and anorectum. As a result, a "cloaca" in the above-mentioned definition does not exist in normal embryos.

How can the female malformation called "cloaca" be explained? In our opinion, this malformation develops in two steps:

1. An anorectal malformation develops as described above.
2. The downgrowth of the vagina is hampered by the abnormal connection between urethra and anorectum (Figure 1.10).

HYPOSPADIAS

Many investigators[103–106] believe that the urethra develops by fusion of the paired urethral folds following the disintegration of the urogenital membrane. Impairment of this process is thought to result in the different forms of hypospadia.[106] However, in our study of normal cloacal development,[107] we were puzzled by the fact that disintegration of the urogenital part of the cloacal membrane could not be observed in rat embryos (Figure 1.11). This finding caused us to call in question the generally assumed concepts of hypospadia formation. Instead we found that

1. The urethra is always present as a hollow organ during embryogenesis of rats, and that it is always in contact with the tip of the genitals.
2. An initially double urethral anlage exists. The differentiation in female and male urethra starts in rats of 18.5 days old. On the other hand, we found no evidence for
   a. The disintegration of the urogenital cloacal membrane
   b. A fusion of lateral portions within the perineum

In our opinion, more than one embryological mechanism is at play in the formation of the hypospadias complex. The moderate degrees, such as the penile and glandular forms, represent a developmental arrest of the genitalia (Figure 1.12). They take their origin from a situation comparable

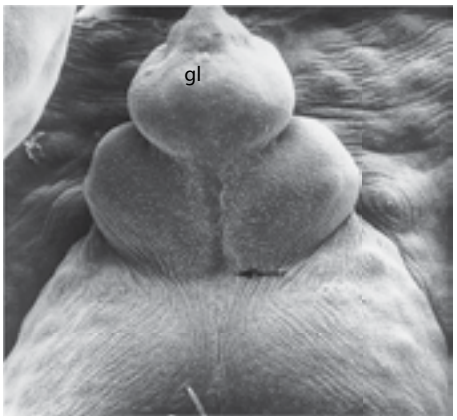

Figure 1.11 Genitals of a normal female rat embryo (approx. 18.5 days old) (gl, glans). Arrow points to future opening of the female urethra. No signs of disintegration of the cloacal membrane.

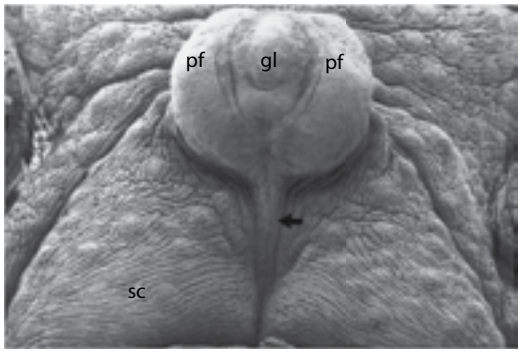

Figure 1.12 Genitals of a normal male rat embryo (approx. 20 days old) (gl, glans; pf, preputial fold; sc, scrotum). Arrow points to the raphe up to this stage; disintegration of the urogenital part of the cloacal membrane was not seen. Note similarity with clinical picture of hypospadia.

to the 20-day-old embryo. Consequently, the penis, not the urethra, is the primary organ of the malformation.

Perineal and scrotal hypospadias are different from the type discussed previously. Pronounced signs of feminization in these forms suggest that we are dealing with a female-type urethra. The origin of this malformation complex is an undifferentiated stage as may be seen in the 18.5-day-old rat embryo.[104]

CONCLUSION

Despite the long history of experimental embryology, we know very little about etiology and pathogenesis of congenital malformations. For decades, hypotheses were abundant while few data existed to support them. The tremendous progress of neighboring biological sciences is now providing powerful tools for researchers in the field, such as recombinant DNA and hybridoma technology. Future investigations will monitor closely how genes are switched on and