Teratology Primer

Birth Defects Research

Education

Prevention

TERATOLOGY SOCIETY

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Some Web Sites of Interest

Site Name	URL
Teratology Society	**http://www.teratology.org**
Organization of Teratology Information Specialists (OTIS)	**http://www.otispregnancy.org/**
Neurobehavioral Teratology Society (NBTS)	**http://www.nbts.org/**
American College of Toxicology	http://www.actox.org/
Canadian Congenital Anomalies Surveillance Network (CCASN)	http://www.phac-aspc.gc.ca/ccasn-rcsac/index-eng.php
Canadian Institutes of Health Research (CIHR) Institute of Human Development, Child and Youth Health (IHDCYH)	http://www.cihr-irsc.gc.ca/e/8688.html
Centers for Disease Control and Prevention	http://www.cdc.gov/
DevTox—A Resource for Developmental Toxicology	http://www.devtox.org/
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)	http://www.nichd.nih.gov/
European Medicines Agency (EMA)	http://www.ema.europa.eu/home.htm
European Teratology Society	http://www.etsoc.com/
FASEB—Federation of American Societies for Experimental Biology	http://www.faseb.org/
Fetal Alcohol and Drug Unit, University of Washington-School of Medicine	http://depts.washington.edu/fadu/
Gene Interactions	http://www.biocarta.com/genes/index.asp
International Conference on Harmonization (ICH) guidelines	http://www.ich.org/cache/compo/276-254-1.html
International Federation of Placenta Associations	http://www.epineux.com/IFPA/home/home.html
Japanese Teratology Society	http://jts.umin.jp/newpage2.html
March of Dimes	http://www.marchofdimes.com/
MedPedia	http://www.medpedia.com/
Mendelian Inheritance in Man	http://www.ncbi.nlm.nih.gov/omim
Middle Atlantic Reproduction and Teratology Association (MARTA)	http://www.e-marta.org/
Mouse Atlas	http://genex.hgu.mrc.ac.uk/
National Birth Defects Prevention Network (NBDPN)	http://www.nbdpn.org/
National Center for Biotechnology Information (NCBI)	http://www.ncbi.nlm.nih.gov/
National Center for Environmental Health/Agency for Toxic Substances and Disease Registry (NCEH/ATSDR)	http://www.atsdr.cdc.gov/
National Institute of Environmental Health Sciences (NIEHS)	http://www.niehs.nih.gov/
National Institutes of Health (NIH)	http://www.nih.gov/
National Institutes of Health (NIH) Office of Rare Diseases	http://rarediseases.info.nih.gov/
National Library of Medicine	http://www.nlm.nih.gov/
PubMed/MEDLINE	http://www.ncbi.nlm.nih.gov/pubmed/
REPROTOX	http://reprotox.org
Society for Developmental Biology (SDB)	http://www.sdbonline.org/
Society for the Study of Reproduction (SSR)	http://www.ssr.org/
Society of Toxicology (SOT)	http://www.toxicology.org/
TERIS	http://depts.washington.edu/terisweb/teris/
Thalidomide Victims Association of Canada	http://www.thalidomide.ca/home/
The Hospital for Sick Children (Sick Kids)	http://www.sickkids.ca/AboutSickKids/who-we-are/index.html
The Multi-dimensional Human Embryo	http://embryo.soad.umich.edu/
TOXNET	http://toxnet.nlm.nih.gov/
U.S. Environmental Protection Agency (EPA)	http://www.epa.gov/
U.S. Food and Drug Administration (FDA)	http://www.fda.gov/
Vaccines and Medications in Pregnancy Surveillance System (VAMPSS)	http://www.otispregnancy.org/vaccines-and-medications-in-pregnancy-surveillance-system-vampss-s13053
Visible Embryo	http://www.visembryo.com/

If you know of web sites that should be listed on this page but are not, or if the URL has changed, please contact tshq@teratology.org.

Editors: Barbara Hales, Anthony Scialli, and Melissa S. Tassinari

The first edition of the *Teratology Primer* was published by the Teratology Society in June 2005. Thousands of copies have been distributed to colleagues and trainees. The fundamental information provided in the first edition is still valid, however, knowledge grows constantly. We have included new topics in this second edition and the chapters that appeared in the original *Primer* have been updated.

The goal of this second edition of the *Primer*, like that of the first, is to give you in a few short pages, a sense of what this field is about. What is teratology? Do I want to be a teratologist? How are new chemicals evaluated for their reproductive risk? What exposures should we be concerned about? This *Teratology Primer* is meant to answer these questions and more. Topics range from how birth defects are diagnosed, to the impact of genes or environmental exposures, to ethical considerations, to the use of systems biology or computational approaches to predict teratogenic risk.

The *Primer* was written by scientists who want to share their fascination for the development of complex organisms from a couple of microscopic cells, and for why and how, things don't always go right in the process. Being a teratologist is having a front row seat for the most exciting and mysterious performances known to this planet. We hope that you will become as excited about working in this field as are the contributing authors. If you find yourself drawn to a topic and you want to learn more, we hope that you will contact the Teratology Society. You are the future of this field.

We are especially pleased that the timing of this second edition of the *Teratology Primer* coincides with the 50th anniversary of the Teratology Society. As we enter this new decade for the Society, we have decided to drop the traditional format of a paper bound book and move to an electronic only version for the *Teratology Primer*. The *Primer* will be freely available on the Teratology Society Web site (http://www.teratology.org) as a single PDF or as PDFs of individual chapters. This approach will allow for a wider scope of circulation of the *Primer*, permit more frequent updates of the content, and help save a forest of trees.

It is our hope that this edition of the *Teratology Primer* lays the common foundation for basic scientists, clinician scientists, healthcare professionals, trainees, policy makers and anyone who has an interest in the discipline to acquire the knowledge that they seek. We have tried to give a balanced presentation of different views but not every scientist whose name is listed as a contributor to this book will agree with every statement made in the book.

We should like to acknowledge the dedicated support of headquarters staff for their roles in the production of this *Primer*. We thank the National Institute of Environmental Health Sciences, NIH for their financial support. Finally, we thank all the contributors and members of the Teratology Society for their valuable contributions and support in making this *Primer* possible.

The Teratology Society
Editors
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What is Teratology?

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"What a piece of work is an embryo!" as Hamlet might have said. "In form and moving how express and admirable! In complexity how infinite!" It starts as a single cell, which by repeated divisions gives rise to many genetically identical cells. These cells receive signals from their surroundings and from one another as to where they are in this ball of cells —front or back, right or left, headwards or tailwards, and what they are destined to become. Each cell commits itself to being one of many types; the cells migrate, combine into tissues, or get out of the way by dying at predetermined times and places. The tissues signal one another to take their own pathways; they bend, twist, and form organs. An organism emerges. This wondrous transformation from single celled simplicity to myriad-celled complexity is programmed by genes that, in the greatest mystery of all, are turned on and off at specified times and places to coordinate the process. It is a wonder that this marvelously emergent operation, where there are so many opportunities for mistakes, ever produces a well-formed and functional organism.

And sometimes it doesn't. Mistakes occur. Defective genes may disturb development in ways that lead to death or to malformations. Extrinsic factors may do the same. "Teratogenic" refers to factors that cause malformations, whether they be genes or environmental agents. The word comes from the Greek "teras," for "monster," a term applied in ancient times to babies with severe malformations, which were considered portents or, in the Latin, "monstra."

Malformations can happen in many ways. For example, when the neural plate rolls up to form the neural tube, it may not close completely, resulting in a neural tube defect—anencephaly if the opening is in the head region, or spina bifida if it is lower down. The embryonic processes that form the face may fail to fuse, resulting in a cleft lip. Later, the shelves that will form the palate may fail to move from the vertical to the horizontal, where they should meet in the midline and fuse, resulting in a cleft palate. Or they may meet, but fail to fuse, with the same result. The forebrain may fail to induce the overlying tissue to form the eye, so there is no eye (anophthalmia). The tissues between the toes may fail to break down as they should, and the toes remain webbed.

Experimental teratology flourished in the 19th century, and embryologists knew well that the development of bird and frog embryos could be deranged by environmental "insults,"
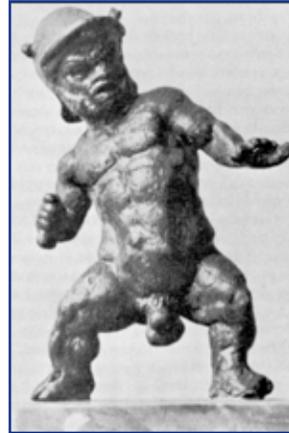
"Achondroplasia Gladiator"— Bibliotheque Nationale, Paris [In "Congenital Malformatons" by J. Warkany, Year Book Medical Publishers, 1971]

such as lack of oxygen (hypoxia). But the mammalian uterus was thought to be an impregnable barrier that would protect the embryo from such threats. By exclusion, mammalian malformations must be genetic, it was thought.

In the early 1940s, several events changed this view. In Australia an astute ophthalmologist, Norman Gregg, established a connection between maternal rubella (German measles) and the triad of cataracts, heart malformations, and deafness. In Cincinnati Josef Warkany, an Austrian pediatrician showed that depriving female rats of vitamin B (riboflavin) could cause malformations in their offspring— one of the early experimental demonstrations of a teratogen. Warkany was trying to produce congenital cretinism by putting the rats on an iodine deficient diet. The diet did indeed cause malformations, but not because of the iodine deficiency; depleting the diet of iodine had also depleted it of riboflavin!

Several other teratogens were found in experimental animals, including nitrogen mustard (an anti cancer drug), trypan blue (a dye), and hypoxia (lack of oxygen). The pendulum was swinging back; it seemed that malformations were not genetically, but environmentally caused.

In Montreal, in the early 1950s, Clarke Fraser's group wanted to bring genetics back into the picture. They had found that treating pregnant mice with cortisone caused cleft palate in the offspring, and showed that the frequency was high in some strains and low in others. The only difference was in the genes. So began "teratogenetics," the study of how genes influence the embryo's susceptibility to teratogens.

The McGill group went on to develop the idea that an embryo's genetically determined, normal, pattern of development could influence its susceptibility to a teratogen—the multifactorial threshold concept. For instance, an embryo must move its palate shelves from vertical to horizontal before a certain critical point or they will not meet and fuse. A teratogen that causes cleft palate by delaying shelf movement beyond this point is more likely to do so in an embryo whose genes normally move its shelves late.

As studies of the basis for abnormal development progressed, patterns began to appear, and the principles of teratology were developed. These stated, in summary, that the probability of a malformation being produced by a teratogen depends on the dose of the agent, the stage at which the embryo is exposed, and the genotype of the embryo and mother.

The number of mammalian teratogens grew, and those who worked with them began to meet from time to time, to talk about what they were finding, leading, in 1960, to the formation of the Teratology Society. There were, of course, concerns about whether these experimental teratogens would be a threat to human embryos, but it was thought, by me at least, that they were all "sledgehammer blows," that would be teratogenic in people only at doses far above those to which human embryos would be exposed. So not to worry, or so we thought.

Then came thalidomide, a totally unexpected catastrophe. The discovery that ordinary doses of this supposedly "harmless" sleeping pill and anti-nauseant could cause severe malformations in human babies galvanized this new field of teratology. Scientists who had been quietly working in their laboratories suddenly found themselves spending much of their time in conferences and workshops, sitting on advisory committees, acting as consultants for pharmaceutical companies, regulatory agencies, and lawyers, as well as redesigning their research plans.

The field of teratology and developmental toxicology expanded rapidly. The following pages will show how far we have come, and how many important questions still remain to be answered. A lot of effort has gone into developing ways to predict how much of a hazard a particular experimental teratogen would be to the human embryo (chapters 9–19). It was recognized that animal studies might not prove a drug was "safe" for the human embryo (in spite of great pressure from legislators and the public to do so), since species can vary in their responses to teratogenic exposures. A number of human teratogens have been identified, and some, suspected of teratogenicity, have been exonerated—at least of a detectable risk (chapters 21–32). Regulations for testing drugs before market release have greatly improved (chapter 14). Other chapters deal with how much such things as population studies (chapter 11), post-marketing surveillance (chapter 13), and systems biology (chapter 16) add to our understanding. And, in a major advance, the maternal role of folate in preventing neural tube defects and other birth defects is being exploited (chapter 32). Encouraging women to take folic acid supplements and adding folate to flour have produced dramatic falls in the frequency of neural tube defects in many parts of the world.

Progress has been made not only in the use of animal studies to predict human risks, but also to illumine how, and under what circumstances, teratogens act to produce malformations (chapters 2–8). These studies have contributed greatly to our knowledge of abnormal and also normal development. Now we are beginning to see exactly when and where the genes turn on and off in the embryo, to appreciate how they guide development and to gain exciting new insights into how genes and teratogens interact. The prospects for progress in the war on birth defects were never brighter.

Suggested Reading

Fraser FC. *Evolution of a palatable multifactorial model.* Am J Hum genetics *1980; 32: 796–813.*

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What Birth Defects Are Common in Humans?
How Are They Diagnosed at Birth?

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A malformation is a structural abnormality with surgical, medical, or cosmetic importance. About 2% of newborn infants have a malformation. The frequency increases to 3% at 1 year of age, as additional abnormalities are identified. The frequency of malformations is higher in spontaneous abortions than in liveborn infants, reflecting the fact that many of the most severe problems are incompatible with survival.

Defects in the formation of the heart are the most common birth defects and are recognized in about 15% of infants with birth defects. Heart defects are found by listening to the infant's heart for the presence of a heart murmur. The examining physician decides whether or not to have additional studies, such as an echocardiogram, to identify a specific heart abnormality. Many heart defects are minor and transients and routine echocardiography would identify up to 5% of babies as having an abnormality, even though only a small fraction of these children have a clinically important problem. Therefore, the rate of detection of birth defects is very dependent on how much effort is put into finding birth defects.

Other common abnormalities include extra fingers and toes (polydactyly), especially on the outer aspect of the hands, webbing between fingers and toes (syndactyly), defects in closure of the developing spine (myelomeningocele), club foot (talipes equinovarus and calcaneovalgus) cleft lip, cleft palate, and incomplete closure of the urethra of the male (hypospadias). Because these abnormalities are readily identifiable by examining the outside of the child, they are less prone to variation based on level of effort of the examining clinician.

Variation by Race and Ethnic Group:

Many common birth defects are much more common in individuals from one race or ethnic group in comparison to another. For example, the dangling extra finger along the fifth finger side of the hand occurs in 1% of African newborn infants in comparison to 10 times less (0.1%) in Caucasian infants born in the United States. Another example is spina bifida, more common among Hispanic infants than in African-American infants. A third example is the fact that Polynesian infants born in New Zealand have a much higher frequency of club foot deformity in comparison to Caucasian infants born in the same country.

CAUSES OF BIRTH DEFECTS	
Genetic Cause	
Dominant or recessive gene	**3%**
Chromosomal aberrations	**10%**
Multifactorial inheritance	**23%**
Familial conditions	**15%**
Teratogens* and uterine factors**	**5.6%**
Twinning***	**0.4%**
Unknown cause	**43%**
	100%

* *Teratogens were insulin-dependent diabetes mellitus in the mother; anticonvulsant and anticoagulant drugs*

** *Uterine factors included amnion rupture which produced limb deformities*

*** *Twinning associated defects were conjoined twins*

(from Nelson K and Holmes LB, 1989)

How Are Malformations Identified?

Many birth defects, such as polydactyly, syndactyly, hypospadias, club foot, cleft lip, and spina bifida, are diagnosed by the infant's doctor in a "surface" examination at birth. Often, the first signs of heart defects are a murmur heard in the initial examination. Many abnormalities of the kidneys, such as hydronephrosis, multicystic dysplasia, and absence of the kidney, can be identified in the routine prenatal screening by ultrasound at 18 to 20 weeks of pregnancy. There are often no signs of these "silent" urinary tract abnormalities at birth. The more testing applied to a pregnancy or a newborn, the more likely an abnormality will be found. Early medical or surgical treatment of some of these defects in important, so liberal use of diagnostic testing may be helpful. In other instances, diagnostic testing creates undue worry about a defect that may be transient or clinically unimportant.

Establishing the Diagnosis and Apparent Cause

The physician examining a child with a common malformation like a heart defect, hypospadias, cleft lip or club foot, determines whether or not the abnormality is "isolated" or is one of several birth defects. Many isolated and common malformations are attributed to multifactorial

inheritance, a process that is thought to include more than one genetic abnormality and other non-genetic factors. The practical significance of this type of inheritance is that there is an increased chance that the brothers and sisters or, when older, the sons and daughters, will have the same abnormality.

When a common malformation occurs in association with other malformations, it is more likely to be associated with a chromosome abnormality or is due to a genetic syndrome (chapter 6). Chromosome abnormalities are diagnosed by routine chromosome analysis or the new chromosome microarray testing. These tests identify extra chromosomes (trisomies), unbalanced translocations, and more subtle chromosome deletions and duplications. If a genetic syndrome is suspected, the testing available can be identified in the listings at www.genetests.org. A brief summary of most genetic disorders is provided by the on-line Mendelian Inheritance in Man, a catalog of autosomal dominant, autosomal recessive and X-linked disorders. For most malformations, the cause of the condition is unknown; however, an increasing number of genetic abnormalities are being described as causes of malformations.

Environmental exposures are occasional causes of birth defects. Although many parents of children with malformations feel guilty about an exposure to a medication or workplace chemical, in most cases, careful evaluation of the exposure history does not suggest exposure as a cause of the malformation. For most malformations, the cause of the condition is unknown; however, an increasing number of genetic abnormalities are being described as causes of malformations.

Treatments

The "isolated" malformations are typically corrected by surgical repair. However, one dramatic exception is the talipes equinovarus club foot deformity. The pioneering work of Ignacio Ponsetti, M.D., an orthopedist at the University of Iowa, showed that over 90% of the affected infants could be treated successfully with serial casting over the first few weeks of life. The treated infant continues to wear corrective braces for several weeks and then, only during naps and while asleep for three years. This non-surgical approach makes successful treatment more readily available in developing countries.

New Research Findings

Many investigators have been searching for the gene mutations associated with the common malformations. Several mutations have been identified and are a factor in the occurrence of cleft lip, cleft palate, heart malformations, club foot, hypospadias, and holoprosencephaly. In general, the testing for these associated mutations has been carried out by research laboratories and is not available in commercial laboratories. Investigators are also identifying non-genetic etiologic factors, such as nutritional deficiencies in the mother of an infant with gastroschisis, an abdominal wall defect.

Even though environmental exposures, including medications, are unusual as causes of malformations, a great deal of effort is expended in identifying possible teratogenic exposures. The evaluation of possible teratogenic exposures involves an interpretation of experimental animal and epidemiology studies. The identification of a new teratogenic exposure informs the at-risk pregnant woman and her clinician and offers the possibility of prevention of birth defects.

Suggested Reading

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Mendelian Inheritance in Man:
www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

What Is the Timeline of Important Events During Pregnancy That May Be Disrupted by a Teratogenic Exposure?

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Pregnancy is typically envisioned as consisting of three sequential trimesters, each lasting three months. Many events occur during prenatal development that are critical for the success of the pregnancy and, ultimately, the birth of a healthy child. These events can be described as milestones of pregnancy, or periods and phases during which essential events occur. Normal development of the conceptus plus its supporting membranes and placenta—can be adversely affected by poor maternal health and nutrition, genetic mutation, environmental exposures, or a combination of these factors. The focus of this chapter is the timing of important events that may be disrupted by a teratogenic exposure (Figure 2-1). Although there are critical periods in which the conceptus is highly susceptible to teratogenic exposure, in fact, the conceptus can be susceptible to exposure prior to and throughout *in utero* development and even postnatally.

In addition to using trimesters to measure the progress of pregnancy, three periods of development are used as milestones: the period of the **egg**, the period of the **embryo**, and the period of the **fetus**. The period of the egg is generally defined as the time during pregnancy that precedes implantation—that is, the time from formation of the zygote until the blastocyst burrows into the wall of the receptive, hormonally primed, uterus. This is initiated by the end of the first week post fertilization. The conceptus at this stage appears grossly spherical. The second period, the period of the embryo, is roughly defined as the time from implantation through the 8th week of development. During this second period, part of the conceptus takes on the shape of what can be readily recognized as an embryo (Figure 2-2), a simple organism composed of a rudimentary head, trunk, and tail, projecting limb buds, a beating heart, obvious eyes, and a primitive segmentation. The remainder of the conceptus contributes to the extra-embryonic membranes, such as the amnion, which enclose and protect the embryo during its development, and to the fetal component of the placenta. The final period, the period of the fetus, extends from the beginning of the 9th week of gestation until birth. This period is characterized by rapid growth, and the differentiation of cells, resulting in the formation of distinct tissue types that become assembled into functional organ systems.
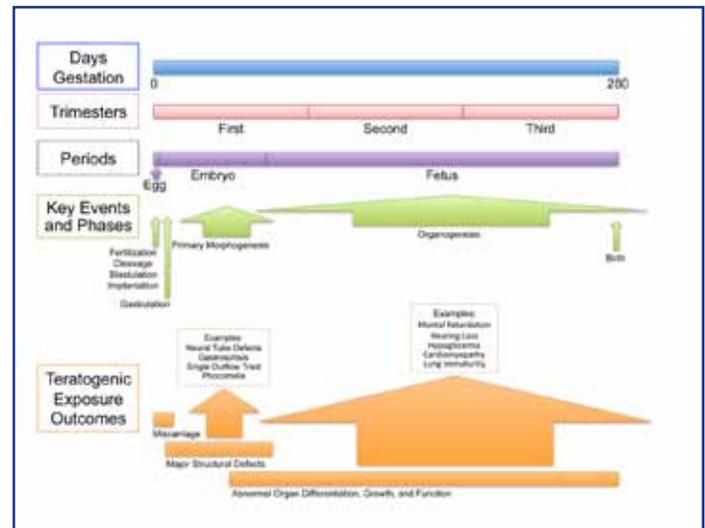
Figure 2-1. *Timeline of important events during pregnancy that may be disrupted by a teratogenic exposure. Shown are the lengths of pregnancy in days (0–280, from conception or fertilization to birth), the span of the three trimesters (3 months each), the three periods of prenatal development (egg, embryo, and fetus), key developmental events (fertilization, cleavage, implantation, gastrulation, primary morphogenesis, organogenesis, and birth), and some outcomes of teratogenic exposure.*
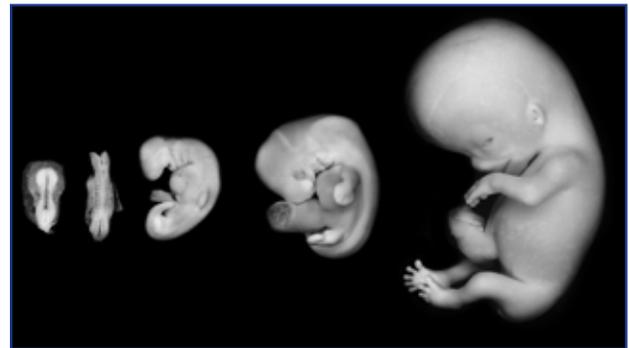
Figure 2-2. *Photographs of human embryos at five stages of gestation reproduced from the collection of the Congenital Anomaly Research Center, Kyoto University Graduate School of Medicine, courtesy of Dr. Kohei Shiota, Ms. Chigako Uwabe, and Dr. Shigehito Yamada. Shown (from left to right) are Carnegie Stages 9, 10, 13, 17, and 23 during the period of the embryo. The Stage 9 embryo has initiated neurulation, which is largely completed by Stage 10 with the exception that the cranial and caudal ends of the neural tube remain open as the neuropores. By Stage 13, body folding has established the tube-within-a-tube body plan of the early embryo, with a distinct head, trunk, and tail, and paddle-like limb buds. By Stage 17, the developing eyes are readily identifiable, and the limb buds now have bulbous distal plate-like structures that will form the hands and the feet. By Stage 23—the last stage in the period of the embryo—all external structures have taken on morphologies similar to those of the adult.*

Periods of development broadly define the structure of the developing organism at three different times during pregnancy. For example, the structure of the pre-blastocyst conceptus prior to gastrulation is rather different than the definitive structure of the embryo proper and its supporting tissues, which are elaborated during the period of the embryo. The embryo contains the rudiments of the organs of the fetus which makes the link between the structure of the embryo and fetus more intuitive, but, for example, the paddle-like limb buds present in the early embryo are non-functional and have a very different structure than that of the upper and lower limbs of the fetus, which at birth are fully functional. Just as the egg contains the precursor cells for the rudiments of the embryo, the embryo contains the precursor cells for all of the tissues and organs of the fetus. Precursor cells—regardless of their period in development—are susceptible to disruption by teratogenic exposures, which can alter their survival, rate of proliferation, migratory activity, ability to differentiate, or to function properly.

Phases of development define not the structure of the developing organism, but the unique developmental events that are occurring at that time. Four major phases are recognized in the prenatal development of humans: **gametogenesis; fertilization, cleavage,** and **blastulation; gastrulation** and **formation of the tube-within-a-tube body plan**; and **organogenesis**, with **cellular and tissue differentiation** and rapid **growth**. The gametes are generated during gametogenesis in the ovaries of the female and the testes of the male. During gametogenesis, germ cells (first identifiable in the yolk sac—an extra-embryonic membrane) migrate to the developing gonads, divide mitotically, and then initiate meiosis, which is completed postnatally after the onset of puberty, resulting in the generation of haploid oocytes and spermatozoa.

During the second phase, fertilization, cleavage, and blastulation, the oocyte and spermatozoon fuse to produce a diploid zygote, which rapidly initiates a series of mitotic divisions (that is, undergoes cleavage) to produce a solid ball of cells called a morula. As the morula passes down the oviduct toward the uterus, it forms an internal cavity, transforming into a hollow cyst-like structure called the blastocyst. The blastocyst is capable of implanting into the wall of the uterus, initiating in utero development of the embryo. During implantation, the blastocyst differentiates two cell regions: the outer trophoblast, consisting of the cells that invade the uterine wall and contribute to the formation of the placenta, and the inner cell mass, the source of the embryo and its extra-embryonic membranes.

As implantation is occurring, the embryo initiates the third phase, gastrulation, a process in which three distinct cell layers (germ layers) form, each of which gives rise to specific derivatives. The ectoderm forms the nervous system, the mesoderm forms most of the muscle and bone of the embryo, and the endoderm forms the lining of the gastrointestinal tract. These three germ layers are stacked upon each other like pancakes. This two-dimensional stack becomes sculpted into a three-dimensional embryo having a tube-within-a-tube body plan and containing rudiments of all of the major organ systems. How this body plan is achieved is referred to as primary morphogenesis and it involves localized changes in the shape, size, position, and numbers of cells in the three germ layers, generating tissue movements such as thickening, folding, delamination, and fusion. As a result of primary morphogenesis, specific organ rudiments are generated as well as an embryonic body that is now largely separated from its surrounding extra-embryonic membranes. For example, the outer tube of the tube-within-a-tube body plan is the future body wall; it is generated by the expansion, folding, and fusion of the edges of the two-dimensional embryo. Its outer wall is in direct contact with amniotic fluid after formation of the amniotic cavity. Simultaneously, the inner tube of the tube-within-a-tube body plan is formed: the future gastrointestinal tract or gut tube, lined with endoderm.

Simultaneously, other primary morphogenetic events are occurring to generate the rudiments of the major organ systems. During a process called neurulation, a portion of the ectoderm thickens, then folds inward and fuses at its edges to form a third tube, the neural tube, the rudiment of the entire adult central nervous system. Some cells left over from the process of neurulation delaminate from the ectoderm to form neural crest cells, an important population of migratory cells that contribute to a number of organ systems such as the mesenchyme of the developing face, the cranial and spinal ganglion of the peripheral nervous system, and the septum that partitions the outflow tract of the heart into two major vessels that separate the systemic and pulmonary circulation: the aortic and pulmonary vessels. Mesodermal cells in the developing trunk of the embryo undergo segmentation to form transient structures called somites, which give rise to the muscles and bones of the trunk, as well as the adjacent dermis of the skin. Some cranial mesodermal cells condense into paired tubes that fuse to form the heart during the process of cardiogenesis. After the formation of a single heart tube, this rudiment rapidly loops upon itself, begins to beat and pump blood, and then partitions to form four rudimentary chambers: the right and left atria, and right and left ventricles. Still other mesodermal cells arrange into tubules, contributing to the developing urogenital system.

The final phase, organogenesis, involves the growth and differentiation of precursor cells and tissues contained within each of the organ rudiments formed during primary morphogenesis. Of the four phases, organogenesis occurs over the longest period of time, extending from about four weeks of development (during the period of the embryo), throughout the fetal period, and even continuing postnatally for some organ systems.

Teratogenic exposure during any period or phase of development can have dire consequences (Figure 2-1). In general, disruption of the earliest developmental stages (gametogenesis; fertilization, cleavage, and blastulation) results in the loss of the conceptus (a miscarriage, often before the woman realizes she is pregnant). Disruption during primary morphogenesis and organogenesis may result in major structural anomalies, what is usually termed a birth defect, (e.g., a neural tube defect such as spina bifida, a ventral body wall defect such as gastroschisis, a heart defect such as the formation of a single outflow tract, a limb anomaly, such as phocomelia, or a facial cleft such as cleft lip or palate). Disruption during the late embryonic and fetal period generally results in abnormal organ differentiation, growth, and function (e.g., mental retardation, hearing loss, neonatal hypoglycemia, lung immaturity). Thus, the timing of a particular teratogenic exposure can result in different outcomes. Examples that illustrate this are discussed in later chapters.

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What Are the Possible Consequences of Pre-Conception Germ Cell Exposures on Pregnancy Outcome?

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Certain exposures during pregnancy can damage the developing embryo or fetus. But can a pre-conception exposure of germ cells, either oocytes or spermatozoa, also result in adverse effects?

Female Germ Cells: Oocytes

Few studies in this area have been done on oocytes although some studies have shown that exposure of female rats or mice, as adults, in the early post-natal window or during gestation, to chemicals such as 4-vinylcyclohexene or to therapeutic agents such as cyclophosphamide, can result in a reduction of primordial, primary, or antral follicles. In the mid-gestation female fetus, oocytes progress through meiotic prophase, undergoing the complex events of synapsis and recombination, and then enter a protracted arrest phase in late prophase. During this time window, *in utero* exposures to specific environmental chemicals may affect the ability of chromosomes to synapse, leading to chromosomal segregation errors. In the post-pubertal adult ovary, oocyte growth resumes to culminate in the completion of meiosis I and the ovulation of a metaphase II–arrested egg. Disturbances in growth may affect these processes and disrupt spindle formation and the alignment of chromosomes. However, it is laborious to obtain mature oocytes from the ovary and relatively few scientists have studied these processes. Due to our growing reliance on *in vitro* fertilization techniques, *in vitro* oocyte maturation procedures are of increasing interest. There are several studies that suggest that oocytes maturing *in vitro* are susceptible to chemical insults. It is clear that additional studies are needed to determine whether these exposures will result in adverse effects on embryo quality and whether exposures *in vivo* may have similar effects.

Targets in the Male Reproductive System

Spermatozoa are much easier to study. They are abundant, replenished daily, and easy to obtain; on average, a man produces 100 million sperm/day. Male germ cells undergo several mitotic and two meiotic divisions, followed by chromatin remodeling to repackage the haploid
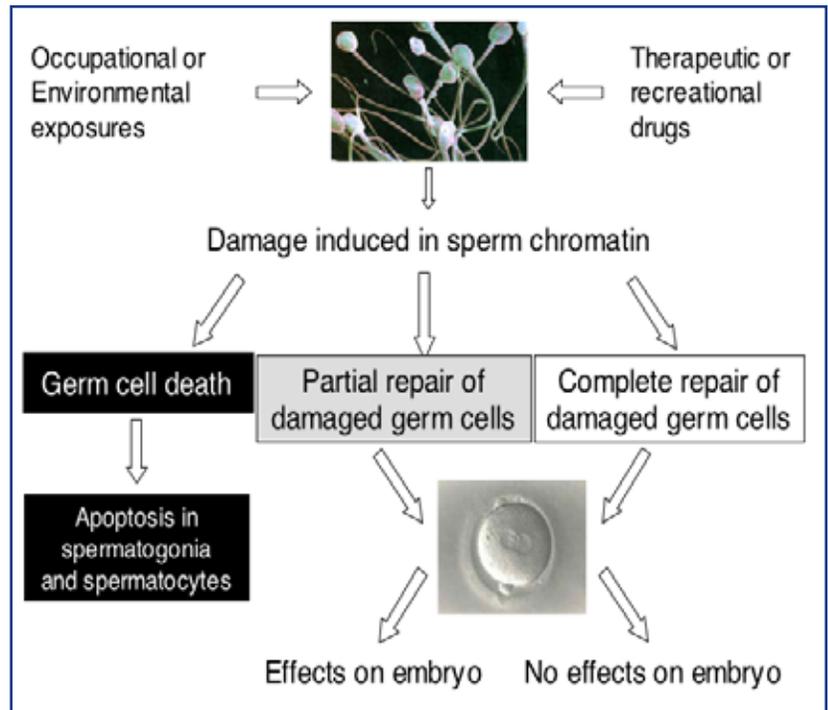
Figure 3-1.

genome for delivery to the oocyte. Male germ cells show differential vulnerability to the action of chemicals and altered environmental conditions during these cell division and chromatin remodeling events.

The extent to which human paternal exposures contribute to infertility and abnormal pregnancy outcomes is not clear. In some studies, being a welder, painter, auto mechanic, or fireman has been associated with an increased risk of male infertility and adverse effects on progeny outcome, including increases in spontaneous abortions, birth defects, and childhood cancer. It is difficult to link paternally mediated adverse effects on pregnancy outcome to any specific chemical or combination of chemicals from these studies of occupational exposures. First, infertility and pregnancy loss are frequent in humans even without chemical exposures. Secondly, there is often little information available on dose, duration of exposure, or potential chemical interactions. One group that is an exception in this respect is the male survivors of childhood cancer; medical records document the selection and dose of chemotherapeutic agents and radiation therapy that these patients received. A large

follow-up study has revealed that survivors who were not sterile were only half as likely to sire a pregnancy as their siblings. Radiation therapy of more than 7.5 Gy to the testes, higher cumulative alkylating agent dose, and treatment with cyclophosphamide or procarbazine were identified as risk factors. Indeed, one recent study has shown that the sperm generated two years after chemotherapy maintain a significant degree of chromatin damage. The establishment of a battery of chromatin quality tests would assist in counselling cancer survivors.

There are three mechanisms by which paternal exposure to a drug theoretically may adversely affect progeny outcome. In animal experiments, high doses of several drugs, including the anticancer alkylating agent cyclophosphamide, given to males before mating were found in the seminal fluid of male rats. Cyclophosphamide was transmitted to the female during mating, and caused a dose-dependent decrease in the number of early embryos. This effect could be due to direct exposure of the early conceptus or through an effect on sperm function.

Since normal testicular function depends on hormonal signals coming from the pituitary, that is, in turn, controlled by the hypothalamus, interference with communication between the hypothalamic-pituitary complex and the testis may have adverse effect on spermatogenesis. Alternatively, chemicals may target the testis directly. Treatment of male rats with high doses of di(2-ethylhexyl) phthalate (DEHP), a commonly used plasticizer, reduces serum testosterone and increases serum luteinizing hormone (LH) levels; the reduction in testosterone is due to an effect on the testis rather than the hypothalamic-pituitary axis. Ethane dimethanesulphonate targets Leydig cells and epididymal principal cells. Sertoli cells are targeted by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) or hexanedione. Disrupting the functions of these cells may alter germ cell quantity or quality; this would be anticipated to affect male fertility, but may or may not directly affect offspring.

The third mechanism is a direct effect of the toxicant on the male germ cell, either during spermatogenesis in the testis or sperm maturation in the epididymis. Damage that arrests sperm production or maturation cannot affect offspring because the germ cells never achieve the ability to fertilize oocytes. But when spermatozoa are affected by drugs or chemicals and still retain their ability to fertilize eggs, trouble may arise.

Male Germ Cells in the Testis or the Epididymis

Rat spermatogonia undergo several mitotic cell divisions to become spermatocytes and then two meiotic cell divisions to form spermatids. Development of spermatids, called spermiation, includes a condensation of nuclear elements, the development of a propulsion mechanism, the shedding of most of the cytoplasm, leading to the production of spermatozoa. The kinetics of progression through spermatogenesis are very tightly controlled. Therefore, we can deduce the stage of spermatogenesis affected by a toxicant based on the time from treatment of the male to adverse effects after mating.

For example, in rats, an effect on progeny outcome during the first week after exposure of males to a drug or X-rays means that spermatozoa in the epididymis were affected. Exposure 2–4 weeks prior to conception represents an effect on germ cell first exposed as spermatids, and exposure 5–6 weeks before conception represents an effect on germ cells first exposed as spermatocytes. Exposures more than seven weeks prior to conception represent an effect on germ cells first exposed as spermatogonia or stem cells.

Spermatogonia are susceptible to exposure to a wide range of certain drugs, including many anti-cancer drugs; these exposures reduce sperm numbers and increase the percentage of abnormal sperm among surviving cells. In mice, exposure to chlorambucil, an anti-cancer drug, causes a peak in mutations in offspring when germ cells are exposed as spermatids. Spermatids and spermatozoa are most sensitive to mutations induced by acrylamide and lethality induced by ethylnitrosourea. The treatment of male mice with clinically relevant doses of anticancer drugs, including cyclophosphamide and procarbazine, results in statistically significant, dose-dependent increases in expanded simple tandem repeat mutations in the germ line.

Cyclophosphamide has very different effects that depend on both the timing of exposure and the dose. When spermatogonia are exposed to cyclophosphamide, a small increase in external malformations and growth retardation is seen in developing rat fetuses. But when spermatids or spermatozoa are exposed to chronic low-dose cyclophosphamide, postimplantation losses are increased, but no malformations or growth retardation is seen. Cyclophosphamide is particularly interesting because effects on postimplantation loss and malformations and even some behavioral abnormalities occur not only in offspring but also in the second generation of the treated rats.

Spermatozoa acquire the ability to fertilize an egg while passing through the epididymis. Either radiation or drugs can affect spermatozoa in the epididymis and vas deferens. Methyl chloride increases embryo death, possibly by causing inflammation in the epididymis; an anti-inflammatory agent reverses the effect. Cyclophosphamide increases post-implantation loss only when the exposed spermatozoa originate in the head or body of the epididymis, but not the tail.

Responses of the Male Germ Cell to Insult

The response of the germ cell to insult depends on both timing and the extent of damage (Figure 3-1). Damage may be completely repaired, in which case everything proceeds normally. Damage may be partially repaired, in which case spermatogenesis may continue and the "wounded" sperm, with its load of damaged chromatin (DNA + associated proteins), could fertilize an oocyte. DNA repair within the oocyte may rescue the zygote. Studies in rodents have revealed that fertilization with a damaged male germ cell triggers a DNA damage response in the zygote; gammaH2AX, a post-translationally modified histone that marks sites of DNA damage, accumulates in the male genome. If repair fails or is incomplete, abnormal progeny may result. Another possibility is that the insult overwhelms repair processes and the damaged male germ cell dies by a form of suicide called apoptosis. Drug-induced apoptosis is most pronounced in spermatogonia and spermatocytes, both pre-meiotic germ cells that have DNA repair mechanisms. Spermatids and spermatozoa, that are post-meiotic germ cells, cannot repair DNA and seem to lose the ability to die by apoptosis. Losing suicidal capability is not a good thing, because damaged spermatids and spermatozoa could soldier on to transfer damaged DNA to an oocyte. The ability of spermatozoon to fertilize an oocyte is not dependent of the quality of the DNA in its nucleus.

Conclusion

Over the past two decades, abundant evidence has demonstrated that male-mediated adverse progeny outcome is a real phenomenon in experimental animals, and several mechanisms responsible for these adverse outcomes are well described. Both genetic and epigenetic mechanisms have been suggested.

Should we be concerned about progeny outcome after the exposure of men to chemical agents? While to date, human studies have not shown an increase in birth defects among the children of men treated with cancer therapies or exposed to ionizing radiation, it is clear that there is an effect on fertility; some studies have shown an increase in germline mutations. More research is needed.

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What Is the Role of the Placenta—Does It Protect Against or Is It a Target for Insult?

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The placenta is the *anchor*, the *conduit*, and the *controller* of pregnancy—and it can also be a target for toxicant action. The placenta attempts to protect the embryo and fetus from insult; however, the placental protector can be compromised and also be the site of toxic action.

The placenta is not just a barrier, but has many functions that are vital to the health of the embryo/fetus. The placenta encompasses not only the chorioallantoic placenta but all of its extraembryonic membranes (chorion/amnion) and the yolk sac. (Figure 4-1). The placenta and its membranes secure the embryo and fetus to the endometrium (uterine lining) and release a variety of steroid and protein hormones that characterize the physiology of the pregnant female.

Alterations in any of these functions can lead to pregnancy loss (miscarriage). Of particular concern is the relative hypoxic environment that is normally present during early embryonic development and the abnormal maternal blood flow, which can produce higher levels of oxygen and lead to miscarriage and pre-eclampsia/eclampsia, as noted in Figure 4-2. The placenta transports nutrients to the embryo/fetus and waste products away from the embryo/fetus but, during this exchange, foreign compounds can hitchhike across the placenta. The placenta stores chemicals and also serves as a site for biotransformation. Substances that may be benign to the pregnant woman can be transformed by metabolizing enzymes in the placenta to agents toxic to the embryo/fetus. Thus, the functions of the placenta that nourish and support the fetus can be compromised to result in placental toxicity, fetal nutrient deprivation, and production of toxic chemicals.

Patterns of placental development are similar among different animal species, but developmental time schedules may differ. The placentae themselves vary widely among species. Marsupials, for example, have only a yolk sac, while sheep have multicotyledonary (multi-lobed) placentation. Rats, mice, and rabbits, the principal species studied in teratology, all have yolk sac placentae, as do humans, but the functions of the yolk sacs are very different. In rodents and rabbits, the yolk sac everts (turns inside out) and becomes an important port of entry for molecules (e.g., immunoglobulins) into the embryo and fetus throughout pregnancy, even though a chorioallantoic placenta develops
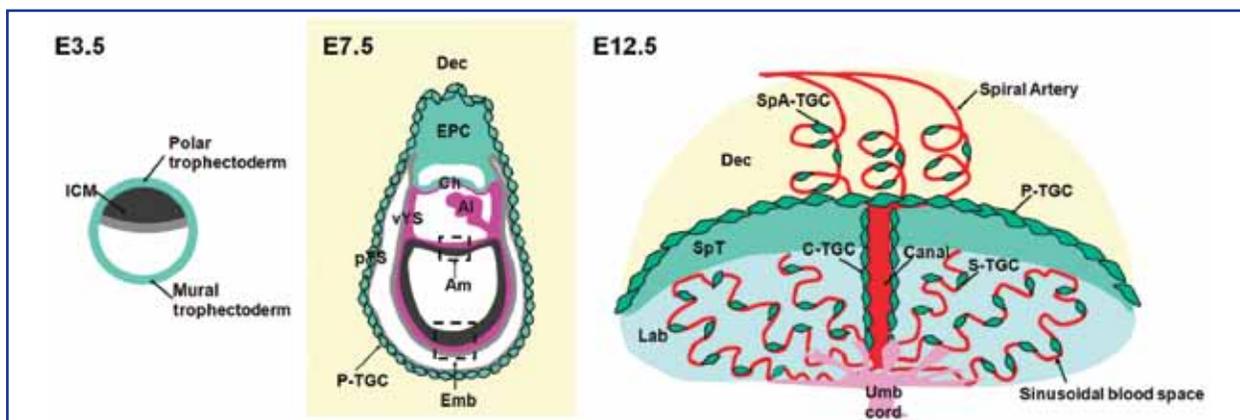
Figure 4-1. *Placental Structure in the Mouse.* *Figures depict early development of the mouse conceptus at embryonic days (E3.5, E7.5, E12.5). In the fetus, the visceral yolk sac (vYS) inverts and remains active throughout the entire gestation providing for transfer of selective large molecules, e.g., immunoglobulins IgG and vitamin B_{12}. Abbreviations: Al, allantois; Am, amnion; Ch, chorion; Dec, decidua; Emb, embryo; Epc, ectoplacental cone; ICM, inner cell mass; Lab, Labyrinth; pYS, parietal yolk sac; SpT, spongiotrophoblast; TCG, trophoblast giant cell; Umb Cord, umbilical cord; vYS, visceral yolk sac; C-TGC, maternal blood canal trophoblast giant cell; P-TGC, parietal trophoblast giant cell; S-TGC, sinusoidal trophoblast giant cell; SpA-TGC, Spiral artery-associated trophoblast giant cell; Cyan-trophectoderm and trophoblast lineage, Black- inner cell mass and embryonic ectoderm; Gray -endoderm, Red-maternal vasculature, Purple-mesoderm, Yellow-decidua, Pink-fetal blood vessels in labyrinth. (From Hu and Cross,* Int. J. Dev. Biol. *54:341–354, 2010.)*
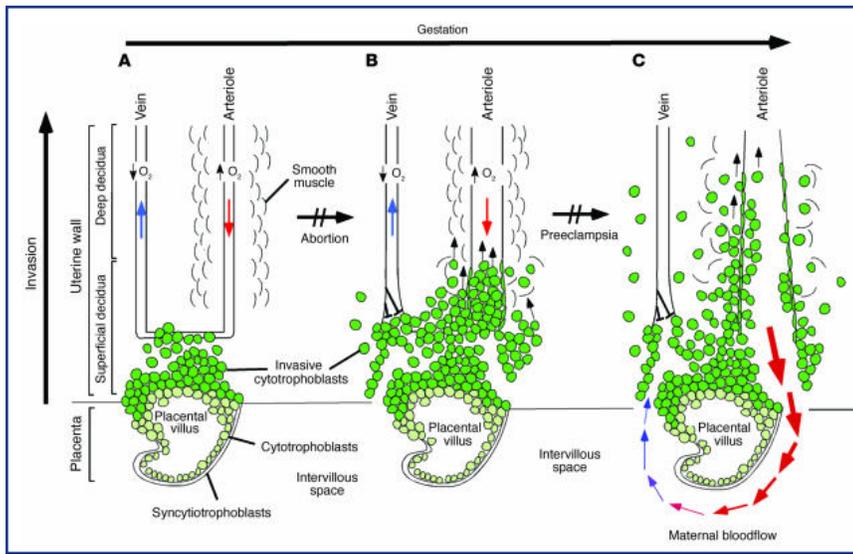
Figure 4-2. *Oxygen tension plays an important role in guiding the differentiation process that leads to cytotrophoblast invasion of the human uterus.* *(A) The early stages of placental development take place in a relatively hypoxic environment that favors cytotrophoblast proliferation rather than differentiation along the invasive pathway. Accordingly, this cell population (light green cells) rapidly increases in number as compared with the embryonic lineages. (B) As development continues, cytotrophoblast cells (dark green cells) invade the uterine wall and plug the maternal vessels, a process that helps maintain a state of physiological hypoxia. As indicated by the blunt arrows, cytotrophoblast cells migrate farther up arteries than veins. (C) By 10 to 12 weeks of human pregnancy, blood flow to the intervillous space begins. As the endovascular component of cytotrophoblast invasion progresses, the cells migrate along the lumina of spiral arterioles, replacing the maternal endothelial lining. Cytotrophoblast cells are also found in the smooth muscle walls of these vessels. In normal pregnancy the process whereby placental cells remodel uterine arterioles involves the decidual and inner third of the myometrial portions of these vessels. As a result, the diameter of the arterioles expands to accommodate the dramatic increase in blood flow that is needed to support rapid fetal growth later in pregnancy. It is likely that failed endovascular invasion leads in some cases to miscarriage, whereas an inability to invade to the appropriate depth is associated with pre-eclampsia/eclampsia and a subset of pregnancies in which the growth of the fetus is restricted. (From Red-Horse et al, J. Clin. Invest. 114:749, 2004.)*

during the last half of pregnancy to provide gas and small molecule exchange. In contrast, the yolk sac shrinks and becomes vestigial (shrunken and nonfunctional) in humans during the latter part of the first trimester.

In laboratory species, immunoglobulins, critical for immune function in the neonate (IgG), are transported only through the visceral yolk sac; in humans, transport occurs via receptors in the chorioallantoic placenta. Both the yolk sac of the rodent as well as the chorioallantoic placenta of the human are important sites for degrading proteins and providing amino acids to the embryo and fetus. Trypan blue and other agents that interfere with protein degradation can kill the rodent embryo or fetus or can cause birth defects.

Many agents, including cadmium, can alter placental function (see Table 4-1). Injecting cadmium in a pregnant rat close to giving birth causes the fetus to die and the placenta to degenerate within 24 hours. Directly injecting fetuses with cadmium near term doesn't kill them, although they do develop hydrocephalus (head enlargement due to excess cerebrospinal fluid in the ventricles of the brain). Cadmium was previously thought to affect primarily the kidney, but it turns out that, at least in rats, cadmium is even more highly concentrated in the placenta. Human placentae definitely concentrate cadmium and can degenerate because of exposure. Early in pregnancy, the effects of cadmium on the placenta can lead to miscarriage; women affected by exposures to high concentrations of cadmium can have repeated pregnancy losses. The ability of the placenta to sequester cadmium protects the fetus or embryo for a while, but when a pregnant woman is exposed to substantial amounts of cadmium, the placenta—and with it, the fetus—may die.

One source of cadmium is cigarette smoke and may be one reason why smoking can compromise the growth of the conceptus. Smoking is associated with pregnancy loss, premature delivery, and decreased birth weight. Smokers' placentas have very high levels of cadmium compared with those of nonsmokers, and these levels may contribute to the adverse outcomes that are associated with cigarette smoke.

The placenta can prevent or at least delay the transmission of viruses that infect the mother. Experiments have shown that a variety of viruses, including cytomegalovirus (CMV), human immunodeficiency virus (HIV), Coxsackie B, and Echo 11 cannot directly cross the placenta. However, these viruses may infect certain cells within the placenta. Whether or not the placenta is infected determines whether the embryo/fetus eventually becomes infected.

In vitro models using dual perfusion of the human placenta, explant culture, and cell cultures have been useful to assess transfer of agents as well as effects on the placenta. Studies using human placental perfusions have been used in lieu of rodent studies for human proteins when FDA approval for these types of new agents has been necessary.

Following delivery, the baby gets all of the attention. The placenta is neglected, often assigned to the trash; however, detailed pathological assessments of the placenta can not only be critical for determining what happened *in utero* but also may be helpful in predicting what may be health issues for the child. During pregnancy, the placenta is the star of the show. Teratology would be incomplete as a science without attention to this important organ.

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Table 4-1.

SOME XENOBIOTICS OBSERVED TO ALTER PLACENTAL AND YOLK SAC FUNCTION	
AGENT	**EFFECT**
DRUGS OF ABUSE	
Cocaine	In humans, reduces amino acid uptake and causes reperfusion injury.
Ethanol (alcohol)	In humans, alters membrane fluidity, and inhibits nutrient transport; in animals, ethanol inhibits pinocytosis.
Narcotics	In humans, narcotics decrease protein incorporation and decrease amino acid uptake.
Smoking	Smoking, in humans alters histology, induces monoxygenases, and increases cadmium levels, and doubles or triples the rate of PROM (premature rupture of membranes). Nicotine decreases glucose utilization, decreases lactate production, decreases amino acid uptake, and increases acetylcholine release, benzo(a)pyrene induces monoxygenases and alters protein hormone secretion.
DRUGS	
Colchicine	In humans, reduces amino acid uptake, inhibits differentiation. In animals, inhibits pinocytosis.
Enalapril	In animals, causes a pathologically hypocellular, small placenta.
Verapamil	In humans, increases COMT (catechol-O-methyl transferase) enzyme activity, and decreases MAO (monoamine oxidase) activity.
Indomethacin	In humans, stimulates progesterone secretion.
Anti-HIV drugs	Zidovudine and nucleoside analogs, in humans, inhibit cell proliferation and differentiation, and alter release of progesterone, HCG (human chorionic gonadotropins), and HPL.
Chloroform	In animals, causes placental necrosis.
Ritodrine	In humans, decreases CAMP.

METALS	
Cadmium	In humans, causes placental necrosis, loss of placental integrity, reduced release of protein hormones, inhibition of trophoblast proliferation, and induction of metallothionein. In animals, cadmium causes placental necrosis, increased mitochondrial calcium, and altered enzymes. Cadmium accumulates rapidly in both humans and animals; the effect is reversible with zinc.
Arsenic	Affects lipid peroxidation in humans.
Mercury	In humans, alters membrane fluidity and impairs amino acid transport. In animals, inhibits transport of zinc and copper.
Nickel	In animals, inhibits glutathione s-transferase and reduces glutathione reductase.
Pesticides/ Environmental Chemicals	In humans, chlordane, *p,p*-DDT and DDE cause alterations in cholinesterase. In humans, methoxychlor, mirex, lindane, p,p DDT and DDE all inhibit Ca^{2+} ATPase. Dinitrophenol, in animals, inhibits pinocytosis and inhibits vitamin B_{12} uptake. Dinitrophenol/iodoacetamide, in humans, inhibits glucose metabolism, inhibits amino acid transport, produces placental leakiness, and decreases ATP levels. In animals, dinitrophenol/iodoacetamide inhibits vitamin B_{12} uptake.
OTHER	
Trypan blue	In animals, inhibits pinocytosis, inhibits lysosomal enzymes, and reduces receptor mediated endocytosis. Zinc does not reverse the effect.
Choline acetyltransfer-ase inhibitor (2-benzolethyl-trimethyl ammonium)	In humans, inhibits amino acid uptake, lowers choline acetyltransferase, lowers acetylcholine levels, and decreases acetylcholine release.
Antisera	Yolk sac antiserum, in animals, inhibits pinocytosis and inhibits intralysosomal digestion of macromolecules. Kidney antiserum, in animals, decreases amino acid transport.
Cortisol	In animals, alters solvent transfer
Ethylnitrosourea	In animals, induces choriocarcinoma.
Hydralazine	In humans, inhibits monoamine oxidase (MAO) and cathechol-O-methyl transferase (COMT)
Hypochlorous acid	In humans, causes oxidant damage of collagen type I in chorioamnion.
Iodoacetate	In animals, inhibits pinocytosis.
Kepone	In humans, inhibits Ca^{2+} ATPase.
Leupeptin	In animals, inhibits lysosomal proteolysis but does not inhibit pinocytosis.
Lipooxgenase inhibitors	In humans, nordihydroguairetic acid (NDGA) inhibits HCG secretion. In animals, N-hydroxy-n-methyl-7-propoxy-2-naphtalin ethanamine alters morphology.
Hyperglycemia	In animals, reduces protein uptake.
Ouabain	In humans, inhibits Na, K, and ATPase, and inhibits amino acid transport.
N-acetoxy-2—acetylamino-fluorene	In animals, causes localization of DNA adducts.
Phthalate	In humans, inhibits cell proliferation and increases trophoblast apoptosis
Phorbol	In humans, stimulates HCG production.
Polychloro-biphenyls (PCBs)	In animals, decreases amino acid transport.
Polycyclic aromatic hydrocarbons	In humans, induces AHH (arene hydroxyl hydrolase) and monoxygenases; alters protein secretion

Serotonin	In animals, decreases sodium transfer
Somatomedin inhibitors	In animals, inhibits pinocytosis, alters protein processing.
Sucrose	In animals, alters ultrastructure.
Suramin	In animals, inhibits pinocytosis.
Trifluoperazine	In humans, inhibits Ca^{2+}ATPase.
Vitamin E deficiency	In animals, causes placental necrosis.

Table 4-1. *Compounds presented in this table have been shown to adversely affect either the chorioallantoic placenta or the visceral yolk sac. Limitations in the data set are the often high concentrations of the compounds being used; few doses are used and the stage of gestation may vary. Thus, this table is presented to the reader as a compilation of responses; however, the reader is referred to the original research for specific responses and doses. This table was adapted and updated from Slikker and Miller, 1994.*

Can Prenatal Exposures Have Long-Term Effects on the Brain and Behavior?

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For decades fetal injuries were conceived largely in terms of structural birth defects. Effects on the central nervous system (CNS) were ignored unless the brain was visibly abnormal. As we all know from personal experience, cognitive development is a long, slow process requiring decades. Yet today, the idea that chemicals can damage the developing brain (neurobehavioral teratogenesis/developmental neurotoxicity) is an accepted fact. What changed? What changed was that a series of environmental and drug exposures were shown to damage the developing brain.

The first example was identified after mercury contaminated Minamata Bay, Japan. The mercury was transformed to methylmercury in plants and was progressively concentrated as it worked its way up the food chain and accumulated in the fish. People around the bay who ate the fish began showing symptoms, the most severe of which were among children exposed in utero (mental retardation, cerebral palsy, blindness, and other CNS impairments). Methylmercury poisoning has since been reported in other places; each time the most severely affected were prenatally exposed children. Even women who ingested moderate amounts had affected children despite being unaffected themselves, demonstrating that the developing CNS is more vulnerable than the adult CNS.

The heavy metal lead is among the best known chemicals to cause CNS impairment after developmental exposure. Adverse effects are seen after prenatal or childhood exposure or a combination of both. As research has advanced, CNS effects have been found at lower and lower levels and an entirely safe level of lead remains to be determined. Lead is illustrative because: (1) it is a pervasive compound, the effects of which are still felt decades after removal from products, (2) it is a worldwide problem, and (3) its effects occur over a long period of development, thereby illustrating how protecting the brain is a long-term and complex proposition. Other environmental agents that cause developmental neurotoxicity include the heavy metal cadmium, the transition metal manganese, polychlorinated biphenyls, and some pesticides.

What about drugs? Drugs are designed to have biological effects so it is no surprise that some drug exposures are teratogenic. It has taken longer to determine that some are developmental neurotoxicants. 13-cis-retinoic acid (Accutane) is for the treatment of severe acne. *In utero* exposure causes birth defects (retinoid embryopathy), and many of the children also had low IQs. In animal experiments, Vitamin A (another retinoid) is known to cause neurobehavioral impairments hence the effects of 13-cis-retinoic acid might have been predicted. The effects of 13-cis-retinoic acid on intelligence and birth defects were not always aligned; i.e., some children had severe birth defects and several mental impairments, but others had barely detectable birth defects and large IQ reductions. When the associations between birth defects and CNS effects are not concordant or nonexistent, proving connections between school performance and emotional problems identified later in life is far more difficult. For this to occur, detailed animal experiments and long-term human studies are required. Government regulations now expect companies to test animals for CNS effects before they are approved if a potential for adverse CNS effects is possible.

The effects of 13-cis-retinoic acid are striking but the medications are used by relatively few. A larger problem occurs in epilepsy. Approximately 1% (~3 million) of the population has epilepsy and most are treated with antiepileptic drugs (AED). The use of AEDs is associated with birth defect syndromes. One example is valproate (Depakote), an AED that when taken during pregnancy leads to 1–2% of exposed infants born with neural tube defects, and a higher percentage born with the Fetal Valproate Syndrome. The syndrome includes characteristic facial features (dysmorphic facies) and impaired intellectual development. Recent studies suggest it also may increase rates of autism. What about other more commonly used drugs, such as antidepressants or statins? While it is reassuring that no evidence of fetal neurotoxicity has yet appeared for these, determination of safety during pregnancy through well designed prospective human studies and specifically focused animal studies still need to be done.

Two recreational drugs that are problematic when overused are alcohol and tobacco. Both have been shown to result in life-long neurobehavioral effects. At the extreme, prenatal alcohol exposure can cause Fetal Alcohol Syndrome, characterized by three clusters of effects: (a) growth impairment (pre- and postnatal), (b) facial dysmorphogenesis, and (c) CNS effects. The latter include changes in structure (visualized using brain imaging techniques) and behavioral

abnormalities (intellectual impairment, attention deficit hyperactivity disorder (ADHD), emotional instability, and antisocial behaviors). There are also less severe effects of alcohol, called fetal alcohol effects, in which one or two of these clusters of effects are observed rather than all three. Cigarette smoking is associated with intrauterine growth retardation, as is widely known, but studies also show that exposed children have IQ reductions of ~10 points, primarily of verbal abilities. Animal experiments verify that prenatal nicotine affects offspring behavior.

What about illegal drugs? We think of cocaine, methamphetamine, LSD, heroin, and PCP as powerful drugs with destructive consequences, so can we assume they damage fetal brain development? We know these drugs cross the blood brain barrier because they are psychotropic. It turns out that the blood brain barrier is far more restrictive than the placenta, so these drugs easily reach the fetus; some (methamphetamine) even accumulate more in the fetus than in the mother. Despite this, effects on development have been difficult to pin down. None of these exposures has been shown to cause birth defects, but animal studies now show that prenatal cocaine alters dopamine systems which are involved in attention, including the principal dopamine receptor, D1, such that the receptor is less available to interact with dopamine when it is released into the synaptic cleft. Changes are also seen in the neurotransmitter GABA and in pyramidal neurons in the cerebral cortex. In children prenatally exposed to cocaine, problems of attention are prominent, which is consistent with a dopamine effect. Cocaine-exposed children also show IQ reductions (~4 points), which, while not easy to notice in one individual, have implications across a population. If the bell-shaped distribution of IQs shifts downward for thousands of children each year, there could be a large impact on schools that educate them and employers that train them. Patterns of use are important too; women using higher doses, using more drugs (polydrug abuse), with use for a longer time during gestation, and with less prenatal care have worse outcomes. Prenatal marijuana has also been documented to result in reductions in visual processing and impulse control.

More recent is the problem of methamphetamine abuse, which leads to changes in brain structure, neurotransmitters, spatial memory, and language development after prenatal exposure. Between 1994 and 2006, pregnant women entering drug treatment programs in the U.S. who identified methamphetamine as their primary drug of abuse rose from 8% to 24%. A human prospective prenatal study of this drug has begun and recent animal experiments reveal long-term effects on brain neurochemistry and behavior, including impaired spatial memory and enduring changes in dopamine and serotonin after developmental exposure to methamphetamine.

For exposures that are teratogenic, the first trimester, perhaps even before pregnancy is recognized, is the major period of vulnerability. For the CNS, the period of vulnerability starts during the first trimester and lasts through adolescence. There are many reasons for this extended period of sensitivity, one of which is the phenomenal complexity of the brain (Figure 5-1). Developmental neurotoxic effects are often described as subtle. While this is accurate to the extent that it is a comparative statement, for example, ADHD is subtle compared to a life-threatening cardiac defect, it is not accurate when it is used to imply that such effects are less important. While developmental neurotoxic effects may not be life threatening, they may have an important impact on how people are able to navigate through life. As we think about drug and chemical safety, the impact of CNS damage should never be forgotten.
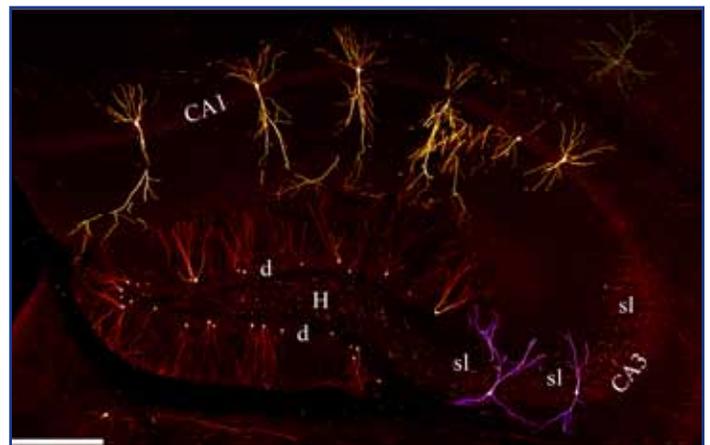
Figure 5-1. *From review by S. Danzer with permission (Danzer, 2008). Photomontage of confocal microscopy images showing principal neurons of the hippocampus. Images are from an adult Thy1-GFP-expressing mouse. Red = dentate granule cells, Purple = CA3 pyramidal cells, Yellow = CA1 pyramidal cells. d = dentate granule cell layer; H = hilus; sl = stratum lucidum; CA3 = CA3 pyramidal cell layer; CA1 = CA1 pyramidal cell layer. Scale bar = 300 μm.*

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How Do Genes Affect the Risk of Having a Child with a Birth Defect?

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Every infant born in the U.S. has at least a 2%–3% risk of being born with a major malformation or deformation, and an even higher risk (approximately 10%) of being born with internal anomalies or functional deficits that may not become apparent until later in life. The cause of most congenital anomalies is unknown. Approximately 40–50% of malformations fall into the group of those defects with unknown cause(s). It has previously been estimated that genetic causes (anomalies arising from alterations in genetic material) account for at least 25% of all human malformations. As we have learned more about the human genome and developed new techniques, the proportion of birth defects attributed to genetic causes has increased.

Figure 6-1.

There are numerous types of genetic alterations, the most common of which are mutations (changes to the DNA sequence of genes) and chromosomal defects (e.g., extra or missing chromosomes, or parts of chromosomes, Figure 6-1). Genetic alterations leading to malformations can be inherited, or can occur spontaneously due to random mutations of DNA. To date, genetically mediated malformations have not been found to result from exposure to any environmental agents, even those agents that have been shown to be capable of causing damage to genetic material in individual cells (mutagen). Radiation is a highly potent mutagen, but even after the atom bombs were exploded over Japan, a careful study of the exposed population over the subsequent generations demonstrated no increase in birth defects or genetic diseases in offspring caused by new mutations, despite the significant radiation exposure (chapter 21). Environmental causes of human malformations (defined as any external influence on fetal development, i.e., not genetic) are thought to be rare. It is estimated that only approximately 1% of all human malformations are related to drug exposures, chemicals, or radiation.
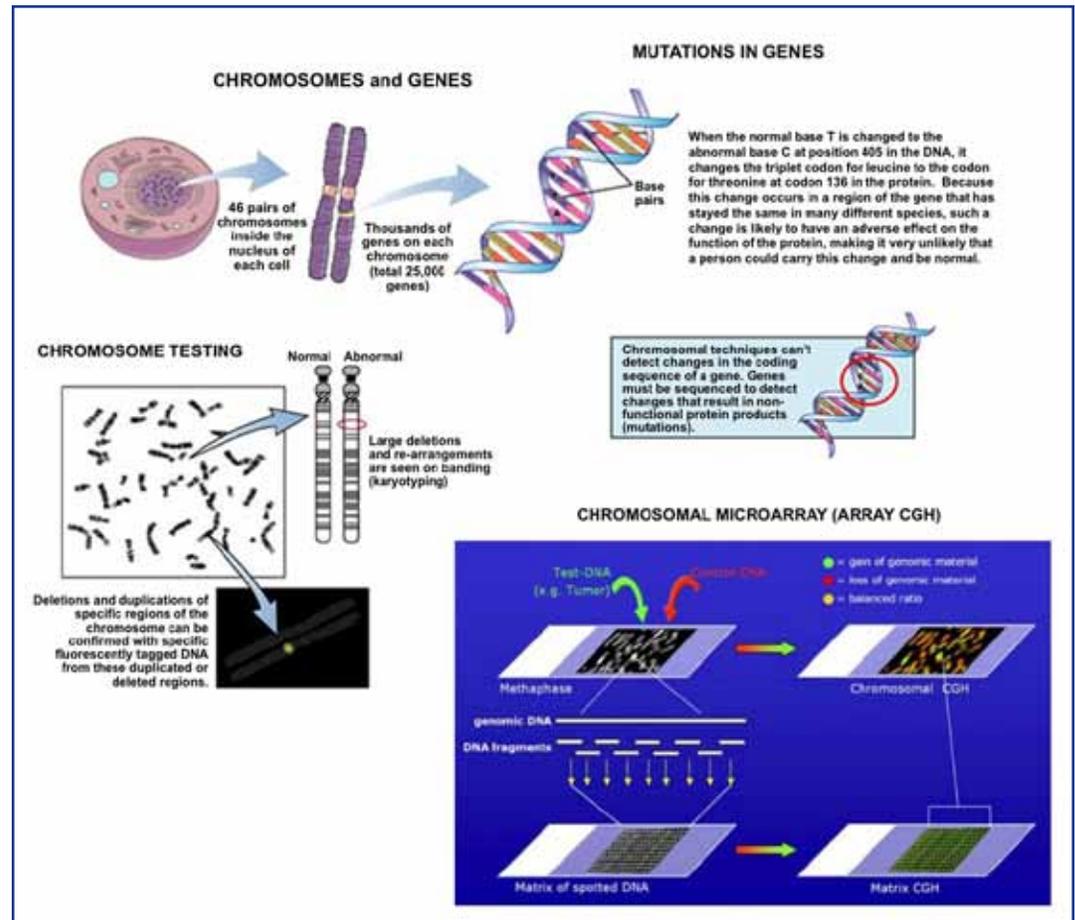
A birth defect can occur singly as an isolated defect. Multiple birth defects can also occur in one individual. When multiple birth defects, especially defects affecting varied organs and systems appear together and are seen in different individuals in different families in a recurrent pattern or combination, they are generally accepted to have a common underlying cause, and are designated a

birth defect syndrome. One example is Down syndrome, or trisomy 21, caused by the presence of all or part of an extra chromosome 21. The risk of having a child with Down syndrome is increased with increasing maternal age. Paternal age may also be a factor when the mother is 35 or older. Even with well-defined and refined syndromes, there is inherent variability in the manifestations of these birth defect syndromes, both in the type and severity of the various structural abnormalities that may appear. As a particular pattern of defects or syndrome is seen in other patients and as more is learned about a syndrome over time, initial descriptions are refined. Patients with the same syndrome will manifest varying degrees of common core features, as well as occasional unusual or infrequent (but related) features. The purpose of syndrome identification and refinement is to enable clinicians to recognize these features as suggesting a specific condition, with a common underlying cause, natural history, and prognosis. Syndrome diagnosis assists clinicians in, among other things, patient counseling and treatment.

There are two broad classes of birth defect syndromes, genetic and non-genetic. Genetic syndromes result from some change in the genetic material of the conceptus, occurring prior to or around the time of conception. Suggested reading at the end of this chapter describes the general approach for determining the difference between these two broad classes of birth defect syndromes has been delineated in more detail. An alteration in the genetic material of the cells may cause a genetic disease or genetic birth defect syndrome. While the same genes and the same genetic alteration will generally appear in each cell of the body, not all genes are expressed (i.e., activated) within each tissue in the body. The expression (or lack of expression) of each gene or group of genes in a given tissue is often controlled by other genes, and is responsible for different functions in different cells. When an error occurs in genetic material affecting a specific gene(s), even though the error appears in each cell of the body, it will only affect those cells (and thus organs) in which those genes are expressed. The functional capabilities of other genes, not affected by the error, help to determine much of the individual variability among different people with the same syndrome.

Thus, syndromes occur within the context of the underlying genetic background for each individual. Among children with Down syndrome, parental background for common genetically determined traits like stature, intelligence, and pigmentation always come through in the child with the syndrome, so that the tallest children with Down syndrome come from the tallest parents. Because there are underlying genetically determined susceptibilities for many common

birth defects, this helps to explain why different individuals with the same syndrome do not have the exact same combination of birth defects.

The type and location of each specific genetic alteration within a given gene will help to determine the variability and severity within a specific syndrome. Likewise, it is believed that all humans carry some genetic alterations that do not cause problems, either because the function of that particular gene product is covered by the other member of the gene pair, because that particular mutation does not alter the function of the gene product that it encodes, or because the particular mutation occurs in a non-essential part of the genetic material.

Genetic alterations can occur in either the sperm or the egg, or both, prior to or at the time of conception. If there is an alteration in the genetic material of the sperm and/or the egg, when the sperm fertilizes the egg at conception, each cell that derives from that fused egg/sperm cell (zygote) will carry that mistake. It is also possible that the genetic material of the sperm and egg could be "normal," but shortly (within hours, or at most days) after conception, a mistake occurs during cell replication. This type of cell replication error would result in "mosaicism," meaning that only some cells of the body will carry the error or mutation (i.e., those cells that derive from the cell where the error first took place). Even when such a mosaic genetic error occurs after conception, the error must occur shortly after conception, since after the first week there would be far too many cells without the mutation for manifestations of the genetic alteration to be apparent. Some genetic mutations may not be compatible with survival of the early embryo and result in an early spontaneous abortion. A mutation that occurs within the male or female germ cells could actually result in the occurrence of a dominantly inherited syndrome, even though neither parent shows signs of the same syndrome. This is estimated to occur in 1–3% of cases.

Genetic disorders can also be inherited from the affected individual's normal parent(s). Within the 25,000–30,000 pairs of genes in the human genome, one member of each pair, or one allele, is derived from each parent. Because genes work in pairs, a person can have a functional gene or allele and a defective gene, and still be "normal." Usually the functional version of the gene pair, where one gene is defective, allows that gene to perform its specific task. The gene that does not function in these cases is called a recessive gene. Carriers for Tay-Sachs disease, cystic fibrosis, or sickle cell disease can be without the condition but carry the defective gene because it is only in one copy. In normal carrier parents these recessive genes only become

apparent when each parent has the same recessive gene, and the child inherits both non-working genes (one mutant gene from each parent). The risk for two carrier parents to have a child with a recessive disorder is 25%.

Dominant conditions result when there is one defective gene in a gene pair, and the normal member of that gene pair cannot complete a specific developmental task by itself. These dominant conditions usually result in a pattern of birth defects, a specific birth defect, or a risk for specific types of cancer because these genes affect developmental pathways or basic cell replication pathways. When a person has a mutation in one of these genes, the chance of passing it to an offspring is 50%; thus, such dominant autosomal conditions are inherited in families. When such conditions appear for the first time in a family, they reflect a sporadic, new occurrence of the condition. In many instances, random or spontaneous mutations, or chromosomal errors, can lead to genetic birth defects and genetic birth defect syndromes. Spontaneous mutations are not uncommon. When neither parent has the dominant genetic problem seen in their child, it is termed a de novo (new) or sporadic occurrence. It is estimated that between 3.0 and 7.5% of all malformations in humans are the result of such fresh dominant mutations in genetic material.

Individuals exposed to some highly mutagenic chemotherapy drugs do not produce offspring with more than the expected incidence of birth defects and other genetic diseases. To the extent of our current scientific knowledge, mutations leading to birth defects occur in the normal course of cell division. There is one exception: advancing age. Advancing paternal age is associated with an increased risk for structural chromosomal defects and gene mutations in the male. It is commonly accepted that fresh dominant mutations occur more commonly in the sperm than in the egg. The susceptibility of sperm stem cells to genetic damage may be an important factor in the accumulation of genetic damage since men produce spermatozoa continuously throughout their reproductive lives. In contrast, oocyte development arrests before birth (chapter 3). The male germ cell begins to show effects of aging at 30 years, and most *in vitro* fertilization centers will discourage a man older than 40 years from donating his sperm.

Clearly genes, and genetic alterations, play a role in the risk of having a child with a birth defect. Tests are available to screen prenatally for some of these genetic alterations (chapter 9). Furthermore, while the genetic make-up of the offspring is critical, the environment also plays a role in determining cell fates. The extent to environmental exposures and interactions between genes and the environment affect the risk of birth defects are active areas of research (chapter 7).

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How Do Gene-Environment Interactions Affect the Risk of Birth Defects?

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A key concept in teratology is that there are individual differences in sensitivity to teratogenic exposures. Only a small percentage of infants with known teratogenic exposures display any adverse effects. After years of study, we have come to appreciate the fact that the more common birth defects represent the interaction between multiple genes with small effects and environmental factors (gene-environment interaction), disrupting development to a degree beyond which these factors could have produced individually.

Genetic variation affects food tolerances and may also influence dietary requirements. A good example is the striking finding about the critical role of a simple vitamin, folic acid, in normal embryonic development. Neural tube defects (NTDs) are common congenital malformations, occurring in approximately 1 per 1000 liveborn infants and known to have both an environmental and genetic component to their development. Epidemiologic and experimental studies demonstrate the benefit of folic acid supplementation in preventing NTDs and other congenital anomalies, though how it provides these benefits is not known (chapter 32).

There are some NTDs that are not preventable by folic acid supplementation, suggesting that a "genetic subpopulation" may exist that is either less responsive to folic acid supplementation or has a different underlying cause for these malformations. Researchers have investigated variants in genes associated with folate metabolism and transport as potential risk factors for NTDs. These genes include: folate receptor alpha (FRα); reduced folate carrier (SLC19A1); 5,10-methylenetetrahydrofolate reductase (MTHFR); cystathionine β-synthase (CBS); methionine synthase (MTR); methionine synthase reductase (MTRR); methylenetetrahydrofolate dehydrogenase (MTHFD1); betaine-homocysteine methyltransferase (BHMT); and thymidylate synthase (TYMS). Interactions between maternal folate intake and variations in folate genes (e.g., MTHFR C677T, MTRR A66G, and SLC19A1 A80G) have been suggested by these studies. For example, in a population-based case–control study conducted in California, infants with an 80GG genotype of SLC19A1 gene born to mothers who did not take vitamin supplementation during early pregnancy had a significantly higher risk of developing spina bifida.
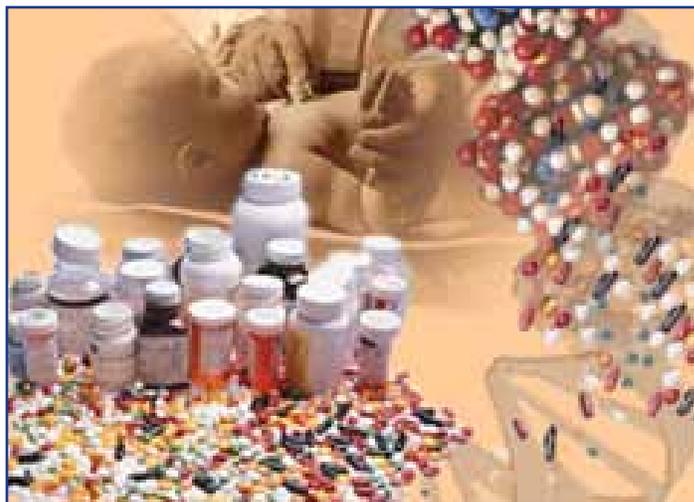
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Another example of gene-environmental interaction involves anticonvulsant drugs, long recognized as causing birth defects in infants exposed *in utero*. However, only about 11% to 20% of these infants will exhibit neurodevelopment impairment with or without structural defects, while about 3% to 10% will be born with structural malformations alone. In animal studies, there are clear differences in anticonvulsant-induced NTD susceptibility between strains. It is likely that a similar situation exists for humans, where an estimated 1% to 2% of infants exposed *in utero* to valproic acid will be born with spina bifida or other forms of NTDs. Detoxification enzymes involved in metabolizing drugs and pollutants, as well as toxic compounds produced by the mother or fetus, may play a role. Variant forms of both Phase 1 (cytochrome P450 enzymes) and Phase 2 (e.g., epoxide hydrolase, glutathione transferases, sulfotransferases, and N-acetyl transferases) enzymes are likely to increase risk of malformations, because poor metabolizers may experience a "build-up" of toxic chemicals in susceptible embryonic tissues or because enhanced, rapid metabolizers for Phase 1 enzymes may produce more toxic intermediates than the Phase 2 enzymes can handle. Many Phase 1-generated intermediates are chemically reactive and bind to protein or DNA, and may be teratogenic, mutagenic, or carcinogenic.

Only a few clinical studies have investigated some of these enzyme variants with respect to risks of structural birth defects. Recently, the MTHFR C677T genotype was studied regarding the rates of major malformations following *in utero* exposure to antiepileptic drugs. Neither the "risk allele" (T) nor the antiepileptic drug-exposure alone had a significant impact on the rate of serious malformations in the offspring, but when these two factors co-existed, the risk increased, suggesting that genetic testing may help predict which infants are at the greatest risk of developing birth defects from exposure to anticonvulsant drugs.

A final example of gene-environment interaction is cigarette smoking and the risk for having an infant with an orofacial cleft. Maternal smoking during pregnancy is associated with cleft lip and/or palate (CL/P). Several animal studies have also demonstrated the adverse effects of cigarette smoking on development of cleft lip and/or cleft palate. Gene-environment interactions have been investigated between maternal smoking and more than 2 dozen genes, including nitric oxide synthase 3 (NOS3), aryl hydrocarbon receptor (AhR) pathway genes, several detoxification genes (CYP1A1, EPHX1), glutathione transferase gene family (GSTs), arylamine N-acetyltransferase gene family (NATs), hypoxia-induced factor-1 (HIF1), folate pathway genes (eg MTHFR), muscle segment homeobox1 (MSX1), and other developmental genes. The most-studied gene in this area is transforming growth factor α (TGFA). A large study involving both nonsmoking and smoking pregnant women found that heavy smokers who carried the rare 'risk' variant of this gene were twice as likely to have a baby affected with cleft lip or palate than nonsmoking women with the more common gene variant. Infants who possessed the rare gene variant were six times as likely to have cleft lip or palate when the mother was a heavy smoker.

Although medicine is still far from individualized preventive measures for birth defects, understanding how specific environmental factors interact with an individual's genetics, or "genotype," may yield critical clues that will ultimately lead to new approaches to prevent preventable birth defects.

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Do Teratogenic Exposures Act through Common Pathways or Mechanisms of Action?

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The term *mechanism of action* refers to the interactions of an agent with the organism that lead to an adverse effect. Mechanisms of action are diverse: agents can interact with a receptor, bind to DNA or protein, degrade cell membranes or proteins, inhibit an enzyme, or modify proteins. If enough of these interactions between an exogenous agent and the organism occur at a biochemical level, changes can occur in the cells and tissues and can lead to changes in cell function, cell fate, or result in cell death. Abnormal development results if the damage is extensive. Different agents can act through the same mechanism of action; producing similar effects. For example, there are a large number of vitamin A derivatives (retinoids) that are used for therapeutic purposes; in the embryo, retinoids interact with the same family of retinoic acid receptors. These agents differ in potency (i.e., the dosage needed to produce an effect) but not in the outcome (malformation or death) they produce.

Graphic courtesy of Barbara F. Hales.

Receptor Interactions

Receptors are proteins within or on the surfaces of cells that are targeted by hormones or other signaling molecules. Receptors perform the same function for cells as our senses perform for our bodies: they inform the cell about its environment and, when activated, bring about changes in cell function. Some teratogens act by interacting with receptors, either mimicking the endogenous signaling molecule or by interfering with the signaling molecule's ability to interact with its receptor. Examples include retinoic acid and diethylstilbestrol (DES), a potent estrogen that was once given to pregnant women in an effort to prevent miscarriage. Retinoic acid, is essential for normal development, and interacts with its receptors that are expressed in certain embryonic structures; too much or too little retinoic acid causes defects in those structures. DES binds to estrogen receptors and causes defects in male and female reproductive organs, as well as a rare form of vaginal cancer in about one of every thousand women whose mothers took DES during pregnancy. The retinoic acid receptor and the estrogen receptor are part of a family of nuclear receptors called cytosolic receptors; many other receptors in this family are known or suspected to be targets for teratogens, such as the androgen receptor and the thyroid hormone receptor.
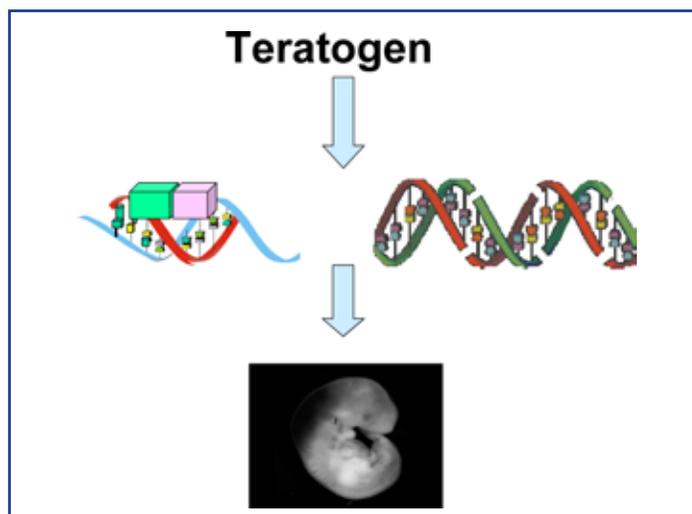
Covalent Binding to DNA or Protein

Some agents are chemically reactive or are metabolized by the body to chemically reactive forms. These reactive forms create covalent bonds to important biomolecules, changing the function of these molecules. For example, cyclophosphamide, a drug used to treat cancer, is metabolized to phosphoramide mustard, a reactive intermediate that covalently binds DNA and other important molecules in the cell, impairing the function of these molecules and ultimately the function of the cell.

Peroxidation

Chemicals that generate highly reactive substances like hydrogen peroxide can oxidize molecules, particularly the lipids that form the foundation for cell membranes. Disruptions to the cell membrane often lead to cell death.

Enzyme Inhibition, Interference with Sulfhydryl Groups

Enzymes are proteins that catalyze chemical reactions, such as the reactions that break down sugars to produce energy for the cell, or that synthesize the large molecules needed for cell structure and function. Inhibiting the function of an enzyme may have teratogenic consequences. For

example, methotrexate, a folic acid antagonist used to treat cancer, psoriasis, rheumatoid arthritis, and ectopic pregnancy, interferes with the synthesis of the nucleotides needed to make DNA, as well as with other metabolic processes that require folic acid.

Sulfhydryl groups, which contain sulfur and hydrogen and are found on the amino acid cysteine, are important in creating the three-dimensional structure of proteins: two sulfur atoms that are distant from each other link together to form a disulfide bridge, creating a loop in the protein. Sulfhydryl groups are used to hold essential minerals like zinc in place in proteins. Sulfhydryl groups are also important in caspases and other enzymes involved in programmed cell death, a normal developmental process. Cadmium, mercury, and other heavy metals can interact with sulfhydryl groups, disrupting the function of the proteins that contain them.

Modification of Proteins

Some proteins require modification in order to carry out their function, and these modifications can be another target of teratogenic exposures. For example, a signaling protein called *sonic hedgehog (Shh)* must first be clipped into two fragments, with the signaling fragment having a cholesterol molecule added to it in order for it to function normally. Shh functions to delineate the ventral portion of the central nervous system. Defects of the central nervous system in which the ventral portion is poorly defined, such as holoprosencephaly or cyclopia, arise when Shh does not function correctly. A number of different agents have been shown to interfere with Shh function, including cyclopamine (an alkaloid in certain range plants in the Western U.S.) and some but not all inhibitors of cholesterol synthesis. These agents appear to act by interfering with the cholesterol modification of Shh. Mutations of one particular gene in the cholesterol synthesis pathway can cause identical abnormalities, as does mutation of Shh itself, an example of how different mechanisms at a biochemical or molecular level can have common outcomes.

Progression of Mechanistic Events to Pathology

If the mechanistic events are sufficiently widespread, they may result in changes at the cell and tissue level. Different exposures can cause the same cascade of events that result in abnormal development. For example, the edema syndrome results when embryos are exposed to low oxygen levels. Heart rate and blood pressure drop, sodium and potassium concentrations in the plasma change, and fluid seeping out of blood vessels causes hollow organs to swell and blisters to form in solid structures. The distortions caused by fluid accumulation disrupt development. But other agents can cause the edema syndrome as well; trypan blue (a biological stain) and other agents that affect the nutrition of the early embryo cause similar effects.

Apoptosis is a form of programmed cell death that occurs in embryos during normal development as a means of sculpting limbs and other structures, to get rid of extra cells or cells that have served their purpose and to remove damaged cells. Apoptosis in normal cells that have not completed their useful lifespan can cause major problems. Zinc deficiency and ethanol (the alcohol in beer, wine, and liquor), for example, extend the size of areas in the developing embryo undergoing cell death beyond what is normal. Other chemicals, such as deoxycoformycin, an antimetabolite that inhibits nucleotide synthesis, produce apoptosis in areas where it does not normally occur. Research into teratogenic mechanisms and pathogenesis is advancing as great strides are made in our understanding of the molecular processes that control embryonic development. Teratologists have only begun to study how chemical agents interact with these processes.

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What Tests Are Available to Screen Prenatally for Birth Defects?

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There are two kinds of tests used in clinical medicine, screening tests and diagnostic tests. Screening tests identify people in the general population who have a higher than average risk of a disease of interest. Diagnostic tests address the question of whether a particular individual is affected. Often in obstetrics, a screening test is applied to all pregnant women to find those women at particular risk of having a baby with a congenital abnormality. Diagnostic tests are then used for those women to see which of them in fact is carrying an affected child. Most diagnostic tests in pregnancy are invasive and have a risk for pregnancy complications and fetal loss, so we do not want to do them on everyone. Screening tests, therefore, refine the population for which diagnostic tests may be worth the risk.

The ability of screening tests to correctly predict abnormalities varies depends on the incidence of the condition in the general population and the reliability (its ability to accurately deliver a result) of the test. Ideally, the test should have few false positive results (predicting that a normal pregnancy is abnormal) and few false negatives (predicting that an abnormal pregnancy is normal).

The background risk for birth defects in liveborn babies is 2–3% at birth. No prenatal screen or diagnostic test that is currently available can identify all of this risk. Available prenatal screening and diagnostic tests use ultrasound, maternal serum, amniotic fluid, chorionic villi, or fetal blood. Each test has specific indications and risks (Table 9-I).

Ultrasound

Ultrasound, also called sonography, uses the reflection of sound waves to make an image of tissue-interfaces. These images can be highly detailed, almost photographic depictions of the embryo and fetus. Ultrasound can confirm a live pregnancy, establish gestational age, and identify twins and other multiple gestations. Ultrasound can also detect fetal abnormalities and is often the only useful prenatal test following a potential or known teratogenic exposure. Ultrasound is useful for evaluating fetal growth and development, but cannot determine the underlying cause of an abnormality. Ultrasound also cannot provide much information about neurological functioning of the fetus.

Ultrasound is used for both screening and diagnosis. For example, during the first trimester, thickening of a fluid compartment in the embryo's neck is associated with an increased risk of certain chromosome abnormalities (Figure 9-1). The test is called nuchal translucency and is not diagnostic, because some embryos with increased neck fluid are normal. If ultrasound images show increased nuchal translucency, this abnormal screening test can be followed by a diagnostic test for chromosome abnormalities (discussed below). In other cases, ultrasound can be diagnostic. For example, the accuracy of ultrasound in detecting anencephaly (incomplete head development) is approximately 100%.

Table 9-1.

PRENATAL DIAGNOSTIC SCREENS AND TESTS			
SAMPLE	**TESTS**	**INDICATIONS**	**RISKS**
Maternal serum	AFP Multiple-analyte screens	Open NTD, abdominal wall defects Down syndrome, Trisomy 18, aneuploidy	Bruising, pain at site of blood withdrawal
Amniotic fluid (amniocentesis)	Karyotype AFP DNA, enzyme, hormone analysis	Maternal age ≥ 35, Abnormal MSAFP or triple screen Indicated as result of genetic counselling	≤1:200 risk for miscarriage
Chorionic villi (CVS)	Karyotype DNA, enzyme, hormone analysis	Maternal age ≥ 35 Indicated as result of genetic counselling Earlier diagnosis desired	≤1:100 risk for miscarriage
Fetal blood (fetal blood sampling)	Karyotype DNA testing		1 to 3 in 100 risk for fetal loss

Figure 9-1. *Ultrasound image of a normal first trimester embryo in profile. The "+" signs mark the nuchal translucency measurement.*

It has become common practice in the U.S. for pregnant women to have at least one or two ultrasound examinations. The first scan is performed in the first trimester to confirm gestational age and the number of fetuses. This first scan can also measure nuchal translucency and can identify some malformations. A second ultrasound examination is performed at 18–20 weeks gestation. This ultrasound includes an anatomic survey that can identify about half of the structural malformations. Sometimes the second ultrasound will be followed by a more detailed examination at a later gestational age to further define suspected structural abnormalities. For example, fetal echocardiography, a specialized ultrasound examination of the fetal heart, can be used to characterize heart abnormalities with good accuracy.

Maternal Serum Screening

Maternal serum alpha-fetoprotein (MSAFP) measurement at 15–20 weeks' gestation is used to determine if the fetus is at risk for an open neural tube defect. Alpha-fetoprotein (AFP) is secreted by the fetal liver and excreted in the fetal urine, but some AFP crosses the placenta and can be measured in maternal serum. The median values of AFP in amniotic fluid and maternal serum change with gestational age, so results are expressed as multiples of the median, or, cutely enough, MoM.

Elevated MSAFP can be due to any of several factors. An abnormal fetal condition such as an open neural tube defect or abdominal wall defect can result in cause excessive AFP in the amniotic fluid, or an abnormal maternal-placental interface could allow excessive AFP to cross into the maternal circulation. Because AFP rises throughout pregnancy, inaccurate gestational age could cause an MSAFP level to seem high. Multiple gestations can also increase MSAFP, because more than one fetus is generating AFP. A less common reason for elevated amniotic fluid and maternal serum AFP levels are some rare inherited renal and skin diseases. Even if no reason can be found for an elevated MSAFP, the pregnancy would be considered at increased risk for preterm delivery or other adverse outcomes.

Nowadays, a "triple screen" or "quadruple screen" is often done, which combines a test for MSAFP with measurement of two or three other molecules. These chemicals currently include estriol, human chorionic gonadotropins (HCG), and inhibin-A, but the search for new and more predictive analytes or combinations of analytes is an area of active research. Initially designed to identify pregnancies at increased risk for trisomy 21 (Down syndrome), these multiple-analyte screens are also useful for detecting trisomy 18 and other less common aneuploidies (abnormalities of chromosome number). For the multiple-analyte screens, maternal blood is drawn between 15–20 weeks of gestation, at which time median values for each marker are well established.

There are also protocols involving maternal blood sampling earlier in pregnancy, and first trimester screening with a nuchal translucency measurement and maternal blood testing is commonly offered to pregnant women. Establishing gestational age is critical for accurate interpretation because the medians for each marker change by the week. Results also have to be adjusted for maternal age.

Diagnostic Testing

Because diagnostic testing in pregnancy is usually invasive, it is reserved for situations where the risk of an abnormal result exceeds the risk associated with the procedure.

Three different procedures can diagnose chromosomal abnormalities. The earliest test that can be done is chorionic villus sampling (CVS), which is performed between 10 and 12 weeks of gestation. CVS involves suctioning bits of placental tissue, called chorionic villi, through a needle or a thin tube. These bits of placental tissue usually have the same chromosomes as the embryo. The cells from the chorionic villi are grown in culture and their chromosomal complement is analyzed. CVS is performed under ultrasound guidance; samples can be obtained either through the cervix or through the abdomen, depending on the operator preference and location of the embryo in the uterus. The risk of miscarriage is generally described as 1/100, but is considerably lower with more experienced operators. The advantage of CVS is that it can diagnose an abnormality earlier in pregnancy than any other test. However, CVS can only be used for chromosomal abnormalities; as we will see, amniocentesis can also be used for evaluating open neural tube and other defects.
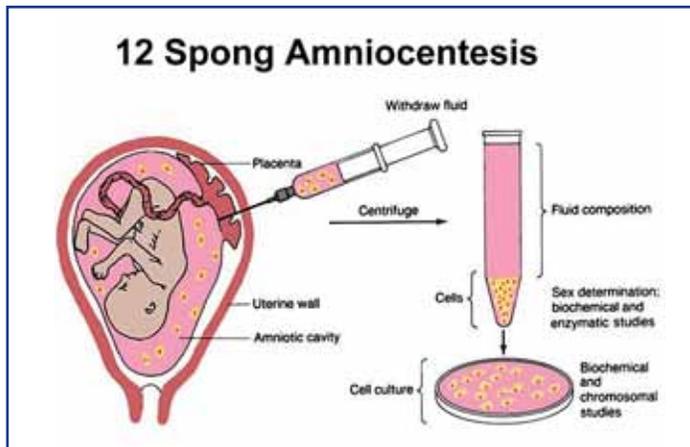
Figure 9-2. *Removal of amniotic fluid for diagnostic screening. Amniocentesis is performed under continuous ultrasound guidance. A needle is inserted through the maternal abdomen into the amniotic sac; the placenta is avoided, if possible. The tip of the needle is visible by ultrasound and is monitored throughout the procedure. Once the needle is within the amniotic sac, care is taken to avoid the fetus and umbilical cord. (In contrast, a fetal blood sampling targets the umbilical cord). A small amount of fluid is removed and discarded to avoid any possible contamination with maternal cells during insertion of the needle. Then the sample of fluid for analysis is removed. After needle removal, the fetal heart rate is checked by ultrasound.*

Amniocentesis, the sampling of amniotic fluid, is commonly performed in the second trimester (Figure 9-2); chromosomal analysis is performed on amniocytes, which are cells that originated in the fetal skin and have the same chromosomes as the rest of the fetus. AFP levels in amniotic fluid are used to test for open neural tube and a few other kinds of defects. Amniocentesis performed under continuous ultrasound guidance is described to patients as having an approximate 1/200 risk for miscarriage, although the actual risk in experienced hands is much lower. First trimester amniocentesis is possible, but carries a greater risk of miscarriage. Amniocytes or amniotic fluid can also be used for DNA-based mutation analysis or enzyme analysis in the diagnosis of many inherited diseases. Tests for unusual genetic diseases are only done when the fetus has been determined by genetic counselling/history taking to be at risk for a specific condition.

The third way to do a chromosomal analysis is by taking a blood sample directly from the fetus after 18 weeks of gestation. This procedure involves the removal of blood from the umbilical vein, preferably close to the placental insertion site, and is associated with a 1–3% risk of fetal loss.

Fetal cells are present normally in the maternal circulation. If isolated, these cells have potential to be useful in prenatal diagnosis, as a method that involves no risk to the fetus. However, the volume of fetal cells in the maternal circulation is very small, and there is some evidence that fetal cells may

persist in maternal cells for many years, significantly beyond the pregnancy. Fetal cells sampled in a woman's second pregnancy, then, might be contaminated with cells from the woman's first child. A better alternative to harvesting fetal cells from maternal blood may be the identification of fetal DNA in maternal blood. DNA is shorter-lived than whole cells. Harvesting of fetal DNA offers an opportunity to test the fetus by drawing blood from the mother, a procedure that carries little if any risk. This test is not yet in clinical use, but may be a common practice in the future.

Are These Tests Useful?

The usefulness of a test depends on what you want the test to tell you. Prenatal screening tests are limited in terms of the conditions detected and cannot be expected to give yes or no answers; they simply identify a population at greater than average risk of a given disorder. For example, chromosome abnormalities occur in about 1 in 1000 pregnancies in the general population. A woman with a triple screen result showing a 1 in 250 chance of an affected pregnancy has a higher than average risk and she may choose to have diagnostic testing. Notice, however, that the screening test does not give a normal/abnormal result. After all, 99.6% (249/250) of women with this "abnormal" result will have an unaffected pregnancy.

Even diagnostic testing is limited to chromosome analysis and AFP testing, and cannot guarantee a perfect baby. An anomaly that is detected early gives a pregnant patient and her partner the option to continue or terminate the pregnancy. When a decision is made to continue the pregnancy, medical management may be altered, and fetal surgery is sometimes possible for certain structural anomalies. Knowing about a problem in advance may be helpful to the family.

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What Sources of Information Are Available on Developmental Risks and Pregnancy Safety?

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Growing public awareness about birth defects has sent many people planning to have children to health care professionals with questions about how their exposures to drugs and environmental agents might affect their pregnancies. The number of published teratology and developmental toxicology studies published has increased over the years and the task of synthesizing and communicating accurate information has become more challenging.

Most risk evaluations that are done are based on the general population's likelihood of having an adverse event. Parents need help in understanding what their individual risk for an adverse pregnancy outcome is relative to that for the general population. For pregnant women who are exposed inadvertently, there is also a great need to understand the possible risks to the fetus. Approximately 15–20% of recognized pregnancies end in spontaneous abortion and between 2 and 3% of pregnancies result in a child with a major birth defect and/or mental retardation. Too often, counseling emphasizes the risks associated with drug therapy without balancing the discussion with the benefits to the mother and baby of treating an illness in the pregnant woman. A healthy mother helps to ensure a healthy fetus, and letting pregnant women remain ill because of fear of drug risks may not be the wisest course. To determine whether drug exposure increases a woman's risk above background levels, health care professionals must integrate scientific information available on the agent and medical factors. A patient's family or medical history may carry more risk than the drug in question, and complete knowledge of the pregnant woman's health status, family history, pregnancy history, and occupational history, is helpful when evaluating teratogenic risk.

What sources of information are best? When health care practitioners have questions about medica-tions during pregnancy, they usually consult the product labeling. For drugs, product labeling is developed at the time a new product is approved by regulatory agencies such as the Food and Drug Administration (FDA) in the U.S. and the European Medicines Agency (EMA) in the European Union. The labeling describes the chemistry, pharmacology, and toxicology of the drug. It provides information on which uses (indications) and for which patient populations the drug has been approved and what the recommended doses are. The label includes results from both clinical studies and experimental animal studies to describe the safety and effectiveness of the drug. The pregnancy section of the label for a new drug is based almost entirely on laboratory animal data. It is a real challenge to practitioners and pregnant patients to understand the information in the labeling and determine the individual risk that taking the drug might have.

The pregnancy section of the labeling in the U.S. currently contains a standardized rating system for pregnancy effects with five categories, each of which is given a letter (A, B, C, D, and X) [Table 10-1]. These categories were introduced by the FDA in 1979 in an effort to make pregnancy information easier to understand by practitioners, but the categories

Table 10-1.

THE CATEGORIES
Category A means that adequate controlled studies in women have failed to show a risk to the fetus. Virtually nothing is Category A.
Category B means either (1) experimental animal studies show risk but human studies do not, or (2) experimental animal studies are negative and adequate human studies do not exist.
Category C means either (1) experimental animal studies show fetal risk or (2) there is no information one way or the other.
Category D means that there is evidence of risk to the human fetus (not necessarily from human studies, by the way), but that the benefit may outweigh the risk.
Category X means either (1) that there is evidence of risk to the human fetus, but the benefit would never outweigh the risk or (2) the drug has no conceivable utility in pregnancy.
Category B is a funny one, because if part (1) is fulfilled (adequate human studies do not show fetal risk), why is the drug not Category A? If part (2) is fulfilled, you have to wonder whether the experimental animal studies were adequately done. The way experimental animal studies are performed, a selection of doses is used in pregnant rodents or rabbits; the highest dose tested is supposed to result in some degree of maternal toxicity. Usually the top dose results in impairment of weight gain by the pregnant animal. In the face of impaired maternal weight gain during pregnancy, there ought to be an effect on the offspring (often a parallel impairment of offspring weight gain). This effect on offspring should count as fetal toxicity.

have not been successful. The category system is erroneously interpreted as representing a gradation of risk and has not helped practitioners because the category designation does not communicate accurately what is known about the effects of a medication on pregnancy nor does it tell practitioners or patients what action to take.

If you look closely at the definitions, you can see why the gradation of risk idea is inaccurate. Category C might be assigned to a drug that has been tested in some animal studies that may or may not have been adequate to assess potential risk, or the drug might be a C because there is little or no information in either animal or human studies. The majority of drugs fall into this category. Category X might be assigned to a drug that does not cause birth defects but simply has no conceivable use during pregnancy. Birth control pills are a great example of a Category X drug that has been shown not to increase the risk of birth defects. Birth control pills have been much better tested than some drugs in Category B or C and are in Category X only because there is no reason to take birth control pills during pregnancy.

Just as the categories do not describe a gradation of risk, the severity of effect is not indicated by the category level. For example, the anticonvulsant phenytoin is a Category D drug and is associated with birth defects in about 10% of exposed pregnancies. Lovastatin, a Category X drug based on lack of a perceived use for it during pregnancy, has not been associated with birth defects in exposed pregnancies. The difference between D and X may be simply whether there could be a reason to prescribe the drug during pregnancy, in spite of the presumed risk. Human data are available for only about 60% of Category X drugs. In other words, the Category X listing can be determined for presumed risk based on experimental animal studies plus a lack of a reason to use the medication during pregnancy.

In 1994, the Teratology Society issued a recommendation that the category system be abandoned in favor of a plain-text explanation of the available information on toxicity during development or on reproduction. Society members viewed the categories as hazardous to the fetus in potentially causing the termination of wanted pregnancies through inaccurate and incomplete information. The FDA also recognized the need to change the labeling and, in response to the Teratology Society, the Organization of Teratology Information Specialists (OTIS), and other concerned groups' published a draft Proposed Rule: Pregnancy and Lactation Labeling that eliminates the pregnancy categories in favor of inclusion of plain text information relevant to determining risk. Based on the many comments received about the draft Rule, the FDA is in the process of finalizing the Rule, which will mandate changes in the pregnancy section of the labels for new drugs and eventually for all drugs.

What are other resources available for clinicians? The drug labeling itself will never provide all the information that might be needed to help make informed decisions for the pregnant patient. Other resources include textbooks, computerized databases, and teratogen information services. Textbooks that provide information regarding the reproductive effects of environmental agents can be found in the Suggested Reading section that follows. Peer-reviewed journals publish original studies, review articles, editorials, and information on upcoming conferences. Computerized databases contain information on thousands of agents, including medications, and summarize information from scientific studies. These databases are available on the Internet. For links to some of these databases, see the website list in the beginning of this *Primer* or go to the Teratology Society Web site at http://www.teratology.org/scientists.asp#tox.

Teratology Information Services (TIS) are comprehensive, multidisciplinary resources that provide free, up-to-date information about the reproductive effects of environmental agents to both health care providers and their patients. Most TIS have at least one full time teratogen information specialist and are directed by individuals with a medical or doctoral degree and expertise in clinical teratology. TIS are usually located at major medical universities or state health departments and can access a variety of resources, including medical libraries, online reproductive databases, and consultants in teratology-related fields such as toxicology, pharmacology, occupational medicine, genetics, radiation biology, infectious diseases, perinatology, and epidemiology. Although TIS operate independently, OTIS was formed more than 15 years ago to facilitate education and training in this area and to establish quality-assurance criteria. Through OTIS, teratology information specialists are in a unique position to quickly respond to public concerns that may be raised when research findings reach the popular press before health care providers can critically evaluate them. For more information and a list of TIS in North America, the OTIS Web site is at http://www.otispregnancy.org.

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How Do Epidemiologic Studies Contribute to the Identification of Teratogenic Exposures?

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Epidemiology is the study of the distributions of, and risk factors for, diseases in human populations. Epidemiologists use a variety of designs and statistical procedures to identify and assess risk factors. Although clinical observations from a single case or case series sometimes play a role in understanding the effects of a particular exposure during pregnancy, in most circumstances, it is difficult to know whether the observed outcome is due to the pregnancy exposure or if the exposure is incidental. However, these clinical observations may raise clues that can be addressed in epidemiologic studies.

Epidemiologists who study potential teratogenic exposures typically conduct *observational* studies on populations. Unless a particular exposure or procedure is thought to be beneficial, most studies cannot ethically be *experimental* in their design, e.g., randomized controlled trials. In experimental studies, the investigator has much more control over the many exposures study subjects may encounter, whereas in observational studies, the investigator is relegated to being an observer of factors to which subjects in a study population are exposed. In some instances, many of these exposures are not known or cannot be adequately controlled. Thus, observational epidemiology studies offer associations and do not establish causation.

The two primary types of observational studies that epidemiologists have at their disposal are cohort and case-control studies. The cohort study approach starts with one group of individuals in a defined population exposed to a particular agent and compares the risk of disease/outcome in that group to a second group of individuals from the population not exposed to the same agent (Figure 11-1).
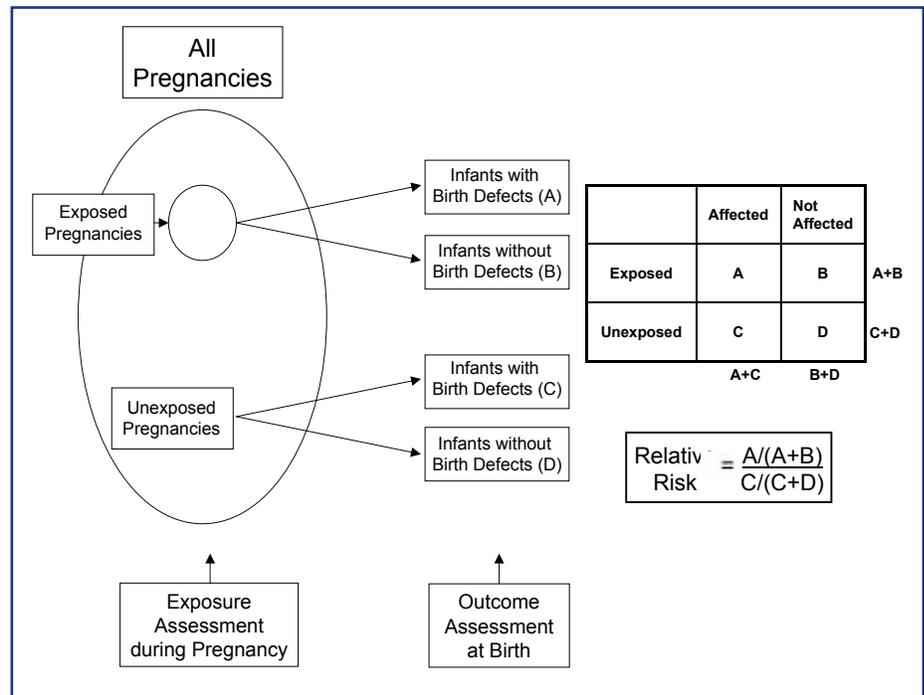
Figure 11-1. *Study design of a cohort study: Pregnancies (exposed and unexposed to the agent of interest) are identified from all pregnancies in a defined population. Birth outcomes (whether the baby has a birth defect or not) are determined. The rate of infants with birth defects among exposed pregnancies is then divided by the rate of infants with birth defects among unexposed pregnancies to determine the relative risk. Adapted from Fletcher* et al*., 2005.*

Cohort studies may include the entire population of interest or at least a large segment of the population, and often require long periods of follow-up time to reliably establish the risk of disease/outcome between the two groups. The second main type of observational study design—the case-control study—includes all cases of the disease of interest in the defined population, but only a random sample of the non-diseased population (Figure 11-2). That is, the epidemiologist determines the ratio of cases to controls that are included in the study, and compares the frequency of the specified exposure/factor between cases (with the disease of

interest) and controls (without the disease of interest). These types of studies tend to be more economical than cohort studies because one does not have to enroll as many individuals as would be needed in a cohort study, many of whom will never get the disease/outcome that is of interest.

Associations in these observational studies are assessed by calculating "risk estimates" for the exposed group relative to the unexposed group. The typical measure used in cohort studies to estimate the size of the association between a factor of interest and a disease/outcome of interest is known as the relative risk— see Figure 11-1. A relative risk of 1.0 indicates that the risk of disease in the group exposed to the risk factor is the same as the risk of disease in the group not exposed. Relative risks more than 1.0 indicate that the risk of disease is higher in the exposed group, whereas relative risks less than 1.0 indicate that the risk is lower in the exposed group when compared to the unexposed. The typical measure used in case-control studies to estimate the size of the association between an exposure or factor of interest and a disease/ outcome of interest is known as the odds ratio—see Figure 11-2. The odds ratio is an approximation of the relative risk when the outcome is rare, and its interpretation is similar to the relative risk. That is, an odds ratio of 1.0 indicates no association between factor and disease, an odds ratio >1.0 indicates an increased risk association and an odds ratio <1.0 indicates a decreased risk association. Confidence intervals are a measure of statistical precision of the relative risk or the odds ratio. Confidence intervals that contain 1.0 indicate that relative risk or odds ratio estimates do not differ statistically from the no-effect value of 1.0.

Conducting epidemiologic studies and drawing inferences from such observational studies requires the epidemiologist to be aware of methodologic issues pertinent to the exposure and the disease being studied. These issues include ascertainment of defects, grouping of defects, choice of control groups, confounding, and chance:

Ascertainment—Some birth defects are much more easily identified, for example, severe heart defects, such as hypoplastic left heart syndrome, are more likely to be ascertained than less severe heart defects, such as ventricular septal defects and atrial septal defects. These less severe defects are much more difficult to ascertain uniformly in
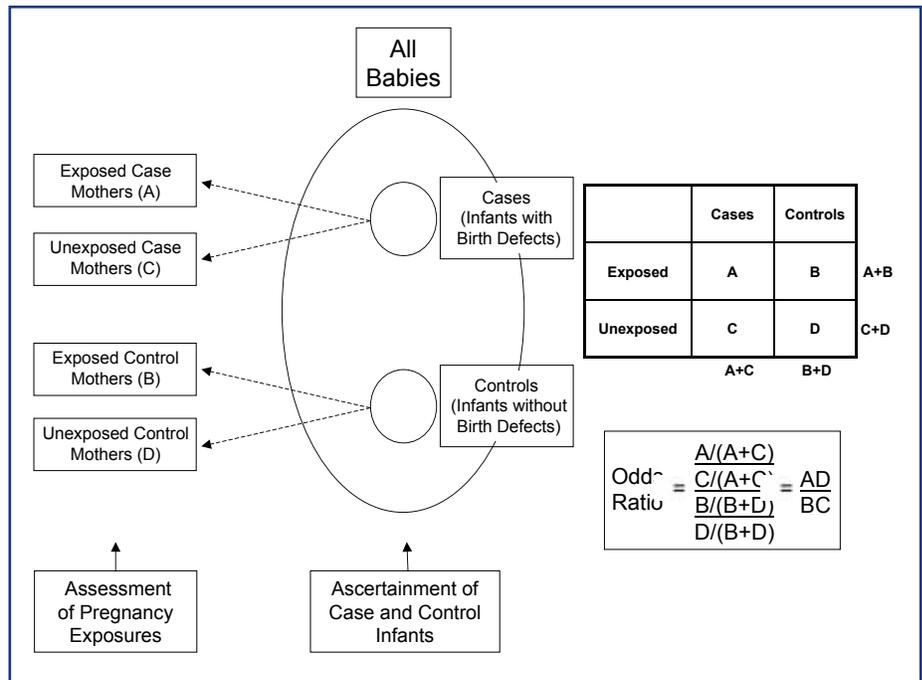
Figure 11-2. *Study design of a case-control study: All infants with birth defects are identified from a defined population and a sample of infants without birth defects is selected from the same defined population. Information is assessed about exposure during pregnancy after the pregnancy is complete and the outcome is known. The odds ratio, which estimates relative risk when the outcome is rare, is calculated as the odds of exposure among cases divided by the odds of exposure among controls. Adapted from Fletcher* et al.*, 2005.*

a population because they may be asymptomatic in early life. Ascertainment of a defect needs to be nearly complete and consistent because if there is variability within a study on how well defects are ascertained between exposed and unexposed individuals, the observed result may be spurious.

Grouping of defects—Human birth defects comprise many different developmental systems and structures, reflecting manifold differences in underlying pathogenesis and etiologies. Typically teratogenic exposures do not increase risks of all birth defects. Even specific groups of defects, e.g., heart defects, are heterogeneous in anatomy, development, and epidemiologic factors. Combining different birth defect types for analyses is a valid approach only if the defects being lumped have an underlying pathogenesis that is similar.

Control groups—A very important consideration in case-control studies is the selection of the control group. An appropriate control group in a study of a specific birth defect is a random sample of mothers/babies who would have been included in the case group if their child had the birth defect being studied. In the circumstance with a medication that is indicated for a limited set of underlying conditions, a further approach might be to choose a control group that had the underlying condition, but did not use the medication. The

latter may not be practical nor may it be ideal because those with the underlying condition may differ in severity of the condition.

Confounding—Studies investigating whether a specific birth defect is associated with a particular exposure need to accurately assess whether other factors associated with the exposure such as maternal age contribute to the results. For a factor to be a confounder, it must be associated with both the exposure and the outcome. If these other factors are known and have been measured, they can be addressed analytically with statistical methods such as logistic regression.

Chance—As noted above, the confidence interval provides a guide to determining the likelihood that a result occurred by chance. Another way to determine such likelihood is by using *p* values. The *p* value is an estimate that the differences observed occurred by chance alone, assuming that there is no difference between exposed and unexposed women. Typically, *p* values of <0.05 are considered statistically significant, although it should be recognized that the selection of 0.05 as a cutoff is arbitrary and other values may sometimes be more appropriate.

Bias—In addition to chance, it is important to consider whether some sort of study bias is an alternative explanation of the results that have been observed. Bias can be introduced in a variety of ways, e.g., mother's recall of an exposure, incomplete case ascertainment, and confounding. If bias is present, it will cause observed results to differ from the truth. Investigators who use observational studies need to be particularly aware of such biases because the "treatment" or exposure under study was not assigned randomly. Statistical methods sometimes, but not always, can be used to address some biases. One factor to consider in case-control studies is whether the results observed could be due to recall bias, the tendency for a woman who had a baby with a birth defect to be more likely to recall prenatal exposures than a woman who had a normal baby.

Absolute Risk *vs.* Relative Risk—Another issue to consider is what study results mean for an individual woman with a particular exposure. It is important to recognize that an elevated relative risk or odds ratio needs to be put into context by taking into account the frequency at which the outcome occurs. For example, a relative risk of 3 means that an exposed woman is at a three-fold risk of the particular outcome. If the outcome is rare (1 in 100,000), her absolute risk if exposed to the medication is 3 in 100,000 (1 in 33,333), whereas if the outcome is more common (1 in 100), her absolute risk would be 3 in 100, or 3%.

Epidemiologic studies provide a valuable approach to better understand effects of certain exposures during pregnancy. However, many factors need to be considered and results from such studies must be combined with existing information before assuming that the effect observed in a study is valid and meaningful for an individual woman.

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What Are the Ethical Considerations for the Inclusion of Pregnant Women in Clinical Trials?

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Women often need to take medications at some point during their pregnancy. Most drugs have not been evaluated in clinical trials that knowingly enroll pregnant women. A woman, as any other patient, should expect that any medicine prescribed during pregnancy has had an evaluation of risks as well as benefits. What are the ethical considerations for testing drugs during pregnancy? Ethical rules applied to medical practice and research are often referred to as medical ethics and include human participant protections, patients' rights, informed consent and privacy/confidentiality. Ethical issues arising from advances in biological and medical sciences that are subsequently applied to human life, e.g., genetics, genetic testing, stem cell research, pharmacogenomics and personalized medicine, DNA databases and sample storage are referred to as bioethics (Figure 12-1). Both medical ethics and bioethics apply to clinical research.

Medical Ethics and Bioethics

Medical Ethics	*Bioethics*
▪ Ethical rules applied to issues in medical practice and research	▪ Ethical issues due to advances in biological and medical science applied to human life
▪ Human participant protections	▪ Genetics, genetic testing
▪ Patients' rights	▪ Stem cell research
▪ Informed consent	▪ Pharmacogenomics, Personalized Medicine
▪ Ethical review	▪ DNA databases, sample storage
▪ Privacy/confidentiality	

Figure 12-1.

The fundamental ethical principles and codes that are instituted for the protection of human subjects are found in 3 key documents. The *Nuremberg Code* in 1947 stated that "certain types of medical experiments on human beings, when kept within reasonably well-defined bounds, conform to the ethics of the medical profession generally. … and that certain basic principles must be observed in order to satisfy moral, ethical, and legal concepts." This code became the prototype to assure that research involving human subjects would be carried out in an ethical manner. In the 1979 *Belmont Report* and in the 2008 version of the *Declaration of Helsinki*, these principles were further defined, clarified and updated.

The Belmont Report summarizes the basic ethical principles according to the nature and definition of informed consent, the assessment of risk-benefit criteria in research involving human subjects, and their fair selection for research. The basic ethical principles for research involving human subjects are respect for persons (protection of pregnant women and fetuses through informed consent and additional safeguards), beneficence (prior evaluation of the risks of medications and/or medical procedures in pregnant women and fetuses), and justice (appropriate distribution of the benefits and burdens of research) (Table 12-1).

THE BELMONT REPORT, 1979	
PRINCIPLE	**APPLICATION**
Respect for persons Individuals should be treated as autonomous agents; those with diminished autonomy are entitled to protection	**Informed consent** Subjects, to the degree that they are capable, must be given the opportunity to choose what shall or shall not happen to them. The consent process must include three elements: information, comprehension and voluntariness
Beneficence Human subjects should not be harmed; Research should maximize possible benefits and minimize possible harms	**Assessment of risks and benefits** Nature and scope of risks and benefits must be assessed in a systematic manner
Justice The benefits and risks of research must be distributed fairly	**Selection of subjects** There must be fair procedures and outcomes in the selection of research subjects

Table 12-1. *The Belmont Report established three basic ethical principles—**respect for persons, beneficence and justice**—which are the cornerstone for regulations involving human subjects.*

Respect for Persons embodies the ethical concepts that individuals should be treated as autonomous persons and that those with diminished autonomy should be protected. This protection speaks to vulnerable populations, i.e., those that are limited in their scope of options, that are often frequently subjected to coercion in their decision making ability, or that may be compromised in their ability to give

informed consent. Vulnerable groups include pregnant women, human fetuses and neonates, children, prisoners, and persons with impaired decisional capacity.

In practice, respect for persons requires that individuals, to the degree that they are capable, be given the opportunity to choose what will or will not happen to them. This choice is provided through the three standard elements of informed consent: information, comprehension, and voluntariness. Respect for persons also safeguards the individuals' right to withdraw at any time from research.

Beneficence refers to the efforts to ensure the well-being of the individual and is related to nonmaleficence, the obligation to do no harm. The Hippocratic maxim "do no harm" is a fundamental principle of medical ethics, but in the realm of research, refers to no harm regardless of the benefits that might come to others. The practical application of beneficence is the assessment of risks and benefits and the careful analysis of the design as well as alternative ways of obtaining the benefits sought in the research. Risks and benefits of research not only affect the individual subjects but possibly affect the families of the individual subjects and society. Hence, there is a requirement that risks to subjects be outweighed by the sum of both the anticipated benefit to the subject, if any, and the anticipated benefit to society in the form of knowledge to be gained from the research.

Justice is framed in the question: Who ought to receive the benefits of research and bear its burdens? This concept speaks to the fairness in distribution. Injustice implies denial of a benefit to which a person is entitled or undue imposition of a burden without good reason. The principle of justice gives rise to the requirement that there be fair procedures for the selection of research subjects.

In many of the early writings on the ethics of research involving human subjects, the burdens of research fell largely on the poor and on unwilling prisoners, while the benefits of improved medical care came primarily to the wealthy. Justice in the selection of subjects requires that potentially beneficial research be equitable and not based on classes of vulnerable subjects to bear burdens without the benefits of the research.

In 1991, a uniform set of regulations for the protection of human subjects was issued as the Federal Policy for the Protection of Human Subjects, informally known as the "Common Rule." The Common Rule applies to all federally funded research and requires that for research with human subjects, the entity conducting the research must assure the federal government that it will provide and enforce protections for research conducted under its sponsorship, assess research proposals as to risks and potential benefits to subjects, ensure requirements for selecting subjects and obtaining informed consent are met, and establish and

delegate to an Institutional Review Board (IRB) the authority to review and oversee human subjects protections for all research conducted.

The main elements of the Common Rule are often referred to by the specific section of the Rule. Subpart A covers the requirements for assuring compliance by research institutions, obtaining and documenting informed consent, and IRB review of research; Subpart B covers additional protections for pregnant women, *in vitro* fertilization, and fetuses; Subpart C addresses the ethical use of prisoners; and Subpart D covers the use of children in research.

Ethical Issues: Prenatal Screening and Exposure

Advances in medical technology allow pregnant women to undergo genetic testing of their unborn child as part of routine prenatal care to potentially detect individuals at increased risk for an abnormal pregnancy outcome. A patient can then choose to continue or terminate the pregnancy, which leads to difficult ethical decisions regarding the best interests of all parties involved. Genetic technology can lead to positive/negative eugenics regarding gender and phenotypic trait selection (designer babies), in some situations resulting in loss of genetic diversity in the gene pool.

Prenatal exposure to many compounds, infectious diseases, stress and nutrition has the potential to negatively affect the developing fetus with preventable defects that can last a lifetime. It is the ethical responsibility of the mother to protect the developing child through appropriate behavior and medical care.

Suggested Reading

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http://www.hhs.gov/ohrp/references/nurcode.htm

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Research Involving Vulnerable Populations at:
http://grants2.nih.gov/grants/policy/hs/populations.htm

Hippocratic Oath:
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Common Rule:
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What is the Role of Post Marketing Surveillance in Detecting Teratogenic Exposure?

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Once a new medication is marketed, if the medication is used at all by women who are in their childbearing years, there are likely to be pregnant women who take the drug. This pregnancy exposure can occur unintentionally during the early weeks of pregnancy before a woman knows that she is pregnant. A pregnant woman may also take a new medication intentionally for some disease or condition that requires treatment. Without human pregnancy clinical trial data to establish the safety of a medication for the developing fetus, post-marketing surveillance studies are an increasingly common method for gathering data on potential teratogenicity as quickly and as efficiently as possible.

One type of post-marketing surveillance study is a called a "pregnancy registry." Pregnancy registries are studies in which pregnant women who have taken a specific medication are asked to enroll in the registry. Information is collected about the woman's pregnancy, her medication and other exposures, and the outcome of that pregnancy. Information is typically collected on all pregnancy outcomes, whether the pregnancy ends in miscarriage, stillbirth, or a live born baby or babies, and information is collected on any complications that occur, including birth defects. The number of specific poor outcomes, such as babies born with birth defects, among women who took the drug of interest is evaluated to determine if these events are more frequent than expected and if it is plausible that the excess number of affected infants might be due to a teratogenic effect.

Every pregnant woman has a small, 2–3% risk of having a child with a birth defect regardless of the medications she takes. In order to determine if a specific new drug exposure might be increasing that risk, pregnant women exposed to the drug under study are compared to another group of pregnant women. This reference group can be the general population of pregnant women, or can be a comparison group of pregnant women who are enrolled in the registry but who have not taken the medication under study.

The objective of a pregnancy registry is to determine as early as possible after a drug is marketed whether or not there is any indication of a teratogenic risk in humans. This approach is appealing to pregnant women and their health care providers because it has the potential to provide clues about safety issues or teratogenic potential so that the best treatment decisions can be made.

A pregnancy registry may be the most efficient method for post-marketing surveillance if a drug is used for a very rare condition or is used only infrequently in the population of women who might become pregnant. A pregnancy registry can also be a good method for identifying a new human teratogen if the medication causes a very unique and severe pattern of birth defects or a very high incidence of specific birth defects.

However, because new medications might be infrequently used in pregnant women and because pregnancy registries rely on women and/or their health care providers to volunteer for the study, the number of women who enroll in any given registry often is very small. Small numbers of participants can limit the ability of a pregnancy registry to conclusively detect human teratogens, particularly if the drug only affects a small proportion of exposed pregnancies. Thus, an important function of a pregnancy registry is to identify potential "signals" or suggestions of an excess risk, and to call for additional studies to confirm or refute that signal. By the same token, pregnancy registries can never definitively establish safety but can provide some reassurance that a specific drug does not carry a high risk for a severe pattern of birth defects.

Another approach to post-marketing surveillance takes advantage of the technological advances in electronic claims data and medical and pharmacy records storage. Large databases that include pregnancy information, such as linked prescription and birth records can compare pregnancy outcomes between pregnant women who have been prescribed a new drug of interest and those who have not within the same healthcare database. This approach offers many of the advantages of a pregnancy registry at potentially far less cost, and need not rely on volunteers to enroll.

Some limitations of healthcare database studies include the difficulty in determining if the drug that was prescribed was actually taken by the mother and when and oftentimes lack of access to information on other important exposures such as whether or not the mother smoked cigarettes or drank alcohol during pregnancy. In addition, just as with pregnancy registries, if the drug of interest is infrequently prescribed to women of childbearing age, even very large databases may have access to only small numbers of

pregnant women exposed to any particular drug. Therefore, large databases may still have difficulty in identifying new teratogens unless the risk is high for a severe and easily recognizable teratogenic effect.

Despite the challenges of performing post-marketing surveillance for human teratogenicity, the public health need for such information is great. In the absence of randomized clinical trials, synthesis of information from post-marketing studies along with population-based studies, pre-clinical developmental toxicity studies, and other predictive techniques, as described in this *Primer*, are needed to optimize the capacity to recognize a potential teratogenic effect with a new pharmaceutical agent or conversely to provide reassurance that a new drug does not pose a substantial risk.

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How Are New Drugs Evaluated for Developmental Toxicity?
Can Animal Studies Predict Human Risk?

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No pregnant woman wants to take chances with the health of her baby, and women expect that medications prescribed during pregnancy have been tested for safety for the embryo or fetus. Product labels for drugs contain a section on use in pregnancy. What is different about this section compared to the rest of the drug label is that it is almost always entirely composed of data from animal studies rather than data from human clinical trials. Most drugs are not tested in pregnant women, and during initial testing, women at risk of pregnancy are intentionally avoided. Only post-menopausal women or women who are using effective contraception are normally enrolled in drug trials before reproductive toxicology tests are conducted. After these tests are completed, reproductive age women are included in large-scale clinical trials.

Since the 1960's, testing in experimental animal models has been required to estimate prenatal risk in humans. These studies are typically performed in one rodent and one non-rodent species. Rats and rabbits are the most often used, unless it is known that one or the other is not considered an appropriate model. In recent years, there is increased use of primate models to test biologic drugs because the traditional rat and rabbit models are not relevant. Worldwide, the International Conference on Harmonization (ICH) guidelines for testing pharmaceuticals are followed. Similar guidelines and regulations from the U.S. Environmental Protection Agency (EPA) and the European Organization for Economic Cooperation and Development (OECD) govern the testing for potential exposures to chemicals in the environment.

The studies that follow these ICH guidelines are intended to test the potential for adverse effects from pre-conceptional exposure through exposure *via* the milk in newborn animals. Developmental toxicity is evaluated in two of these studies. The first is the embryo-fetal toxicity study, which exposes pregnant female animals during the period of organogenesis, typically defined as the period from implantation to palate closure. Rats are dosed on gestational days 6–17 and the fetuses are examined on day 21, just prior to term. Rabbits are dosed on gestational days 7–19 and the fetuses are examined on gestational days 29 or 30. Fetuses are examined for external malformations and internal malformations of the organs
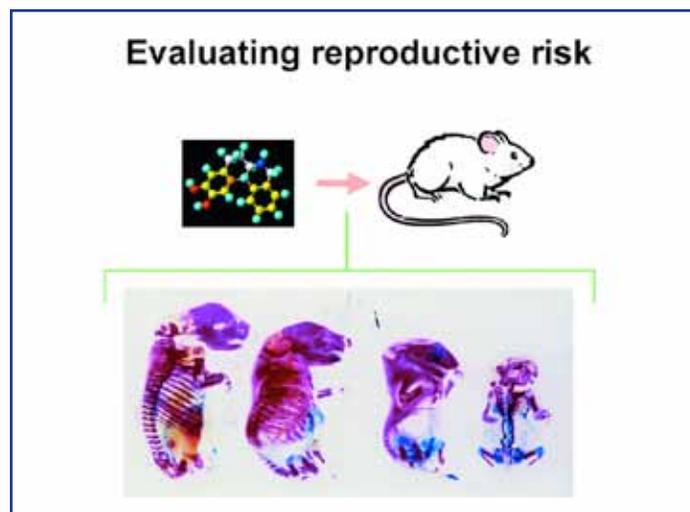
Graphic courtesy of Barbara F. Hales.

(viscera) and skeleton. Growth is evaluated by body weight and in some cases by crown-rump (body) length. The doses used in these studies are carefully selected to cover a range of concentrations including exposures at or above expected human exposures. Animals are exposed to at least three different doses and outcomes are compared with a control group, exposed only to water or another inactive vehicle. The agent is tested over a range of doses up to a dose that stresses the system by producing some degree of maternal toxicity. The highest dose of the agent is usually chosen as one that will produce maternal toxicity, for example, a small decrease in pregnancy weight gain. The lowest dose of the agent is one that is close to the anticipated human exposure level. Middle doses are chosen between these two levels. The lowest dose of the agent that produces abnormal development is called the LOAEL (lowest observed adverse effect level). The next lower dose is called the NOAEL (no observed adverse effect level). These levels can be compared to the anticipated human exposure level. In general (in the absence of other modifying information about the toxicity of the agent), if the anticipated human exposure level is 100 times lower than the NOAEL, adverse effects on human development are considered unlikely. Some researchers believe that this 100-fold "safety margin" is unnecessarily

high, and that much smaller margins, perhaps between 4-fold and 100-fold, would be just as protective. In instances where no abnormal effects on the offspring, even when the agent is given at maternally toxic levels, the evidence suggests that abnormal effects on human development are unlikely. Any testing scheme, however, cannot categorically define a medicinal product, as safe or unsafe because this expectation ignores the importance of the exposure level in determining toxicity. A chemical that is toxic at one dose will be nontoxic at another dose.

Embryo-fetal toxicity studies evaluate the potential for structural malformations and developmental delays of the offspring but are not designed to assess effects on function. Another ICH guideline study is used to do this. The pre- and post-natal study covers exposure during both the prenatal and early postnatal stages of development. Rats are dosed from gestational day 6 (pre-natal) through lactation day 21, the day of weaning (post-natal). Physical endpoints used in developmental toxicity studies are examined in this study, but additional tests are done as well. Body weight changes in pups are measured to evaluate growth. Offspring are observed for the achievement of functional developmental landmarks, including the development of the air righting reflex (the ability for the pup to land on its feet). Vision and hearing are tested, and water mazes and other tests evaluate learning and memory. Once the animals attain sexual maturity, functional reproductive ability is evaluated by mating the offspring. The dams are observed to evaluate the potential impact of exposure on parturition and the ability to care for her young during the lactation period.

The data from all of these studies form the basis for assessing risk during pregnancy. Data from animal models are not absolutely predictive of human risk but do provide a good assessment of potential problems. The ability of a drug to cause developmental toxicity is usually related to the concentration in blood and tissues. Concentrations that cause developmental toxicity in one species will usually cause developmental toxicity in other species, although the malformations may be different across species. Study outcomes include death or malformations (cleft lip/palate, spina bifida, etc.), but it is much more common for studies to indicate effects on overall development, such as lower fetal body weights, delayed skeletal ossification, or delayed maturation of the kidneys or other organs.

Completion of these studies is only half the task; appropriate interpretation of these data is key and should be done by scientists trained in the concepts and principles of teratology. It is extremely important to factor in maternal toxicity when interpreting findings, because illness or stress in a dam will affect her pups (chapter 24). Malformations or
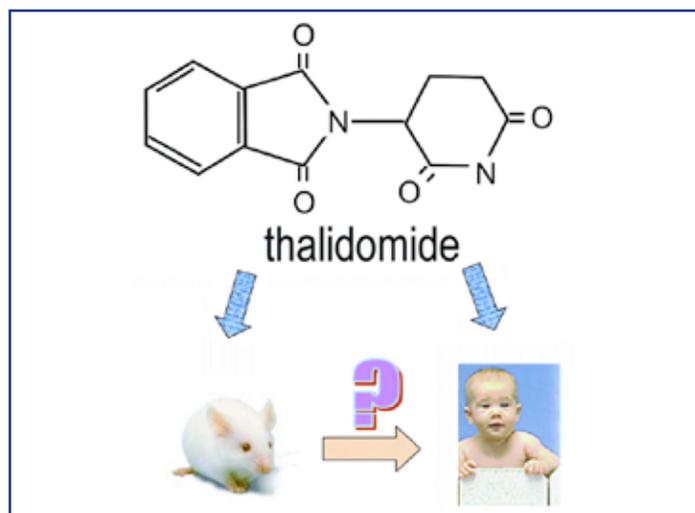
Graphic courtesy of Anthony R. Scialli.

significantly delayed fetal development without any effects on the dam may indicate a direct effect of the drug on the fetus. It is also important to know the baseline incidence of specific malformations in animal models to determine the effects of an agent over the spontaneous background risk. Data are also reviewed to determine if there is a dose-response relationship, meaning that the incidence and severity of adverse outcome increases with increasing dose. Assessing patterns is important in assessing risk. A study that shows no effect of low and high doses, but shows an effect at a middle dose, is less convincing than a study that relates increasing risk to increasing dose. Understanding the pharmacologic action of the drug and the mechanisms for toxicity are also important. If, for example, the malformations observed are unique to the species tested, then the data may not be relevant to human risk. Finally, risks must be weighed against benefits. A drug that is a member of a class known to be teratogenic in animal experiments would carry a warning against use in pregnancy, but in a pregnant woman with a chronic disease, it may be important to use the drug in spite of the possible risk. In these circumstances, testing remains an important part of the risk evaluation to characterize the margin of safety for humans. To date, there has not been a single example of a chemical that produces harm to the developing human without producing adverse effects on development in rats or rabbits at doses high enough to produce maternal toxicity. In other words, human embryos and fetuses are not uniquely sensitive provided that the agent has been tested at sufficiently high doses in experimental animals. The converse is not true: if a drug or chemical produces toxicity in experimental animal studies, it is not necessarily a risk at typical human exposure levels. There are many drugs and chemicals that produce abnormal development at the

high doses used in experimental animal studies but not at the exposure levels encountered by humans. Experimental animal testing is designed to be conservative for use of drugs in pregnancy.

While human data on developmental toxicity is increasingly included in drug labels, the majority of the drugs approved for use in the United States still have no human data in the pregnancy section of their label. For most drugs, clinical trials are not conducted in pregnant women. Consequently, we must rely on animal data to understand potential risk for both newly approved drugs and existing drugs. The pregnancy section of the drug label is often the major source for the information used by the clinician in estimating risk (chapter 10). Developmental toxicity studies in animals provide informative but complex data for assessing potential risks of drug use in human pregnancy. Extrapolation of information from reproductive toxicology studies to humans requires more data than is normally available in the product label. Animal studies are designed to elicit adverse events in order to provide a margin of safety for human use. Individual factors, including genetic background and the risks of the underlying disease being treated are weighed in order to make rational decisions about the use of any drug during pregnancy.

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Can *In Vitro* Methods Contribute to the Identification of Teratogenic Exposures?

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Teratogenic effects range from embryo death to malformations, from growth problems to long-term functional impairments such as mental retardation. The advantage of experimental animal tests in teratogenicity risk assessment is that the pregnant animal comprises all of the potential endpoints for adverse effects of an exposure—i.e., the mother, placenta and embryo. So, how can *in vitro* methods contribute to the identification of teratogenic exposures?

All *in vitro* teratogenicity tests have a biological component that can undergo differentiation or organogenesis *in vitro*. This biological component could be cultured cells, an organ, or a whole embryo. Each of these levels of complexity has both advantages and disadvantages. The European Centre for the Validation of Alternative Methods (ECVAM; http://ecvam.jrc.it/) has led an initiative to establish and validate *in vitro* tests for embryotoxicity. The ideal *in vitro* teratogenicity test should be rapid, reproducible, inexpensive, technically easy to perform, and not involve experimentation on animals. Cell culture involves few animals, especially if the cells have been maintained in culture over many generations as in established cell lines. A whole organ or embryo culture system, however, is more likely to involve all the processes in development that are likely to be susceptible to interference after a teratogenic exposure. No one system is perfect for every context, and a combination of several tests may be recommended. The advantage of *in vitro* testing is that exposure can be controlled very precisely. Species differences in how a chemical is metabolized can be overcome by adding the metabolite or an appropriate metabolizing system.

This chapter will focus on three mammalian embryotoxicity test systems: the culture of embryonic stem cells, limb bud or limb bud micromass cultures, and whole embryo cultures. Many signalling pathways that control embryo development occur across species, so non-mammalian systems, using embryos of zebrafish, for example, may also have potential to evaluate teratogenic risk.

Embryonic Stem Cells

Embryonic stem cells are pluripotent cells, capable of differentiating into a wide variety of cell types, that are derived from the blastocyst inner cell mass (Figure 15-1). In the embryonic stem cell test, the effects of a test chemical
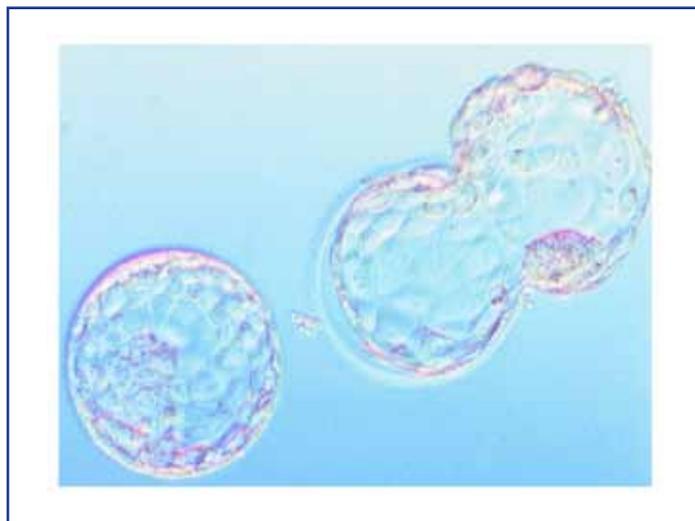
Figure 15-1. *The embryonic stem cell test evaluates the embryotoxicity of a chemical on the basis of its ability to inhibit embryonic stem cell differentiation and its cytotoxicity to embryonic relative to adult cells.*

on a mouse embryonic stem cell line (D3) are compared to those on adult fibroblast 3T3 cells. The endpoints measured are the ability of the chemical to inhibit growth of the embryonic stem cells or their differentiation into cardiac myocytes (muscle cells), for example, compared to their effect on adult 3T3 cells. Thus, this test compares the effects of a test chemical on embryonic cells relative to adult cells, and also looks at the ability of the chemical to inhibit differentiation. The use of established cell lines means that additional vertebrate animals are not needed.

Limb Bud or Limb Bud Micromass Cultures

In the limb bud culture system (Figure 15-2), fetal limbs, usually from mice, are removed on day 11 or 12 of gestation and cultured for 6 to 9 days, with or without the agent under study. During culture the limbs undergo extensive changes in morphology (size and shape), and biochemical differentiation, which can be assessed quantitatively. Changes in size and shape can be measured using a scoring system or image analysis. Measurable biochemical changes include DNA, RNA, and protein content as well as creatine phosphokinase (related to muscle development) or alkaline phosphatase (involved in pre-bone formation).
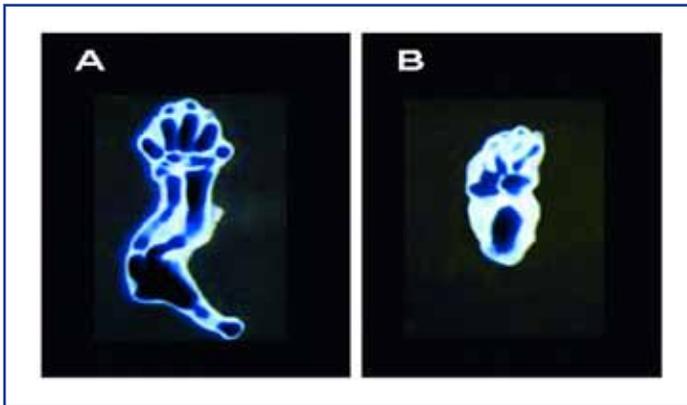
Figure 15-2. *Limb buds removed from mouse embryos on day 12 of gestation were cultured for six days in a roller bottle system with chemically defined medium in the absence (A) or presence (B) of retinol acetate (10IU/ml) and stained with toluidine blue.*

Figure 15-3. *In the whole embryo culture system gestation day 10.5 rat embryos (A) are cultured in a roller bottle system in the presence of rat serum for up to two days (B). In (C) compare the control embryo on the left to the embryo exposed* in vitro *to a DNA damaging agent, 4-hydroperoxycyclophosphamide, on the right.*

The limb bud micromass culture system is based on the ability of undifferentiated limb bud mesenchyme cells to form foci, or clusters, of differentiating chondrocytes (cartilage cells). Cell proliferation, differentiation, and interactions between cells, or between cells and the extracellular matrix, are all implicated in this process. Limb buds are isolated from day 14 rat embryos, and made into a cell suspension, which is placed into wells (plates or trays dotted with small round indentations). The cells are incubated for 5 days with medium and a test chemical; control wells contain only medium. The total number of viable cells, differentiated cells and foci are determined. Chemicals that reduce the number of foci, or the number of cells within foci, are considered potentially problematic.

Whole Embryo Cultures

In the mammalian whole embryo culture system (Figure 15-3), rat or mouse embryos with intact yolk sacs are removed during early organogenesis and cultured in rat serum. During culture, the embryos undergo tremendous growth and differentiation, advancing from the early somite stage to 30 to 45 somites by the end. The embryos turn and rotate, the neural tube closes, and the brain, heart and other organs begin to develop. The effect of chemicals on the number of dead and live embryos and the number of malformed versus normal embryos is noted, but many other measurements are taken as well. Effects on yolk sac diameter, crown-rump length, head length, and number of somite pairs are scored. Biochemical criteria, including DNA content, gene expression, protein content, and hemoglobin,

are also usually assessed. Malformations induced in intact animal models by cadmium, retinoic acid, and hydroxyurea cause very similar effects in whole embryo culture, although adding chemicals directly to an embryo's environment may not replicate the effects of maternal exposure to and metabolism of an agent.

Use of Non-Mammalian Embryos in a Screening Assay

Embryogenesis in the zebrafish (*Danio rerio*) is very similar to that of higher vertebrates. Zebrafish females can lay hundreds of eggs per week in the lab. The shell of their eggs is transparent, making them easy to study. The embryos development very rapidly, in just a few days, and are easy to handle. Thus, it is not surprising that screening assays using zebrafish embryos are attractive as an alternative method for the analysis of developmental toxicity.

Role of *In Vitro* Methods or Non-Mammalian Embryos in the Identification of Teratogenic Exposures

The ECVAM international validation study concluded that the whole embryo culture test, the micromass test on limb bud cells, and the embryonic stem cell test were scientifically validated and acceptable tests for regulatory use. Test chemicals were classified correctly at a rate that often exceeded 70%; for agents that were strongly toxic to the embryo, these tests were almost 100% predictive. The ECVAM Scientific Advisory Committee recognized that these methods could not replace intact animal tests for assessing reproductive toxicity, but concluded that they could provide suitable means for reducing and refining the use of animal

procedures. Thus, *in vitro* tests have a role in screening chemicals, but are not viewed as replacements for *in vivo* whole animal developmental toxicity tests.

Are there instances in which *in vitro* tests or non-mammalian embryos are more useful than current *in vivo* mammalian animal tests in identifying teratogenic exposures? Scientists have begun to acquire new information about the genes that regulate developmental events, and about the chemical messenger systems involved in establishing spatial information or position in the early embryo. *In vitro* systems will be particularly useful in investigating the role of a specific gene or gene family during organogenesis. Transgenic animal (one whose genome contains gene(s) from another species) approaches are laborious and frustrating, especially because sometimes deleting the gene of interest kills the embryo. *In vitro* approaches may help to determine the role of specific signaling molecules or pathways.

The National Research Council vision, described in "Toxicity Testing in the 21st Century" is the future of toxicity testing will be built on the use of cell-based assays and the knowledge of pathways by which cells respond to toxicants. Moving from effects of a chemical on the behavior of cells in a culture dish to predicting the ability of this substance to increase the incidence of birth defects *in vivo*, in animals or humans, is quite a challenge. In the mean time, it is likely that *in vitro* assays will complement but not completely replace *in vivo* animal testing. These assays can be valuable in elucidating mechanisms by which teratogenic exposures affect development, and may be particularly useful in testing chemicals that are metabolized in a species-specific manner. *In vitro* tests should be useful as screening tests to winnow compounds for further research and development.

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How Might Systems Biology Contribute to the Prediction of Teratogenic Risk?

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Development is a collection of interacting and dynamic processes that form an embryo, fetus, and ultimately a child. Much of what we understand has come about through study of isolated individual events occurring at an organ, cellular, or molecular level but in the dynamic events such as those that form an embryo, fetus, and ultimately a child, the interplay of individual events must also be understood. Systems biology seeks to study the relationships and interactions between various parts of a biological system (metabolic pathways, organelles, cells). This approach can contribute to our understanding of normal development and how it may be perturbed by a teratogenic exposure.

Teratologists think as systems biologists, either consciously or intuitively. In order to understand development, the conceptus is thought of as the maternal-child unit from the very start of life. While this approach is not unique to teratologists as many systems biologists and engineers model and understand processes as a whole and thereby optimize their understanding of dynamic systems by looking at interacting processes leading to an end state (developmental maturity), it is unique that teratologists have developed both disciplinary and scientific approaches that allow for such an integrated examination of normal and altered development.

Early embryologists used hierarchical and temporal approaches to understand the origin of tissues and cells. For example, as the developing organism moves from the blastula to gastrula to neurula stages, or when organs form from three tissues, **ectoderm** (neural plate, neural crest and epidermis), **mesoderm** (dorsal –cephalic and trunk notochord and somites, ventral-blood islands and lateral plate organs including heart and kidney), and **endoderm** (yolk cells and alimentary canal organs such as lungs, liver, and stomach). Such tissue hierarchies are highly relevant for predicting impacts across species.

Systems biology provides a framework to follow the interconnectedness and dependencies of the different processes of development. Recent research has emphasized the importance of using cell, organ, and embryo cultures to understand the details of tissue and cell interactions; however, only by looking at how these interactions build upon levels of biological complexity, moving from genetic and epigenetic, molecular, cellular, multicellular, tissues, organs, organ systems, to whole organisms, can we understand overall development. There is genomic conservation so observations

made at these levels are highly conserved and relevant for similar levels across species. Examples include the relatively few (17) cell signaling pathways that have been characterized in all bilateral organisms that are able to explain most of development. For example, hedgehog signaling pathways, present in both vertebrate and Drosophila development, direct spermatogenesis in vertebrates and oogenesis in Drosophila. Hence, there is both a conserved but also a species-specific component that requires a systems approach in order to interpret impacts.

Recent advances in computational approaches have allowed systems biologists to become increasingly sophisticated in their ability to quantify impacts at one level for outcomes observed at more complex levels, birth and functional development (chapter 18). In particular, such computational approaches have shown promise for answering more detailed questions about mode of action for teratogenic exposures, for improving cross-species extrapolation, for quantitative structure activity relationships, and for improving our understanding of gene-environmental interactions and responses. A systems biology approach also allows for evaluation across levels of potential biological observation at the molecular, cellular, organ, conceptus, or population level and can allow for better extrapolation across biological levels of observation. There has been tremendous progress made in use of cell systems and organ culture to examine various effects on development. Linking the knowledge about the toxicokinetics and dynamics of chemical impacts has allowed for better prediction of potential for impacts at the organism level.

An important implication of a systems biology approach is that in order to understand normal as well as altered development, teratologists are needed from diverse scientific and clinical disciplines. Clinicians such as obstetricians who are teratologists follow the course of pregnancy and may see birth defects early in gestation using ultrasound imaging. Dysmorphologists are trained to look at developmental processes and to diagnose syndromes and alterations in development that represent deviations from such processes resulting in malformations. Developmental biologists study details on the mechanisms of organ and tissue development. Molecular biologists look at comparable cellular and molecular processes in order to follow alterations that result in birth defects. Developmental

toxicologists and pharmacologists study how chemicals or drugs can alter normal development and cause birth defects and developmental toxicity. An integration of knowledge from these many disciplines using the principles of systems biology will speed the understanding of teratogenic risk and an increased ability to minimize or prevent birth defects.

In order to properly understand the big picture, everyone should fear becoming mentally clouded and obsessed with one small section of truth.
Xun Zi

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Can Chemical Structures Predict Teratogenic Risk?

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The structure of a chemical determines its size, shape and physiochemical properties. These characteristics in turn influence the effect of a chemical on biological systems including developing organisms. Going from this assertion to the description of chemical characteristics that influence developmental toxicity represents a challenge in computational toxicology, a subset of computational biology. However, because of the substantial strides in chemistry, computer sciences, biostatistics, mathematics, and developmental biology it is now possible to imagine that the structure of a chemical will allow us to predict its potential developmental toxicity (chemoinformatics). These areas of research offer exciting opportunities for the young scientist to change the way we think about developmental toxicology and may allow us to predict drugs and chemicals that can produce birth defects.

Chemists and biologists began exploring the relationship between biological activity and chemical properties more than 150 years ago, observing that the toxicity of chemicals was related to their lipid solubility. Within a group of chemicals, physicochemical parameters such as solubility and structural features like the presence of nitrogen or oxygen can be used to explore relationships with biological activity such as developmental toxicity. Because our understanding of (Quantitative) Structure Activity Relationships ((Q)SAR) has grown, the pharmaceutical industry uses (Q)SAR models in drug discovery and in the design of new medicines. Regulatory agencies around the world have also invested in the development of (Q)SAR resources such as datasets of chemicals and biological activity and (Q)SAR tools allowing scientists to explore the relationships between chemical characteristics and biological responses.

Chemicals can be characterized by size, shape and physicochemical properties as well as structural attributes. Physicochemical parameters are classified as electronic, hydrophobic, or steric. Electronic parameters describe a molecule's potential for engaging in interactions with other molecules. Chemical reactions are an example of this kind of intermolecular interaction. Hydrophobicity (water avoidance) is a description of where the compound can be found when it distributes between water or the more oil-friendly n-octanol. Hydrophobicity is important in both
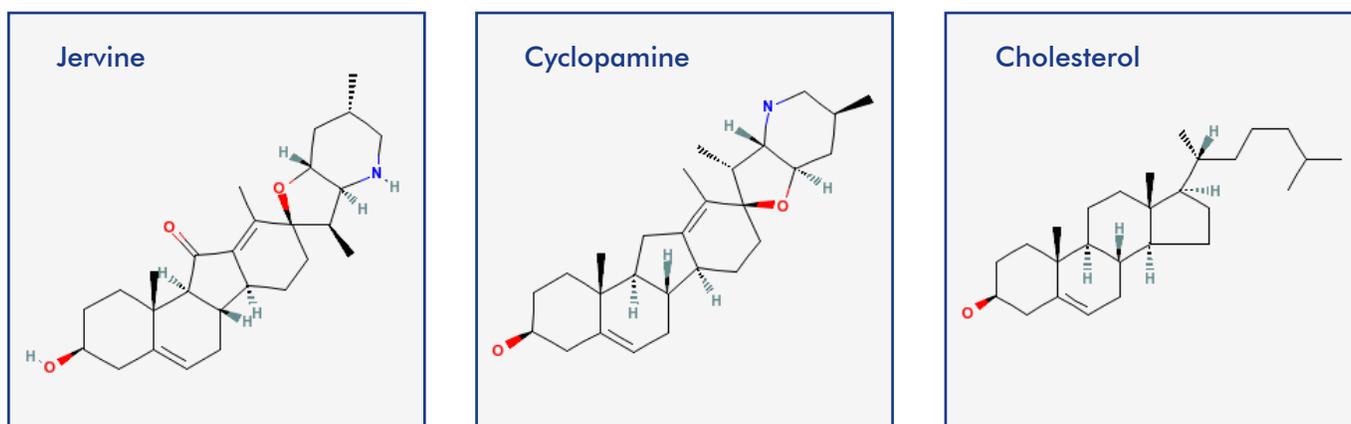
Figure 17-1. *Convergence between chemoinformatics and bioinformatics (chapter 19) allows us to better understand how chemicals produce developmental toxicity. One example of this was an observation in the 1950's that sheep grazed on the field lilly* Veratrum californicum *during early gestation (gestation days 10–15, normal gestation is ~150 days) had a higher risk of delivering a lamb with cyclopia and holoprosencephaly. Ingestion of* Veratrum californicum *prior to day 10 had no effect on embryonic development and intake after day 15 produced fetal death and limb malformations. The molecules which produce these malformations have structural similarity to cholesterol, suggesting that jervine and cyclopamine might alter cholesterol concentrations during embryonic development, producing the malformation. The potency to produce malformations is jervine ~100, cyclopamine ~50 and cholesterol ~0. Research has demonstrated that jervine and cyclopamine interact with Sonic Hedgehog protein altering its ability to interact with the Patched receptor and change the critical gradients necessary for normal brain and eye formation.*

drug development and toxicology because it may correlate with how well a compound is absorbed and transported to its site of action. Steric parameters include molecular volume and surface area, measures of a molecule's "bulkiness" that may reflect size requirements at a receptor site or target molecule. Chemicals that have similar steric parameters may activate the same receptor. Many size, shape, and physicochemical properties have been characterized for chemicals that are thought to cause human developmental toxicity at commonly encountered exposure levels.

Chemicals can also be described by their structure, for example the number and type of atoms and the way they are connected. In analysis of chemical structures and developmental toxicity, the chemical is "fragmented" electronically into contiguous heavy atoms (atoms with molecular weights greater than hydrogen) from 2 to 10 atoms in length and the distribution of those fragments among the chemicals is analyzed to determine if they appear more frequently in chemicals with that act as toxicants. Within the structure of a chemical there are fragments of connected atoms that may be predictive of the biological activity of the chemical. Other fragments may simply reflect scaffolding for the chemical, atoms contributing to the structure but not responsible for the biological activity. Chemical structure evaluation has been used in analysis and prediction of chemicals thought to alter endocrine function in developing or adult animals as well as for chemicals that produce developmental toxicity at some exposure level.

Investigators using either physicochemical or structural features to explore developmental toxicity need to be extremely careful in constructing datasets and performing (Q)SAR analysis. This is especially critical as the mechanism or mode of action resulting in developmental toxicity following chemical exposure is not always well understood. In developing datasets for (Q)SAR analysis clear definition of the testing systems and developmental endpoints utilized (death, structural abnormality, functional abnormality or altered growth) is critical to allow other investigators the ability to analyze data and explore utility of the developed models. The criteria for assessment of the literature to create the developmental toxicity assessment must be explicitly described and the way the data in the dataset are utilized to make a prediction must be transparent.

The (Q)SAR validation approach starts with the assumption that chemicals can be characterized as toxic or nontoxic, either categorically or quantitatively. The principles and techniques of (Q)SAR have been applied to developmental toxicology. Several different chemical-biological relationships have been explored for chemicals with similar structures, including phenols, retinoids, aliphatic, heteroaromatic and carboaromatics, carboxylic acids, and bromochloro-haloacetic acids. Using a list of chemicals identified as developmental toxicants or nontoxicants in rats, mice, rabbits, and humans, SAR models correctly predicted results for 77–82% of randomly constructed test sets. Species-specific differences were observed for some structurally similar fragments. Carboxylic acid esters (e.g., $COO-CH_3$ and $COO-CH_{2-}$) for example, contributed to developmental toxicity in rats and mice, presumably by hydrolysis to the carboxylic acid. In humans, however, fragments referring to carboxylic acid esters were found to be benign in terms of developmental toxicity.

(Q)SAR will never entirely replace research using animal models, and it remains to be seen whether these approaches can be reliable enough to have a place in assessing the developmental risk of new or unknown agents. There is exciting potential for further computational investigations of the relationship among chemical structure, physicochemical properties, and developmental toxicity endpoints. In the future, (Q)SAR will be an important tool for (a) achieving insights into mechanisms, (b) rapid and economical screening of compounds for hazard identification, (c) prioritizing chemicals for regulatory, research, and remedial actions, and (d) establishing guidelines for the design of industrial chemicals and pharmaceutical agents with minimal potential for human developmental toxicity.

Suggested Readings

U.S. EPA National Center for Computational Toxicology
http://epa.gov/ncct/index.html

This site provides resources relevant to computational toxicology within the U.S. EPA, including datasets which can be used in research. Also available at the EPA Web site is a tool for ecological structure activity analysis (EPISuite) which also calculates many of the physiochemical properties of chemicals (http://www.epa.gov/oppt/newchems/tools/21ecosar.htm).

Chemoinformatics.org http://www.cheminformatics.org/
This site provides links to sites providing datasets and programs for QSAR. The datasets are described and literature citations describing documentation are provided.

National Cancer Institute resources. http://129.43.27.140/ncidb2/
This site provides access to more than 250,000 chemical structures and resources for predicting various types of biological activity.

European Commission, Joint Research Centre, Computational Toxicology http://ecb.jrc.ec.europa.eu/qsar/

The European Commission has invested in development of resources for (Q)SAR analysis and those resources are available for downloading at this site. One of the links in this site is to the Danish Ministry of the Environment QSAR web site where toxicology predictions on more than 160,000 chemicals by more than 70 (Q)SAR models are available (http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=DDB).

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Can Computational Models Be Used to Assess the Developmental Toxicity of Environmental Exposures?

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Prenatal Vulnerability

Environmental causes of birth defects include maternal exposure to drugs, chemicals, or physical agents. Environmental factors account for an estimated 3–7% of birth defects although a broader contribution is likely based on the mother's general health status and genetic blueprint (chapter 6). This chapter will focus on 'environmental chemicals' are natural and man-made compounds to which human populations are continually exposed as a matter of individual lifestyle, local geography and community life. The Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) samples the U.S. population every two years to determine which chemicals get into people and at what concentrations [CDC, 2009]. NHANES measured 212 environmental chemicals in blood or urine. The National Children's Study (NCS) which began its pilot program in 2009 will examine the effects of broad environmental influences on the health and development of 100,000 children across the United States, following these kids from before birth until age 21 [http://www.nationalchildrensstudy.gov]. These 'biomonitoring programs' will help identify vulnerable populations and guide further assessment of chemical exposure sources.

Yet, we are all exposed to many more chemicals daily. We also lack developmental effects data for most of the chemical landscape. The U.S. Environmental Protection Agency's (EPA) Aggregated Toxicology Resource (ACToR) database indicates that developmental effects data is available for less than ~30% of 9,912 chemicals in commerce or relevant environmental interest. EPA's Toxicity Reference Database (ToxRefDB) reveals potential developmental toxicity for 53 of 283 (18.7%) environmental chemicals tested in pregnant rats or rabbits. It is clear that a new approach is needed if we are to be able to assess the potential for developmental toxicity of the remaining 70% of the chemicals of interest today. And there are more chemicals synthesized on a daily basis.

Biological Susceptibility

To understand developmental susceptibility, teratologists use "biological models" to extrapolate data from high-dose effects in animal studies to low-dose predictions in human populations. Two general kinds of predictive models are kinetic models and dynamic models.

Kinetic models predict what happens to the chemical once it enters the body: **A**bsorption following oral intake, breathing dust or contact with the skin; **D**istribution and partitioning to different body compartments; **M**etabolism to chemical forms that are more or less biologically active; and **E**xcretion from the body. **ADME** factors vary by individual, species, and chemical based on the particular xenobiotic (foreign chemical) metabolizing enzymes in the organ. Kinetic models can predict internal dose to a human embryo when the mother is exposed to low concentrations of the chemical for prolonged periods of time. Such information is necessary to determine the range of exposures that may be relevant for potential human toxicity. These models do not, however, address mechanisms.

Dynamic models predict what happens once a chemical or active metabolite reaches the target tissue. Because the embryo is a dynamic system, environmental chemicals may disrupt the timing of critical events in morphogenesis, growth, and differentiation. For example, cyclopamine, the natural plant product consumed by foraging sheep, blocks cholesterol esterification of the sonic hedgehog protein (SHH), disrupting forebrain development and in turn leading to formation of a single midline eye (cyclopia). In attempting to analyze a complex system like the embryo, teratologists face the challenge of untangling the pathway leading to a birth defect such as cyclopia into its primary initiating mechanism, a perturbation in the SHH pathway, from the myriad of secondary consequences leading to a birth defect. This information may be more readily available for drugs that have been designed with a specific therapeutic target

in mind than for environmental chemicals to which human exposure is unintended.

Can *in vivo* databases that track exposure-disease correlations, as well newer technologies that permit 'high-throughput screening' of the molecular and cellular pathways of toxicity help to fill in our knowledge gap with respect to the potential developmental toxicity of environmental chemicals?

High-Throughput Screening (HTS)

In 2007, the National Academy of Sciences recommended an HTS paradigm to understand human toxicology of large numbers of chemicals. Automated technologies, originally developed for pharmaceutical screens, are now being used to profile cellular effects of thousands of chemical compounds in commerce or potentially entering the environment. In an HTS drug development paradigm, chemical libraries containing thousands of unique structures may be rapidly screened for target biological activity against specific cell lines in a high-throughput mode (Figure 18-1). Active compounds may be used as leads to design compounds that would have the desired *in vivo* efficacy; they are then evaluated in animal tests, clinical trials, and post-marketing surveillance. For example, the National Chemical Genomics Center (NCGC) of the NIH has the capacity for automated HTS of 100-200K compound libraries across tens of *in vitro* assays.

Applying the pharmaceutical HTS paradigm to toxicity testing turns the drug discovery process upside down (Figure 18-1). Compounds of unknown or suspected human toxicity are tested through hundreds of *in vitro* assays. Computers are then used to look for patterns of biological activity across the assay portfolio and chemical library. These profiles may be compared with reference compounds of well-characterized biological activity or interrogated in different ways, looking for *in vitro* patterns of signals that are potentially diagnostic of *in vivo* toxicities. EPA's ToxCast™ project is providing HTS data on 309 chemicals to examine predictiveness of 467 assays and data for ~1408 chemicals to evaluate potential toxicities based on weight-of-evidence. The broader "Toxicity Testing for the 21st century" (Tox21c) federal consortium is ramping up to test ~10,000 chemicals of importance to commerce and the environment [http://www.epa.gov/ncct/toxcast/]. Alternative assays, such as embryonic stem cells and free-living zebrafish embryos, can be used to rapidly test chemical effects in systems undergoing morphogenesis, growth, and differentiation [http://www.alttox.org/].

Computational (*In Silico*) Models

Several challenges face this type of predictive modeling. These challenges cycle back to a need for computational models to merge high-throughput screening data with the vast literature on embryonic development. How does *in vitro* concentration-response correlate with internal dose-response kinetics, how do *in vitro* bioactivity profiles extrapolate from one cell-type or technology platform to another or how do individual targets of *in vitro* bioactivity link into pathways of developmental toxicity? A deep understanding of embryological development, intuition for new technologies and approaches to the study of birth defects, and a concern for environmental influences on human development are all driving the new vision on where environmental health protection needs to go.

Whereas numerous models have been developed for cell signaling pathways in the embryo, fewer models exist for understanding how chemical lesions are propagated during developmental toxicity. Computational (*in silico*) models that execute a morphogenetic series of events may help in this regard, bridging the gap between *in vitro* profiling and *in vivo* response at different concentrations of chemical. The general idea is to model the cell of an embryonic tissue as an agent, i.e. the smallest fundamental unit capable of an autonomous decision.

Figure 18-1. *Paradigm for pharmaceutical HTS and toxicity testing.*

Row labels: **drug development** / **toxicity testing**

Top row: target identification → HTP *in vitro* screening → design actives → animal tests efficacy, safety → clinical trials surveillance

Bottom row: targeted tests (verification) ← computer models ← target identification ← HTP *in vitro* screening ← unknown toxicity (or suspected)

Individual agents and their interactions are coded into the model based on biological knowledge. The multicellular simulation is run and the emergent properties are recorded. These agent-based models can simulate the potential effects of environmental chemicals based on various inputs. The models can be exercised across conditions not practical experimentally due to cost, time, scale, or complexity. EPA's Virtual Embryo will build computational (*in silico*) models that may make high-throughput screening data useful in a quantitative risk assessment of developmental toxicity [http://www.epa.gov/ncct/v-Embryo/]. So, to return to the original question, "Can computational models be used to assess the developmental toxicity of environmental exposures?" the answer may be not yet but the future is open. Much effort is bring expended today to realize this goal.

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How Can We Use Bioinformatics to Predict Which Agents Will Cause Birth Defects?

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The availability of genomic sequences from a growing number of human and model organisms has provided an explosion of data, information, and knowledge regarding biological systems and disease processes. High-throughput technologies such as DNA and protein microarray biochips are now standard tools for probing the cellular state and determining important cellular behaviors at the genomic/proteomic levels. While these newer technologies are beginning to provide important information on cellular reactions to toxicant exposure (toxicogenomics), a major challenge that remains is the formulation of a strategy to integrate transcript, protein, metabolite, and toxicity data. This integration will require new concepts and tools in bioinformatics.

Bioinformatics applies principles of information sciences and technologies to make vast, diverse, and complex life sciences data more understandable. The applications of bioinformatics approaches may be particularly challenging in the embryo because of the complexity of the data, particularly with regards to the relative plasticity of precursor target cell populations in the embryo and the realization that morphogenetic processes have critical timing for inductive events and for regulative growth. No single technology is likely to resolve the complex problem of teratogenesis. Teratologists will need to maximize the amount and quality of input data from both experimental models and human epidemiological studies. High-throughput genomics has made many advances but cannot yet predict toxicity when the experimental parameters are unknown.

Bridging Gaps

Data collected from research must be amenable to high performance computing. As emerging technologies continue to provide tools for comprehensive studies of large-scale gene expression, they will continue to raise key issues. Foremost of these is the storage and access to vast digital information, the annotation of large-scale gene expression profiles, the interpretation of the causal and functional relationships between genes upwardly or downwardly regulated, and the ability to uncover biomolecular pathways across species in the context of disease. Bridging the gap between transcriptome regulation (all mRNA molecules-transcripts- produced in a cell) and cellular function, for example, demands high-resolution data on cell function, gene promoter informatics, post-translational protein modifications, and biophysical interactions. Biologically significant changes at these information levels may go undetected by gene expression profiling alone and therefore must be integrated across the different technology platforms. Teratologists will increasingly learn to mine public data (knowledge) bases using bioinformatics tools that can deliver concept-driven outputs from data-driven inputs.

International efforts are underway to build a high quality public knowledge base for toxicogenomics, referred to as the Chemical Effects in Biological Systems (CEBS) database. Partners in the design and construction of this database include the National Center for Toxicogenomics and National Toxicology Program (NIH/NIEHS), the European Bioinformatics Institute, and the International Life Sciences Institute and Health and Environmental Sciences Institute. CEBS will provide an on-line repository for relevant data from molecular expression and conventional toxicology studies. By assimilating and refining information from multiple public databases and scientific literature, the knowledge base will provide a comprehensive resource on genes, gene clusters, pathways, toxicities, and diseases.

Modeling frameworks represent the basis for computational algorithms that ultimately allow for data-driven simulations. For such predictive models to be effective, they must link adverse developmental outcomes to user-specified exposure scenarios, or vice-versa, through a cascade of biomolecular and physiological changes resulting from the interaction of chemicals with critical molecular targets in the embryo. We do not yet know whether mathematical models can accurately describe developmental processes and toxicities. The initial step is to discern unique molecular signature profiles to improve connections between input (exposure) and output (disease) endpoints as "complex systems" in the exposure-disease continuum. In nature, the organization of complex cellular systems requires fundamental inputs of energy, robustness, and control. Understanding this is at the cutting edge for addressing key questions in biology and medicine.

Systems biology is defined broadly as the application of systems theory to biological problems, where systems theory refers to the analysis of composite systems that may divided

into subsystems to facilitate understanding and modeling (chapter 16). Central issues deal with the identification of component subsystems (modules), characterization of subsystem behavior (responses), and determination as to how different components are connected (networks) and how they relate to observable behavior of the system as a whole (controls). Mathematical models allow for the characterization of these subsystems. It remains to be determined whether this engineering-based approach will allow us to simulate mammalian development and mathematically characterize the adverse outcome scenarios in response to different environmental stressors. In moving toward the realm of digital biology there are certain generic questions with which we may begin. How do elements of system structure fit into a hierarchy for control? Given some members of the module, can we use bioinformatics to complete the list and identify pathways that predict the behavior of the altered (stressed) system?

Birth Defects Systems Manager (BDSM)

The 'Birth Defects Systems Manager' (BDSM) is a web-accessible data base designed to facilitate discovery of associations across developmental stages, organ systems, and disease phenotypes. BDSM contains embryo-based, high-content gene expression data from NCBI's Gene Expression Omnibus (GEO). BDSM is being modified to become a machine-readable knowledge base that can supply data to support EPA's new 'Virtual Embryo' project (http://www.epa.gov/ncct/v-embryo). The data query tool, QueryBDSM (Figure 19-1), allows specific queries across experiments to facilitate analysis of developmental genomics data and compare data generated across technology platforms and study types. To demonstrate, BDSM-derived data were used to compare expression profiles across six unrelated studies for developing systems. The analysis was restricted to 160 gene arrays in the BDSM library based on well annotated experiments published on normal mouse embryogenesis using Affymetrix/Agilent technology platforms. A virtual meta-chip was constructed for probes common to all five technology platforms represented in these studies, yielding 346 genes. The biggest limitation in combing different platforms is the small number of genes found in common between these platforms. Using tools available with BSDM, as shown in Figure 19-2, the gene-expression profiles correctly ordered the samples first by organ system and then by developmental sequence within each system.
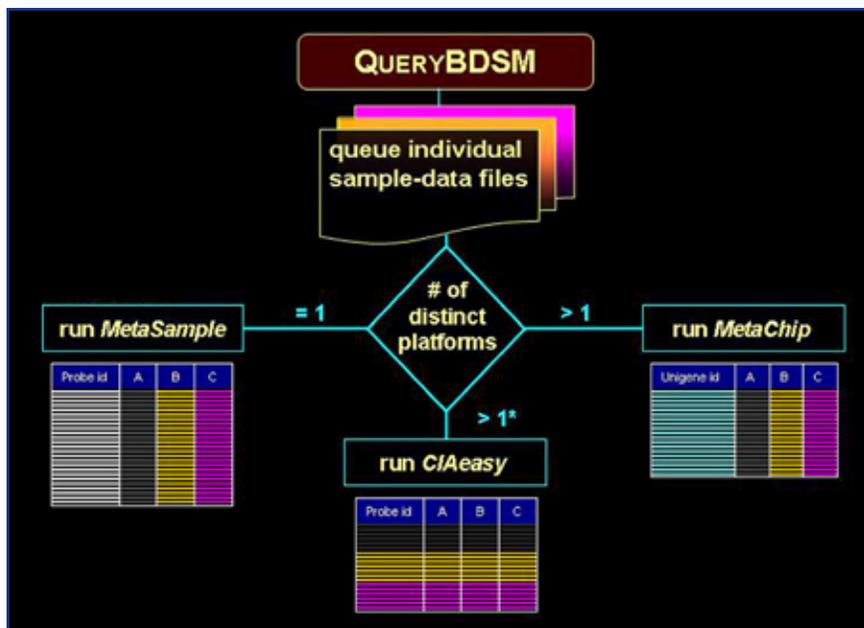
Figure 19-1. *Workflow schema for the QueryBDSM module of BDSM. Individual files of normalized microarray data are selected from the GEO library. QueryBDSM determines the number of distinct microarray platforms in the sample queue and merges the data as follows: if all samples come from the same platform, then MetaSample is used; if multiple platforms are represented, then MetaChip is used. CIAeasy compares joint trends in expression data for the same samples run on different platform [Singh, Knudsen and Knudsen, 2007].*
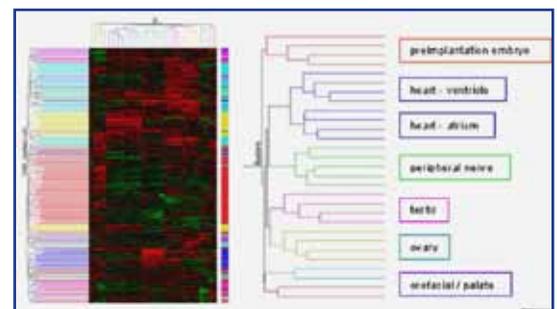
Figure 19-2. *Hierarchy of molecular phenotypes in developing mouse embryos. In five GEO platforms (160 samples), 346 genes were found differentially expressed in BDSM profiles: preimplantation mouse embryo (GSE1749) [16], heart (GSE1479) GD10—GD18 [14] nerve (GSE972) GD9.5—birth [3]; ovary (GSE1359) and testis (GSE1358) GD11.5—birth [13]; orofacial (GSE1624) [9] and palate [2] between GD13-15. Each developing organ system was properly ordered by its natural chronology, based on the 346 gene-expression signature and an unsupervised clustering algorithm with Pearson correlation. Gene colors are mapped by K-means clustering (6 sets) [Singh, Knudsen and Knudsen, 2007].*

Comparative bioinformatics analysis using QueryBDSM provides teratologists a tool to develop hypotheses connecting genetic perturbations to phenotype and enabling comparison to other developmental systems entered into the BDSM database.

Path forward

The U.S. National Library of Medicine's Pubmed site includes 19 million citations and abstracts and continues to grow. The BDSM team is now working on assembling the literature's unstructured data into a structured database and linking it to BDSM within a system that can then be used for testing and generating new hypotheses. This effort will generate data bases of entities (such as genes, proteins, metabolites, gene ontology processes) linked to PubMed identifiers/abstracts and providing information on the relationships between them. The end result will be an online/standalone tool that will help researchers to focus on the papers most relevant to their query and uncover hidden connections and obvious information gaps.

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Is There a Safe Dose of a Teratogen?

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> *The dose makes the poison.*
> *Paracelsus*

If a compound causes birth defects, it should not be administered to pregnant women. However, it is not feasible to simply exclude drugs during pregnancy. Chronic conditions such as asthma, high blood pressure, epilepsy, and diabetes can also have adverse effects on pregnancy, so withholding drugs to treat these conditions during pregnancy can be bad for both the woman and the embryo or fetus. Additionally, pregnant women will continue to be exposed to various contaminants in their environments throughout their pregnancies.

It is generally accepted that low doses of some compounds may not pose a significant risk to the embryo or fetus. However, any agent may be harmful if the dose is high enough. Establishment of a safe level of exposure to a teratogenic agent is an important area of research, both for counseling women inadvertently exposed during pregnancy and for determining which drugs are appropriate for use in pregnancy.

The idea that safe doses of known teratogens may exist is based on the threshold concept. This theory holds that embryotoxicity depends on multicellular injury and that there is a threshold dose below which no risk exists (for an example see chapter 21). Dose-response relationships in humans for most teratogenic agents are not known because drugs prescribed for a given condition are usually taken over a very small dose range. However, it has been suggested in several studies that doses of valproic acid above 1000 mg/day are associated with an increased risk of birth defects. Valproic acid, originally approved to prevent seizures, but can also be prescribed for the treatment of bipolar disorder.

If one assumes repair mechanisms are functioning to correct damage to embryonic cells, then it makes sense that an insult to the multicellular embryo can be overcome. Indeed, apoptosis, or programmed cell death, is a normal and necessary part of development. The knowledge that cells can be repaired or replaced supports the theory that there may be a threshold dose, below which no ultimate adverse effect occurs.
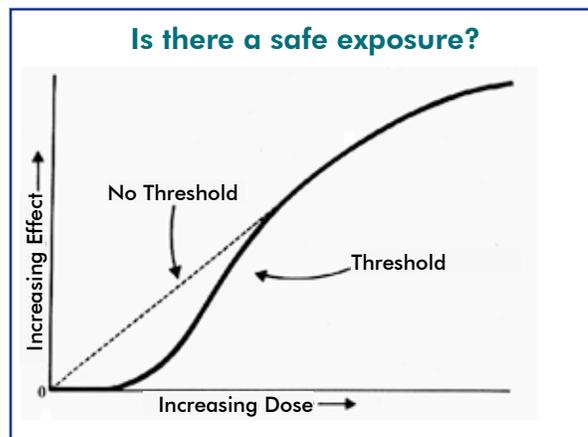
Figure 20-1.

There are some who do not agree with the concept of a threshold dose. Only about one third of the malformations that occur in humans can be traced to genetic or environmental causes. Most defects are categorized as spontaneous malformations, meaning that we don't know what causes them. But if internal factors can cause birth defects, and environmental or drug-induced factors affect the same internal factors, then a drug-induced influence on that factor could tip the balance in the direction of embryo malformation. In this scenario, no safe level of exposure exists; instead, there is a continuum of probability for malformation, no matter how miniscule the drug exposure. An example could be a drug that alters the level of Vitamin A. Too little vitamin A produces birth defects and too much vitamin A produces birth defects. A drug that changes the level of vitamin A could tip the balance either way and cause a birth defect.

If the relationship between dose of an agent and its response is continuous, can there be a safe exposure to a teratogenic agent? If the relationship between dose and outcome is known from animal studies or from human cases of accidental exposure, then the uncertainty about risk of malformation can be reduced by administering lower doses that are unlikely to cause embryotoxicity. Safe doses may be estimated by using safety factors (reducing problematic doses by multiples, usually of 10) or by using quantitative biologically based dose-response models to mathematically estimate doses with a very low risk for the developing embryo.

We can also use our knowledge about critical periods when the embryo is most vulnerable to possible teratogenic events, to reduce or eliminate exposures during those times. The critical period, however, is not the same for all drugs. For example, limb malformations caused by exposure to thalidomide appears to require exposure during the first trimester of pregnancy, while the teratogenic effects of angiotensin-converting enzyme inhibitors (a class of blood pressure medicines) occur during the second two-thirds of pregnancy.

Protecting the embryo or fetus from the effects of toxic agents requires knowledge of the target site of the agent, the dose at which toxicity occurs, and the mechanisms by which toxicity occurs. If we know enough about these three areas, safe use of important medications may still be possible.

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What are the Reproductive and Developmental Risks of Ionizing Radiation?

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Many pregnant women are concerned about the potential reproductive and developmental risks of radiation exposures. With the advent of the Internet, consulting has become more rapid and efficient. In 2008, the pregnancy website of the Health Physics Society (HPS), ATE (Ask the Expert), received approximately 2,400,000 hits. Over 700,000 prepared answers to questions were downloaded. Over 1,600 contacts were still anxious after reading the Website answers and requested a personal consultation. From this experience we have learned that many physicians and other counselors are not prepared to counsel patients concerning reproductive and developmental radiation risks. Approximately 8% of the contacts have provided inaccurate information that could have resulted in an unnecessary interruption of a wanted pregnancy.

Evaluating developmental risks requires attention to two important elements in order to provide scientifically and medically appropriate counseling. First, quality epidemiological studies are the foundation for determining human risks. It is rare that *in vitro* studies or animal studies can refute either negative or positive findings in epidemiological studies if an adequate and well-performed number of epidemiological studies are available. However, there have been instances when animal studies have been more reliable predictors of developmental effects. Secondly, biological plausibility is a powerful tool in evaluating alleged human risks; however, it is dependant on being knowledgeable in all the basic sciences and teratology principles.

There are five areas of radiation embryology with which counselors should be knowledgeable, as well as being familiar with the basic principles of teratology, in order to provide scientifically and medically appropriate counseling.

1. Can the fetus be harmed by ionizing radiation if it is not directly exposed? In other words if diagnostic radiological studies are performed on the head, neck, chest or extremities, is the embryo in the uterus at risk for an increase in adverse effects on development? The effects of radiation have been studied in animal models; these data indicate that radiation exposures in the diagnostic dose range (less than 0.1 Gy, or 10 rad) do not increase the risk of adverse developmental effects because the exposure to the embryo is very small. Diagnostic radiological studies that do not expose the embryo will not increase the risk for birth defects or miscarriage above the background risk of 3% for birth defects and 15% for miscarriage.

2. Is mental retardation produced as a consequence of radiation during pregnancy a threshold phenomenon? There is no doubt that exposure of the developing human fetus to high doses (1–2 Gy) of ionizing radiation can result in mental retardation and microcephaly. The most vulnerable stage for the induction of mental retardation and severe microcephaly is reported to be from the 8th to 15th week of human gestation. During mid-gestation the brain can be depleted of neurons and when the neurons are killed at this stage they are not replaced, resulting in the induction of mental retardation and microcephaly. There is little disagreement about the vulnerability of the brain during organogenesis and fetogenesis. Although most radiation embryologists assumed that the exposures to diagnostic radiological studies were too small to produce mental retardation, there were few data in the human to confirm or refute this assumption. In 1984, Otake and Schull reanalyzed the data pertaining to the children who were irradiated *in utero* in Hiroshima and Nagasaki (RERF, Radiation Effects Research Foundation). They concluded that the most vulnerable period for the induction of mental retardation was from the 8th–15th week of gestation and that 40% of the fetuses who received 1 Gy were mentally retarded (I.Q. <70). They also concluded that mental retardation could be produced with exposures below 0.1 Gy and that radiation-induced mental retardation was a stochastic effect (non-threshold effect). Clinical analysis, the application of the concept of biological plausibility and animal studies did not support the concept that radiation induce mental retardation was a stochastic effect. Reanalysis of the A-bomb data indicated that the threshold for radiation induced mental retardation was approximately 50 rad (0.5 Gy). There was no increased risk of mental retardation or decrease in I.Q. from exposures of 10 rad (0.1 Gy) or below.

3. Does fractionation and protraction of radiation decrease the magnitude of the reproductive and developmental risks? Animal studies were very helpful

in evaluating whether fractionation decreased the teratogenic and growth retarding effects of ionizing radiation. Fractionation and protraction of the radiation exposure reduced the developmental effects of the radiation. Developmental risks were reduced when diagnostic x-ray studies occurred over a period of days, occupational exposures occurred over a period of weeks to years and when flying at high altitudes occurred over a period of hours.

4. Is there a period during pregnancy when radiation will result in an increased mortality but not an increase in malformations? The "all or none" phenomenon was described in the 1950s. Irradiation of rats and mice with up to 1.5 to 2.0 Gy during the pre-implantation and pre-organogenesis stages resulted in embryo lethality; however, malformation rates in the surviving fetuses at term were similar to the controls; at this early stage of pregnancy, high exposures induced cell loss or chromosome abnormalities that most likely resulted in zygote death or malformations that were lethal.

5. How vulnerable is the fetus to the oncogenic (cancer inducing) effects of radiation? This is the most controversial and difficult area to evaluate, because the epidemiological studies are not consistent. In 1999 Boice and Miller published their interpretation of the data pertaining to the oncogenic risks of low-level intrauterine radiation. They noted, "Evidence for a causal association derives almost exclusively from case-control studies, whereas practically all cohort studies find no association, most notably the series of atomic bomb survivors exposed *in utero*. Learned debate continues as to the causal nature of low-level intrauterine radiation exposure and subsequent cancer risk. The association is not questioned, but the etiologic significance is. Different scientists interpreting the same data have different opinions as to the causal nature of the association and the possible level of risk." The most recent publication based on the 60 year follow-up of the *in utero* population exposed to the A-bomb in Japan indicates that the embryo is actually less sensitive to the oncogenic effects of radiation than the child, with a suggested threshold at 20 rad (0.2 Gy). The population of *in utero* survivors numbers approximately 1,500, which is a small population for oncogenic studies and this population is only in their 60s, so we have to wait another 20 years to finalize the risk of cancer among those who were exposed to the A-bomb as embryos. In the mean time, parents of patients who were exposed *in utero* to diagnostic radiation can be told that the oncogenic risk of those amounts of *in utero* radiation is very low.

We utilize the scientific information obtained from studies in these five areas to counsel patients concerning radiation risks during pregnancy. The willingness and persistence of scientists to debate the controversial aspects of this research and apply the best available scientific information to assist patients in turmoil about the risks of embryonic radiation to themselves and their offspring have saved thousands of lives and changed family histories.

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Do Assisted Reproductive Techniques Increase the Risk of Birth Defects?

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About 15% of couples meet the clinical definition of infertility by not becoming pregnant after trying for one year. In some cases, these couples are normal and just need more time, but in other cases, there is a problem for which medical attention may be helpful. Causes of infertility affect three general features of the reproductive process (Figure 22-1):

- Normal ova may not be ovulated, or ova may not be ovulated normally.

- Normal sperm may not be introduced into the female genital tract or may not survive once they are introduced.

- There may be an obstruction in the genital tract, preventing the ova and the sperm from reaching one another.

These 8-cell human embryos were fertilized *in vitro*. They will soon be ready to place into the uterus.

Figure 22-1. *Photo courtesy of Shady Grove Reproductive Science Center, Rockville, MD.*

The following are some of many therapies in use to overcome these abnormalities.

- Hormones or hormone-like compounds that induce ovulation.

- *In vitro* fertilization (IVF). This technique harvests ova from the ovaries through a needle placed into the ovary through the vagina. The ova are mixed with sperm in a laboratory dish. The resultant embryo is placed into the uterus through the cervix.

- Gamete intrafallopian transfer (GIFT) and Zygote intrafallopian transfer (ZIFT). These techniques are similar to IVF; however, in GIFT, the ovum and sperm meet before fertilization in the fallopian tube, rather than a laboratory dish. The fallopian tube is where the ovum and sperm would join under normal circumstances, but in GIFT, the ovum and the sperm are injected into the fallopian tube together, through a thin instrument inserted into the belly through a tiny incision. In ZIFT, the fertilized ovum, called a zygote, is placed into the fallopian tube. Thus, fertilization has occurred in the laboratory, but early development happens in the fallopian tube, which may provide a more natural environment.

- Intracytoplasmic sperm injection (ICSI). A spermatozoon is injected directly into the ovum using a microscopically tiny pipette. The technique is otherwise exactly like IVF. ICSI eases the job for the sperm by ferrying it across the zona pellucida, the membrane that surrounds the ovum.

It has now been 30 years since the first baby was born following assisted reproductive techniques or ART. The use of this technology has increased to the extent that to date over 1 million babies have been born following ART. However, there is concern that ART may increase the risk of an abnormal pregnancy. These concerns are based on two possibilities that the medications or physical procedures that are involved could injure a normal gamete or embryo, or that an abnormal gamete, ordinarily incapable of fertilization, will be helped to achieve fertilization and will give rise to a child with birth defects.

The good news for couples using these techniques is that little if any increase in birth defects has been shown to occur in the resulting children, although not all researchers agree on this point. Experimental animal studies support the safety of these techniques; also, animal breeding programs have used these assisted reproductive methods extensively for many years without an apparent increase in birth defects. The news is not entirely reassuring however; there may be other reproductive consequences.

Possibilities include:

Miscarriage

It has been suspected that some assisted reproductive techniques might increase miscarriages. Accurate miscarriage rates are almost impossible to determine because many miscarriages occur so close to the time of an expected period that a woman would never have suspected that she was pregnant. Miscarriage occurs in at least 30% of all pregnancies, but only about half of those pregnancies are recognized. In a group of women undergoing fertility treatments, detailed monitoring of hormone levels will catch even very early pregnancies, and an episode of bleeding will be correctly identified as a miscarriage. So the reason that miscarriage rates among women undergoing fertility treatments appear higher than in the general population may be because it is closer to the true rate.

This argument notwithstanding, at least one ovulation inducing medication, called clomiphene, may increase the miscarriage rate slightly. Clomiphene stimulates maturation of the oocyte in the ovary. During maturation of the oocyte granulosa cells that surround the oocyte produce primarily estrogen. After ovulation, these cells produce primarily progesterone. Both estrogen and progesterone prepare the lining of the uterus for implantation of the fertilized egg. Clomiphene may alter the function of the granulosa cells so that hormone production is inadequate to support implantation. Under these circumstances, a fertilized egg might fail to implant or might implant poorly. How often clomiphene causes early miscarriage due to faulty implantation is unknown, but we do know that only half of women who ovulate in response to clomiphene are recognized to be pregnant. Stimulating ovulation with clomiphene clearly does not completely restore the reproductive system to normal.

Ectopic Pregnancy

Sometimes an embryo implants outside the uterus, usually in the fallopian tube. In early pregnancy, fingerlike projections called chorionic villi dig into the tissue where the embryo is implanted, in order to set up the placenta. When a pregnancy occurs outside the uterus, the invading villi can erode into blood vessels, causing bleeding that can be life-threatening. Ectopic pregnancies may result from scarred fallopian tubes that trap the fertilized ovum before it can pass freely into the uterus. It is not clear whether assisted reproductive techniques increase the risk of ectopic pregnancies, or whether these techniques are used in women who already have a higher rate of tubal damage.

Chromosome Abnormalities

Abnormal chromosome numbers occur commonly in early pregnancies. In pregnancies that miscarry, about half have an abnormal number of chromosomes, which is presumed to be the reason for the subsequent miscarriage. A naturally-conceived pregnancy that miscarries very early would not be checked for chromosome abnormalities. It has been noted that chromosomal abnormalities appear to be more common in embryos created through assisted reproductive techniques that involve fertilization in the laboratory, compared to the general population; however, when embryos are placed into women without pre-testing, chromosome abnormalities at birth are not increased. It appears likely, then, that chromosomally abnormal embryos are largely eliminated by natural processes.

Imprinting Defects

Case reports have associated ICSI with an unusual group of genetic abnormalities called imprinting defects. Imprinting refers to the methylation of cytosine in GpC rich regions of the genome, and can change the way the DNA is read. Imprinting defects include Angelmann syndrome, associated with severe mental retardation, ataxia, and seizures, or Beckwith-Wiedeman syndrome, associated with macrosomia (an abnormally large body), omphalocele (a defect in the abdominal wall at the umbilicus) and other clinical features. DNA methylation occurs during the development of sperm and oocytes, and ICSI techniques are believed by some researchers to interfere with normal methylation. It is not known, however, whether imprinting abnormalities are increased by assisted reproductive techniques, or whether they are associated with the underlying infertility problems.

Multiple Gestations

Hormone-like medications that induce ovulation may cause more than one egg to be released in a cycle, resulting in twins, triplets, and higher-order multiple gestations. The rate of twins in the general population is about 1%, but between 8 and 25% of pregnancies achieved with ovulation induction result in twins. The rate of triplets in the general population is about 0.01%. With some ovulation induction medications, this rate can reach a few percent. With optimal monitoring of the induced ovulation cycle quadruplets and higher do not occur very often. IVF, GIFT, and ZIFT have the potential for producing high order multiples (more than two babies at once) if more than one embryo or zygote is placed in the woman's reproductive tract. Multiple embryos may be used in order to increase the chance that at least one of them will implant and develop. If four embryos are

placed in the uterus and all of them happen to implant, the result is quadruplets. Many fertility doctors now recommend implanting no more than one or two embryos at a time, in order to avoid multiples of a higher order than twins.

Although having many babies at one time may sound like an efficient way for a couple to complete its family, multiple gestations are associated with pregnancy risks, including increased birth defects. Perhaps the most important risk is prematurity, because the more babies there are in the uterus; the earlier in pregnancy the uterus will sense that it is overfull. Labor may start weeks before the babies are due. Prematurity is an important cause of death and disability in children arising from these pregnancies.

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What Are the Effects of Alcohol Use During Pregnancy?

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Thousands of research studies over the past 40 years have demonstrated that alcohol (ethanol) used during pregnancy is teratogenic. Prenatal exposure can cause growth deficiency, malformations, and neurobehavioral deficits. Whether a conceptus is affected depends on the dose, timing, and conditions of exposure. Different strains of mice respond differently to similar doses of alcohol, and it is very likely that women also differ in how alcohol intake affects offspring. Individual variations in offspring can also make a difference; even twins can be differentially affected. In general, dizygotic (non-identical) twins show more disparate responses to prenatal alcohol than do monozygotic (identical) twins.

There appear to be several mechanisms for the teratogenicity of alcohol. Alcohol has a directly toxic effect on the developing brain; it can kill brain cells and interfere with the transport of amino acids (the building blocks of proteins) and glucose (the main energy source for cells). Alcohol can also impair placental-fetal blood flow, causing hypoxia or disrupting the hormonal and chemical regulatory systems in the brain that control the maturation and migration of nerve cells.

The teratogenic properties of alcohol, our most frequently used "social" drug, came to attention originally through independent clinical observations in 1986 by Lemoine in France and in 1973 by Jones and Smith in Seattle. Noting a pattern of malformation, growth deficiency, and central nervous system effects in children of alcoholic mothers, the term Fetal Alcohol Syndrome (FAS) was coined in 1973 and drew immediate attention to alcohol as a teratogen. Four decades of research have increased recognition of the types of damage caused by prenatal alcohol and the long-term consequences. Our understanding of the breadth of prenatal alcohol damage derives from studies of children with Fetal Alcohol Syndrome (FAS), experimental animal models, and long-term prospective studies of diverse human populations.

In general, larger doses cause greater effects. Both animal and human studies show that a "binge" dose (many drinks in a short time period) is particularly damaging for the conceptus. The brain appears to be the most vulnerable organ: brain effects are widespread, and can occur across a wide range of doses and patterns of exposure. In general, morphological (structural) abnormality occurs at the highest

Figure 23-1. *The discriminating features of FAS are those on the left side. However, as the facial features often coarsen with puberty, not all adolescents and adults will retain these features. Graphic from Streissguth AP and Little.* Alcohol, Pregnancy, and the Fetal Alcohol Syndrome, *2nd edition, Unit 5, Project Cork Slide Lecture Series, Dartmouth Medical School, available from Milner-Fenwick, Baltimore, 1994.*

doses or highest peak doses, while behavioral deviations can occur at lower doses or lower peak doses. Direct cell death, aberrant neuronal migration and development, and disrupted neurochemical balance of the brain can result from exposure during all periods of embryonic and fetal development. The typical facial appearance of FAS, however, arises from exposure early in pregnancy.

Prospective epidemiologic studies link alcohol to increased risk of miscarriages, stillbirths, low birth weight, and increased neonatal morbidity. Alcohol abuse is often associated with poor nutrition, which can compound results. Experimentally, animal studies have shown that these adverse birth outcomes result from prenatal alcohol even when diet and rearing conditions have been controlled; poor diet and poor rearing conditions, however, can aggravate adverse effects.

Alcohol crosses the placenta, so that minutes after a pregnant woman consumes alcohol, the fetal blood alcohol level is similar to that of the mother. Alcohol can cause a temporary pause in fetal respirations. Autopsies of deceased children with FAS, imaging studies, and experimental animal

Figure 23-2. *First child identified with FAS at birth, shown here on day 1, 8 months, 4 1/2 years, 8 years and 18 years. Note the short palpebral fissures (eye openings), thin upper lip, smooth philtrum (between upper lip and nose), and microcephaly. The first four photos from; CIBA Foundation book: "Mechanisms of Alcohol Damage* in utero" *Ciba Foundation Symposium 105, Pitman, London, 1984.*

studies have shown us that many brain regions, including the hippocampus, cerebellum, corpus callosum, and basal ganglia, are affected by prenatal alcohol. A large and compelling experimental animal literature links prenatal alcohol to disruptions in learning, memory, emotional responsiveness, and behavior. Associated brain-behavior deficits include optic nerve hypoplasia with impaired vision, reduced cerebellar size with impaired motor development and ability, decreased callosal size with hyperactivity, and decreased serotonin synthesis with impaired instinctive maternal behaviors. See Table 23-1 for the comparability of alcohol-induced behavioral problems in humans and laboratory animals.

The consequences of prenatal alcohol exposure can last a lifetime. Rodents exposed prenatally to alcohol have higher rates of sickness and early death. Some (but not all) studies have found that early growth deficits attenuate with time, at least in some individuals. Problems with maintaining attention and focus are not only measureable in day-old newborns, but also manifest throughout childhood, adolescence, and

TABLE 23-1.

BEHAVIORAL EFFECTS FOLLOWING PRENATAL ALCOHOL EXPOSURE	
HUMANS	**ANIMALS**
Hyperactivity, Reactivity	Increased Activity, Exploration and Reactivit
Attention Deficits, Distractibility	Decreased Attention
Lack of Inhibition	Inhibition Deficits
Mental Retardation, Learning Difficulties	Impaired Associative Learning
Reduced Habituation	Impaired Habituation
Perseveration	Perseveration
Feeding Difficulties	Feeding Difficulties
Gait Abnormalities	Altered Gait
Poor Fine and Gross Motor skills	Poor Coordination
Developmental Delay (Motor, Social, Language)	Developmental Delay
Hearing Abnormalities	Altered Auditory Evoked Potentials
Poor State Regulation	Poor State Regulation

From: Driscoll, Streissguth, & Riley. J. Stud. Alcohol *59:292–304. 1990.*

early adulthood. Learning problems (particularly in abstract thinking and arithmetic) are not easily measurable until early childhood, but have lifelong consequences. Some of the most marked behavioral deficits in humans are observed in adaptive behavior and "executive function" as measured by communication, socialization, problem-solving, and daily living skills.

Follow-up studies show that children with FAS do not fare well as they reach adolescence and early adulthood. They often cannot live independently, are impulsive, exhibit poor judgment, do not respond appropriately interpersonally, are frequently depressed, and often socially isolated. Lifespan studies of patients with FAS, or other fetal alcohol effects related to significant prenatal alcohol exposures, show a wide array of "secondary disabilities." These include social, behavioral, psychiatric, and legal problems that stem from the primary disability of prenatal brain damage from alcohol but may be complicated by the failure of an appropriate early diagnosis, the impact of high-risk environments associated with parental alcohol abuse, and the failure of society to adequately provide appropriate services to this population of disabled adolescents and adults.

FAS and other Alcohol-Related Neurodevelopmental Disabilities (ARND) represent many neurobehavioral deficits besides mental retardation. Epidemiologic studies in the U.S. and some Western European countries have revealed an FAS prevalence of at least 2 to 7 per 1000. The prevalence of a relatively new more inclusive term for patients with and without FAS facial features, "Fetal Alcohol Spectrum Disorders" (FASD), has been recently estimated at 2–5%.

The U.S. Surgeon General's 1981 recommendation for "no alcohol use for women who are pregnant or considering pregnancy" was re-issued in 2005. Recently, France and England have issued comparable warnings. Bottle labeling, begun in the U.S. in 1989, is now commencing in France.

In the past decade increasing international collaborations in fetal alcohol studies and increased identification of affected individuals has spurred prevention/intervention efforts and the development of local and national plans for identifying and serving affected families. In 2009 The World Health Organization (WHO) began developing the first global strategy on reducing health damage from alcohol abuse. Preventing FASD should be at the top of the list.

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Is Stress a Developmental Toxicant?

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Stress is a term that can mean very different things under different circumstances. In toxicology, stress refers to any external challenge that disturbs the constancy of the internal environment, or homeostasis. This definition encompasses the neurochemical, physiological, and behavioral reactions to novel, dangerous, or upsetting situations. Stress isn't necessarily bad; the endocrine and neural adaptations typically referred to as the "stress response" may cause a person to, for example, escape from the path of a moving car. However, these responses can be maladaptive in certain situations, especially in cases of chronic stress.

Stressors can be environmental conditions or events, including excessive heat or cold, trauma, noise, starvation, chemical exposures, or physical exertion. Stress can even include domestic violence or abuse, conflicts with family members, or a hostile work situation, and psychological stressors include anxiety and depression. Although a considerable amount of experimental animal research has been done on the topic, far fewer human studies have been attempted. Because women and their life situations differ widely, such studies are difficult to conduct and interpret.

Laboratory animal studies, most often with rodents, have tested the effects of such stressors as physical restraint, hypothermia, electric shock, noise, visual stimuli, vibration, shipping, and crowding on development. Deprivation of food and water has also been used as a stressor, but the possible effects of malnutrition confound these studies. Studies in pregnant rodents have demonstrated that certain maternal stressors can increase the adverse effects of chemicals known to cause malformations or other manifestations of developmental toxicity. Moreover, it is also possible that the stress caused by maternally toxic exposures to chemicals might exacerbate the harmful effects of those chemicals on the embryo or fetus.

Numerous experimental animal studies have examined effects of maternal stress or excess glucocorticoids (hormones produced in response to stress) on the physiology (especially endocrine alterations) or behavior of the young at various times after birth. Some investigators have noted increases in the likelihood of disorders of cardiovascular function, glucose homeostasis, and anxiety related behaviors in the animal as an adult. Other investigators have noted mortality, decreased growth, or development of extra ribs in the

Cleft palate (right) can be induced in fetuses of susceptible mouse strains by maternal stress during pregnancy.

embryo or fetus, and a few studies have reported stress-related malformations. These malformations include encephalocele and exencephaly (different degrees of defective development of the brain and skull) caused by failure of the neural tube to close normally during development of the embryo.

There are clearly significant differences in susceptibility to maternal stress during development among species and even among strains of the same species. For example, it is generally easier to induce adverse effects of stress on growth, mortality, and anatomical development in mice than in rats. And in some strains of mice, maternal stress is associated with cleft palate, while other strains are unaffected.

Several human studies have attempted to evaluate the potential for effects of maternal stress on development. Various clinical studies have associated such an adverse environment during pregnancy with the development in the offspring of metabolic disorders and neuroendocrine dysfunction as well as an increased risk of psychiatric diseases in later life. Some studies have reported findings such as increased incidences of low birthweight or preterm delivery, and a few studies have found diminished scores on neonatal neurological examination. However, these human studies often have methodological shortcomings, including arbitrary measures of psychological stress or inappropriate comparisons between groups. These studies must also deal with possible biases, especially recall bias. Recall bias is attributable to selective memory. Women, who miscarried, gave birth prematurely, or who gave birth to abnormal

children may be more likely than women with normal pregnancies to remember events that they believe may have contributed to such outcomes. This differential reporting may occur even when the life experiences of the two groups were not truly different.

Confounding factors are also common in human studies. Stressful life events are typically more common in women of low socioeconomic status, and poverty itself can be a stressor. Stressed mothers are commonly younger, more likely to smoke, abuse alcohol or other drugs, or have poor nutrition; any of these exposures could affect the results of a study on development. Thus, compelling human evidence for stress-induced effects on the offspring has been difficult to obtain.

Although there has been much speculation, we know relatively little about mechanisms by which maternal stress could adversely affect pregnancy. The response to stress is a basic adaptive mechanism that is protective in times of crisis. Stress activates the sympathetic nervous system and results in the release of specific hormones, including catecholamines and glucocorticoids. In mice, it is the increase in glucocorticoids that appears to be the cause of stress-induced cleft palate (see Figure). Stressors may also alter activities of the immune, neural, and renal systems, as well as having additional endocrine effects. Any of these influences may, in turn, affect various aspects of a mother's physiology and behavior. Further, epigenetic mechanisms are increasingly being invoked as likely causes of postnatally manifested effects on offspring.

Stress responses are complex and can vary depending on the stressor and species involved. Although adverse effects have been shown to occur in laboratory animals, determining possible mechanisms by which maternal stress might negatively affect human embryonic or fetal development has been problematic.

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What Is the Risk of Treating or Not Treating a Pregnant Woman with Antidepressants?

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The risk of major depression among women is highest during childbearing age with an estimated prevalence of up to 20% during pregnancy. Untreated depression during pregnancy is associated with several adverse reproductive outcomes, including cesarean or premature delivery, low birth weight, and neonatal intensive care admission. Furthermore, maternal stress has been linked in some studies to a higher rate of cranial neural-crest malformations, orofacial clefts and various other congenital anomalies.

Selective serotonin reuptake inhibitors or SSRIs account for over 80% of antidepressant prescriptions in the U.S. Commonly used SSRIs include fluoxetine (Prozac), sertraline (Zoloft), paroxetine (Paxil), and citalopram (Celexa). All share a similar mechanism of action in depression, but their chemical structures and pharmacokinetic properties vary. Although many studies of pregnancy outcome treat these drugs as a class, different SSRIs may actually affect the developing fetus differently.

Data on human teratogenicity associated with maternal SSRI use during the first trimester of pregnancy are inconsistent. Most studies have limited sample sizes and power, and many of the associations found may be attributable to chance despite their nominal statistical significance owing to multiple comparisons made within the studies. Available epidemiological studies (Chapter 14) are of four main types:

1. The majority of studies assessing risk of first trimester SSRI use are retrospective cohort studies based on linked administrative records (data bases from prescription records, insurance claims, hospital records, etc). Five of the 10 published studies based on this design show positive findings: In one study, maternal use of any SSRI early in pregnancy was found to be a risk factor for any congenital malformation or any heart malformation. In two studies, maternal paroxetine use in particular was found to be associated with such risk. An increased risk for septal heart defects was found in the infants of mothers who took sertraline or citalopram in early pregnancy in another study. Maternal fluoxetine treatment in combination with benzodiazepine therapy was associated with any congenital anomaly, while associations of maternal combined SSRI and benzodiazepine therapy or citalopram monotherapy with congenital heart defects were reported in one other study. Other cohort studies did not detect any significant association of congenital malformations in general (four studies) or any heart malformation (two studies) with maternal use during pregnancy of any SSRI (four studies) or of paroxetine in particular (one study).

2. Four exposure cohort studies have been performed through teratogen information services. One study found a significantly increased risk for cardiac anomalies among the infants of women treated with fluoxetine early in pregnancy. No significant association of maternal SSRI use during pregnancy and birth defects was found in the other three studies.

3. One prospective population-based cohort study found an association of maternal paroxetine treatment during pregnancy with cardiovascular malformations in the infant. Another study of similar design found a significant increase in risk for cardiac defects with use of any SSRI.

4. Retrospective case-control studies have found a tripling of the frequency of right ventricular outflow tract heart defects among infants with maternal paroxetine treatment in early pregnancy and an increased frequency of maternal sertraline treatment among infants with cardiac septal defects or omphalocele. Increased risks for omphalocele were also found among infants whose mothers had been treated with any SSRI, and increased risks for right ventricular outflow tract heart defects were associated with paroxetine treatment in another study. The risks for many other birth defects analyzed were not increased.

Other data suggest that maternal SSRI treatment late in pregnancy is associated with various neonatal complications, including symptoms of neonatal withdrawal and toxicity, premature delivery, low birth weight and persistent pulmonary hypertension of the neonate. Data on the risk of neurobehavioral and long-term cognitive problems among children of women who were treated with SSRIs during pregnancy are limited. Given the pharmacological effects of these medications on the developing brain, the possibility of functional abnormalities is one to be studied.

Bupropion is the next most commonly used antidepressant in the U.S. Congenital anomalies were reported in 3.6% infants of women identified prospectively to have taken bupropion in the first trimester and reported to a pregnancy registry (chapter 16) operated by the manufacturer. Congenital heart defects were observed in more than 1/3 of these infants. Results from a more recent population-based case-control study indicated a doubling of the risk for one particular kind of cardiac malformation (left ventricular outflow tract defects) among infants of mothers who used bupropion in the first trimester of pregnancy. Relatively little is known about the risks of newer antidepressants, such as selective norepinephrine reuptake inhibitors.

None of the antidepressant treatments during pregnancy is known to be associated with a high risk of birth defects in the infant. There is a lack of consistency among studies that suggests there may be additional explanations other than the drugs that contribute to those findings that are statistically significant. Any increase in risk, if it exists, is small and difficult to detect against a background risk of 2–3% of having a malformation identified at birth (or 3–4% identified by 1 year of age) in any pregnancy.

The clinician must be aware of the concerns that a pregnant woman might have in taking a medication. Concern may be heightened as well, because discontinuation of antidepressant treatment during pregnancy may pose a substantial risk of relapse and consequent pregnancy complications. Clinicians can counsel pregnant women and those of childbearing age who require antidepressant treatment that some but not all studies suggest a slightly increased risk of malformations in children whose mothers take SSRIs or bupropion early in pregnancy, while at the same time highlighting the fact that these studies show most such babies are normal and that discontinuation of treatment may pose serious risks to the pregnancy.

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What Are the Effects of Antiepileptic Drugs during Pregnancy?

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Exposure to an anticonvulsant drug occurs in 1 in 250 (0.4%) pregnancies. Since exposure to many of these drugs has been shown to be harmful to the fetus, this makes these medications the most common potentially harmful fetal exposures for pregnant women. The dilemma, however, is that diseases that require treatment with antiepileptic drugs, if untreated, often present risks for a pregnant woman and her fetus as well.

There are over 30 anticonvulsant drugs prescribed in the United States. Some drugs, like lamotrigine, valproate, and topiramate, are used for several medical conditions, such as preventing seizures, treating the symptoms of bipolar disorder, or preventing migraine headaches.

Human research studies have not been carried out on the fetal effects of each anticonvulsant drug. The information available has shown that the risks vary for each drug. Because of these risks, women with epilepsy (or other medical conditions) who take these medications are urged to meet with their physicians to discuss their treatment before beginning a pregnancy. The crucial issues are:

1. Does she need to take an anticonvulsant drug?

2. Is the medication she is taking more potentially dangerous to her unborn infant than another drug?

3. If she is taking two anticonvulsant drugs, can she eliminate one? The reason is that taking two is often more dangerous to the fetus than taking one.

4. Can she take a lower dose of the anticonvulsant? There is a dose-response relationship for any medication, which means the lower the dose, the lower the risk to the fetus.

Potential fetal effects of anticonvulsant drugs

These effects have been identified for several drugs:

1. Major malformations: The range of effects has been for a doubling (for drugs like carbamazepine, phenytoin and lamotrigine) to a 4- to 6-fold increase (for phenobarbital and valproate). Specific malformations that are more common include cleft lip, cleft palate, heart defects, and spina bifida.

2. More subtle effects, including mid-face hypoplasia and digit hypoplasia, occur in some anticonvulsant-exposed infants. Systematic studies have shown that these physical features occur in 10 to 15% of infants exposed to phenytoin, phenobarbital, and carbamazepine. The child with these physical changes is more likely to have IQ deficits than the anticonvulsant-exposed infants who do not have these physical effects from the medication.

3. An increased frequency of low birth weight.

4. Lower intelligence and developmental delay. This effect has been most severe after exposure to the drugs valproate and phenobarbital. More subtle effects may occur after exposure during pregnancy to other anticonvulsant drugs. Studies to assess these potential effects are needed for most of the "new" anticonvulsant drugs which have been marketed since 1990.

Can the fetal effects of anticonvulsant drugs be prevented by folic acid supplements during pregnancy?

Very few systematic studies have addressed this important question. Studies of the occurrence of major malformations in women enrolled in pregnancy registries have shown that each of the mothers of affected infants was taking a vitamin (including folic acid) supplement. It is current practice to recommend to each woman taking an anticonvulsant drug to take a vitamin supplement that includes 4 mg folic acid per day. The hope is that this high dose could be beneficial. There is no evidence of a risk for harm to the fetus from this dose.

Is there a genetic risk for the harmful fetal effects of anticonvulsant drugs?

Clinical studies have suggested that the pregnant woman who has taken an anticonvulsant drug and whose drug-exposed child has the physical abnormalities attributed to these drugs has a greater risk of having a second affected child. However, no systematic studies have been carried out to identify the presumed genetic basis for the harmful fetal effects of anticonvulsant drugs.

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Can Chemicals in the Environment That Affect Hormone Function Disrupt Development?

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Hormones, including estrogens and androgens, regulate the expression of genes that play critical roles in guiding the development of organ systems in the embryo. Changes in either the amount or the timing of hormone exposure can lead to altered human development. For example, humans with a defect in the androgen receptor gene have androgen insensitivity syndrome; although they are genetically male, they look female, because androgens cannot activate the receptor to masculinize the reproductive tract during critical periods of prenatal development.

Hormonally-active chemicals were first shown to have effects on development in humans in the early 1970s, when vaginal adenocarcinoma, a rare cancer, was shown to be linked to fetal exposure to diethylstilbestrol (DES). During the 1950s, the mothers of these young women had been given DES, a synthetic estrogen, in an effort to prevent miscarriage and preterm delivery. In Taiwan in 1979, many people consumed rice oil contaminated with high levels of polychlorinated biphenyls (PCBs) and dibenzofurans. The offspring of exposed women were smaller at birth and had delays in neurological development. Several more recent studies on PCBs have shown neurological effects in other populations exposed to lower amounts. Although we know that PCBs and related compounds can interact with various components of the endocrine system, the exact cause for the developmental disorders observed after these exposures is not known. In 1992, a publication of the proceedings of a conference organized by Theo Colborn concluded, "We are certain of the following:...A large number of man-made chemicals that have been released into the environment... have the potential to disrupt endocrine systems of animals, including humans".

Chemicals that affect development through hormonal processes are called endocrine disruptors, endocrine modulators, or, simply, hormonally active substances. The International Programme on Chemical Safety, part of the World Health Organization (WHO), adopted the term endocrine disruptors and defined them as "exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations." Evidence supporting the endocrine disruptor hypothesis is derived from studies in wildlife living in contaminated environments, studies of livestock foraging on phytoestrogen-containing

Hypospadias is a malformation in which the urinary opening (the urethra) opens on the underside of the penis instead of at the tip. Some scientists believe that hypospadias can be caused by environmental chemicals with hormonal activity.
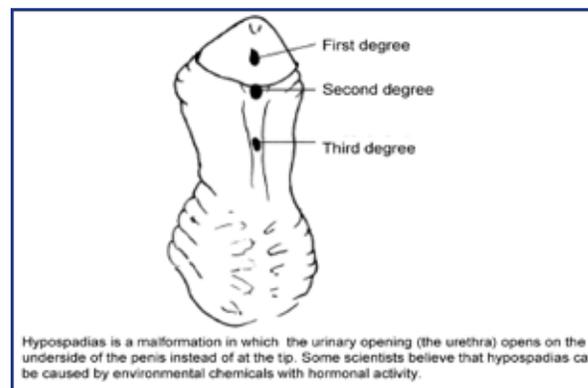
Reprinted from Advances in Neonatal Care, Volume 4, Stokowski LA, Hypospadias in the neonate, 206–215. Copyright (2004), with permission from National Association of Neonatal Nurses.

plants, laboratory animal studies, and reports of increasing rates of health effects in hormonally sensitive tissues, including tumors of the testes, breast, and prostate, birth defects that affect the reproductive tract (hypospadias and cryptorchidism), and diminished semen quality.

The most convincing evidence to support the endocrine disruptor hypothesis has been obtained in laboratory experiments in which animals are exposed during critical developmental periods. Female rodents exposed as embryos or fetuses to a sufficient dose of an estrogenic chemical typically develop accelerated puberty and delayed preputial separation; males have reduced accessory sex gland weights and reduced sperm production. Exposures with anti-androgenic effects affect primarily male offspring, causing reduced anogenital distance, hypospadias, retained nipples, reduced testes and sex accessory gland weights, and reduced sperm counts. For example, in experimental animals, the DDT isomer o,p-DDT binds to the estrogen receptors and feminizes hormonally sensitive tissues, while the DDT metabolite p,p=-DDE demasculinizes development by binding to and inhibiting the androgen receptor. Other chemicals that disrupt development by endocrine-based modes of action include the fungicides vinclozolin, the plasticizer diethylhexylphthalate, a group of surfactants called alkylphenols, and 2,3,7,8-tetrachlorodibenzodioxin (commonly called dioxin or TCDD). Exposures that alter the function of the thyroid gland during development affect the

differentiation of central nervous system and sensory organs (e.g., hearing). In rodents, testis size is inversely proportional to the thyroid status in the neonate. Many of the effects of hormone disruption are not obvious at birth but become apparent on the functional level as the individual matures.

Organizations that have reviewed the evidence concerning the impact of endocrine disruptors in the environment on human health have generally concluded that additional research is needed before cause-and-effect determinations can be made with any degree of confidence. The link between exposure to endocrine disruptors and effects in humans is largely based on the concurrent rise in the use of many chemicals with hormonal activity and the increase over time in certain health outcomes that have at least a partial endocrine basis. However, data on the impact of low level environmental exposures on the rates of endocrine-sensitive health outcomes have been conflicting and there are some who do not believe that human endocrine disorders are caused by low level environmental exposures. The Endocrine Society was one of the most recent organizations to weigh in on the controversy, when it released a position paper that made a number of recommendations regarding research to increase our understanding of the effects of endocrine active chemicals and advocated involvement of individual and scientific society stakeholders in fostering changes in public policy and awareness of the issue.

The United States Congress included in the Food Quality Protection Act of 1996 a provision that the U.S. Environmental Protection Agency begin a program to screen and test chemicals for ability to interact with the estrogen, androgen, and thyroid hormone activity. This program, which is arguably the largest testing program to be adopted since the 1970s, will provide a much more comprehensive evaluation of the chemicals that can interfere with endocrine mediated processes. The latest information on the status of this requirement can be found at: http://www.epa.gov/endo/pubs/edspoverview/index.htm.

One of the most controversial contemporary issues relates to the plasticizer bisphenol A (BPA), which is produced in very large quantities and used in a number of consumer products. BPA binds to the estrogen receptor, although its' potency is several orders of magnitude less than that of estradiol, the most active human estrogen. While traditional toxicology studies have shown that BPA is a weak estrogen in exposed animals, other studies have reported adverse effects in tissues at very low, environmentally relevant concentrations. Similar low dose findings have also been reported for the phthalate plasticizers. These types of findings have fueled the concern that exposures to endocrine disrupting chemicals well below those previously considered safe can cause harm to the developing fetus. Recently, the National Institute of

Environmental Health Sciences (NIEHS), a part of NIH, has launched a $30M grant program (http://www.niehs.nih.gov/news/releases/2009/bisphenol-research.cfm) in an attempt to clarify whether current human exposure to BPA affects development.

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Is Herb Use during Pregnancy a Reason for Concern?

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Women commonly use medicinal herbs during pregnancy; the National Birth Defects Prevention Study found that 9.4% of 4239 women reported herbal use during pregnancy primarily in the first trimester. Teas made from raspberry leaf (*Rubus idaeus*) are used throughout pregnancy in many cultures, and teas made from ginger (*Zingiber officinale*), peppermint (*Mentha x piperita*), or spearmint (*Mentha spicata*) are common folk remedies for morning sickness. Some midwives incorporate herbs into their practices, including oral or topical oil of evening primrose (*Oenethera biennis*) to speed cervical ripening, or a mixture of blue cohosh (*Caulophyllum thalictroides*) and the unrelated black cohosh (*Cimicifuga racemosa*) to treat stalled labor.

The most common herb intentionally ingested by pregnant women is red raspberry leaf even though there is no evidence that raspberry leaf eases morning sickness or labor; a placebo-controlled clinical trial of raspberry leaf extract administered from 32 weeks gestation until labor found no significant differences between groups in pregnancy outcomes.

Dried ginger root, commonly used for morning sickness, has been tested in numerous clinical trials in doses up to 1 g/d and appears to be effective. No adverse pregnancy outcomes have been linked to ginger, and reproductive toxicology studies of ginger in rats have identified no problems. Rates of major malformations were no higher in 54 women who took St. John's wort (*Hypericum perforatum*) during pregnancy than for a comparator group.

Evening primrose oil, a source of gamma-linolenic acid, may be used in an effort to soften the cervix; it is more commonly topically applied than ingested orally. In a clinical trial, orally ingested evening primrose oil from the 37th gestational week until birth did not shorten gestation or decrease the overall length of labor.

Not all herbs are benign. Licorice, an herb that is used medicinally but is consumed most commonly in the form of candy or chewing gum, appears to shorten gestation in humans. A questionnaire study in Finland, where licorice candy consumption is so common that participants could be separated into low, medium, and high-exposure groups, found that heavy exposure to licorice (more than 500 mg/ glycyrrhizinic acid per week) slightly shortened

Blue cohosh is sometimes used by herbalists to stimulate labor.

Thomas G. Barnes @USDA-NRCS PLANTS Database/Barnes, T.G. & S.W. Francis. 2004, Wildflowers and ferns of Kentucky, University Press of Kentucky. Reprinted with permission.

gestation and more than doubled the risk of delivering a baby before 38 weeks. Birth weight and head circumference were unaffected. A study of offspring in this cohort at age eight found dose-related cognitive defects and attention problems in eight-year- olds exposed to high levels of licorice *in utero*.

Maternal use of blue cohosh (*Cimicifuga racemosa*) in high doses has been associated with cases of stroke, heart attack, and hypoxic-ischemic symptoms in exposed infants. Blue cohosh rhizomes contain several vasoconstrictive compounds, including methylcytisine, caulosaponin and caulophyllosaponin. A woman who took blue cohosh in an effort to induce abortion developed tachycardia, abdominal pain, vomiting, and muscle weakness, symptoms consistent with toxicity mediated through the nicotinicacetylcholine receptor.

Lack of adequate regulation of herbal products in the United States complicates the use of herbs. Herbal products may contain different herbs than are stated on the label, be adulterated with other drugs, or be contaminated with heavy metals or bacteria, some of which might cause adverse effects during pregnancy. Misidentification of an herb was associated with neonatal hirsutism in a baby born with hair on its forehead, pubic hair, swollen nipples and enlarged testes. The mother had taken a product that purportedly contained eleuthero, also called Siberian ginseng (*Eleutherococcus senticosis*) throughout pregnancy and during lactation. In a two-generation rat study, *Eleutherococcus senticosis* caused

no adverse effects on reproductive performance. Subsequent analysis of the product taken by the mother showed that the herb consumed was actually Chinese silk vine (*Periploca sepium*), which is contraindicated during pregnancy.

Surveys of pregnant women and midwives have not revealed reckless use of problematic herbs. Some alarmist articles in the medical literature include lists of plants to avoid in pregnancy, but these lists often include plants that are never used in pregnancy, never used medicinally, or never ingested intentionally except by those attempting suicide. Such lists are not helpful to clinicians.

On the other hand, the safety of many herbs commonly used during pregnancy has not been established. Even where some data in humans exist, no studies specifically designed to pick up adverse reproductive effects have been performed to date. There are many unknowns about the safety of herbs in pregnancy. The popularity of some herbs makes further research an important public health issue.

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Does Maternal Diabetes or Obesity Increase the Risk of Having a Child with a Birth Defect?

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Given the increasing frequency of diabetes and obesity among women of childbearing age in recent years, understanding the effects of these disorders on the embryo or fetus is critical. Many epidemiologic studies using different study designs have provided strong evidence for maternal pregestational diabetes (type 1 and type 2) as a risk factor for numerous types of birth defects, and results from experimental animal studies have supported these epidemiologic findings. In most studies, the overall risk for birth defects among infants of diabetic mothers is about two to four times that of infants of non-diabetic mothers. The degree of increased risk varies by the type of birth defect and by whether a defect is "isolated" or occurs with other unrelated major birth defects ("multiple") (Figure 29-1). For certain rare birth defects, such as sacral agenesis, the risk among diabetic mothers is estimated as more than 100 times higher than among non-diabetic mothers, whereas the increase in risk for other defects is more modest and the risk is not at all increased for a few defects. Women who achieve strict control of their blood glucose before conception can substantially reduce their risk for having an infant with a birth defect to that approaching the risk in the general population. Although diabetes is a well-recognized risk factor for birth defects, the mechanisms by which the increased risk occurs are not well understood. Better understanding of these mechanisms could provide strategies for intervention among women with poorly controlled diabetes and is an important area for future research.

The risk for having an infant with birth defects associated with gestational diabetes, diabetes that is first diagnosed during pregnancy, is more controversial. Some studies have shown an increased risk for certain birth defects among women with gestational diabetes, while others have not. Some studies have suggested that gestational diabetes may present a higher risk for women who are obese. Given the timing of gestational diabetes (with onset after the critical period of development of most organs), some investigators have suggested that the increased risk for birth defects observed in some studies might be due to inclusion of women who actually have pregestational diabetes in whom
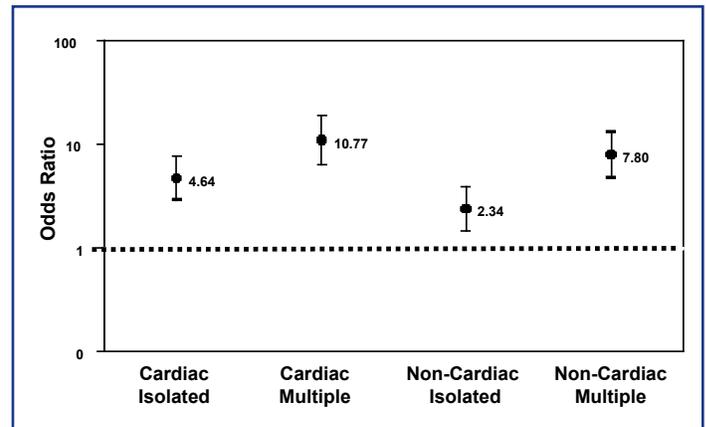
Figure 29-1. *Graph showing adjusted odds ratios and 95% confidence intervals for the association between maternal pregestational diabetes and types of birth defects—cardiac and non-cardiac, and infants with isolated defects and with defects occurring with other major unrelated birth defects (multiple defects). Data are from National Birth Defects Prevention Study (NBDPS); of note, not all cardiac or non-cardiac birth defects are eligible for inclusion by the NBDPS. (Correa et al., 2008)*
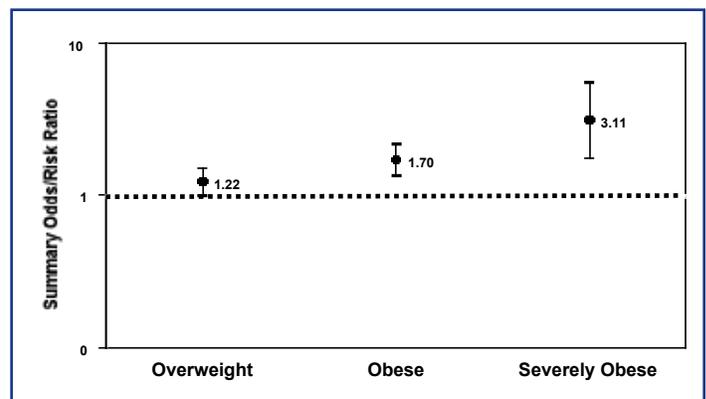
Figure 29-2. *Graph showing summary odds ratios and 95% confidence intervals from a meta-analysis examining the association between maternal obesity and neural tube defects. This meta-analysis demonstrated a dose-response relationship between degree of overweight or obesity and odds of neural tube defects. (Rasmussen et al., 2008)*

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[1]*The findings and conclusions in this report are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention.*

the diagnosis was not made until undergoing screening for gestational diabetes during the second or third trimester of pregnancy. Of note, in the United States, about one-third of diabetes goes undiagnosed.

Many studies have shown that women who have a baby with a neural tube defect, such as spina bifida or anencephaly, are more likely to be obese, suggesting that obesity is a risk factor for these defects. In addition, a dose-response relationship has been observed; that is, the risk appears be higher as the degree of overweight or obesity increases. In a recent meta-analysis, odds ratios for having a pregnancy affected by a neural tube defect were 1.22 for overweight women, 1.70 for obese women, and 3.11 for women who were severely obese (Figure 29-2), compared with women of normal weight (an odds ratio of 1.0 would indicate no increased risk). An increase in the risk of other defects has also been suggested, with the most convincing evidence for congenital heart defects in which a meta-analysis identified an odds ratio of 1.30 (95% confidence intervals 1.12–1.51), suggesting a modestly increased risk. Although several defects appear to occur more frequently among obese women, one defect, gastroschisis (a defect in which the intestines and sometimes other organs protrude through a defect in the abdominal wall), appears to occur less frequently.

The reasons for the association between maternal obesity and certain birth defects are not known. One possible reason is that women who are obese might have different nutritional requirements than women who are of normal weight. Lower levels of serum folate, have been associated with some types of birth defects and also have been observed among obese women, providing some evidence for this hypothesis. Another possible reason is that obese women may have metabolic abnormalities (e.g., increased levels of insulin or estrogen) that may increase the risk for birth defects. Another possibility might be related to undiagnosed diabetes. Women who are obese are more likely to have pregestational diabetes, a known risk factor for birth defects. In most previous analyses of the association between obesity and birth defects, women known to have pregestational diabetes have been excluded, but it is possible that some women with pregestational diabetes were not diagnosed before pregnancy and thus

were inadvertently included, potentially raising the observed risk associated with obesity. Finally, there may be other differences in obese women (e.g., related to diet or physical activity) that may increase the risk for birth defects.

In summary, maternal prepregnancy diabetes is a well-recognized risk factor for birth defects, but the effects of gestational diabetes are less well understood. Maternal obesity is associated with increases in several types of birth defects, with the evidence being the strongest for neural tube defects and perhaps congenital heart defects. Better understanding of these conditions and how they increase the risk for birth defects is essential to the development of strategies to decrease risks for birth defects to infants born to women with these conditions.

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Which Infections Increase the Risk of Birth Defects?

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Most infections that a woman contracts during pregnancy do not harm the developing embryo or fetus. However, a few infectious diseases can kill an embryo, fetus, or newborn, cause birth defects, trigger a premature delivery, or inhibit fetal growth. Only eight infectious agents are generally considered to increase the risk of birth defects in humans. These include six viruses: the rubella virus, Venezuelan equine encephalitis virus, cytomegalovirus, varicella zoster virus, herpes simplex viruses, and lymphocytic choriomeningitis virus; one bacterium, *Treponema pallidum*; and one protozoal parasite, *Toxoplasma gondii*.

Rubella virus causes German measles. An exposed fetus may develop a congenital rubella syndrome, which includes cardiovascular defects, microcephaly (abnormally small head), microphthalmia (abnormally small eyes), cataracts, glaucoma, retinopathy, mental retardation, growth retardation, motor impairment, an enlarged liver and spleen, and skin rash. The risk of birth defects from rubella exposure during the first trimester of pregnancy may be as high as 85 percent. Exposure between weeks 13 and 18 of pregnancy has about a 25 percent risk of birth defects. There is little risk of birth defects after the 18th week of pregnancy. The incidence of congenital rubella syndrome has decreased dramatically since the introduction of the rubella vaccine.

Venezuelan equine encephalitis virus is related to the rubella virus. First trimester infection can cause spontaneous abortion of the embryo or fetus. Fetal infection during the second or third trimester of pregnancy may cause severe damage to the brain and fetal death. The virus also causes brain and eye malformations in rhesus monkeys infected during gestation.

Cytomegalovirus (CMV) can cause serious effects in babies infected anytime during pregnancy. About 5 to 10 percent of infected infants show serious effects at birth. Even if infants appear normal at birth, 10 to 15 percent develop handicaps within a few years. Adverse effects of CMV include microcephaly, cerebral atrophy, enlarged cerebral ventricles, enlarged liver and spleen, intracranial calcifications, chorioretinitis (an inflammation of the choroid, the thin pigmented vascular coat of the eye, and the retina), skin lesions, blindness, hearing loss, mental retardation, intrauterine growth retardation, and neurologic dysfunctions.

Child with congenital rubella syndrome (South 1966).

Decrease in rate of rubella cases in the U.S. between 1966 and 1982. A complementary reduction in congenital rubella symdrom was also observed (South1986).

Infant's brain damaged due to toxoplasmosis induced hydrocephalus (Larsen, 1986)
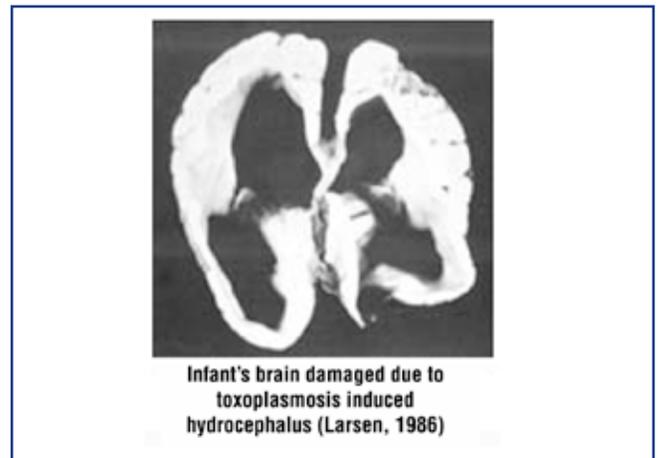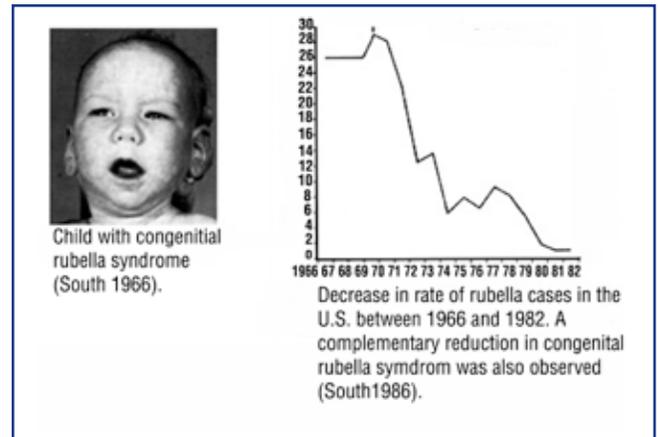
Graphics from www.teratology.org.

Varicella zoster virus is related to cytomegalovirus and herpes simplex viruses. Varicella zoster causes chickenpox (varicella), and reactivation of the virus causes shingles (herpes zoster). A fetus infected during the first 20 weeks of pregnancy has about a one percent risk of developing congenital varicella syndrome. Newborns with the syndrome may have atrophy of the cerebral cortex, enlarged cerebral ventricles, nerve fiber abnormalities, microcephaly, mental and psychomotor retardation, learning disabilities, cataracts, microphthalmia, chorioretinitis, hypoplastic (underdeveloped) limbs and digits, intrauterine growth retardation, and scarring of the skin.

Herpes simplex viruses (HSV) cause blisters and ulcers of the skin and can infect the central nervous system, eyes, and liver. HSV Type 1 usually produces cold sores around or in the mouth, whereas HSV Type 2 usually affects the genitalia, causing genital herpes eruptions. When a woman is infected with herpes for the first time during a pregnancy, the fetus can be severely affected. HSV can cause microcephaly, hydrocephaly, hydranencephaly (absence of the cerebral hemispheres), porencephaly (cavities in the brain), intracranial calcification, microphthalmia, chorioretinitis, skin aplasia (failure of skin to develop), skin lesions, and psychomotor retardation. The most serious effects appear following infection during the third trimester, but abnormalities can occur from infection any time during pregnancy. Many effects are likely due to disruption of existing structures rather than malformation. Most HSV abnormalities are caused by the Type 2 virus, but some are caused by the Type 1 virus.

Lymphocytic choriomeningitis virus (LCMV) is acquired from exposure to infected rodents and causes lymphocytic choriomeningitis virus syndrome. About one-third of fetuses infected during pregnancy die, and about 85 percent of surviving infants have serious problems including microphthalmia, cataracts, chorioretinitis, scarring and atrophy of the eyes, macrocephaly (abnormally large head), hydrocephaly, and microcephaly due to dysplasia or atrophy of the cerebral cortex. Congenital LCMV mimics congenital cytomegalovirus and congenital toxoplasmosis.

Treponema pallidum is the bacterium that causes syphilis. A fetus can contract congenital syphilis anytime during pregnancy. Untreated syphilis infection during early pregnancy causes up to 40 percent of pregnancies to end in spontaneous abortion, fetal death, or perinatal death. Congenital syphilis can cause fetal hydrops (abnormal accumulation of fluid in the entire body), hydrocephaly, defects of the teeth and skeleton, enlargement of the liver and spleen, vision loss, hearing loss, mental retardation, hemiplegia (paralysis of one side of the body), and skin lesions and scarring.

The protozoan parasite *Toxoplasma gondii* causes toxoplasmosis. Infants infected during pregnancy may develop hydrocephaly, microcephaly, macrocephaly, cerebral calcifications, enlarged liver and spleen, vision loss, hearing loss, mental retardation, cerebral palsy, seizures, neurologic problems, encephalitis, chorioretinitis, and inflammation of multiple organs. The highest risk for severe effects occurs with maternal infection between 10 and 24 weeks of gestation. Up to 40 percent of fetuses infected during the first trimester of pregnancy develop severe effects.

Human birth defects have been reported to occur in association with other infectious agents including HIV, parvovirus B19 (which causes fifth disease), *Borrelia burgdorferi* (which causes Lyme disease), cocksackie virus, Epstein-Barr virus (which causes infectious mononucleosis), influenza viruses, mumps virus, Mycoplasma, and Salmonella. However, there is presently insufficient evidence to conclude that the infections caused the congenital abnormalities.

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How Does Nutrition Influence Development

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A growing fetus needs food. Severely restricted intake of calories or protein can cause decreased fertility, fetal death, premature delivery, and growth restriction. Deficiencies in some essential minerals or vitamins increase the risk of malformations and other adverse pregnancy outcomes.

Extreme caloric restriction leads to irregular menstrual periods or amenorrhea (lack of menstruation), both of which decrease fertility. During the last six months of World War II, a Nazi-occupied area of the Netherlands was completely cut off from food supplies, in retaliation for a Dutch rail strike supporting the Allies. The average daily food ration decreased from 1400 calories to less than 600 calories (almost exclusively from bread and potatoes) during the last two months. Women who were pregnant or became pregnant during this famine suffered an excess of premature deliveries, very low-birth weight infants, and infant deaths. Similar poor outcomes were reported during the siege of Leningrad. The "fetal origins of adult disease" hypothesis holds that under-nutrition during fetal life, infancy, and early childhood followed by a rapid increase in body mass index can increase the risk of chronic diseases such as coronary heart disease, hypertension, and type 2 diabetes, underscoring the importance of good maternal nutrition.

Deficiencies in certain micronutrients cause congenital malformations in animals, including humans. The relationship between iodine deficiency, goiter, and cretinism, a neurologic disorder characterized by severe mental retardation, was identified during the nineteenth century and represents the earliest observation of the interconnection of diet and birth outcome. While iodine supplementation (primarily in the form of iodized salt) has eliminated goiter and cretinism in developed countries, about 1.6 billion people (24% of the world population) still suffer from iodine deficiency disorders. Iodine deficiency during pregnancy also can cause fetal death, severe growth restriction, abnormal bone development, and varying degrees of mental impairment.

Vitamin A deficiency was first shown to be teratogenic in swine in the 1930s. Warkany and his colleagues extended these findings by detailing the defects that vitamin A deficiency produces in virtually every organ system of the rodent. More recently, vitamin A has been shown to be critical in establishing anterior–posterior body axis patterns in the embryo. Sporadic reports in the literature have linked eye abnormalities and other adverse birth outcomes to severe maternal vitamin A deficiency. Malformations induced by

Nutritional deprivation can result in undergrown babies (right).

Blickstein I. Normal and abnormal growth of multiples. Seminars in Nematology 2002; 7(3): 177-185. Reprinted with permission.

vitamin A deficiency in humans are rare, but in developing countries, vitamin A deficiency remains the leading cause of visual impairment and blindness. Even though it is important to get enough vitamin A during pregnancy, too much vitamin A can also be teratogenic (discussed in the last paragraph of this chapter).

Deficiencies in many B vitamins adversely affect development. In animals, riboflavin, niacin, folic acid, and pantothenic acid deficiencies cause structural malformations; pyridoxine and thiamine deficiencies increase embryonic mortality and decrease fetal growth. Folate deficiency induced by a folic acid antagonist, causes structural malformations in animals. The neural tube defect rate among offspring of women taking folic acid supplements at the time of conception is reduced by as much as 50% compared to that among unsupplemented pregnancies (chapter 32). The U.S. Public Health Service recommends that all women receive 400 μg of folic acid daily, and since January, 1998, all grain products are required to be fortified with folic acid at a level designed to provide an additional daily intake of 100 μg/day folic acid. It is controversial whether supplemental folate overcomes the effects of subclinical deficiency in the pregnant woman or a metabolic problem of the embryo. Low maternal Vitamin B_{12} status can lead to neurological developmental delay and megaloblastic anemia in the offspring and has been reported to be an independent risk factor for the occurrence of neural tube defects.

Choline has been deemed an essential nutrient as the body cannot always produce enough to meet its needs. Choline functions in the synthesis of membrane phospholipids and the neurotransmitter acetylcholine and participates in methylation reactions including DNA methylation, which can affect gene expression. In experimental animals, low choline intake during late pregnancy alters brain structure and function whereas *in utero* choline supplementation can improve cognitive performance or behavioral tests in the offspring. Long chain polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) found in fatty fish, have also garnered interest. Reports from animal and human studies suggest a possible relationship between DHA and visual acuity in the offspring.

Low maternal vitamin D status is associated with reduced infant growth, neonatal hypocalcemia, and poor bone mineralization. Vitamin D can be obtained from diet, supplements and is generated in the skin from 7-dehydrocholesterol upon UVB radiation. Dark skin, concealing clothing, sunscreen use, and northerly latitudes can limit sunlight exposure and reduce vitamin D synthesis. Recently, a high prevalence of maternal hypovitaminosis D has been identified internationally, fueling the concern that vitamin D deficiency is an increasing public health problem. Vitamin E deficiency in rats produced litters in which approximately 30% of pups had brain anomalies (exencephaly or hydrocephalus). However, there is no evidence that vitamin E deficiency is teratogenic in humans. In contrast, vitamin K deficiency in humans (usually as a result of therapy with warfarin, an oral anticoagulant) results in a high percentage of miscarriage and prematurity. Infants have characteristic bone abnormalities, optic atrophy, and mental retardation.

Zinc deficiency is teratogenic in animals, affecting the development of virtually every organ system. After only 24 hours of dietary deficiency, plasma zinc decreases by 40%, and only a few days of deficiency during the embryonic period can produce malformations. Offspring of women with acrodermatitis enteropathica, a genetic disorder of zinc absorption, have a higher rate of malformations. Epidemiological studies also suggest a relationship between zinc deficiency and central nervous system malformations in humans. Although severe zinc deficiency is uncommon in developed countries, mild deficiency is common: the average dietary intake is only about half of the Recommended Daily Allowance (RDA) for pregnant and lactating women. Also, some drugs, chemicals, and physiological or environmental stressors can significantly alter zinc metabolism. These two facts, along with the observation that even transitory zinc deficiency can have adverse effects, suggest that unrecognized, subclinical zinc deficiency may play a role in some human embryonic morbidity.

The adverse effects of copper deficiency during pregnancy have been shown in numerous species, including humans. Copper-deficient lambs exhibit neonatal ataxia and myocardial atrophy. The effects of prenatal copper deficiency in humans have been reported in offspring with Menkes' disease, an X-linked disorder of copper metabolism. These infants have mental retardation and severe cardiovascular and connective tissue defects that generally cause death by three years of age. Many of the defects can be linked to decreased activity of copper-requiring enzymes. Copper-chelating drugs, including D-penicillamine, used to treat Wilson's disease (a genetic copper overload condition) and rheumatoid arthritis can induce copper deficiency in humans.

Vitamins may be good for you, but more is not necessarily better. Megadoses of vitamins may be harmful in some instances. In animal models, an excess of vitamin A is teratogenic, affecting the development of many organs. The expression of many genes responsible for establishing the embryonic body pattern is controlled by retinoic acid, the active form of vitamin A. Vitamin A levels are tightly controlled in the embryo; excess vitamin A can overwhelm these control mechanisms, leading to abnormal development. It is likely that vitamin A excess would be teratogenic in humans, but the minimum teratogenic dosage is not clear. The latest thinking is that this level is more than 30,000 International Units (IU)/day; the RDA for pregnancy is 2567 IU/day). The Teratology Society, in a position paper on vitamin A issued in 1987, reasonably advocated that vitamin A intake in pregnant women be restricted to the RDA.

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What Is the Impact of Dietary Folic Acid Fortification on the Risk of Birth Defects?

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Whether taking folic acid around the time of conception could prevent birth defects of the brain and spinal cord known as neural tube defects (NTDs) was highly controversial until the publication of two landmark studies in the early 1990s, one by Czeizel and Dudas, and the other by the Medical Research Council. These studies proved conclusively that folic acid could dramatically reduce the risk of NTDs and led the U.S. Public Health Service (USPHS) to recommend that all women of childbearing age capable of becoming pregnant take 400 micrograms of folic acid daily. It is unfortunate that this recommendation was followed by only 30–40 percent of American women. The USPHS soon recognized that another strategy was needed to get folic acid to all women at risk and began fortifying enriched cereal grains (bread, pasta, rice) with 140 micrograms of folic acid per 100 grams of grain.

The impact of this effort has not been easy to measure. In the U.S. most NTDs are diagnosed prenatally and many pregnancies are terminated without being identified in vital records. Thus, looking at rates of NTDs reported on birth certificates results in missing many cases. In Canada, which has a very similar fortification program, more information is available on prenatally diagnosed cases. The rates before and after fortification in various countries are shown in Figure 32-1. Comparing rates in U.S. and Canadian studies showed that the drop in rates was greater in areas where ascertainment was more complete. As shown in the figure, the drop in rates is also related to the starting rate; higher risk populations show greater reductions, probably due to a combination of factors: some populations have low folate diets and some are genetically at high risk. A demonstration project in China showed that northern China, for example, had far higher NTD rates than southern China, probably because folate containing foods were scarce in the north. Newfoundland had higher rates than most areas of Canada, probably because the Irish and Scottish background of the inhabitants put them at high risk because of genetic factors.

It is not clear how much of a reduction in rates should have been seen. The Medical Research Council trial found a 72% decrease in NTD rates but the confidence interval extended from 29% to 88% and the women in the trial were not typical because all had a history of previous affected
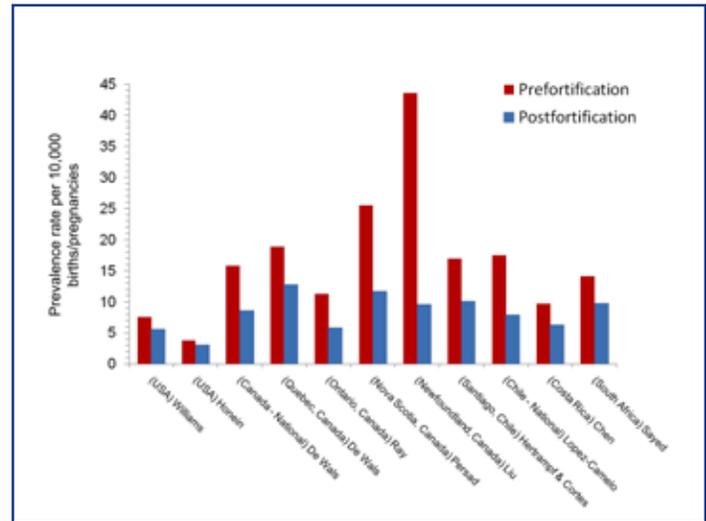
Figure 32-1 Legend. *Change in neural tube defect (NTD) prevalence rates following mandatory fortification of food with folic acid in selected populations. Ascertainment of cases varies by area; some include prenatally as well as postnatally diagnosed cases. Therefore, the denominator may be births or pregnancies.*

children. Other trials did not have a sufficient number of subjects to determine what the expected benefit should be. Case control studies generally showed around a 50% reduction in risk in those who took folic acid-containing vitamins. Case control studies, however, compared women who elected to take folic acid containing vitamins to women who did not. This design could introduce a bias in that vitamin takers could be more health conscious in general. Thus, it is uncertain exactly what the target reduction is.

The actual drop in NTD rates since fortification began averages around 40 to 50% (Figure 32-1). This drop is consistent with the effect expected based on the case control studies. It is also consistent with the effect predicted by modeling based on giving women various doses of folic acid to determine how much was needed to reach blood folate levels known to be protective against NTDs.

Are there more folic acid-preventable NTDs? A recent publication by Mosley *et al.* indicates that there may not be. The investigators interviewed women who had an NTD

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affected child and women who had unaffected children. They found that women who had an affected child were not significantly less likely to have used folic acid supplements. Their data suggest that, because folic acid supplement use was not a factor, the amount of folic acid in fortified food is sufficient to prevent folate-related NTDs. Although it is well known that some NTDs occur for reasons unrelated to folic acid (chapter 7), such as genetic syndromes and obesity, it is not completely clear what the minimum NTD rate achievable by folic acid is.

Older case control studies suggested that periconceptional use of multivitamins containing folic acid might reduce the risk for defects other than NTDs. These findings led a number of investigators to compare other defect rates pre and post-fortification. The results are mixed: some reports show a significant decline in congenital heart defects (atrial septal defects, conotruncal and other severe defects), diaphragmatic hernia, isolated cleft palate, pyloric stenosis, limb defects, and omphalocele. Other reports, however, have failed to confirm these findings, suggesting that the positive results could be chance findings. The failure to find consistently lower rates of other defects since the institution of food fortification may well mean that only NTDs are preventable by folic acid at the levels present in fortified food.

It is important to note that there is considerable debate over other benefits and risks of food fortification. Food fortification can almost eliminate folate deficiency. Hopes that folic acid could prevent cardiovascular disease by reducing homocysteine, a known marker for cardiovascular disease, have not been realized as shown in many clinical trials. Moreover, some, but not all, trials have found an increase in cancers or pre-malignant conditions in subjects who received folic acid. This finding has raised concerns that folic acid may be promoting the growth of cancerous or pre-cancerous lesions. There is hope that as data from other folic acid trials become available, this question will be resolved.

Folic acid has been a major success story. Food fortification with folic acid is one of very few modalities that can actually prevent a serious birth defect. Much more needs to be learned about the other benefits and risks associated with food fortification with folic acid.

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Can *In Utero* Exposures Program An Increased Risk For Diseases Decades Later In Life?

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In the early 1990's, David Barker and his colleagues studied the relationship between the incidence of coronary heart disease and birth weight in a population of adult men and women in Hertfordshire, England. They found an inverse correlation between the incidence of coronary heart disease and birth weight—the lower the weight at birth, the higher the risk of coronary heart disease in adulthood. Importantly, this was not simply a problem of low birth weight or premature birth, as the inverse relationship was evident among full-term births within a normal birth weight range (i.e., 5–10 pounds). Subsequent studies by this group and others expanded the range of adult diseases inversely correlated with birth weight to include hypertension, diabetes, and obesity. These are components of the *metabolic syndrome*, and all contribute to increased risk of coronary heart disease. Since that time, a number of studies around the world have corroborated these findings. The "Barker hypothesis" postulates that organs and metabolic pathways undergo programming during embryonic and fetal life, which determines the set points of physiological and metabolic responses that carry into adulthood.

A corollary hypothesis, the *thrifty phenotype hypothesis*, focused on maternal and fetal nutrition as the basis for the observed inverse relationship between birth weight and risk of adult disease. This hypothesis states that during the prenatal (and perhaps early postnatal) period, the developing child can adapt to a low nutrient environment by permanently altering its metabolism to become more efficient at storing and using nutrients. The developing organism is making metabolic adaptations for a life of low nutrient availability, as predicted by its developmental environment. This "predictive adaptive" response would be advantageous if, indeed, the undernutrition experienced during development continued throughout life.
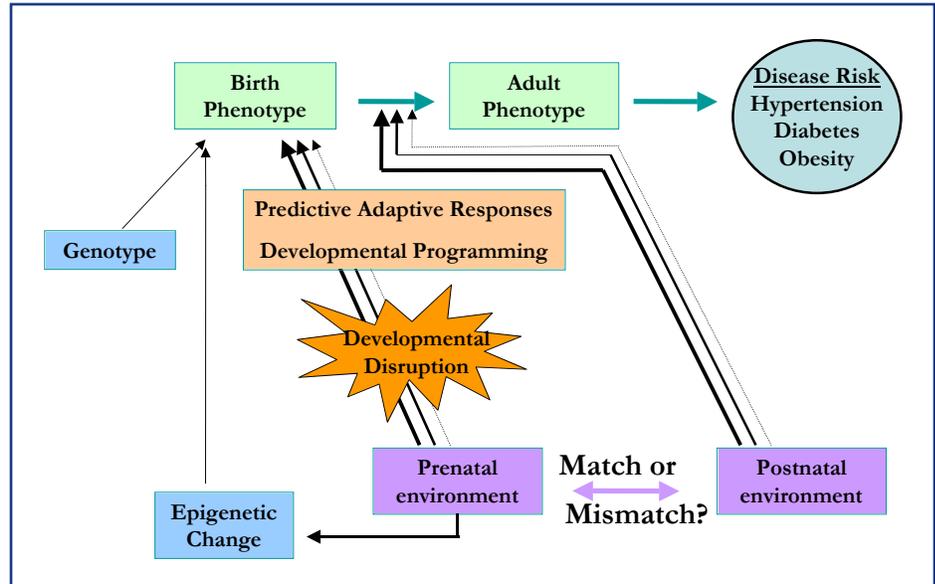
Figure 33-1. *The mismatch hypothesis.* *Predictive adaptive responses to the prenatal environment (developmental programming) produce a birth phenotype adapted to a similar postnatal environment (e.g., nutrient deprived). Developmental programming probably occurs in part by epigenetic alterations to the DNA. If the postnatal environment does not match the prenatal environment, the individual is maladapted and may be at increased risk of diseases later in childhood or adulthood. Adapted from Gluckman and Hanson, 2008.*

But what if this developmental prediction of the adult world is wrong? What if prenatal undernutrition is followed by a postnatal environment of adequate or excess nutrition? The ability to efficiently use and store nutrients is now a maladaptation, one that will favor accumulation of excess energy stores, putting the individual at risk for obesity along with insulin resistance, hyper-insulinemia, and hypertension. A *mismatch* (Figure 33-1) between the developmental environment and the environment in later life may be a key factor in increasing the risk of developing the metabolic syndrome in childhood or later in life. At present it is not clear what, if any, role the prenatal environment has played in the rampant increase in the incidence of childhood-onset obesity and diabetes seen in developed countries in the past few decades.

Studies in laboratory animals, including rats, mice, and sheep, have recapitulated the findings in human epidemiology studies. Pregnant rats either underfed or fed a low-protein

diet during pregnancy have offspring that are more prone to obesity, hyper-insulinemia, and elevated blood pressure than are offspring of pregnant rats fed a nutritionally complete diet. Offspring of rats on the deficient diets are born smaller but tend to catch up to their normally-fed controls by weaning and then are more likely to become obese, hyper-insulinemic, and hypertensive, especially if fed a high-fat diet after weaning. Likewise, offspring of pregnant ewes fed a low protein diet demonstrate insulin resistance and elevated blood pressure. Vascular reactivity is elevated and the number of nephrons in the kidney is reduced in these offspring; both conditions contribute to elevated blood pressure. The periods of development most sensitive to the long-term effects of undernutrition have not been well defined in humans or animals.

There is mounting evidence that some chemical exposures during development may have some of the same long-term effects that undernutrition has on health. Chemicals, including phthalates, bisphenol A, organo-tins and diethylstilbestrol, have been called *environmental obesogens* because developmental exposures to these chemicals can induce obesity in offspring. These chemicals may alter energy balance through their ability to interfere with fatty acid metabolism and/or nuclear receptor signaling in adipocytes and other cells. The developmental period may be particularly sensitive to such effects because the environment during development can determine life-long metabolic patterns in the individual. Recently, a variety of chemical exposures during pregnancy in rats has been demonstrated to lead to elevated blood pressure.

What are the mechanisms by which undernutrition, toxicant exposure, or other adverse developmental environments permanently affect health? Alterations of the hypothalamic-pituitary-adrenal axis may be a common mechanism underlying fetal programming. Plasma cortisol levels in adulthood are correlated with birth weight and risk of developing the metabolic syndrome. Levels of glucocorticoids in the fetus can be elevated by under-nutrition and other stressful insults that have programming effects. These changes in hormone levels are paralleled by altered expression of glucocorticoid receptors and affect expression of enzymes, ion channels, and transporters regulated by the glucocorticoids. Thus, these endocrine changes may be both the cause and the consequence of intrauterine programming.

The molecular basis for developmental programming is likely to be epigenetic modification of DNA. Epigenetic changes are those that alter the expression of genes without altering the genetic sequence of the genes. Two prominent forms of epigenetic changes are DNA methylation and histone modifications. For example, extensive methylation of cytosine nucleotides in gene promotors tends to turn genes off, and this silencing of genes can be permanent. Conversely, acetylation of histones (proteins associated with the DNA) can open the structure of the chromatin and enhance gene expression. There are periods during gametogenesis and embryonic development during which the methylation pattern of the genome is largely erased and re-established, and there are probably other developmental periods during which particular genes important for regulating growth and metabolism may be epigenetically altered, leading to developmental programming. Insights into these processes are emerging rapidly.

So yes, the *in utero* environment can affect life-long health, and a healthy start lasts a lifetime. A society devoted to the study of the Developmental Origins of Health and Disease (DOHaD) has a Web site with further information for those interested in this area: http://www.mrc.soton.ac.uk/dohad/.

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A

Adduct—A compound formed by a chemical addition reaction.

ADME—<u>A</u>bsorption, <u>D</u>istribution, <u>M</u>etabolism and <u>E</u>xcretion; the four functions that determine the fate of a chemical or drug in the body.

Agenesis—Usually occurring at birth, the absence or partial development of an organ or body part.

Allantois—The extraembryonic membrane formed early in development as an outpouching of the yolk sac into the area of the future umbilical cord. It is the site of blood formation for the embryo and the blood vessels of the allantois become the umbilical artery and veins.

Allele—Alternative form of a gene. Alleles are usually found in pairs at a specific site on a chromosome.

Alpha fetoprotein—A protein produced by fetal tissues. An abnormally high amount of this protein in the amniotic fluid or maternal serum may signal a neural tube defect, or other abnormal opening in the fetus.

Amino acid—One of a group of organic compounds containing an amino group and a carboxyl group which are the building blocks of protein.

Amniocentesis—A procedure in which a small amount of amniotic fluid is removed and analyzed to detect genetic abnormalities of the fetus.

Amnion—The extraembryonic membrane that lines the amniotic cavity (sac).

Amniotic cavity—The fluid filled cavity that surrounds the developing embryo.

Anencephalus (anencephaly)—Congenital absence of the upper part of the brain and the flat bones of the skull. See also Exencephaly.

Angiotensin converting enzyme (ACE) inhibitors—A class of drugs that inhibit the proteolytic enzyme that converts angiotensin I into angiotensin II; used to treat high blood pressure.

Aneuploidy—An abnormal number of chromosomes.

Anotia—Congenital absence of the ears.

Antepartum—Before birth.

Anterior—A descriptive term meaning situated in the front.

Anti-mitotic—Refers to inhibition of cell division.

Apoptosis—Programmed cell death; a type of cell death in which the cell uses its own specialized machinery to kill itself.

ARND—Alcohol Related Neurodevelopmental Disabilities—A spectrum of functional neurologic (behavioral) defects resulting from *in utero* exposure to alcohol.

Ataxia—A loss of voluntary muscle coordination.

ATPase—An enzyme that hydrolyzes ATP to ADP and phosphate.

Atrophy—Wasting or decrease in size of a tissue or organ.

B

Basal ganglia—Several large clusters of nerve cells, including the corpus striatum and the substantia nigra, deep in the brain below the cerebral hemispheres; participate in the regulation of motor performance.

Bioinformatics—The science of managing and analyzing large amounts of biological data using advanced computing techniques, especially in genomics.

Biotransformation—The conversion of a compound from one form to another by the actions of enzymes.

Blastocyst—An early stage of the embryo; a fluid-filled cavity surrounded by a single celled membrane, the trophoblast, and containing the inner cell mass, which will become the embryo.

Blastulation—The process by which the early embryo transforms from a solid mass of cells, the morula, to the blastocyst.

C

Caspase—A member of a group of protease enzymes that mediate apoptosis.

Cataract—Partial or complete opacity (clouding) of the lens of the eye; a common cause of blindness but curable by surgery.

Catecholamine—One of a group of hormones (e.g. epinephrine) that affects the sympathetic nervous system.

Caudal—A descriptive term meaning towards the tail; inferior.

Cerebellum—A part of the brain that is important for a number of cognitive and motor functions, including balance and coordination of movement.

Cerebral cortex—The layer of unmyelinated neurons (the gray matter) forming the cortex of the cerebrum.

Cerebrum—The largest part of the brain important for integration of motor, sensory, and other mental functions, such as thought, reason, emotion, and memory.

Cerebral palsy (spastic paralysis)—A condition resulting from brain damage before, at, or shortly after birth, that is marked by lack of muscle control.

Cerebrospinal fluid—The fluid that fills the spaces in and around the brain and spinal cord.

Chondrocyte—A cartilage cell.

Chorioallantoic placenta—The placenta developed from the allantois and chorion; establishes a nutritive and excretory connection between the blood of the fetus and that of the mother.

Chorion—The outermost membrane enclosing the fetus.

Chorionic villus—Any of the tiny extensions from the chorion that contain fetal blood vessels and combine with the uterine tissue to form the placenta.

Chorioretinitis—Inflammation of the choroid layer behind the retina of the eye.

Chromatin—Genetic material composed of DNA and proteins that condense to form chromosomes.

Chromosome—An organized structure of genes formed from condensed chromatin. In humans, there are 46 chromosomes and 2 sex chromosomes (an X or Y).

Cleft palate—A congenital fissure along the midline of the hard palate.

CNS—Central nervous system: The brain and spinal cord, olfactory bulbs and optic nerves.

Conceptus—An embryo or fetus.

Congenital—Present at birth.

Corpus callosum—A band of white neural tissue that joins the left and right hemispheres of the cerebrum.

Cranial—Relating to the cranium or skull; also a term used for directionality meaning towards the head.

Cranial placodes—Thickenings in the surface ectoderm of the embryo associated with the future eye and ear regions.

Craniosynostosis—Premature fusion of the cranial bones leading to abnormal head shape.

Cretinism—A developmental disorder caused by deficiency of thyroid hormone, and characterized by severe mental retardation, sometimes resulting from maternal iodine deficiency.

Cryptorchidism—Failure of the testes to descend into the scrotum.

Cytotrophoblast—The inner cellular layer of the trophectoderm (trophoblast); part of the mammalian placenta.

D

Developmental Neurotoxicity—Adverse effects on the development of the nervous system.

Diploid—Having a pair of each type of chromosome.

Distal—Farther or farthest from the center or trunk.

Down Syndrome—A disorder caused by an extra chromosome 21 (trisomy 21) and characterized by mental retardation and distinguishing physical features.

Dysmorphia (also dysmorphic, dysmorphogenesis)—A descriptive term, often referring to a birth defect, that indicates a difference in appearance of a body part or organ.

E

Ectoderm—The outermost layer in an embryo which will develop into the skin and nervous system.

Encephalitis—Inflammation of the brain.

Encephalocele—Protrusion of brain tissue through a fissure or defect in the skull.

Endocrine—Belonging to the endocrine glands or their secretions.

Endocytosis—A process by which extracellular materials are taken into cells.

Endoderm—The innermost layer of an embryo that will develop into the lining of the digestive tract and respiratory tract.

Endometrium—The inner lining of the uterus that is shed during menstruation.

Embryo—The developing organism from the stage after gastrulation when the central long axis appears until all major anatomical structures are present. In humans, this is from about the second week after fertilization to about the end of the seventh week of pregnancy.

Epiblast—The primitive ectoderm of the early embryo.

Epididymis—The tightly-coiled, thin-walled tube that conducts sperm from the testis to the vas deferens.

Epigenetic—Refers to changes in gene expression that are not the result of changes in the DNA sequence. The changes are stable and potentially heritable.

Epoxide hydrolase—A detoxification enzyme that modifies epoxides by adding a molecule of water and converts them to a molecular structure that can be more rapidly excreted.

Epstein-Barr virus—The herpes virus that causes infectious mononucleosis.

Estriol—One of the three naturally occurring forms of human estrogen. It is produced in significant amounts during pregnancy.

Ethanol—Ethyl alcohol.

Exencephaly—An open brain resulting from failure of the neural tube to close. In humans this is followed by degeneration of the brain, resulting in anencephaly.

External genitalia—The external sex organs.

Extracellular matrix—A non-cellular mesh of fibrous proteins and carbohydrate molecules (glycosaminoglycans) in body tissue that helps maintain and support the cells of that tissue.

Extraembryonic membranes—Membranes that surround the embryo; the chorion, yolk sac, allantois, and amnion.

F

Fetal alcohol syndrome—Characteristic facial changes and impaired mental development resulting from maternal alcohol intoxication during pregnancy.

Fetus—An unborn baby from the 8th week after conception until birth.

Folic acid—A B vitamin involved in DNA synthesis that is essential for growth and reproduction.

Frontonasal dysplasia—Also known as median cleft face syndrome, a rare craniofacial disorder.

G

Gamete—A sex cell. In higher animals, a sperm or an egg.

Gastroschisis—A malformation in which the intestines and sometimes other organs protrude through a defect in the abdominal wall.

Gastrulation—A stage of embryo development in which a two-layered embryo (ectoderm and endoderm) develops a third layer (mesoderm) through the movement of specific cells.

Gene—A hereditary unit of DNA that codes for a protein, found in a specific location on a chromosome. Each human chromosome contains many thousands of genes.

Genital folds—The embryonic structure that will differentiate into the penis in boys or the labia in girls.

Genome—All the genetic material in the chromosomes of an organism.

Genomics—The study of genes and their function.

Genotype—The genetic make-up of an individual. Expressions of genotype result in the phenotype which is how the individual looks. In the case of a recessive gene, such as that for albinism, persons who carry one albino gene and one normal allele and persons who carry two normal alleles, have the same (normal) phenotype, but different genotypes.

Germ cells—Sperm and egg cells and their precursors.

GIFT (Gamete Intrafallopian Transfer)—A technique to treat infertility by fertilizing an egg in the laboratory and placing the resulting embryo into the fallopian tube. The embryo is expected to travel through the fallopian tube and implant in the uterus much as it would have had natural fertilization occurred.

Glaucoma—A disease caused by increased pressure of the fluid within the eye, resulting in damage to the optic nerve; advanced disease is a common cause of blindness.

Glial cell—A kind of connective tissue cell in the brain and spinal cord. Glial cells provide structural support and nourishment to nerve cells.

Glucocorticoids—A class of hormones, including cortisol, produced by the adrenal glands. Glucocorticoids mediate a response to stress and affect protein and carbohydrate metabolism.

Glycolysis—The breaking down of glucose, a simple sugar, to produce energy.

Growth hormone releasing factor—A hormone, made in the hypothalamus, that causes the pituitary to release growth hormone. Growth hormone is involved in growth and in energy metabolism.

H

Haploid—Having only one of each chromosome (see diploid).

HCG (human chorionic gonadotropin)—A hormone made by those cells of the embryo that form the placenta. HCG is the hormone that is detected by pregnancy tests.

Hepatosplenomegaly—Enlargement of the liver and spleen.

Hippocampus—A portion of the brain, located in each temporal lobe, and associated with memory.

Hirsutism—Hair growth in excessive amounts and in unusual places.

Holoprosencephaly—A birth defect in which the embryonic forebrain fails to divide completely into the cerebral hemispheres; results in varying degrees of mental impairment and abnormal development of the eye, nose, and lip.

Hormone—A chemical messenger produced by one organ and transmitted through the blood to initiate or alter the function of another organ or tissue.

Hydranencephaly—A rare condition in which the brain's cerebral hemispheres are replaced by sacs filled with cerebrospinal fluid.

Hydrocephalus—Accumulation of excess cerebrospinal fluid within the ventricles of the brain; head enlargement and brain damage may occur.

Hypoblast—The innermost of the three primary germ layers, adjacent to the blastocyst cavity, which develops into the endoderm.

Hypospadias—A birth defect in which the urethra opens on the underside of the penis instead of at its end.

Hypoxia—Lack of oxygen that may lead to tissue damage.

Hydrops—Accumulation of fluid in body tissues or cavities.

I

ICSI (Intracytoplasmic sperm injection)—An infertility treatment in which the sperm is injected through the membrane of the egg into its cytoplasm.

Implantation—The embedding of the early embryo in the lining of the uterus.

Imprinting (Genetic)—Differential expression of a gene, depending on whether it was transmitted through the sperm or the egg; thought to be regulated by attachment of methyl groups to the DNA, and by chromatin structure.

Ischemia—Loss of blood flow that may lead to tissue damage.

Isotretinoin—A vitamin A-like medication (13-cis retinoic acid).

IVF (*in vitro* fertilization)—Fertilization outside the body, used as a treatment for infertility.

K

Karyotype—A picture of an individual's chromosomes, arranged in order from largest to smallest, to make it easier to look for extra, missing, or rearranged chromosome material.

Ketoacidosis—Abnormally high levels of ketones and acids in the blood; may occur in a diabetic person who does not get enough insulin.

L

Leydig cells—Cells in the testes that produce testosterone in the presence of luteinizing hormone (LH).

Leprosy—A disease caused by infection with the bacterium *Mycobacterium leprae*, often affecting the skin and nerves and causing body parts to become deformed.

LOAEL (Lowest Observed Adverse Effect Level)—In a toxicology study, the lowest tested dose that produces detectable damage.

Luteinizing hormone (LH)—A hormone made by the pituitary gland that acts on the ovary to control egg maturation and triggers ovulation and acts in the testes for the production of testosterone.

Lysosome—A cell organelle that contains enzymes for intracellular digestion of proteins and other molecules.

M

Macrosomia—An abnormally large body or body part.

Malformation—A structural defect due to abnormal development.

Meiosis—The cell division used to make germ cells from body cells. The diploid number of chromosomes is reduced to a haploid number; for example, in humans with 46 chromosomes, meiosis results in germ cells with 23 chromosomes each.

Membrane—A thin layer of tissue separating or connecting structures or organs.

Mendelian inheritance—Passing of genetic traits from parents to offspring, as expected when they are determined by single genes.

Meningomyelocele—A birth defect following failure of the neural tube to close; results in protrusion of a sac of nerve tissue and its covering membranes.

Mesoderm—A middle layer of cells in the embryo, lying between the ectoderm and the endoderm.

Metabolism—The chemical processes necessary for life that occur in the body.

Metallothionein—A protein in the body that binds metals such as zinc.

Methylation—Attachment of a methyl group to a molecule. Methylation of DNA is an epigenetic event that alters gene expression which affects cell function.

Microarray—A two dimensional display, typically on a glass, filter, or silicon wafer, upon which hundreds of DNA or protein samples are deposited or synthesized in a high-density matrix, in a predetermined spatial order, allowing them to be tested with labeled probes in a high-throughput, parallel manner. Used to study how large numbers of genes interact with each other and how a cell's regulatory networks control vast batteries of genes simultaneously.

Micromass culture—A laboratory technique in which dispersed cells of an embryonic organ such as the brain are allowed to reaggregate in culture.

Minimata disease—A syndrome of mental deficiency and neurologic impairment caused by exposure of a fetus to methylmercury.

Mitochondria—Cellular organelles that generate ATP molecules, the chemical energy source for the body.

Mitosis—Cell division that creates two genetically identical daughter cells by duplicating the genetic material of a parent cell.

Microcephaly—A small head.

Microphthalmia—A small eye.

Morphogen—A chemical message that directs tissue development in the embryo. An example of this has been described during development of the limbs.

Morphological—Pertaining to structure or form.

Morula—an early multi-celled stage of the embryo from which the blastocyst is formed.

MSAFP—<u>M</u>aternal <u>S</u>erum <u>A</u>lpha-<u>F</u>etoprotein. Alpha-fetoprotein is a protein made in the fetus that normally leaks, in small amounts, into the mother's circulation. If there is an abnormal opening in the fetus, such as a neural tube defect, larger amounts appear in the mother's serum, providing a screening test for such fetal anomalies.

Multicotyledonary placentation—Formation of a placenta with many lobes

Multifactorial inheritance—The transmission of a trait from parents to offspring that is determined by multiple genetic and environmental factors, each with a small effect.

Mutagen—An agent that increases the mutation rate.

Mutation—A permanent change in the genetic material.

Mycoplasma—A kind of minute microorganism that sometimes causes disease in humans.

Myelination—Coating of certain nerve fibers with a fatty sheath that enhances nerve signal transmission.

Myocarditis—Inflammation of the heart muscle.

Myositis—Inflammation of muscle.

N

Necrosis—Abnormal cell or tissue death.

Neural—Pertaining to nerves.

Neural crest—A band of cells on either side of the neural tube. Cells from these regions migrate to form parts of the nervous system, face, skin, and heart.

Neural plate—A flat area in the middle of the early embryo that will roll up to form the neural tube.

Neural tube—The embryonic tube that becomes the brain and spinal cord.

Neurobehavioral—Pertaining to the function of the nervous system as it relates to behavior.

Neuroendocrine—Pertaining to the nervous and endocrine systems in anatomical or functional relationship.

Neuron—A nerve cell.

Neuropore—An opening at the cranial or caudal end of the neural tube before it completes closure.

Neurulation—The formation of the neural plate and its rolling up into the neural tube.

NOAEL (No Observed Adverse Event Level)—In a toxicology study, the highest dose used that fails to produce evidence of damage.

Nucleotide—One of the basic building blocks of DNA and RNA, consisting of a nitrogenous base, a phosphate group, and a sugar molecule.

O

Omphalocele—The abnormal presence of abdominal contents in the umbilical cord. It results from failure of the normal withdrawal of the intestines from the cord into the abdomen during development.

Oocyte—A female germ cell in the ovary; precursor of the ovum.

Organogenesis—Formation and development of organs.

Orofacial cleft—The failure of the lip or palate to fuse properly.

P

Palate (secondary)—The roof of the mouth, consisting of the hard palate, soft palate, and uvula.

Pharmacogenetics—The study of single gene interactions with drugs.

Pharmacogenomics—The study of the relationship between an individual's genetic make-up (genome) and drug response.

Phenotype—How an individual looks as a function of their genetic make up (see genotype).

Phenylketonuria—A recessively inherited condition in which metabolism of an amino acid, phenylalanine, is blocked; increased phenylalanine in the infant causes nerve and brain cell damage, and mental retardation.

Phocomelia—A birth defect with hands and feet attached to underdeveloped limbs; this and other severe malformations are associated with prenatal thalidomide exposure.

Pinocytosis—The engulfment of liquid droplets by a cell through minute invaginations of its membrane.

Placenta—The organ that is formed in pregnancy from both fetal and maternal tissues and functions in the growth and protection of the fetus.

Pluripotent—Able to differentiate into a variety of cell types; examples are the ovum and embryonic stem cells.

Polydactyly—The presence of extra fingers or toes.

Polymorphism—The occurrence of two or more genetically different forms of a gene in the same population, where the less frequent form has a frequency of 1% or more.

Porencephaly—A cystic cavity in the brain; may result from brain tissue destruction or maldevelopment.

Posterior—A descriptive term meaning situated at the back.

Post-implantation—Occurring after the early embryo embeds into the lining of the uterus.

Postpartum—After birth.

Prader-Willi syndrome—A condition resulting from a deletion in chromosome 16; it is associated with short stature, mental retardation, small hands and feet, obesity, overeating, and underdeveloped gonads.

Pre-Implantation—Occurring before the early embryo embeds in the lining of the uterus.

Progesterone—A steroid hormone produced in the ovary by the corpus luteum; essential for the maintenance of pregnancy.

Protein kinase—An enzyme essential for protein phosphorylation; often involved in the signal transduction pathways activated by stressors.

Proteomics—Analysis of protein expression and function.

Psychomotor retardation—Retardation of both mental and motor development.

Q

QSAR—Quantitative Structure Activity Relationship; the study of the relationship of the structure of a chemical to its biological effect.

R

Receptor—A cell component that combines with a drug or other substance and thereby alters cell function.

Retinoid—A group of compounds that includes many metabolites of vitamin A.

Retinopathy—Disease of the retina, the innermost layer of the eye that receives and transmits images.

S

Sacral agenesis (caudal regression syndrome)—Absence or significant underdevelopment of the lower part of the spine and lower limbs. This congenital malformation is associated with maternal diabetes.

Salmonella—Gram negative rod shaped motile bacteria, some of which cause intestinal inflammation.

SARS—Severe Acute Respiratory Syndrome; a respiratory illness caused by a coronavirus.

Sertoli cells—Somatic cells within the seminiferous tubule which support germ cell development and form tight junctions to create the blood testis barrier.

Signal Transduction—Within a cell, any process by which one kind of signal or stimulus is converted to another.

SiRNA—Short or Small interfering RNA molecules that decrease the expression of a specific gene by degrading its messenger RNA.

Somatic—Pertaining to the body (excludes reproductive cells).

Somatomedin—A growth factor produced by the liver upon stimulation by somatotropin that acts directly on cartilage cells to stimulate skeletal growth.

Somatotropin—A hormone produced in the pituitary gland the acts in the liver to produce somatomedin.

Somite—One of paired, segmented blocks of mesodermal cells on either side of the neural tube of the embryo which give rise to connective tissue, bone, muscle, and the dermis of the skin.

Spermatid—A haploid male germ cell resulting from the division of a spermatocyte; the precursors of spermatozoa.

Spermatocyte—A male germ cell arising from the division of a spermatogonium during meiosis.

Spermatogonium—An undifferentiated male germ cell located close to the basement membrane of the seminiferous epithelium in the testis; gives rise to spermatocytes.

Spina bifida—A defect in which part of the vertebral column is absent, allowing the spinal membranes and sometimes the spinal cord to protrude; a result of failure of the neural tube to close.

Steroid—Any of a number of hormones with a common molecular structure that regulate body functions.

Syncytiotrophoblast—The layer of trophoblast cells that invades the endometrium during implantation.

Syndactyly—Fusion or webbing of fingers or toes.

T

Tay-Sachs disease—A recessively inherited disease, in which a deficiency of hexosaminidase A causes abnormal storage of a ganglioside. There is progressive mental deterioration and early death.

Teratogen—An agent that may induce abnormal embryo/fetal development when administered during pregnancy.

Teratology—The study of malformations or serious deviations from the normal type in organisms. It is the branch of science concerned with the production, development, anatomy, and classification of malformed fetuses.

Teratogenesis—The process by which birth defects arise.

Teratogenetics—The study of how genes and teratogens interact to cause birth defects.

Tetralogy of Fallot—A complex congenital heart disease involving four abnormalities: a ventricular septal defect, pulmonary stenosis, right ventricular hypertrophy, and an overriding aorta, which means that the aorta lies directly over the ventricular septal defect.

Thalidomide—A sedative, antinauseant, and hypnotic drug that causes abnormalities of limbs, heart, ear, and craniofacial structures when taken by pregnant women.

Threshold dose—The dose at which an agent has begun to have an effect.

Thrombocytopenia—An abnormally low number of platelets in the blood.

Toxoplasmosis—A disease caused by the protozoon *Toxoplasma gondii*. Infants infected during gestation may have hydrocephaly, microcephaly, encephalitis, cerebral palsy, mental retardation, loss of vision, deafness, and other problems.

Transcripts—Messenger RNAs that carry the genetic information from DNA to the ribosome to produce protein.

Transgenic organism—An organism derived by the transfer of one or more genes from another organism.

Trimester—A period of three months; human pregnancy is divided into three trimesters.

Triple screen—A combination of three tests (levels of MSAFP, estriol and HCG) which, if abnormal, indicate that the health of the fetus may be at risk.

Trisomy 18—The presence of an extra chromosome 18; Edwards syndrome.

Trisomy 21—The presence of an extra chromosome 21; Down syndrome.

Trophoblast—The outer layer of flattened cells forming the wall of the blastocyst.

U

Ultrasound—Sound waves of frequency higher than the range audible to the human ear used to delineate body structures by measuring the reflected waves.

Urogenital—Relating to the organs of the urinary and genital tracts.

V

Vas deferens—The tube that conveys sperm from the epididymis to the ejaculatory duct.

Ventral—On the belly side of the trunk; in humans, to the front of the body.

Ventricular septal defect—A defect in the wall dividing the two ventricles of the heart.

W

Whole embryo culture—A technique in which embryos undergoing organogenesis are cultured *in vitro*.

Williams syndrome—A syndrome resulting from a deletion in chromosome 7, which is associated with an elf-like face, mental retardation, short stature, and cardiac abnormalities.

X

X-linked—Refers to a gene that is located on one of the sex chromosomes, which is carried by the female in a double dose (XX) and the male in a single dose (XY).

Xenobiotic—A compound that is foreign to a living organism. Examples of xenobiotics include drugs, carcinogens or compounds that have been introduced into the body.

Y

Yolk sac—A fluid filled sac on the ventral side of the early embryo. Important in the transfer of nutrients during the second and third weeks of development.

Z

ZIFT (Zygote Intrafallopian Transfer)—The transfer of an *in vitro* fertilized zygote into the fallopian tube.

Zona pellucida—The membrane that encloses the mature ovum.

Zygote—The fertilized ovum.