Genetics in obstetrics and gynecology / Joe Leigh Simpson [and others].

GENETICS IN OBSTETRICS AND GYNECOLOGY

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Gynecologic Disorders: Differential Diagnosis & Therapy
Edited by Carl J. Pauerstein, M.D.

This textbook provides a unique and highly practical approach to the diagnosis and treatment of gynecologic disorders. Whereas most books discuss specific disease entities and their symptomatology and therapy, this book teaches the reader to work with the *symptoms* presented by the patient in arriving at the correct diagnosis. Each chapter presents important didactic information, discussions of clinical presentations, diagnostic methods, and the appropriate therapy for various conditions. An important feature is that two or three representative cases and the proper therapy for each are examined in each chapter.

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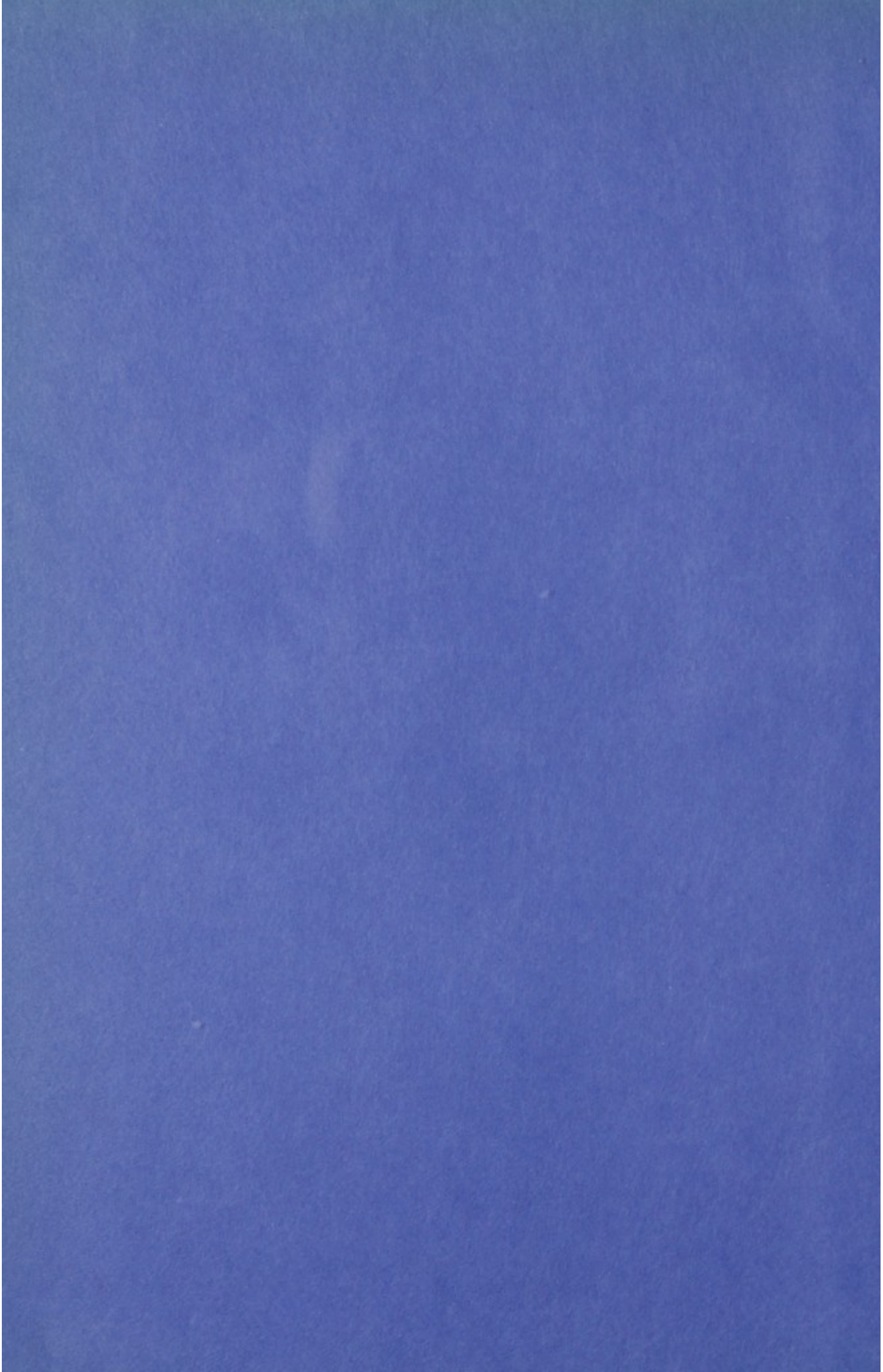

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Contents

Preface

Advances in human genetics have had a major impact on many aspects of medicine. In particular, obstetrician–gynecologists must be aware of the role of genetics in reproductive failure, spontaneous abortion, and congenital anomalies. Furthermore, the availability of antenatal diagnostic techniques makes it obligatory for obstetricians to appreciate the heritability of many disorders as well as their potential for diagnosis in utero.

Despite the increasing importance of genetics, many physicians are not thoroughly informed about genetics and its applications to clinical practice. Most physicians, especially obstetrician–gynecologists, have received no formal genetic training. Moreover, most available books do not address the particular needs of this specialty. To be sure, several volumes lucidly review genetic principles, and still others discuss clinical aspects of rare genetically determined conditions; however, these volumes are usually of interest primarily to pediatricians. Thus, such books are often less than immediately relevant to obstetrician–gynecologists and family physicians. In fact, no book on genetics has previously been written expressly for obstetrician–gynecologists.

Because of this lack, we have attempted to prepare a practical yet accurate volume on genetics and teratology as they relate to obstetrics and gynecology. Specialists in other areas, such as surgery and general medicine, will also find this book useful. Absolutely no prior knowledge of genetics is supposed, for which reason a review of basic genetics is provided in Chapter 1. Throughout the rest of the book, genetically determined disorders of clinical interest are surveyed. Emphasis is placed upon genetic counseling and those clinical features requiring attention by obstetrician–gynecologists. The current status of prenatal diagnosis is considered, as are genetic aspects of spontaneous abortion and twinning. Both clinical and genetic aspects of common gynecologic disorders, pelvic cancers, and disorders of sexual differentiation are discussed. Finally, potential human teratogens—radiation and physical agents, viruses and infectious agents, and chemicals (drugs)—are considered in detail.

Obstetrician–gynecologists who are aware of the information in this volume should feel comfortable in handling most genetic problems that arise in clinical practice. With the help of this volume, physicians will be prepared to appreciate future genetic advances having major impact upon our specialty.

1

Principles of Human Genetics

Both normal phenotypic variation, such as blood pressure, and abnormal phenotypic variation, such as Down syndrome or cystic fibrosis, may be considered in terms of several etiologic categories. These categories include (1) cytogenetic disorders, which result from changes in the number or structure of the chromosomes; (2) Mendelian disorders, which result from mutations at a single genetic locus; (3) polygenic (multifactorial) disorders, which result in part from the cumulative effects of more than one gene; and (4) teratogenic disorders, which depend primarily upon environmental factors. These categories are useful in classifying anomalies and other disorders (Lancet, 1974a). Other genetic factors, such as cytoplasmic inheritance, are of less immediate relevance to physicians.

MOLECULAR ASPECTS OF THE GENE

The principles of inheritance were discovered in 1865 by Mendel and were subsequently "rediscovered" around 1900 by others. In 1902, Sutton and Boveri first suggested that chromosomes served as the physical basis of inheritance. The chemical basis of inheritance was not elucidated until several decades later. In experiments involving genetic transformation of pneumococci (Griffith, 1928; Avery et al., 1944), deoxyribose nucleic acid (DNA) was shown to be the hereditary material of all living organism except certain viruses. Subsequently, several types of ribonucleic acid (RNA) were shown to be required to translate the information inherent in the genes.

Structure and Replication of DNA

In 1953, Watson and Crick proposed that DNA existed in the form of a double helix; this structural configuration helped explain how DNA could replicate. The double helix may be envisioned as a twisted ladder (Fig. 1-1). Each vertical column consists of alternating deoxyribose sugar residues and

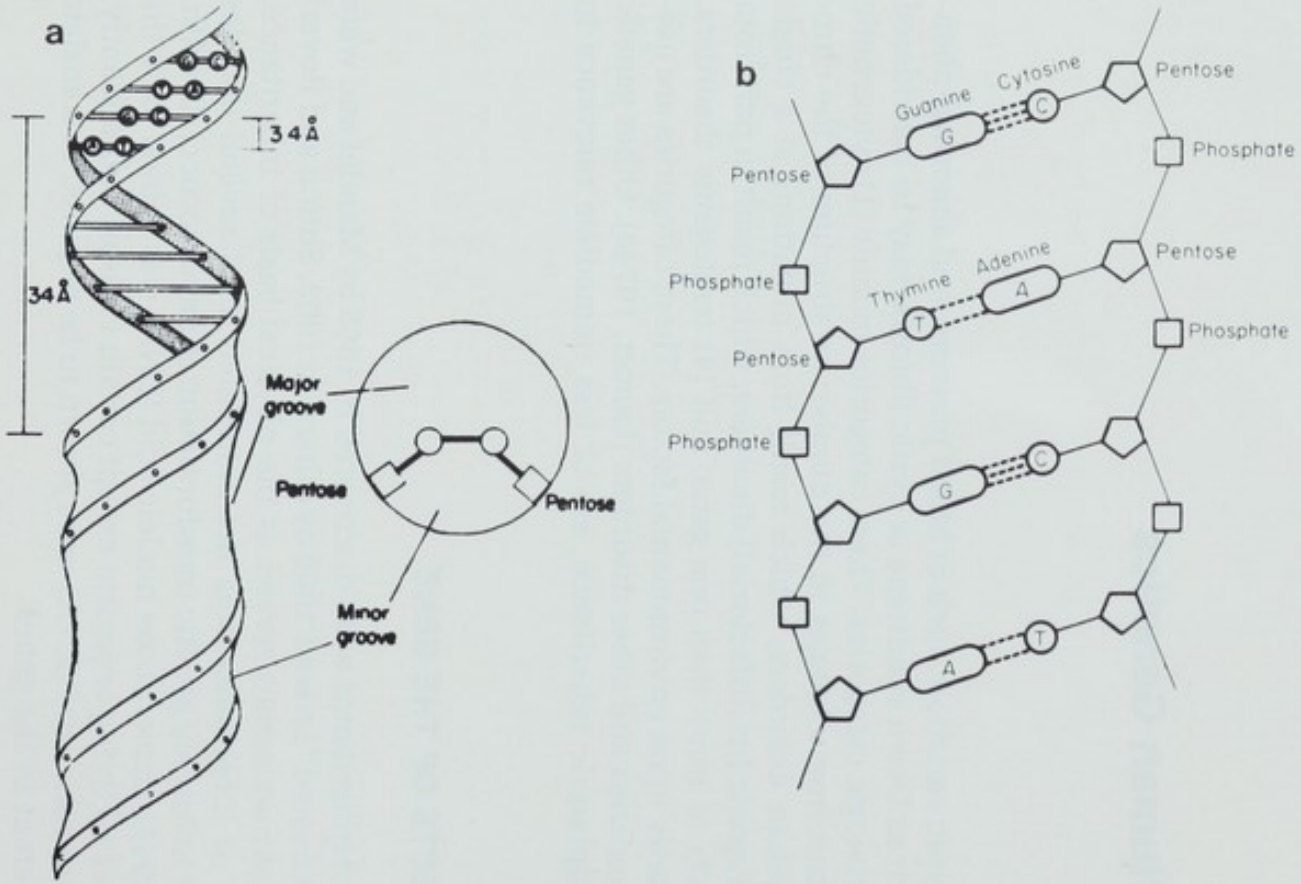


Figure 1-1. Schematic representation of the double helix configuration of DNA, illustrating helix (a) and base pairing (b). Each vertical column consists of alternating deoxyribose sugar (pentose) and phosphate. The pentose residues of opposite sides are connected transversely by nitrogenous bases: the purine adenine (A) or guanine (G) and the pyrimidine thymine (T) or cytosine (C). Each transverse connection consists of one purine and one pyrimidine, (AT or GC), held together by hydrogen bonds, which are represented by dotted lines. (From Ford EHR: Human Chromosomes. New York, Academic Press, 1973, pp. 95, 97. Reprinted with permission.)

phosphate residues. The sugar and phosphate residues of opposite sides are connected transversely—like ladder rungs—by various nitrogenous bases called nucleotides. In DNA the nucleotides are composed of the purine adenine (A) or guanine (G) and the pyrimidine thymine (T) or cytosine (C). Each transverse connection consists of one purine and one pyrimidine, joined by hydrogen bonds (base pairing). Adenine is bound to thymine by two hydrogen bonds. Cytosine is bound to guanine by three hydrogen bonds. Thus, the ratio of adenine to thymine, or guanine to cytosine, is always 1:1. The ratio of adenine–thymine (AT) pairs to guanine–cytosine (GC) pairs varies not only between species but also between different chromosomes in the same species.

The genetic information is coded by the sequence of nucleotides. Because of the base pairing described above, the two DNA strands are complementary. For example, if the sequence of bases on one strand is ATTGC (adenine–thymine–thymine–guanine–cytosine), the sequence on the opposite strand is TAACG. A codon, a sequence of three bases, signifies one and only one of the twenty amino acids. The sequence of codons determines the amino acid composition of a polypeptide. However, there are more codon permutations, namely $4^3 = 64$, than there are amino acids; thus, different codons can signify the same amino acid. Some codons initiate protein synthesis, and still others apparently code for nothing (nonsense codons).

In humans and other mammals DNA is carried by the chromosomes. Chromosomes consist of (1) at least two general classes of DNA—one class either moderately or highly repetitive with respect to its nucleotide sequence (repetitive DNA), and another class not repetitive (unique-sequence DNA); (2) histone proteins, which are believed to provide structural integrity for the chromosomes, and (3) nonhistone proteins, whose function is uncertain. The basic structure probably consists of a single long strand of DNA that is tightly coiled and branching. Unique sequence DNA is interspersed with intervening sequences of DNA. Prior to completion of translation (see below), the intervening sequences (introns) must be removed. DNA replication occurs at different times in different chromosomes and can be initiated at several different points along a single chromosome.

RNA and Protein Synthesis

RNA directs polypeptide synthesis in the cytoplasm (translation). RNA is structurally similar to DNA; however, the sugar in RNA is ribose rather than deoxyribose, and one pyrimidine is uracil rather than thymine. There are several types of RNA: messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), and heterodisperse RNA (hRNA). The function of hRNA is not entirely clear, but it appears to be a precursor of mRNA. Messenger RNA consists of a single-stranded molecule complementary to one of the two strands of the DNA double helix. After its synthesis by complementary pairing from a DNA strand, mRNA moves into the cytoplasm to associate with the ribosomes, structures that consist of protein and a nonspecific high molecular weight RNA (rRNA) (Fig. 1-2). Amino acids are prepared for protein synthesis by the reaction of adenosine triphosphate (ATP) with the α-carboxyl end of an amino acid to form an activated, amino acid–specific tRNA. This amino-acyl tRNA moves toward the surface of the ribosome. Protein synthesis progresses by movement

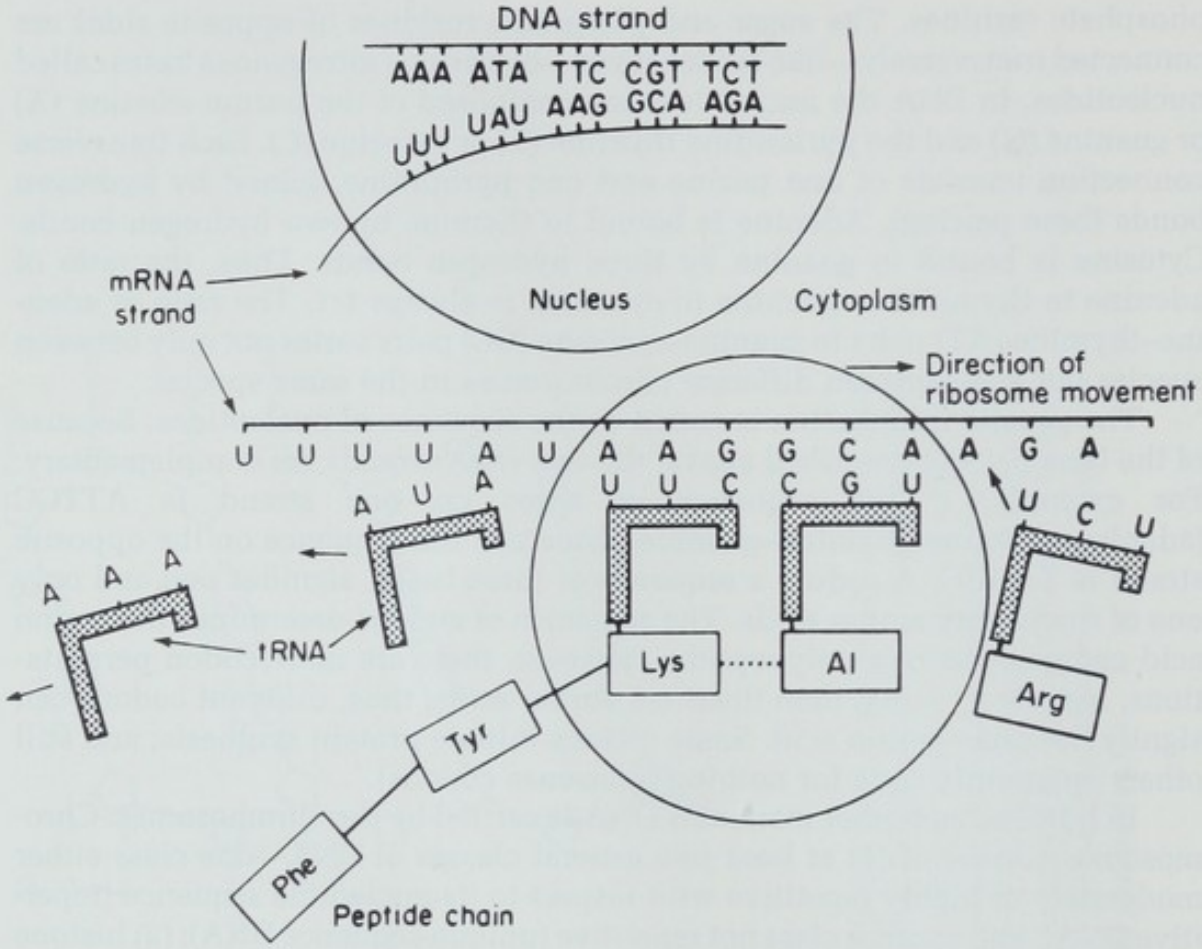


Figure 1-2. Schematic representation of the transcription and translation of DNA. One DNA strand serves as a template for mRNA, which is complementary to DNA in its base pair sequence. Thus, a DNA sequence of TTC corresponds to an RNA sequence of AAG. In RNA uracil is present rather than thymine. After its synthesis mRNA moves into the cytoplasm to associate with ribosomes. Protein synthesis is completed with the aid of activated structures specific for each amino acid (amino-acyl rRNA). When a given triplet sequence on the mRNA strand is ready for translation, the appropriate amino-acyl tRNA joins the ribosome complex. For example, the DNA codon for lysine (TTC) is transcribed by mRNA in complementary fashion (AAG). This complementary sequence is then recognized, again through complementary pairing (UUC), by the amino-acyl tRNA for lysine. (From Ford EHR: Human Chromosomes. New York, Academic Press, 1973, p 103. Reprinted with permission.)

of the mRNA along the ribosome. When a given codon is recognized, the amino-acyl tRNA joins the ribosome complex at the point specified by the appropriate codon (Fig. 1-2). The amino acids comprising a polypeptide chain are thus brought into their correct sequence. After bonds form between the amino acids, the polypeptide chain is completed, free to detach from the ribosomes and exert its action. Because a given protein is usually not coded by continuous unique-sequence DNA, the introns must be spliced before protein synthesis can proceed.

The enzyme reverse transcriptase allows complementary single-stranded DNA (cDNA) to be synthesized from mRNA. This technique not only is useful for nucleotide analysis but is also of clinical significance. Radioactively labeled

cDNA can serve as a "probe" that can pair (anneal) with DNA native to a given cell, perhaps of unknown genetic constitution. Failure to anneal indicates absence of the DNA that should code for the gene. This technique allows diagnosis of α-thalassemia (Kan et al., 1976b). Restriction endonucleases are a series of enzymes, each of which recognizes specific sequences of four to six nucleotides. Eco RI recognizes the sequence GAATTC; other endonucleases recognize different sequences. Exposing DNA to a given restriction enzyme thus cleaves the DNA into fragments of differing lengths that can be separated according to size. Using a specific cDNA probe, the fragment containing nucleotides coding for a given gene (e.g., α-globin) can be identified. This knowledge can be exploited to achieve antenatal diagnosis of certain hemoglobinopathies through analysis of amniotic fluid cells. (Recall that all cells contain the same DNA, even though ordinarily only red blood cells synthesize hemoglobin.) If the single-stranded α-globin cDNA fails to hybridize as expected with an unknown amniotic fluid DNA fragment expected to contain α-globin nucleotides, deletion of the α-globin locus exists (α-thalassemia).

Exposing DNA to the restriction endonuclease Hpa *I* led to the unexpected discovery that the fragment of DNA containing hemoglobin S was longer (13.0 kb) than the fragment containing hemoglobin A (7.0 or 7.6 kb) (Kan & Dozy, 1978). Linkage analysis taking advantage of this observation can be applied to diagnose sickle cell anemia through DNA analysis of cultured amniotic fluid fibroblasts. Restriction endonucleases can also be used to diagnose hemoglobinopathies caused by point mutations, i.e., alteration of a single nucleotide without deletion of DNA. Restriction endonucleases also form the basis for recombinant DNA work, a topic beyond the scope of this volume (Miller, 1981).

Regulation of Protein Synthesis

The regulation of mammalian protein synthesis is not fully understood. In fact, the only well-understood mechanism for the control of protein synthesis is in *Eschericia coli*. Jacob and Monod (1961) showed that synthesis of a given protein in *E. coli* requires a regulatory gene, an operator gene, and a series of structural genes. The control of protein synthesis is usually envisioned in a negative fashion. A regulatory gene is thought to elaborate a repressor that inhibits the operator gene from directing protein synthesis, which proceeds only if the repressor is absent or if an effector prevents the repressor from inactivating the operator gene. A repressor may or may not require other substances such as an end product that exerts negative feedback (corepression). In mammals the situation is unclear, but probably is more complicated because related genes are not always even on the same chromosome. For example, α-globin is coded by No. 16, and β-globin by No. 11.

CHROMOSOMAL CONSTITUTION

The genetic information is carried by chromosomes, a characteristic number of which is present in each species. In humans there are 46 chromosomes: 22 pairs of autosomes (Nos. 1–22) and one pair of sex chromosomes (XX in

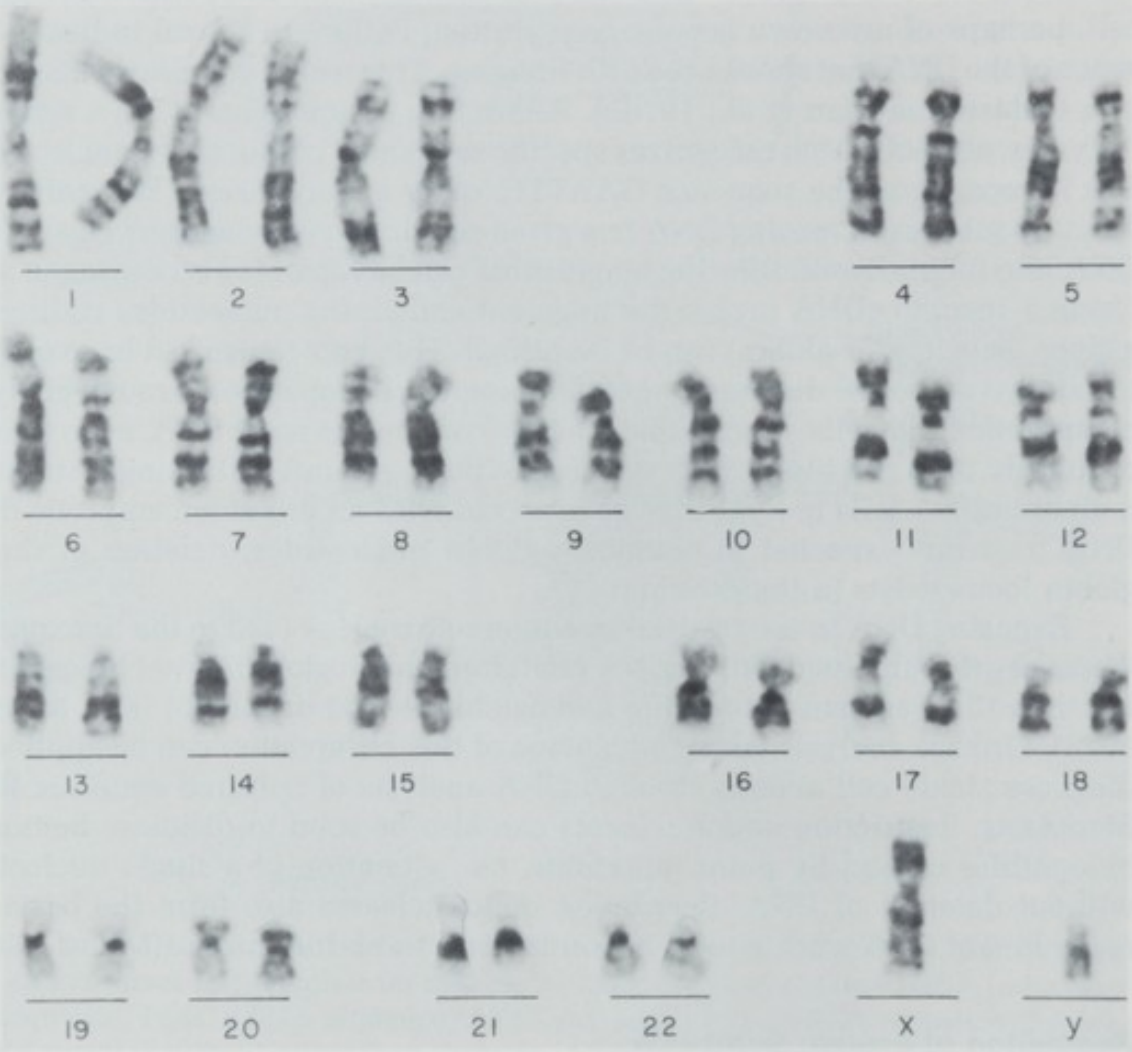


Figure 1-3. Metaphase figure and karyotype of a normal male (46,XY) (G-banding technique).

females, XY in males) (Figs. 1-3 and 1-4). The chromosome number is different in most other mammals. The aggregation of many genes in a limited number of chromosomes facilitates the ability of parental cells to transmit identical genetic information to daughter cells (mitosis), and to reduce their chromosome number by half (meiosis) so that each gamete contributes a haploid (n) number of chromosomes to the diploid (2n) zygote. (In humans n = 23). Errors of meiosis and mitosis lead to abnormal differentiation.

Chromosomal analyses are usually performed on peripheral blood (lymphocytes) or fibroblasts cultured from skin, gonads, or amniotic fluid cells. Cells are grown in nutrient media to which fetal calf serum and antibiotics are usually added. After a period of growth appropriate for a given tissue (48–72 hours for blood, 2–4 weeks for skin or amniotic fluid fibroblasts), cells are prepared for chromosomal analysis by the sequential addition of (1) colchicine (or desoxymethylcolchine); (2) a hypotonic solution that causes cells to swell; (3) an acetic acid–methanol fixative; and (4) a dye to enhance chromosome visibility. Other procedures may be used to produce horizontal banding patterns that allow a given pair of chromosomes to be distinguished from all others.

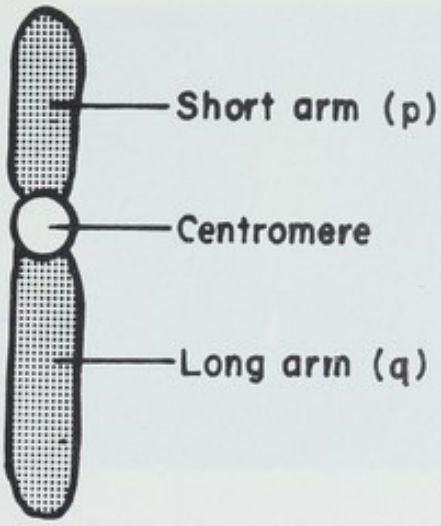


Figure 1-4. Parts of a chromosome.

Such bands are defined either by regions of fluorescent intensity differences (Q-bands) or by regions that stain lighter or darker than adjacent regions (G-, R-, or C-bands).

Additional cytogenetic information concerning sex chromosome constitution can be obtained by analysis of interphase nuclei derived from buccal epithelial cells and other tissues; however, these studies almost never suffice without additional genetic studies. X chromosomes in excess of one become inactivated and form X-chromatin masses (formerly known as sex chromatin or Barr bodies) (Fig. 1-5). The manner by which inactivation is accomplished is unknown, but it involves coiling because inactive X chromosomes are the last of the complement to replicate their DNA (late-replicating X).

The number of Y chromosomes can also usually be determined from interphase cells. After addition of quinacrine and analysis with fluorescent microscopy, the distal two thirds of the long arm of the Y chromosome fluoresces brilliantly in interphase nuclei, in metaphase, and throughout the cell cycle. The fluorescent spot produced by the Y during interphase is called Y-chromatin (Fig. 1-6), and the number of Y chromosomes equals the amount of Y-chromatin per cell. However, the testicular determinants are located not in the fluorescent but in the nonfluorescent region of the Y, near the centromere (German, Simpson, & McLemore, 1973b; Simpson, 1976), so males lacking Y-chromatin may

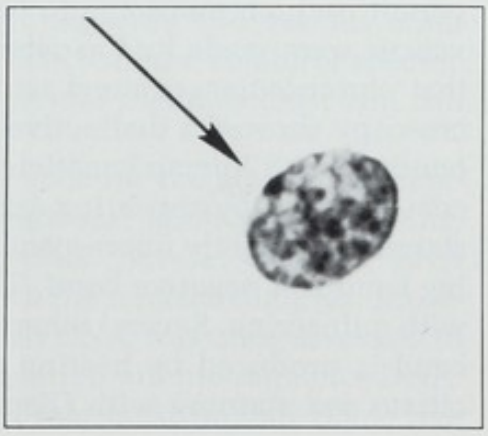


Figure 1-5. Interphase cell showing X-chromatin (arrow). (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 29. Reprinted with permission.)

Figure 1-6. Interphase cell showing Y-chromatin (arrow). (From Simpson JL: Disorders of Sexual Differentiation, Etiology and Clinical Delineation. New York: Academic Press, 1976, p 31. Reprinted with permission.)

be normal. In addition, regions of autosomal fluorescence may be mistaken for Y-chromatin. Analysis of cells from the parents of the fetus or person being studied could minimize these potential sources of error, but complete cytogenetic studies are still preferable.

CHROMOSOME IDENTIFICATION

Chromosomes are numbered according to their size and centromeric position (Fig. 1-3.). The centromere divides a chromosome into a short arm (abbreviated p) and a long arm (q). Based upon the position of the centromere, one can classify a chromosome as metacentric (p and q equal in length), submetacentric (q slightly greater than p), acrocentric (q much greater than p, the centromere being nearly terminal), or telocentric (the centromere being terminal). Prior to 1969 Nos. 1, 2, 3, 16, and Y could be identified. Other chromosomes could only be placed in one of several groups: A (1–3), B (4–5), C (6–12 and X), D (13–15), E (16–18), F (19–20), and G (21–22). The X is a submetacentric chromosome intermediate in size between Nos. 7 and 8. The Y is usually slightly larger than Nos. 21 and 22. With unbanded staining techniques, chromosomes belonging to one group can be distinguished from chromosomes of another group; however, within a given group chromosomes cannot always be distinguished from one another.

Banding techniques (Table 1-1) can now produce horizontal stripes that permit each chromosome to be distinguished from any other. The initial advances were made by Caspersson, Tomakka, and Zech (1971), who observed that chromosomes stained with quinacrine and analyzed by fluorescent microscopy showed a distinctive staining pattern. Each chromosome consists of *bands*, which human geneticists define as a region distinguishable from adjacent segments by appearing darker or lighter (Paris Conference, 1971). A darkly staining or brightly fluorescent band is a *positive band*, whereas a lightly staining band is a *negative band*. Thus, a positive Q-band fluoresces after staining with quinacrine. Several other methods are available (Table 1-1). A positive G-band is produced by heating chromosomes in sodium chloride and sodium citrate and staining with Giemsa or by pretreating cells with proteolytic en-

Table 1-1
Selected Characteristics of Some Banding Techniques Commonly Used in Human Cytogenetics

Name	Technique	Pattern
Q-banding	Staining with fluorescent dyes (e.g., quinacrine dihydrochloride); analysis by fluorescent microscopy	Positive bands fluorescent; negative bands nonfluorescent.
G-banding	Pretreatment by (1) concentrated salt solutions at high temperature (e.g., 60°C), (2) proteolytic enzymes, or other method.	Positive bands darkly staining; negative bands lightly staining; pattern corresponds closely to that of Q-bands.
R-banding	Pretreatment by high temperature and controlled pH; staining with Giemsa. Fluorescent methods may also produce R-bands.	Positive bands darkly staining; negative bands lightly staining. Pattern usually the reverse of that seen with Q- and G-bands.
C-banding	Pretreatment by bases, acids, or other method; followed by staining with Giemsa.	Positive bands darkly staining; negative bands lightly staining. Positive bands usually present only at centromeric regions and Yq.

Modified from Simpson JL, Martin AO: Cytogenetic nomenclature. Am J Obstet Gynecol 128:167, 1977.

zymes like trypsin (GTG-bands). Q-bands are usually, but not always, present in the same locations as G-bands. By a different technique, bands (R-bands) complementary to Q- and G-bands can be produced. Thus, a dark (positive) G-band or a brilliant Q-band corresponds to a light (negative) R-band. Another commonly used technique (C-banding) utilizes denaturation with NaOH, followed by Giemsa staining, to define darkly staining regions called C-bands. In particular, the distal two thirds of Yq shows positive Q- or C-bands.

The banding techniques used by a given laboratory depend upon the availability of equipment and nature of the investigations to be undertaken. A given pattern of bands (e.g., G-bands) can be produced by a variety of methods. In addition, the same method of pretreatment (e.g., NaOH) can produce different banding patterns, depending upon the length of exposure and various other parameters. The obstetrician–gynecologist need not recall the details of specific banding techniques, but should be aware that several methods exist and can be used in complementary fashion.

Some components (DNA) of a chromosome code for the genetic information, whereas other components (histone proteins and nonhistone proteins) preserve the physical integrity of the chromosome. Several types of RNA (mRNA, tRNA, and rRNA) are also associated with the nuclear complex. Moreover, there are several types of DNA. Most human DNA was once assumed to be capable of coding for proteins, hence its designation unique-sequence DNA. However, as noted previously, it is now known that most human DNA, like

DNA of most eukaryotes, contains aggregates of repeated nucleotide sequences that are not complex enough to code for proteins. This *repetitious DNA* may protect DNA that codes for proteins or may synthesize simple structural components like rRNA or histones.

The mechanism of chromosome banding has been reviewed by several authors (Comings et al., 1973; O. J. Miller et al., 1973a, 1973b; Dutrillaux & Lejeune, 1975). Positive C-bands probably represent one of several kinds of repetitive DNA, but no single explanation is universally accepted for Q-, G-, and R-bands. Banding may be related to variations in DNA base sequences along chromosomes; in particular, positive Q- and G-band regions appear to be rich in adenine–thymine (AT) pairs (O. J. Miller et al., 1973a). However, base sequence changes cannot entirely explain banding patterns (Comings, 1974). Thus, Q-, G-, and R-bands may reflect variations not only in base composition but also in the nature or quantity of proteins bound to DNA; that is, bands may reflect DNA–protein interaction.

CYTOGENETIC NOMENCLATURE

An official chromosomal nomenclature exists, adherence to which not only permits the chromosomal complement to be designated in a standard manner requiring no additional explanation, but also increases accuracy of communication.

Table 1-2 lists some symbols used to designate parts of chromosomes and certain rearrangements. Chromosomes are identified by distinctive *landmarks*. The areas between adjacent landmarks are called *regions*. Chromosome bands are numbered according to a particular system, an example of which is shown in Figure 1-7. A band is designated by listing the chromosome, the arm (p or

Table 1-2
Some Symbols Recommended by the Paris Conference (1971) and Supplement (1975)

Centromere	cen	Ring	r
Short arm	p	Dicentric	dic
Long arm	q	Duplication	dup
Isochromosome	i	Inversion	inv
Deletion	del	Break without reunion (e.g., terminal deletion)	:
Translocation	t	Break and join	: :
Reciprocal translocation	rcp	From . . . to . . .	→
Mosaicism	mos		
Chimerism	chi		

From Simpson JL, Martin AO: Cytogenetic nomenclature. Am J Obstet Gynecol 128:167, 1977. Reprinted with permission.

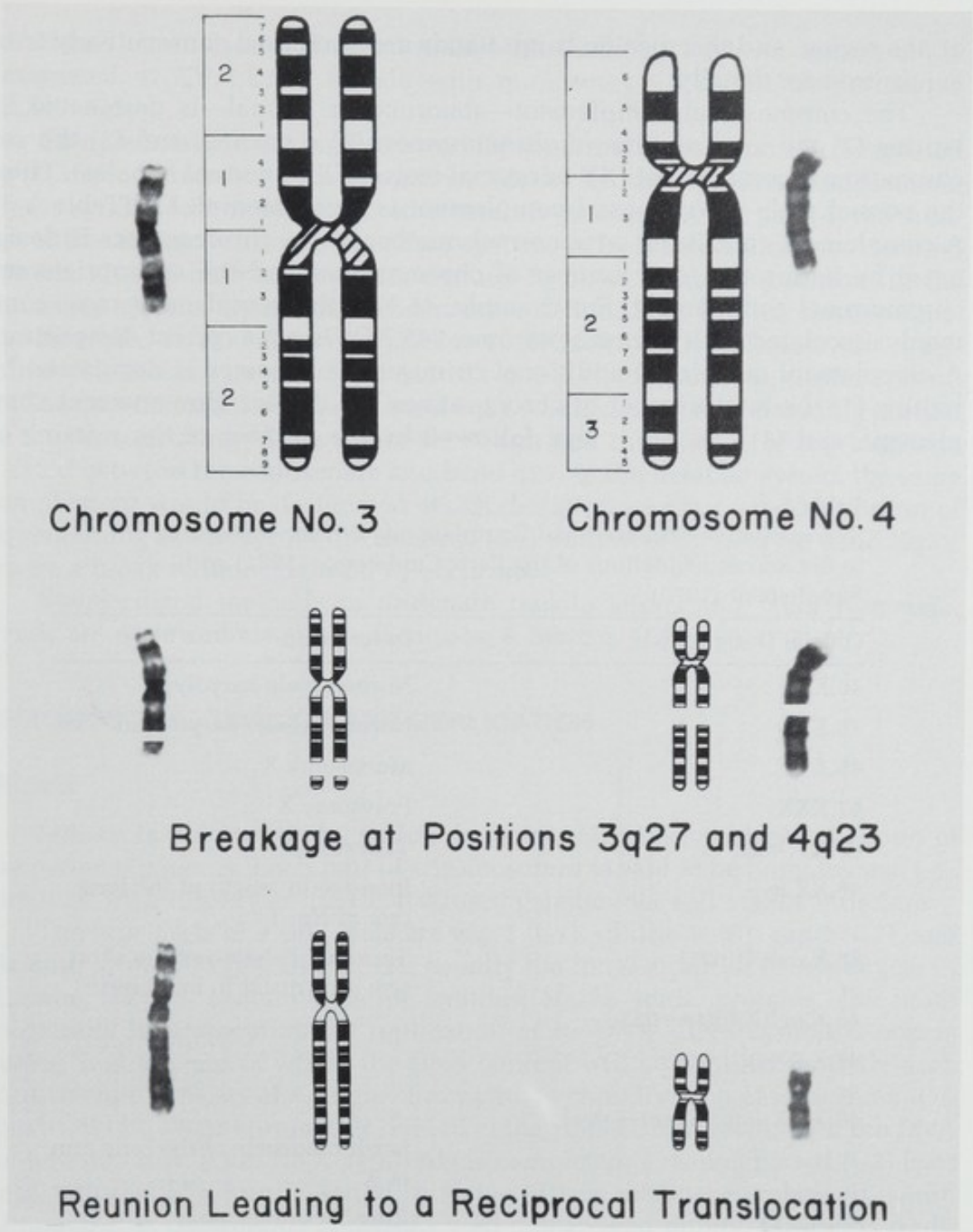


Figure 1-7. A reciprocal translocation between chromosome Nos. 3 and 4. Normal chromosomes are shown at the top, along with diagrammatic representation of the expected G- or Q-banding patterns. In the middle row the positions of the breaks are designated by gaps, and the sections interchanged after breakage shown. The two translocation chromosomes formed as result of reunion and reciprocal exchange are shown in the bottom row. An individual with both these translocation chromosomes, as well as normal Nos. 3 and 4, is said to be a carrier of the translocation in its balanced state (translocation heterozygote). Such an individual is phenotypically normal because neither duplication for deficiency of genetic material has occurred. (From Simpson JL, Martin AO: Cytogenetic nomenclature. Am J Obstet Gynecol 128:167, 1977. Reprinted with permission.)

q), the region, and the specific band. Bands are numbered consecutively from the centromere distally.

The chromosomal complement—abnormal or normal—is designated by writing (1) the total number of chromosomes, (2) a comma, and (3) the sex chromosomal complement (XY in normal males; XX in normal females). Thus, the normal male chromosomal complement is designated 46,XY (Table 1-3). A complement containing an abnormal number of sex chromosomes is designated by listing the total number of chromosomes and the appropriate sex chromosomal complement. For example, 45,X is the complement most commonly associated with Turner syndrome; "45,XO" is not a correct designation. A complement containing additional or missing autosomes is designated by writing (1) the total number of chromosomes, (2) the sex chromosomal complement, and (3) a + or − sign followed by the number of the missing or

Table 1-3
Representative Chromosomal Complements Written According to the Recommendations of the Paris Conference (1971) and Supplement (1975)

Official Designation	Description
46,XY	Normal male karyotype
46,XX	Normal female karyotype
45,X	Monosomy X
47,XXX	Polysomy X
47,XY,+21	Trisomy 21
46,XX,lq+	Increase in length of the long arm of No. 1
46,X,del(X)(p21) or 46,X,del(X)(qter→p21:)	Terminal deletion of the short arm of X distal to band p21
46,X,i(Xq) or 46,X,i(X)(qter→cent→qter)	Isochromosome of the long arm of X
46,X,r(Y)	Ring Y chromosome
46,X,t(X;3)(q21;q31)	Balanced translocation between band 21 of the long arm of X and band 31 of the long arm of No. 3
45,X/46,XX or mos 45,X/46,XX	45,X/46,XX mosaicism

The shortened system is illustrated for each complement; the detailed system is also illustrated for deletions and isochromosomes. (From Simpson JL, Martin AO: Cytogenetic nomenclature. Am J Obstet Gynecol 128:167, 1977. Reprinted with permission.)

additional chromosome. Thus, a male with trisomy 21 (Down syndrome) is designated 47,XY,+21; a female with monosomy 21 would be designated 45,XX,−21.

Complement containing a structurally abnormal chromosome is designated by (1) the total number of chromosomes, (2) the sex chromosomal complement, (3) the symbol for the particular structural aberration present, and (4) the number of the aberrant chromosome. Two systems exist (Table 1-3). In the detailed system, altered chromosomes are defined by their band composition; in the shortened system, altered chromosomes are defined only by their break points. For example, in the shortened system 46,XX,del(5)(q21) indicates that a terminal deletion occurred at band q21 of No. 5 (i.e., long arm (q), region 2, band 1). The genetic material distal to band 5q21 was lost, with the remaining portion of the 5q consisting of the entire short arm and the portion of the long arm located between the centromere and band q21. In the detailed system the same complement would be designated 46,XX,del(5)(pter→q21:), i.e., the portion of 5q remaining extends from the terminal (ter) portion of 5p (pter) to band Xq21, where a break without reunion (:) occurred.

Standardized methods to designate translocations and other rearrangements are reviewed elsewhere (Simpson & Martin, 1977).

CHROMOSOMAL DIVISION AND REPLICATION

Mitosis

Mitosis is the process by which daughter cells receive identical copies of the parental genome. Each pair of chromosomes is said to be homologous, i.e., identical with respect to their constituent genetic loci and visible structure.

The four parts of a cell cycle are gap 1 (G1), synthesis (S), gap 2 (G2), and division or mitosis (D). During G1, usually the longest part of the cell cycle in humans, the cell accumulates nucleotides, amino acids, proteins, and other substances in preparation for replication of its DNA. DNA synthesis occurs during S, at the end of which the DNA content will be doubled. Initially each chromosome consists of a single chromatid; eventually each chromosome will consist of two sister chromatids, joined at the single centromere, with the DNA content doubled. After DNA synthesis is completed, a resting period (G2) lasts until mitosis. The fourth period, division (D) or mitosis, is relatively short. Although it is continuous and lacks well-demarcated points, mitosis is traditionally divided into four stages: prophase, metaphase, anaphase, and telophase (Fig. 1-8).

Prophase. At the onset of prophase, chromosomes are elongated, thereafter becoming shorter, more compact, and more darkly staining. Each chromosome, which has already replicated its DNA prior to prophase, now consists of two sister chromatids; however, by light microscopy a chromosome appears to be composed of only a single unit. Toward the end of prophase a structure called the centriole divides into two daughter centrioles, each of which migrates to opposite poles in preparation for formation of the mitotic spindle, onto which chromosomes will become oriented for division.

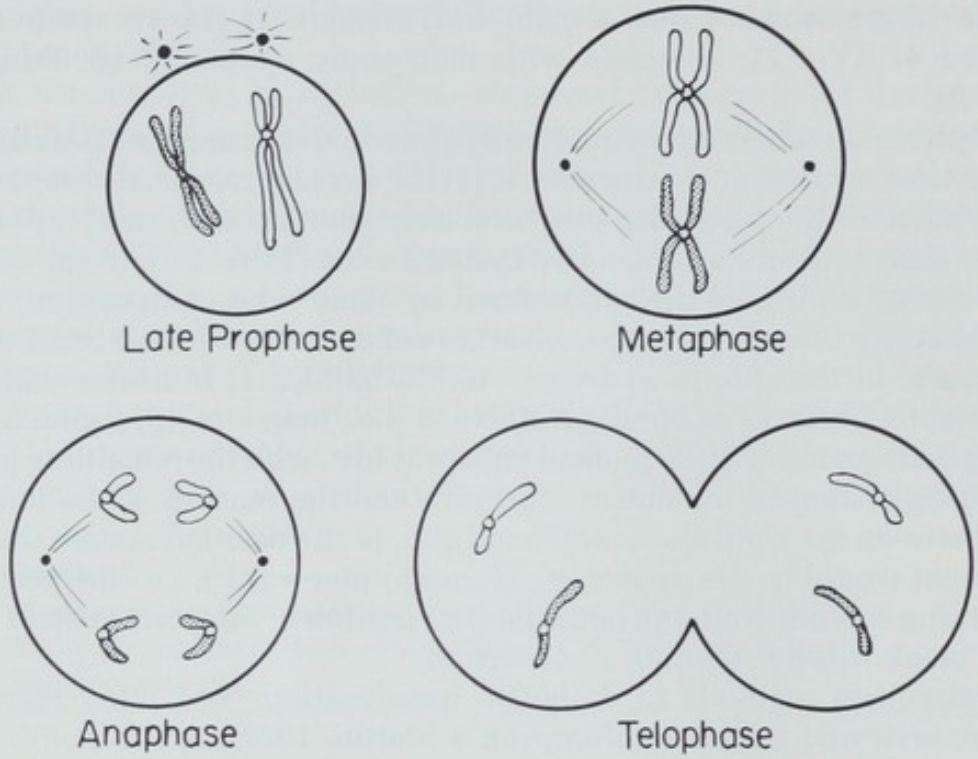


Figure 1-8. Schematic representation of the stages of mitosis. Two chromosomes are represented. After DNA synthesis, each chromosome consists of two sister chromatids. After centromeric division in the longitudinal place, each chromatid passes to different daughter cells. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 10. Reprinted with permission.)

Metaphase. This stage begins when the nuclear membrane disappears and the mitotic spindle forms. The spindle, a structure synthesized predominately from proteins, extends between the two centrioles. It can now be seen for the first time by light microscopy that chromosomes consist of paired sister chromatids connected by a centromere. The chromosomes are arranged on the spindle roughly equidistant between the centrioles, i.e., in the equatorial region. At this stage mitosis can be arrested by addition of colchicine, which deleteriously affects the spindle. This technique allows accumulation of metaphase figures for chromosomal analysis. Following division of the centromere along the longitudinal plane of the chromosome, sister chromatids pass to opposite poles (Fig. 1-8).

Anaphase. This is the process of chromosome (chromatid) movement to opposite poles.

Telophase. Telophase begins when the chromatids reach opposite poles. The mitotic spindle disappears and nuclear membranes reform. Following *cytokinesis* (division of the cytoplasm), two complete cells are formed, each of which is again in G1.

Mitotic division is a relatively exact process, usually allowing a parental cell to pass an identical copy of its genetic information to daughter cells. However, major errors in division can occur, such as failure of chromosome

separation (nondisjunction), loss of a chromosome at anaphase (anaphase lag), centromeric misdivision, and chromosome breakage. These errors are important causes of abnormal development, and they will be discussed in the section Chromosome Errors later in this chapter.

Meiosis

The diploid number (2n) of chromosomes is derived from both paternal and maternal sources. Since each parent contributes a haploid (n) number to the zygote, there must exist a process by which a gamete (oocyte or spermatozoon) receives a haploid (n) number of chromosomes; otherwise, the chromosomal number of offspring would be twice that of its parent. This process is called *meiosis*. Meiosis also provides a mechanism, called crossing-over, for exchange of portions of homologous chromosomes. This generates genetic variability, which can serve as the basis for natural selection. When crossing-over occurs, daughter chromosomes have combinations of genes different from those of either parent (recombination).

Meiosis has two divisions: I and II. During meiosis I the chromosome number is reduced from 2n to n; during meiosis II each haploid germ cell divides into two other haploid cells (Fig. 1-9). From a single diploid germ cell, four haploid cells are formed. In males, all four cells form potentially functional spermatozoa; in females one ovum and either two or three nonfunctioning polar bodies are formed.

Meiosis I

A germ cell passes through the same periods of the cell cycle as somatic cells: G1, S, G2, and D. Except for a small amount of DNA synthesis that occurs during zygotene (a subdivision of meiotic prophase) possibly related to recombination, DNA replication is completed prior to meiosis. Meiosis I has four stages—prophase, metaphase, anaphase and telophase—analogous to the four mitotic stages.

Prophase is longer and more complex than mitotic prophase, and during meiosis homologous chromosomes are first attracted and then repulsed. Several subdivisions of meiotic prophase can be identified. In an early stage, leptotene, chromosomes are long, slender, and darkly staining. Although consisting of two sister chromatids, each chromosome appears as a single unit. During zygotene, homologous chromosomes pair longitudinally with each other, a process known as synapsis. Synapsis occurs at homologous loci, that is, between allelic forms of a gene (see Mendelian Inheritance later in this chapter). (The human X and Y chromosomes have traditionally not been believed to synapse; however, the X and Y short arms undergo end-to-end association that might represent synapsis.) Prior to leptotene, each chromosome consists of two sister chromatids; however, not until pachytene are the two sister chromatids of each chromosome distinguishable. Pachytene chromosomes are characterized by tetrads, groups of four chromatids, which are actually sister chromatids of two homologous chromosomes. At some sites genes are exchanged between nonsister chromatids of a tetrad (recombination). The sites of contact are called *chiasmata*. Chiasmata are thus the visibly obvious sites of interchange, whereas

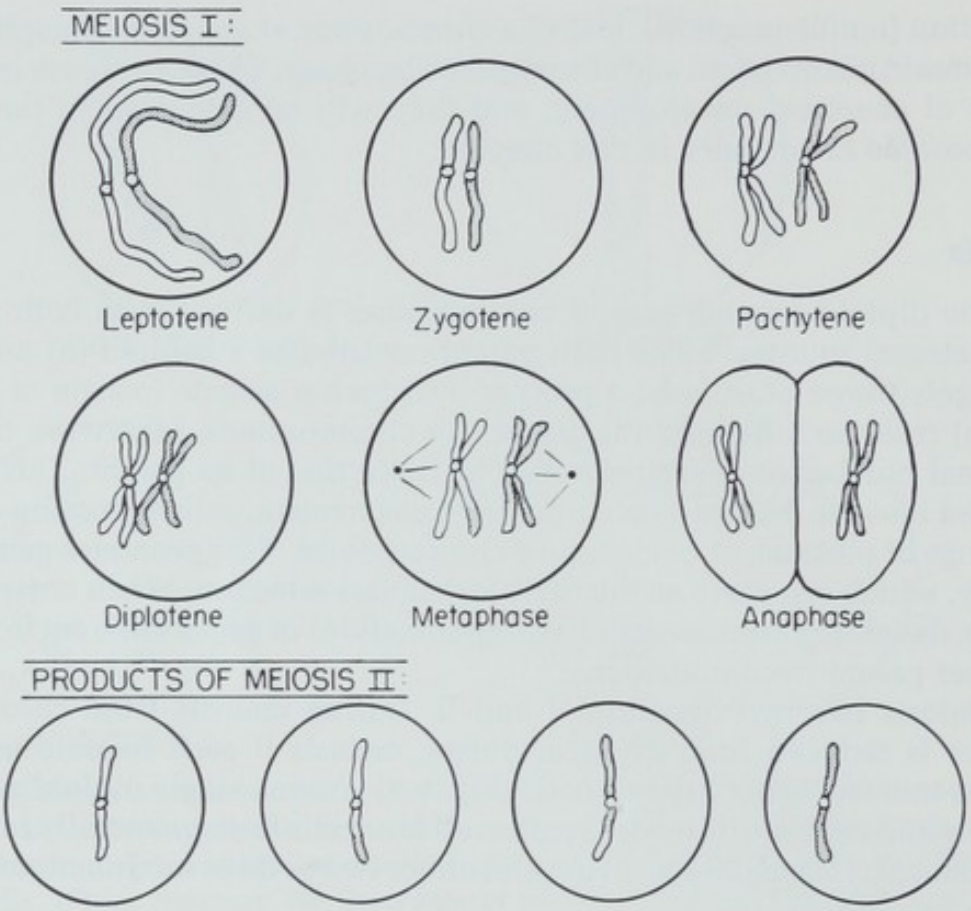


Figure 1-9. Schematic representation of the stages of meiosis I and the gametic products present after meiosis II. The behavior of one pair of autosomes is shown. At zygotene homologous chromosomes pair with each other along their longitudinal planes by a process known as synapsis. Synapsis occurs between the various alleles at a single locus. At some sites segments are exchanged between nonsister chromatids. During diplotene chromosomes begin to separate. If crossing-over occurs, no two of the four chromatids of a given chromosome pair are genetically identical, as illustrated by the four different products of meiosis II. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 12. Reprinted with permission.)

the term recombination is properly applied only to the process of exchange of genetic information. Recombination permits exchange of genes between homologous chromosomes, providing a source for genetic variability; chiasmata prevent homologous chromosomes from separating prematurely prior to metaphase. Premature separation could potentially lead to nondisjunction. During diplotene one pair of sister chromatids separates from the other, premature separation having been initially prevented by chiasmata. During diakinesis the chromosomes shorten and chiasmata move toward the telomeres (terminalization) to permit normal disjunction.

Metaphase. Onset is characterized by disappearance of the nuclear membrane and orientation of the chromosomes on the mitotic spindle. In every tetrad there are two chromosomes and two centromeres. In mitosis, centromeres divide during each cycle, but in meiosis I, homologous chromosomes instead

only repulse one another and move toward opposite poles *(anaphase)*. *Telophase* may or may not occur following meiosis I, its occurrence being species-specific.

Meiosis II

Between meiosis I and meiosis II, an interphase G1 may or may not occur. Little or no DNA synthesis (S) is required, and the occurrence of G2 varies from species to species. The stages of meiosis II—metaphase, anaphase, and telophase—are similar to the corresponding stages of mitosis. However, meiosis II lacks a prophase stage, chromosomes passing directly into metaphase. After completion of meiosis II, a single original diploid cell will have divided into four haploid cells. If recombination occurs, as it usually does, no two cells will be genetically identical (Fig. 1-9).

CHROMOSOME ERRORS

Chromosomal errors may be divided into those characterized by numerical changes (deviations from the normal number) or by structural changes (abnormalities in chromosome morphology).

Numerical Errors

If a haploid gamete or a diploid cell lacks the expected number of chromosomes (n or 2n, respectively), *aneuploidy* exists. If the complement contains one additional whole chromosome (2n + 1), *trisomy* exists. This term can be applied to both autosomal or sex chromosomal anomalies. If the number of sex chromosomes is increased, the term *polysomy* is sometimes used. If one entire chromosome is lacking (2n − 1), *monosomy* exists. *Polyploidy* refers to the presence of more than two haploid complements within a single cell; *triploidy* (3n = 69) and *tetraploidy* (4n = 92) are the most common types of polyploidy in humans.

Trisomy and monosomy may arise by several mechanisms. First, aneuploidy may arise de novo, presumably following a meiotic or mitotic error, De novo aneuploidy on this basis is called *primary nondisjunction*. During mitosis, sister chromatids may not disjoin properly, one daughter cell receiving both sister chromatids. Nondisjunction during mitosis can lead to more than one cell line (mosaicism) (Fig. 1-10); nondisjunction during meiosis causes aneuploid gametes, but the embryo will contain only one cell line (Fig. 1-11). Trisomic or monosomic parents would be expected to produce equal numbers of normal (n) gametes and either n + 1 or n − 1 gametes, depending upon the parental complement. If either of these gametes had been fertilized by a normal gamete, the zygote would be chromosomally abnormal as a result of *secondary nondisjunction*. This is a rare cause of aneuploidy in mammals, as aneuploid mammals are usually either sterile or fail to produce aneuploid offspring in the expected proportion. However, offspring of mothers with Down syndrome may show trisomy 21, and 47,XXX may produce 47,XXX or 47,XXY offspring (Simpson, 1981a). A third source of monosomic cells is *anaphase lag*, a situ-

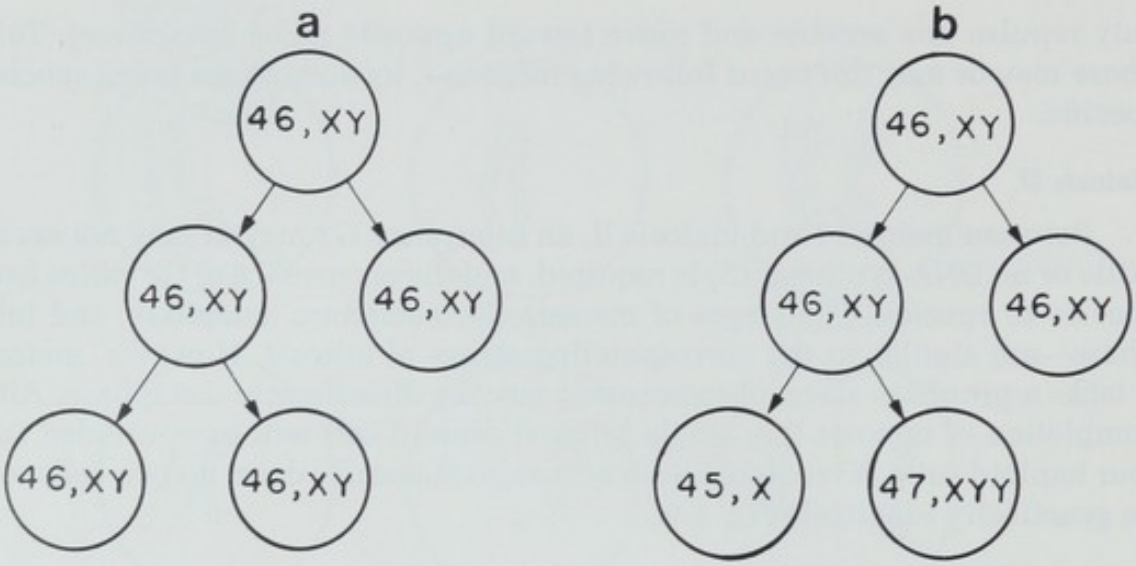


Figure 1-10. Diagrammatic representation of the products of (a) normal mitosis and (b) mitosis characterized by nondisjunction of an X chromosome. If all daughter cells survived, the complement would be 45,X/46,XY/47XYY. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 20. Reprinted with permission.)

ation in which one or more chromosomes fail to pass to daughter cells. Chromosomes lacking a centromere (acentric chromosones) are usually eliminated by anaphase lag.

Errors of ploidy may arise by several mechanisms, principally double fertilization or incorporation of the second polar body into the zygote. Triploidy is detected frequently among spontaneous aborted fetuses but rarely among neonates, and tetraploidy is even rarer.

Structural Errors

Some structural variation *(polymorphism)* exists in human somatic chromosomes without apparent phenotypic consequence. However, clearly abnormal variations *(aberrations)* are normally associated with phenotypic abnormalities. Aberrations may lead to genetic imbalance through deficiency or duplication of genetic information. In deficiency and duplication only portions

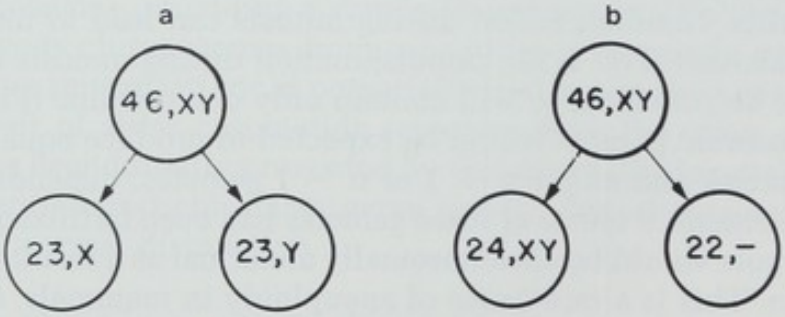


Figure 1-11. Diagrammatic representation of the product of (a) normal meiosis and (b) meiosis in which nondisjunction produced two aneupolid gametes: 24,XY and 22,-. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 20. Reprinted with permission.)

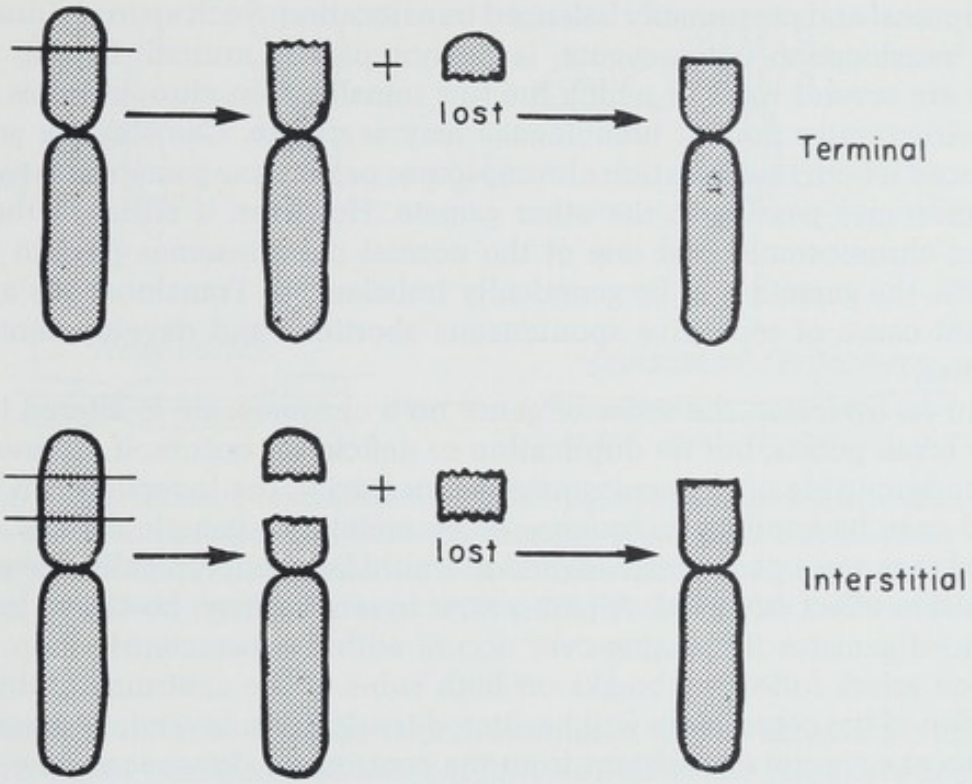


Figure 1-12. Diagrammatic representation of the origin of terminal and interstitial deletions. (From Simpson JL: Disorders of Sexual Differentiation: Etiology and Clinical Delineation. New York, Academic Press, 1976, p 22. Reprinted with permission.)

of a chromosome are involved. Nonetheless, if the change is large enough to be recognized by light microscopy, many genes are duplicated or deficient. A phenotypic abnormality may also result if the position of a gene with respect to its neighboring genes is altered *(position effect)*.

A *simple deficiency* involves loss of one portion of a chromosome. A deficiency can either be terminal or interstitial; only with banding can these be distinguished (Fig. 1-12). Deficiency in an autosome often results in death or malformation of the embryo, but deficiency in a sex chromosome is usually less deleterious.

A *ring chromosome* arises following a break in both the long arm and the short arm. The centromeric ends fuse; the telomeric fragments are acentric, and thus are lost, causing a portion of each arm to be deleted. Ring chromosomes are unstable because their replication can lead to double-sized rings, dicentric rings, or loss of the entire chromosome.

Duplication may originate by duplication of a portion of a single chromosome or by unequal crossing-over between nonsister chromatids. Duplication may also arise if a portion of one chromosome is interchanged with a portion of another *(translocation)*. Duplications are usually less lethal than deficiencies, especially in lower animals; however, in humans duplications are usually associated with phenotypic abnormalities.

If, following chromosome breakage, material is exchanged between two or more chromosomes, a translocation is said to have occurred (Fig. 1-7). Both chromosomes may appear structurally abnormal, one showing a duplication and the other a deficiency. If no genetic material is lost, an individual carries

a reciprocal and presumably balanced translocation. Such an individual, known as a translocation heterozygote, is phenotypically normal. During meiosis I there are several ways in which the two translocation chromosomes and their two structurally normal homologues may segregate. Gametes are genetically balanced if both translocation chromosomes pass to one gamete, the two normal chromosomes passing to the other gamete. However, if either of the translocation chromosomes and one of the normal chromosomes pass to the same gamete, the gamete will be genetically imbalanced. Translocations are an important cause of repetitive spontaneous abortions and developmental abnormalities.

In an *inversion* the order of genes on a chromosome is altered by two or more break points, but no duplication or deficiency occurs. If all breaks occur on the same side of the centromere *(paracentric)*, the inversion can be recognized only by banding techniques or by meiotic or genetic studies. An individual carrying a paracentric inversion would be phenotypically normal unless a position effect occurred. A paracentric inversion may, however, lead to unbalanced gametes if crossing-over occurs within a paracentric loop. If an inversion arises following breaks on both sides of the centromere, the relative position of the centromere will be altered *(pericentric inversion)*, assuming the two breaks are not equidistant from the centromere. Inversions may also lead to repetitive spontaneous abortions, because crossing-over within an inversion may lead to unbalanced gametes.

Isochromosomes have identical arms. They arise following horizontal, rather than longitudinal, division of the centromere (Fig. 1-13). The acentric portion, composed of one entire arm, is lost; the remaining telocentric portion is unstable, replicating at the next cell division to form a metacentric chromosome. The two arms of an isochromosome are mirror images, both structurally and genetically. An isochromosome thus consists of complete duplication of one arm and complete deficiency of the other arm. An isochromosome can be composed of identical long arms (e.g., 46,X,i(Xq)) or identical short arms (e.g., 46,X,i(Xp)), depending upon which arm remains with the centromere. An isochromosome for the X long arm is the most common X-structural abnormality in patients with gonadal dysgenesis.

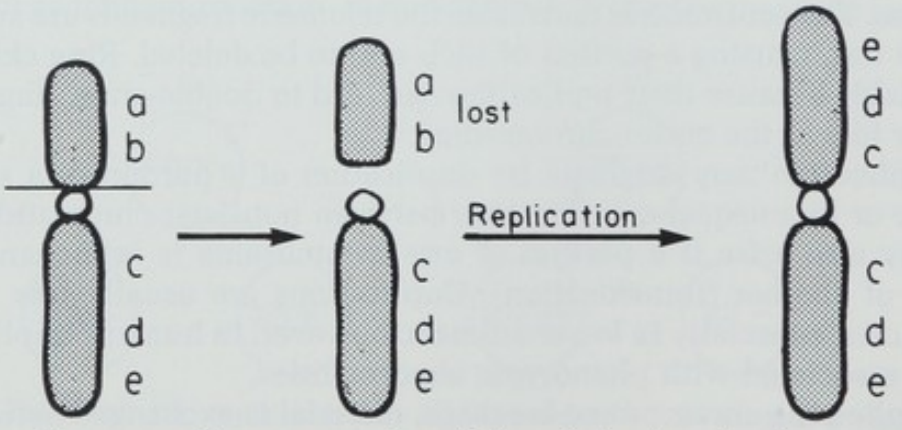


Figure 1-13. Diagrammatic representation of the origin of an isochromosome. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 25. Reprinted with permission.)

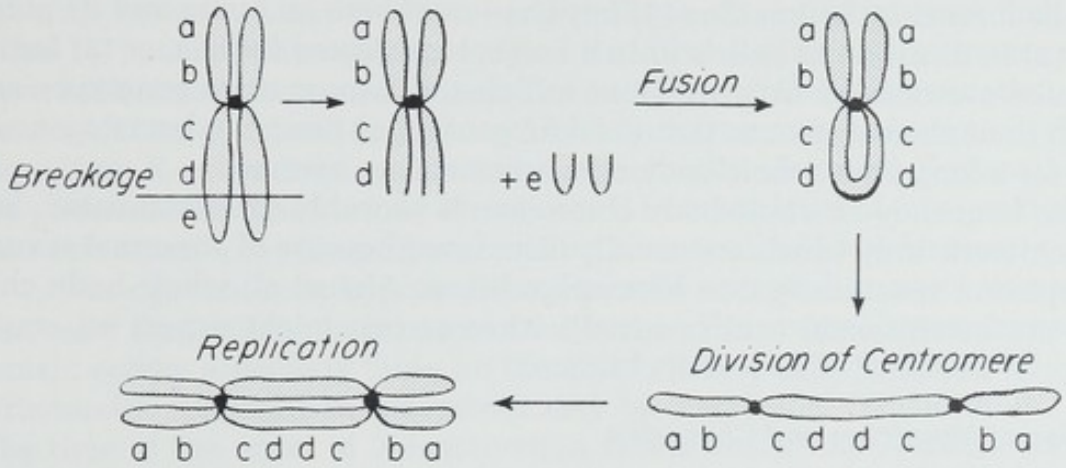


Figure 1-14. Diagrammatic representation of one possible origin of a dicentric chromosome. A dicentric can also arise following crossing-over within a paracentric inversion loop. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 26. Reprinted with permission.)

A *dicentric chromosome* has two centromeres, and is usually thought to arise following an isochromatid break (Fig. 1-14). After the telomeric acentric fragment is lost, sister chromatids join. Following longitudinal division of the centromere, the chromosome consists of a duplicated portion, a deficient portion, and two centromeres. The presence of two centromeres (dicentric) is associated with mitotic instability because the two might migrate to opposite poles during telophase, stretching until breakage, followed by loss of chromosomal material or secondary rearrangements (breakage–fusion–bridge cycle).

Mosaicism

A mosaic individual has two or more genotypes, each originating from a single zygote. Mosaicism usually arises following mitotic nondisjunction or anaphase lag, with survival of at least two cell lines characterized by different complements. Nondisjunction can result in as many as three cell lines (Fig. 1-9), although loss of certain daughter cells may result in a less-complicated mosaicism. Individuals with chromosomal mosaicism usually, but not invariably, differ phenotypically from nonmosaic individuals, the differences presumably reflecting the distribution of various cell lines in different tissues.

Chimerism

Chimerism connotes the presence in a single individual of cells from different zygotes. Benirschke (1972a) distinguished three types of chimerism: (1) blood chimerism, often due to interchange of blood cells between cotwins through placental anastomoses or intrauterine transfusion; (2) transplacental chimerism, due to interchange of fetal and maternal blood cells; and (3) whole-body chimerism, presumably due to fusion of zygotes, and probably responsible for 46,XX/46,XY true hermaphroditism.

Whole-body chimerism probably results from fusion of two zygotes or embryos, which more often develop separately as dizygotic twins. A chimera

could be formed by fusion after (1) fertilization of both an ovum and its polar body, (2) fertilization of both within a single binucleated follicle, or (3) fertilization of ova derived from different follicles. If two or more genotypes are present in nonhematogenous tissues (skin, gonads) or persist in hematogenous tissue for a long time, whole-body chimerism can be assumed.

The frequency of whole-body chimerism is probably underestimated, because chimeric individuals are usually discovered because of abnormal sexual development, specifically true hermaphroditism. Almost all whole-body chimeras are heterosexual (unlike-sexed), whereas one might expect an equal number of isosexual (like-sexed) chimeras.

Detection of Mosaicism and Chimerism

Detection of mosaicism or chimerism depends upon many factors, but most importantly upon the number of cells analyzed per tissue and the number of tissues analyzed.

The number of cells that needs to be analyzed depends upon the percentage of the minority cell line, which itself depends upon the time the mosaicism originates and whether any of the mosaic lines have a selective disadvantage or advantage. Several investigators have calculated the number of cells statistically necessary to exclude a minority cell population. For example, analysis of fifty cells without detection of at least one cell representing a minor line excludes any population comprising 10 percent or more of all cells in the tissue studied ($p < 0.05$) (Ford, 1969).

If mitotic nondisjunction occurs relatively early in organogenesis, yet at a time when the embryo contains at least several hundred cells (1- to 2-week-old embryo), analysis of a single tissue might suffice, assuming absence of cell selection. If, on the other hand, monosomy X originates by nondisjunction or anaphase lag in the older fetus, mosaicism may pass undetected if only a single tissue were studied. Thus, ideally, multiple tissues should be studied; however, practicality will more often dictate analysis of a single tissue.

CYTOLOGIC PROPERTIES OF SEX CHROMOSOMES

X Chromosome

The X chromosome is a submetacentric chromosome intermediate in length between Nos. 7 and 8. Its banding pattern distinguishes it from all other chromosomes. In addition, the late-replicating X (inactive) and the early-replicating X (active) can be distinguished from one another by autoradiography or by exposing cells to bromodeoxyuridine (BUdR), staining with the fluorochrome 33258 Hoechst, and analyzing by fluorescent microscopy.

In females one of the two X chromosomes is the last of the complement to complete DNA synthesis, probably because during interphase it is more tightly coiled than other chromosomes. During interphase, this chromosome, said to represent the heterochromatic X, forms a planoconvex body called X chromatin (Fig. 1-5). In diploid lines the number of X-chromatin masses equals the number of X chromosomes minus one.

All X chromosomes in excess of one are genetically inactive. The rela-

tionship between X-inactivation and cytologic condensation was first elucidated by Lyon (1961), who recognized that X chromosomes in excess of one were inactivated, forming X-chromatin masses, and that determination of the inactive X in a given cell is random if both X chromosomes are normal. (Except for certain X-autosome translocations, structurally abnormal X chromosomes are preferentially inactivated.) First derived from analyses of mice with mutant coat color genes, the Lyon hypothesis was later extended to humans.

Although the Lyon hypothesis is generally accepted, some of its parts are less valid than others. In humans some loci on the normal heterochromatic X remain active, especially those on the distal short arm. By contrast, all loci on structurally abnormal chromosomes may be inactivated (Polani et al., 1970). The time of the onset of X-inactivation is also uncertain. X-inactivation has been demonstrated in mammalian blastocysts by cytologic (Park, 1957) and biochemical techniques (Epstein, 1969; Migeon and Kennedy, 1975); inactivation earlier in embryogenesis has not been demonstrated. Whether X-inactivation occurs in all cells is also unknown. Indeed, electrophoretic studies of oocytes from adult females heterozygous for glucose-6-phosphate dehydrogenase (G6PD) show a G6PD-A band, a G6PD-B band, and a band of the dimer that indicates active synthesis of both G6PD-A and G6PD-B. Thus, both X chromosomes appear to be active in oocytes (Gartler et al., 1972), explaining the deleterious effect of a 45,X complement upon ovaries.

These caveats notwithstanding, X-inactivation probably accounts for the relative viability of monosomy X and polysomy X embryos, compared to the nonviability of embryos carrying a duplication or deficiency of autosomal material.

Y Chromosome

The normal Y chromosome is a submetacentric chromosome whose overall length is slightly longer than a group G (Nos. 21 and 22) chromosome. The distal two thirds of the long arm are brilliantly fluorescent, and can be identified during interphase (Fig. 1-6) as Y-chromatin.

The Y may vary in length from individual to individual. Length variations are heritable. Variant Y chromosomes may be unusually long or unusually short; the portion responsible for polymorphism is the distal Yq, specifically the brilliantly fluorescent portion. In unrelated individuals the lengths of Yp and nonfluorescent Yq are relatively constant, whereas the length of the fluorescent portion of Yq varies in a fashion proportional to the total length of the Y (Bobrow et al., 1971). These observations imply that the fluorescent portion of Yq is genetically inert or at least not essential for normal testicular and somatic development.

MENDELIAN INHERITANCE

There are thousands of genes and only 23 pairs of chromosomes. Thus, each chromosome must carry many genes. If a chromosomal aberration is large enough to be detected by light microscopy, many genes are probably duplicated

or deficient. Thus, the phenotypic consequences of chromosomal errors are several steps removed from the cellular abnormality, because many genes and their interactions can be considered responsible for an abnormal phenotype.

Mendelian inheritance involves gene mutation, which may involve only a single genetic locus. A chromosome carrying a mutant gene usually appears structurally normal because the change involves only a minute deletion or a change in a single nucleotide sequence. Detection of mutant genes is, therefore, accomplished not by cytogenetic studies, but rather by pedigree analysis or biochemical assays that directly measure a gene product or its metabolites.

Linkage

Genes are located on chromosomes at given locations and in definite linear sequences. Genes on the same chromosome are said to be linked or to exhibit linkage. Two genes show linkage if during meiosis, with its opportunity for recombination, they are more likely to remain on the same chromosome in parental combination than to behave as if they were not on the same chromosome. If the frequency of recombination between two linked genes is 1 percent, those genes are defined as being one map unit or one centimorgan apart (percent recombination $\times$ 100 = centimorgans). Genes 50 centimorgans apart on the same chromosome fail, by definition, to show linkage, being indistinguishable in segregation from genes on nonhomologous chromosomes. However, the term *linkage group* has traditionally been used to designate all genes on the same chromosome, including those more than 50 centimorgans apart. (Two genes that show independent assortment can, nonetheless, be shown to be on the same chromosome if each fails to assort independently with another intermediate gene. For example, gene A might be 30 centimorgans from gene B and 70 centimorgans from gene C. If B were 40 centimorgans from C and 30 centimorgans from A, A and C would be in the same linkage group.)

The semantic confusion evident in the above has led some geneticists to apply a new term, *synteny*, to describe all genes located on a single chromosome, irrespective of distance. In this monograph we are interested in linkage because linkage analysis may permit antenatal diagnosis even if the trait in question cannot directly be assayed by biochemical or other methods. Incidentally, closeness of linkage is proportional to the propensity to form chiasmatic (crossover points) between those genes.

Homozygosity and Heterozygosity

Chromosomes exist in pairs, one member maternally derived and its homologue paternally derived. Thus, genes exist in pairs. *Alleles* are different states of a gene. Alleles occupy identical loci on homologous chromosomes; thus, at a given autosomal locus a gene ordinarily has at least two alleles. The normal or native state of a gene, its most common state, is traditionally said to be the *wild type;* however, this term is rarely used in human genetics. An abnormal allele is a *mutant*. If both homologues possess identical alleles, *homozygosity* exists; if homologues possess dissimilar alleles (e.g., one mutant and one wild type), *heterozygosity* exists. If the alleles are dissimilar but both abnormal, *compound heterozygosity* exists; its effect can be similar to homozygosity, and

in fact the phenomena may be relatively common. An allele that can be expressed in its heterozygous state is *dominant*, whereas an allele that can be expressed only if homozygous is *recessive*. A recessive trait whose allele is located on the X chromosome is expressed by all males (46,XY) carrying the allele. Affected males are said to be hemizygous. The terms dominant and recessive are not intrinsic characteristics of genes but depend upon our methods of detection of the gene product.

Familial Transmission of Mutant Genes

The familial patterns followed by Mendelian traits not only depend upon whether the trait is dominant or recessive, but also upon whether the allele controlling the trait is located on an autosome or a sex chromosome. The usual patterns of transmission are autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive, and Y-linked.

Autosomal Dominant Inheritance

The likelihood is 50 percent ($p = 0.5$) that an individual carrying a mutant autosomal dominant gene (allele) will pass that allele to any given offspring, male or female (Table 1-4). Therefore, an autosomal dominant allele might be recognized by its ability to be expressed from generation to generation (Fig. 1-15). If penetrance (see below for definition) is complete, no unaffected individual will have an affected offspring. Males and females usually have an equal probability of having an autosomal dominant trait. Unfortunately, autosomal dominant patterns are not always associated with the characteristics described above. First, some phenotypically normal individuals almost certainly carry a mutant autosomal dominant allele because one parent and one or more offspring have the same rare dominant trait. Such a mutant allele is said to show incomplete penetrance if present in a phenotypically normal individual.

Penetrance is a measure of the ability to recognize a mutant allele. It is the frequency with which a heritable trait is manifested by individuals carrying

Table 1-4
Autosomal Dominant Inheritance*

Gametes		
	Affected Parent	
Unaffected Parent	*A*	*a*
a	*Aa*	*aa*
a	*Aa*	*aa*

From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 34. Reprinted with permission.

*Progeny expected of a mating between an affected parent (*Aa*) and an unaffected parent (*aa*). *A* represents the mutant allele, *a* the normal. The likelihood is 50 percent that an affected parent will pass the mutant allele to any given offspring.

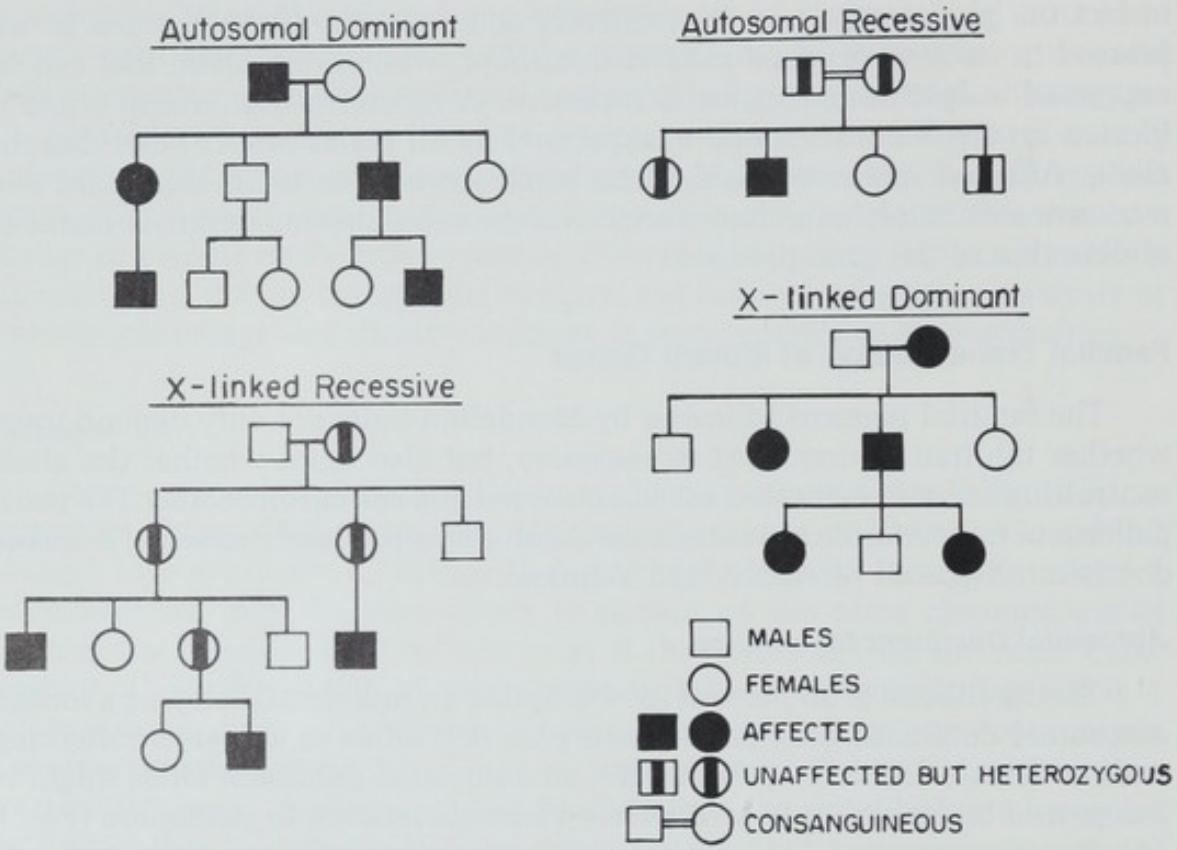


Figure 1-15. Patterns of familial transmission expected of autosomal dominant, autosomal recessive, X-linked recessive, and X-linked dominant traits. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 34. Reprinted with permission.)

a gene coding for it, irrespective of variation in degree of expression. If a trait is detected in half the individuals who carry the allele controlling that trait, the allele displays 50 percent penetrance. A single allele may be responsible for several different phenotypic effects; this is *pleiotropy*. Likewise, phenotypic variation may be associated with a given mutant allele, a phenomenon known as variable expressivity. Variable expressivity occurs not only between families but also among affected members of a single family (intrafamilial variability). An autosomal dominant allele may affect individuals of only one sex (sex limitation), a phenomenon of particular importance to gynecologists. Sometimes it is difficult to distinguish a male-limited autosomal dominant trait from an X-linked recessive trait. Male-to-male transmission excludes X-linked inheritance.

At least 736 human genes, and probably many more, are autosomal dominant (McKusick, 1978). An autosomal dominant allele in humans usually has one of three clinical characteristics. (1) It does not interfere with reproductive ability (e.g., polydactyly). (2) It is manifested only after the usual age of reproduction (e.g., Huntington chorea). (3) It is capable of variable expressivity (i.e., a minimally affected parent might have severely affected progeny). The more severe the trait, the more likely that an affected individual has a new mutation. All individuals with a trait causing sterility represent a new mutation. For common autosomal dominant traits, mutation rates are usually estimated to be 10^{-5} to 10^{-6}/gamete/generation.

Autosomal Recessive Inheritance

An autosomal recessive trait is not expressed unless an individual is homozygous for the appropriate allele; that is, both alleles at a given genetic locus have an identical mutation or are both dysfunctional. An individual with a recessive trait is usually the product of a mating between parents heterozygous for the same mutation (Fig. 1-15, Table 1-5). If two heterozygotes mate, the likelihood is 25 percent that an offspring will be affected. If multiple siblings of both sexes but no other relatives are affected, autosomal recessive inheritance should be considered.

In plants and animals, recessive inheritance can be verified by analyzing progeny from appropriate breeding experiments. For example, if a gene is recessive, mating a homozygous son or daughter to a heterozygous parent (backcrossing) should result in 50 percent affected progeny. In humans this method is obviously not applicable; however, the probability of autosomal recessive inheritance can be assessed by various statistical methods. Consanguineous parents are more likely to carry an identical allele (mutant or normal) than nonconsanguineous parents. An individual with a recessive trait is therefore more likely to arise from a consanguineous than a nonconsanguineous mating. The rarer a trait, the higher the proportion of affected individuals who arise from consanguineous union.

At least 521 autosomal recessive traits are known to exist in humans, (McKusick, 1978), and many more are suspected. Relatively few clinical generalizations concerning recessive traits are valid, but one that is valid is that enzyme deficiencies usually result from recessive genes, autosomal or X-linked. Presumably a single gene codes for enough protein (enzyme) to permit normal biosynthesis; thus, a deficiency does not become evident unless both alleles are mutant. Variation in expression is also more characteristic of dominant than recessive genes, and in homozygous form recessive genes are rarely nonpenetrant.

One clinically important concept is the relationship between the frequencies of homozygotes and heterozygotes. This relationship is expressed by the Hardy-Weinberg equilibrium, which was derived independently in 1908 by the British mathematician Hardy and the German physician Weinberg. The normal allele *A* has a frequency of p, and the mutant allele *a* has a frequency of q. Since the total of all alleles is one, $p + q = 1$. By squaring both sides, $p^2 +$

Table 1-5
Autosomal Recessive Inheritance*

Parental Gametes	*A*	*a*
A	*AA*	*Aa*
a	*aA*	*aa*

From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 36. Reprinted with permission.

*Progeny expected of a mating between two individuals heterozygous for the same mutant allele (*a*). *A* represents the normal allele. The probability is 0.25 that a given offspring will inherit both mutant alleles (*aa*) and be affected. The probability is 0.50 that a given offspring will be heterozygous (*Aa*).

$2pq + q^2 = 1$; p^2 is the frequency of individuals homozygous for allele *A (AA)*; q^2 is the frequency of individuals homozygous for allele *a (aa)*; and 2pq is the frequency of the heterozygote *(Aa)*. The frequency of a mutant allele (q) is usually much less than the frequency of a normal allele. If q is less than 0.5, q^2 is much less than 2pq because p is nearly equal to 1. Thus, a rare recessive disorder almost always results from matings between heterozygous phenotypically normal individuals. It is rarely immediately related to a new mutation. Likewise, most of the genetic load for a deleterious recessive trait lies in the heterozygotes, not in the relatively few homozygotes. These data are relevant to proposals to eliminate mutant alleles from the population by elimination of homozygous or even heterozygous fetuses. Such proposals are not only impractical and ethically unwise, but also theoretically unwise because heterozygous individuals might posess an advantage over homozygously normal individuals that was responsible for maintaining the mutant allele in the population (see Simpson & Gerbie, 1977). In fact, all individuals may be heterozygous for at least 5 to 6 deleterious recessive genes.

X-Linked Recessive Inheritance

A mutant recessive gene located on the X chromosome is expressed by all males (46,XY) who carry it. Such individuals are *hemizygous*. Females are usually affected only if homozygous. A 46,XX individual can be affected if her mother is heterozygous and her father hemizygous, but this mating occurs very rarely for deleterious traits.

An X-linked recessive allele is transmitted through phenotypically normal heterozygous females (Fig. 1-15, Table 1-6). The *proband* (the person through whom a pedigree is ascertained) might have affected male sibs, affected maternal uncles, affected maternal nephews, affected maternal first cousins, and

Table 1-6
X-Linked Recessive Inheritance*

	Gametes	
	Normal Male	
Heterozygous Female	X	Y
X	**XX** Heterozygous daughter	**XY** Affected son
X	XX Normal daughter	XY Normal son

From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 38. Reprinted with permission.

*Progeny expected of a mating between a normal male (XY) and a female heterozygous for an X-linked recessive trait (**X**X). **X** represents an X chromosome carrying a mutant allele; X represents the X chromosome carrying the normal allele. The probability is 0.5 that a given male offspring will inherit **X** and, hence, be affected. Likewise, the probability is 0.5 that a female offspring will inherit **X** and, hence, be heterozygous like her mother.

Table 1-7
X-Linked Dominant Inheritance*

Affected Male†			Affected Female‡		
Gametes			Gametes		
Normal Female	Affected Male		Affected Female	Normal Male	
	X	Y		X	Y
X	**X**X	XY	**X**	**X**X	XY
X	**X**X	XY	X	XX	XY

From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 39. Reprinted with permission.
*X carries an X-linked dominant allele; X does not carry the allele.
†100 percent females affected; 0 percent males affected.
‡50 percent females affected; 50 percent males affected.

perhaps other affected maternal relatives. The probability is 0.5 that a heterozygous female will pass an X-linked recessive allele to any given offspring. Males receiving the allele will be affected; females will be heterozygous like their mother. An affected male passes the allele to all his daughters but none of his sons; all his offspring would be phenotypically normal, assuming his mate is not heterozygous for the same allele. There are approximately 100 X-linked traits, most bearing no apparent relationship to sexual development. Examples include hemophilia and Duchenne muscular dystrophy. For genetic counseling it is important to realize that many males have X-linked recessive traits as result of a fresh mutation in a maternally derived X chromosome.

X-Linked Dominant Inheritance

A male carrying an X-linked dominant allele transmits the allele to all his daughters but to none of his sons (Fig. 1-15, Table 1-7). The probability that a female with an X-linked dominant allele will pass the allele to any offspring, male or female, is 0.5. In X-linked dominant traits, females are twice as commonly affected as males, although sometimes less severely. Relatively few X-linked dominant traits are known. The best example is vitamin-D resistant rickets. Of interest to gynecologists is that Stein-Levanthal syndrome may be an X-linked dominant (Wilroy et al., 1975).

Y-Linked Inheritance

A male passes a Y-linked gene to each of his sons but to none of his daughters. Y-linked inheritance has been postulated for many traits, but proved for none. Several characteristics are controlled by factors on the Y, notably testicular determinants and the H-Y antigen (see Chapter 10).

POLYGENIC OR MULTIFACTORIAL INHERITANCE

Most disorders discussed in this monograph result from chromosomal errors or from single-gene mutations. However, these factors cannot explain every congenital abnormality, nor can they explain the heritability of normal ana-

tomical and physiologic variation (e.g., age of menarche). The recurrence risk of many anatomic anomalies indicates a heritable tendency. For example, following the birth of one child with a neural tube defect (anencephaly or spina bifida), the likelihood that any subsequent progeny will be similarly affected is approximately 2 percent in the United States, assuming unaffected and nonconsanguineous parents. Similar recurrence risks have been empirically derived for many other common anomalies unassociated with mental retardation. That heritable tendencies, rather than shared intrafamilial environmental factors, are responsible for familial aggregates can be deduced from twin studies. Monozygotic twins are more likely than dizygotic twins to be concordant for anomalies limited to a single organ system, although in each circumstance both twins were subject to a similar intra-uterine environment. However, the relatively low recurrence risks (2 to 5 percent) suggest that either more than one gene is involved or that both genetic and environmental factors are involved. By contrast, if neural tube defects were inherited in an autosomal recessive fashion, the recurrence risk would be 25 percent; if inherited in an autosomal dominant fashion, 50 percent.

One explanation for a trait whose recurrence risk is 2 to 5 percent is that the trait is influenced by several genes. This assumes that within the population there is continuous variation with respect to a given trait. This model is attractive because a relatively small number of genes can produce continuous phenotypical variation. For example, consider the number of possible genotypes if one (*A*) gene, versus two (*A* and *B*) genes, controls a single trait.* If the gene frequency of *A* equals the gene frequency of *a*, then 25 percent of the population is *AA* ($p = q = 0.5$; $p^2 = q^2 = 0.25$), 25 percent is *aa*, and 50 percent is *Aa* ($2pq = 0.50$). If two genes influence the trait, nine genotypes are possible: *AABB*, *AABb*, *AAbb*, *AaBB*, *AaBa*, *Aabb*, *aaBB*, *aaBb*, *aabb*. The population will contain only five classes of individuals if *A* exerts the same effect as *B* and *a* exerts the same effect as *b*. The population will contain nine classes of individuals if *A* and *B*, as well as *a* and *b*, exert dissimilar influences (Table 1-8). As the number of genes controlling a trait increases, the histographic representation of the proportion of individuals in each class will more closely approximate a gaussian (normal) distribution. Figure 1-16 shows the situation for three genotypes; for more genotypes, there would be more bars, eventually showing gaussian distribution.

A trait controlled by more than one gene is said to be *polygenically inherited*. Continuous variation may also result from many alleles at a single locus or from a single locus influenced by environmental factors. If both environmental and genetic factors influence a trait, many geneticists apply the term *multifactorial inheritance*. Thus, continuous variation could result from (1) more than one gene, (2) more than two alleles at a single locus, or (3) a single gene influenced by environmental factors (multifactorial). Polygenic and multifactorial inheritance usually cannot be distinguished, although comparisons between monozygotic and dizygotic twins theoretically permits a distinction.

Polygenic or multifactorial inheritance can explain the inheritance of normal anatomic and physiologic variables that display continuous variation

*Assume each gene has only two allelic forms, *A* and *a*, or *B* and *b*.

Table 1-8
Relationship Between Numbers of Genes Controlling a Trait and Numbers of Classes of Individuals in a Population

Number of Genes	Classes of Individuals	Number of Classes
1 (*A,a*)	*AA,Aa,aa*	3
2 (*A,a;B,b*)	*AABB, AABb, AAbb* *AaBB, AaBb, AAbb* *aaBB, aaBb, aabb*	9
n		3^n

From Simpson JL (1976): Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 39. Reprinted with permission.
A and *a* represent alleles at one locus, *B* and *b* at another. If one gene controls the presence or absence of a given trait the population consists of three genotypes; if two genes control a trait the population consists of nine genotypes. If there are more than two alleles at a given locus, the number of genotypes would increase.

(height, skin color, hair color, blood pressure, age of menarche, the ability to metabolize a given drug). However, polygenic inheritance cannot explain discontinuous variation, in which the population consists of two discrete groups, one obviously affected and one unaffected (e.g., cleft palate—there is relatively little variability in the degree of clefting, although some variation exists in all traits.) To explain such dichotomies, one might postulate a threshold beyond which the accrued liability for developing a specific trait is so great that a

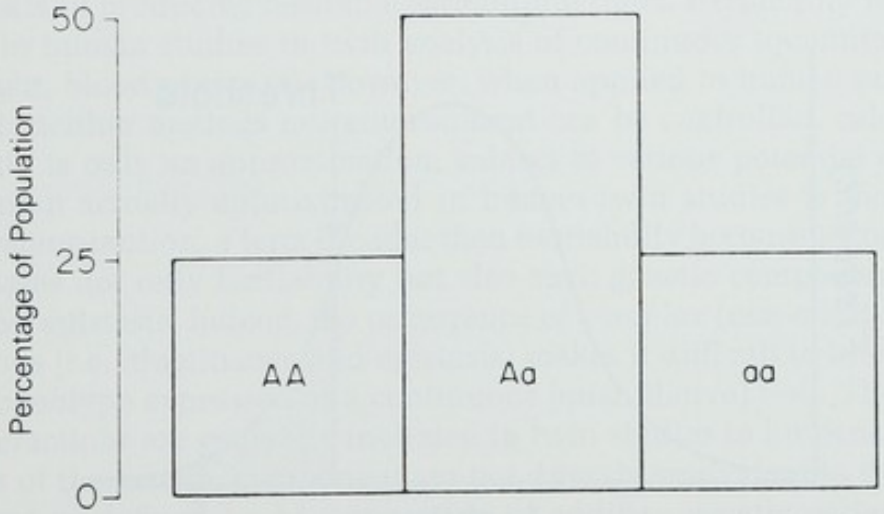


Figure 1-16. Histogram showing the relative proportions of individuals with various genotypes (*AA,Aa,aa*) if a trait is influenced by a single gene that can exist in two allelic forms (*A* or *a*). If $A = a = 0.5$, $A^2 = a^2 = 0.25$ and $2pq = 0.50$ (Hardy-Weinberg equilibrium). Thus, 25 percent of the population is *AA*, 25 percent is *aa*, and 50 percent is *Aa*. If $A = 0.9$, 81 percent are *AA*, 18 percent are *Aa*, and 1 percent are *aa*. (From Simpson JL (1976): Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, p 43. Reprinted with permission.)

malformation occurs (Fraser, 1976) (Fig. 1-17). Phenotypically normal parents with an affected child thus presumably have liabilities nearer the threshold than do most individuals in the general population. The liability might reflect the rate of embryonic growth. If growth occurs too slowly, some essential embryonic step might not be accomplished leading to anomalous development. For example, if the two palatine shelves reach the midline before a given day of development, they fuse and form the secondary palate. After that day the shelves may be too widely separated to fuse, causing cleft palate. The inherited factor (liability) might thus be the rate of growth, with the presence or absence of an anomaly the consequence. Clearly, other factors—e.g., mandible size, tongue size—also influence fusion or nonfusion of the shelves.

In humans, several empirical and statistical observations may lead one to suspect that a trait is inherited in polygenic or multifactorial fashion and is manifested only beyond a certain threshold:

- The trait, whose incidence is usually about 1 or 2 per 1000 births, usually involves a single organ system.
- After one affected child, the risk of recurrence for subsequent sibs is usually 2 to 5 percent, depending upon the particular trait. The risk may increase after two affected progeny but to less than the 25 percent expected for recessive traits or the 50 percent expected for dominant traits.
- If the trait is more frequent among members of one sex, the risk for relatives is higher if the proband belongs to the less frequently affected sex. For example, pyloric stenosis occurs more frequently in males than females. Thus, the recurrence risk for subsequent progeny is higher if the proband is female.
- The more serious the anomaly, the higher the recurrence risk. For example, bilateral cleft palate carries a higher recurrence risk than unilateral cleft palate.

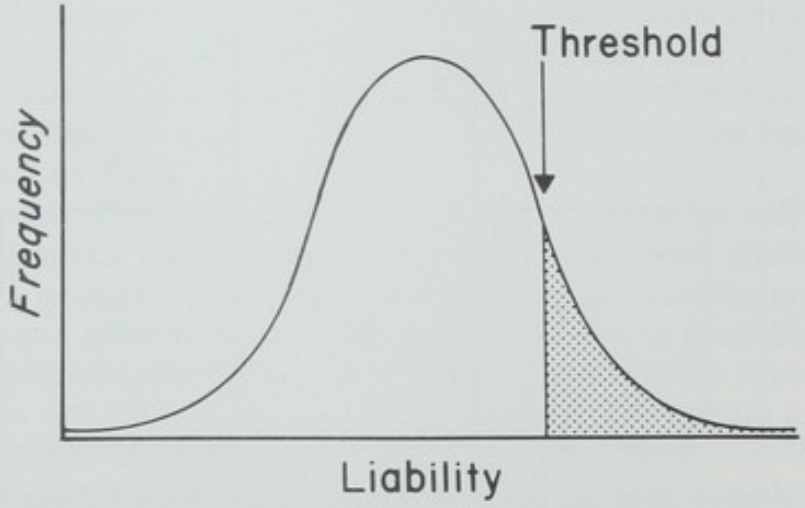


Figure 1-17. Schematic representation of one model for polygenic or multifactorial inheritance, assuming a threshold beyond which liability is so great that an abnormality is manifested. Parents of affected individuals presumably have a greater liability (i.e., are closer to the threshold) than most other individuals in the population. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York: Academic Press, 1976, p 43. Reprinted with permission.)

- The concordance rate (frequency of similarly affected cotwins) is higher among monozygotic than dizygotic twins, although discordance (dissimilarly affected cotwins) nonetheless occurs among monozygotic twins.
- As the degree of relation decreases, the recurrence risk to relatives decreases more rapidly than for autosomal dominant traits.
- Following the birth of one affected child, the probability that a sibling will be affected is approximately $\sqrt{x}$, where x equals the incidence of the trait (Edwards, 1960). Such estimates have especially proved useful in congenital heart anomalies (Nora et al., 1970a).

In humans it is nearly impossible to prove that a given trait is polygenic rather than multifactorial in etiology. Medical geneticists are, moreover, often guilty of loosely using the term polygenic to refer to any trait whose inheritance is complex. In this volume we shall often invoke the concepts polygenic inheritance and multifactorial inheritance, but we are fully aware that the genetic complexities have usually not been elucidated.

Special mention should be made of *heritability*, a concept developed for use in plant and animal breeding systems, in which matings and environment may be controlled. Often applied in the context of polygenic inheritance, *heritability* refers to that part of the variation of a trait due to additive genetic variation, i.e., the degree of genetic control preserved in gametes, the expression of which is independent of dominance or epistasis. (*Dominance* refers to interaction between alleles at a single locus as expressed in the phenotype. *Epistasis* refers to interaction of alleles at different loci.) Dominance and epistasis are created anew with the formation of each successive generation and are not transmitted per se in gametes. Thus, they are *not* included in heritability (h^2), which rather is restricted to that portion of a trait which is genetically controlled and can be transmitted as such from generation to generation. The concept of h^2 has proved useful in predicting the success of selective matings in plants and animals in producing desirable agricultural traits. Heritability is also often invoked in human studies in twin analysis of continuous (quantitative) traits (e.g., height, blood pressure). However, when applied to human populations, for which neither matings nor environment can be controlled, calculation of heritability is only an approximation, subject to various potential errors. The phenomenon actually approximated in human twin studies is the degree of *genetic determination*, a term broader than heritability because it connotes and encompasses not only heritability but also such genetic components as dominance and epistasis. Indeed, the occurrence of complex (non-additive) genetic interactions (i.e., dominance and epistasis) makes it difficult to infer genotype from a phenotype expressed as a continuous (quantitative) trait. The results of these interactions are generally included in twin studies in humans; therefore, estimates of the genetic component are not directly equivalent to the heritable component as defined by h^2 (proportion of additive genetic variance). However, such estimates are often referred to as "heritability." Assuming environmental influences are equivalent for the two types of twins and genotypic–environmental interactions do not exist, the degree of genetic determination is often estimated as

$$H = \frac{V_{DZ} - V_{MZ}}{V_{DZ}}$$

where V refers to the variance of the differences between twin pairs for a particular measurement being analyzed. However, no specific genetic interpretation can be assigned to this value. The formulas used to compute heritability and genetic determination for quantitative (continuous) traits that employ variances of the measurement differences between MZ and DZ twin pairs exist also in more sophisticated forms (see Cavalli-Sforza & Bodmer, 1971; Vogel & Motulsky, 1979). If there is a detectable genetic component to a measurable trait, MZ twin pairs would be expected to differ less on the average than DZ twin pairs. The variance in DZ twins should be due to both genetic and environmental variation, whereas variances in MZ should be due only to the latter. Therefore, subtracting V_{MZ} should theoretically result in an estimate of the degree of genetic variation relative to total variation (V_{DZ}). The interpretation of these estimates of heritability ("genetic determination") is, however, subject to many assumptions, including (1) common environmental variation for both MZ and DZ twins and (2) lack of genotype–environmental interaction. However, both assumptions are probably violated. Moreover, even if it could be correctly estimated, heritability is not a fixed value even in experimental populations, but may vary with age, sex, genetic, and environmental background. In other words, heritability estimates need not be the same for the same trait in different populations, or in the same population at different times, as is well known from plant and animal breeding (Falconer, 1960). Thus, the absolute value of heritability estimates for human population should not be accepted too rigidly. Nonetheless, relative values for different traits or diseases may identify conditions which might be fruitful to explore genetically with other methodologies.

2

Mendelian Disorders and their Influence on Reproduction

The rapidity and intensity with which medical genetics has developed and has come to influence the specialty of obstetrics and gynecology is remarkable. This growth is mirrored in the numerical status of genetic nosology over the last two decades; Table 2-1 lists the traits believed to be inherited in Mendelian fashion in humans, which has increased sevenfold in the past 20 years, from a synergism between the research laboratory and the clinician. Obviously, it would be impossible to survey all of these Mendelian traits and their influence on reproduction in one text, let alone in one chapter. Our more modest goal is to discuss the more common, more noteworthy, and best understood Mendelian traits causing disease states that can influence reproductive function. Further details are available in a recently published volume (Schulman & Simpson, 1981).

HEMOGLOBINOPATHIES

Background

Hemoglobin is a tetrameric protein consisting of four *globin chains*. In humans there are six structurally different types of globin chains, designated alpha (α), beta (β), gamma (γ), delta (δ), epsilon (ε), and zeta (ζ) (Bunn, Forget, & Ranney, 1977). The early embryonic globins ε and ζ are rapidly replaced as the fetus develops (Huehns et al., 1964). Two α and two non-α chains combine to form each of the different hemoglobins.

The thalassemia syndromes are named and classified by the type of hemoglobin chain that is inadequately represented. The thalassemias are quantitative disorders of synthesis; the globin chains are structurally normal. The two major groups are the α- and β-thalassemias, both of which affect the synthesis of HbA, which contains two α and two β chains ($\alpha_2\beta_2$). There is no single etiology for

Table 2-1
Mendelian Traits in Humans*

	Year					
Inheritance Mode	1959	1966	1968	1971	1975	1978
Autosomal dominant	285	837	793	943	1218	1489
Autosomal recessive	89	531	629	783	947	1117
X-linked	38	119	123	150	171	205
Total	412	1487	1545	1876	2336	2811

Adapted from McKusick VA (1978): Mendelian Inheritance in Man (ed 5). Baltimore, Johns Hopkins Press.
*In approximately half of the above traits, Mendelian inheritance is considered proved, and in the remaining half it is quite possible.

these diseases. On the molecular level, a variety of defects have been demonstrated, each involving a different step along the pathway of transcription of DNA into RNA and translation of mRNA into globin chains.

The most common forms of α-thalassemia lack one or more of the four structural genes that code for α-globin (Ottolenghi et al., 1974). In the homozygous condition with all four alleles deleted, no chains are produced and the fetus is unable to synthesize HbF ($\alpha_2\gamma_2$) or any of the adult hemoglobins (Weatherall, Clegg, & Wong, 1970). The result is high-output cardiac failure, hydrops fetalis, and stillbirth. The most severe form of α-thalassemia compatible with extra-uterine life is HbH (β_4) disease, which results from the deletion of three α genes (Kan et al., 1975a). Abnormal quantities of HbH and Hb Bart (γ_4) accumulate, leading to a moderately severe hemolytic anemia. In α-thalassemia minor (α-thalassemia-1), two genes are deleted, causing a mild hypochromia and microcytic anemia, which must be differentiated from iron deficiency. A single gene deletion (α-thalassemia-2) is clinically undetectable (Orkin & Nathan, 1976). The etiology of α-thalassemia in blacks is somewhat modified. In blacks with α-thalassemia-1, the deleted genes are in trans (on the opposite chromosome) rather than in cis (on same chromosomes) position, which makes both HbH disease and α-thalassemia per se rare (Dozy et al., 1979).

In β-thalassemia, no gene deletions have been demonstrated. There are two forms of β-thalassemia, designated β^+ or β^0, depending on whether β-chain production directed by the one β^- is reduced or entirely absent (Conconi et al., 1972; Forget et al., 1974; Kan et al., 1975c). β-thalassemia major is the homozygous state, in which there is little or no production of β-chains. The fetus is protected from severe disease by γ-chain production. However, this protection disappears rapidly after birth. The affected infant is anemic by three months of age, develops hepatomegaly, requires blood transfusions every three to four weeks, and dies by the third decade, usually of myocardial hemosiderosis. Those female patients surviving until puberty are usually amenorrheic with severely impaired fertility (Necheles, 1973).

β-thalassemia minor, the heterozygous state, results in a variable degree

of illness, depending upon the rate of β-chain production. The condition may be clinically subtle and frequently is diagnosed only after the patient fails to respond to iron therapy or delivers a child with homozygous disease. Findings include an increased erythrocyte count, microcytosis, hypochromasia, elevated HbA_2 ($\alpha_2\delta_2$) concentration, elevated serum iron concentration, and an iron saturation over 20 percent.

Several hundred structural variants of α, β, γ, and δ chains have been identified. Most differ from normal by only one amino acid. The molecular location of the substitution has a marked influence on the functional effect. If an individual is homozygous for a mutated β chain (e.g., the S gene), all her adult hemoglobin will contain S (β^S) globin chains instead of normal β^A chains. If heterozygous, one β^S chain will be joined with a normal β^A chain (e.g., $\alpha^2\beta^A\beta^S$).

Reproductive Implications

Approximately 10 percent of American blacks are heterozygous for the hemoglobin S gene; this state is referred to as the sickle cell "trait" (Motulsky, 1973). Whalley and colleagues (1963, 1964), Pritchard et al. (1973) and Blattner, Dar, and Nitowsky (1977) have compared the outcomes of pregnancy in women with normal hemoglobin and with sickle cell trait. They noted comparable rates of spontaneous abortions, low birth weight infants, toxemia, low Apgar scores, and perinatal mortality. However, gravidous women with sickle cell trait had twice as many urinary tract infections as non-sickle-cell women. These women do well during pregnancy and labor, but caution must be observed when using anesthesia to assure good oxygenation and thus avoid sickling.

Patients homozygous for the HbS gene have sickle cell anemia (SCA) and suffer from lifelong complications, in part due to the markedly shortened life span of their red blood cells. Although maternal mortality is quite rare on modern obstetrical services, the perinatal mortality (approximately 28 percent) and the incidence of infants born weighing less than 2500 grams (approximately 45 percent) are high (Freeman & Ruth, 1969; Perkins, 1971a; Pritchard et al., 1973). The spontaneous abortion rate does not appear to be increased. Most observers feel that a woman's prepregnancy health is a good indication of how she will do in pregnancy. The complications of SCA during pregnancy include sickle cell crises, infections, toxemia (in 33 percent of pregnancies associated with SCA, according to Pritchard et al., 1973), hemosiderosis, and folic acid deficiency anemia. There is no support for early elective induction of labor, and, in fact, some studies have shown the use of oxytocin to be associated with decreased bone blood flow (Singer et al., 1951; Fraser & Watt, 1964). Anesthesia-related hypovolemia and/or hypoxia can lead to serious complications, so regional anesthetics should be administered with great caution.

Women who are heterozygous for both the β^S and β^C genes are said to have HbSC disease (HbSCD). HbSCD during pregnancy has a 4 to 5 percent maternal mortality rate (Golbus & Laros, 1981). Pregnant patients with HbSCD experience rapid and severe anemia crises due to splenic sequestration. The clinical manifestations of HbSCD are otherwise similar to SCA but milder. Pregnancy com-

plications include urinary tract infections (70 percent), gross hematuria (30 percent), pyelonephritis (21 percent), pulmonary infection (50 percent), and toxemia (20 percent) (McCurdy, 1964). Patients with HbSCD require increased folate intake, as in SCA. However, those with HbSCD also require iron supplements, and if they do not take them, they may develop iron deficiency anemia.

Hemoglobin C trait (HbAC) is an asymptomatic state without reproductive consequences. Hemoglobin C disease is a mild hemolytic anemia with the hematocrit in the range of 25 to 35 percent and a constant reticulocytosis. There is no morbidity or mortality associated with pregnancy.

In hemoglobin S-β-thalassemia the patient is heterozygous for both HbS and β-thalassemia. The mutations are located on different homologues. Pritchard et al. (1973) described no maternal deaths and 91 percent fetal survival in his study of 37 pregnancies. Laros and Kolstone (1971), on the other hand, described one maternal death in 38 pregnancies (2.6 percent) and an 81 percent rate of fetal survival. The problems are similar to those seen with HbSCD, and the clinical severity usually is directly proportional to the HbS concentration.

Pregnant women with β-thalassemia minor, α-thalassemia minor, and HbH disease appear to have normal deliveries and infants (Alger, Golbus, & Laros, 1979).

The major risks to a pregnant woman with SCA, HbSCD, and HbS-β-thalassemia occur during the third trimester, intrapartum, and postpartum. Hypothetically, complications of the hemoglobinopathy might be avoided if one were able to remove a significant portion of the patients' whole blood and replace it with fresh packed red blood cells at a critical time during gestation. Experience with 36 patients undergoing partial exchange transfusion was reported by Morrison and Wiser (1976a, 1976b). There were two cases of maternal hepatitis, one associated with infant mortality. Overall, the results indicated reduced prematurity and fewer low birth weight infants in the treated groups, and a dramatic decrease in the perinatal death rate.

HEMOLYTIC ANEMIAS

In normal humans erythrocytes survive approximately 120 days. In a hemolytic state the in vivo life span of the erythrocyte is shortened. The inherited hemolytic anemias may be caused by either a structural or an enzymatic defect in the red cell. The most common form of inherited (autosomal dominant) hemolytic anemia is due to spherocytosis, and is characterized by intermittent jaundice, splenomegaly, cholelithiasis, and spherocytes in the peripheral blood smear. Over 650 pregnancies occur annually in the United States in women with hereditary spherocytosis. A hemolytic crisis may be induced by pregnancy and may be treated with replacement transfusions (Johnson & McAllister, 1951) or with splenectomy (McElin, Mussey, & Watkins, 1950; Moore, Sherman, & Strongin, 1976). Prenatal care of women with spherocytosis who have not had a splenectomy should include folic acid supplementation for the increased erythrocyte production and careful monitoring of the hemolytic state. In the absence of severe untreated anemia, hereditary spherocytosis does not contribute to prenatal or neonatal morbidity.

Hereditary elliptocytosis is a somewhat milder hemolytic anemia state caused by an autosomal dominant structural defect of the erythrocyte. The symptoms and signs are similar to those of spherocytosis, albeit not as severe. Hemolysis may be precipitated by pregnancy, with no symptoms or signs between pregnancies. Only transfusion therapy has been required in the few reported cases involving pregnancy (Weiss, 1963; Breckenridge & Riggs, 1968).

The most prevalent red blood cell enzyme deficiencies are those involving the hexosemonophosphate shunt. Because this pathway supplies only a minor component of the cell's energy, these deficiencies are associated with episodic hemolysis secondary to oxidative damage. The most common deficiency involves glucose-6-phosphate dehydrogenase (G6PD) and is an X-linked trait. Pregnancy in patients with G6PD deficiency is associated with a number of specific complications. There is decreased G6PD activity in one third of patients in the third trimester (Vergnes & Clerc, 1968), predisposing to hemolytic episodes at this time. A study of 180 G6PD-deficient pregnant women reported that 62 percent had a hematocrit of less than 30% (Silverstein et al., 1974). Urinary tract infections were more common, which poses an additional risk, because many drugs commonly employed to treat urinary tract infections are oxidants. Exposure of the G6PD-deficient fetus to maternally ingested oxidants may produce fetal hemolysis, hydrops fetalis, and death (Perkins, 1971b; Mentzer & Collier, 1975). A reasonable course would be to screen pregnant black women (3 percent are heterozygous for G6PD deficiency) who have urinary tract infections for G6PD deficiency prior to starting therapy. The neonate is at risk for anemia, hyperbilirubinemia, and kernicterus. The incidence of severe jaundice in randomly selected G6PD-deficient newborn males is approximately 5 percent, but rises to 50 percent if there was a prior icteric sibling (Fessas, Doxiadis, & Valaes, 1962).

Red cell enzyme deficiencies in the glycolytic pathway, which generates 93 percent of the cells' ATP (Oski & Naiman, 1972), are rarer but are usually associated with more severe hemolysis. Pyruvate kinase deficiency, an autosomal recessive trait, is the most common red cell glycolytic defect. A hemolytic crisis may be precipitated by pregnancy (Collier, Ashford, & Bell, 1966; Kendall & Charlow, 1977), and there are at least two reports showing that oral contraceptives intensify the hemolytic process (Nixon & Buchanan, 1967; Kendall & Charlow, 1977). Accentuation of hemolysis results in severe jaundice in the newborn, often requiring exchange transfusion.

The genes for the other enzymes in the glycolytic pathway are inherited in an autosomal recessive mode, except for phosphoglycerate kinase, which is X-linked. There is insufficient experience with defects of these enzymes to allow conclusions regarding their effect on reproduction.

DISORDERS OF HEMOSTASIS

Plasma Coagulation Factor Deficiencies

Hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency) are X-linked traits that are similar in clinical appearance but that can be differentiated by laboratory tests. Nonpregnant carriers of a hemophilia gene

have, on the average, 50 percent of the deficient factor that control women have. Since the carrier values approximate a normal distribution, some will have levels low enough to cause a clinical disorder. The clinical problems are mild and usually consist of bleeding associated with surgery, trauma, or dental work. However, there are rare instances of women with clinical and laboratory findings indistinguishable from those of males with hemophilia. The mechanisms causing a female to manifest an X-linked disease include (1) homozygosity for the deleterious gene, (2) heterozygosity in which the normal gene is not functioning because the X chromosome bearing the normal gene is structurally abnormal and has been preferentially inactivated or because the X chromosome bearing the normal gene has been randomly inactivated in almost all of the cells producing the clotting factor, or (3) hemizygosity with only one X chromosome.

The female heterozygote with a low factor VIII or IX level must be prepared for surgical procedures in the same manner as is a mild male hemophiliac. A pregnant woman whose factor level has not risen over 40 percent of normal by the third trimester may require cryoprecipitate transfusion during and immediately following delivery. The hemophiliac fetus does not appear to be at risk during gestation. Problems due to birth trauma are rare, but intracranial bleeding and cephalohematomas have been reported in hemophiliac neonates (Rausen & Diamond, 1961).

Detection of the heterozygous state for hemophilia has been reviewed recently by Graham (1978). The considerable overlap between the range of factor levels for heterozygous and homozygous normal women made carrier detection difficult until recently. Zimmerman, Ratnoff, and Littell (1971) developed an immunologic test that quantitates factor VIII-related antigen, which is present in normal amounts in hemophilia. Testing heterozygotes consists of comparing the clotting activity and antigen activity, since a ratio of less than one indicates the production of nonfunctional factor VIII. This technique allows identification of 80 to 90 percent of heterozygotes for factor VIII deficiency (Klein et al., 1977). Testing for the carrier state may also be performed during pregnancy despite the fact that factor VIII clotting activity rises substantially, because factor VIII-related antigen levels appear to rise concordantly. Carrier detection for factor IX deficiency is not as reliable, since the distribution of factor IX activity levels in carriers is wide and there is substantial heterogeneity of factor IX antigen levels among patients with hemophilia B (Barrow, Bullock, & Graham, 1960; Roberts et al., 1968). There is no reported experience in attempting to diagnose the factor IX-deficient heterozygote during pregnancy.

Von Willebrand disease (VWD) is an autosomal dominant trait characterized by deficiency of factor VIII (causing a decrease in both coagulant activity and factor VIII-related antigen levels) and deficiency of a plasma factor required for normal platelet function. Bleeding in VWD usually involves the mucous membranes and skin, in contrast to the deep tissue hemorrhages common in hemophilia. Menorrhagia is a serious problem for many women with VWD but is often controllable with oral contraceptives. Postpartum hemorrhage occurs in approximately 25 percent of parturients (Noller et al., 1973). The treatment of choice is cryoprecipitate, since it can correct the bleeding time as well as the factor VIII deficiency and poses the least risk of hepatitis.

For most of the plasma coagulation factor deficiencies, there is little or no experience with pregnant patients. Deficiencies appear to be inherited as autosomal recessive traits. No clinical problems have been reported in heterozygous women. Homozygous affected women with factor X, XI, or XIII deficiency may hemorrhage postpartum (Czapek, 1973; Rizza, 1976). A pregnancy in a factor XIII-deficient woman resulted in a spontaneous abortion due to severe decidual bleeding (Fisher, Rikover, & Noar, 1966).

Platelet Disorders

Qualitative platelet defects demonstrate reduced platelet adhesion or aggregation. The Bernard-Soulier syndrome appears to be due to the absence of a glycoprotein complex on the platelet surface that serves as the Von Willebrand factor receptor (Nachman, 1977; Phillips, 1977). This autosomal recessive trait results in moderate to severe membrane bleeding, excessive bruising, and menorrhagia. The risk of an affected woman having postpartum hemorrhage is considerable, and platelet transfusions may be required.

Thrombasthenia probably represents a heterogeneous group of disorders inherited in an autosomal recessive mode. The severity is variable, but menorrhagia may require hormonal suppression of menses (Cronberg & Nilsson, 1968; Vinazzer, 1974). Profuse bleeding at delivery has been described (Cronberg & Nilsson, 1968). An obstetrically indicated cesarean section has been performed following platelet transfusion (Vinazzer, 1974).

The hereditary thrombocytopenias are all rare disorders. Danger during pregnancy is directly related to the platelet count, and the therapy, if needed, is platelet transfusion. There is virtually no literature on the effects of platelet disorders on reproduction.

METABOLIC DISORDERS

Many metabolic disorders cause premature death, severe mental retardation, or impaired fertility. However, other metabolic disorders have been present in pregnant women with no observable influence on either the mother or fetus (e.g., acid maltase deficiency, arginosuccinic aciduria, galactosemia, Gaucher disease, Hartnup disorder, hydroxyprolinemia, hyperornithinemia, iminoglycinuria, McArdle syndrome, and xanthuria) (Lamon et al., 1981). There are also a few metabolic disorders in which a maternal or fetal effect has been demonstrated, and about which the obstetrician should be more knowledgeable. These disorders will be discussed in this section.

Phenylketonuria

Phenylketonuria (PKU) is an autosomal recessive trait usually caused by a deficiency of the enzyme phenylalanine hydroxylase, which allows too much phenylalanine to accumulate in the body, resulting in mental retardation and other manifestations. However, there are other causes of hyperphenylalaninemia. A low-phenylalanine diet begun in the first few days of life prevents

most of the neurologic and intellectual damage. In fact, the use of such diets, in conjunction with newborn screening for PKU begun in the 1960s, has allowed many individuals with PKU to reach adulthood without having experienced any symptoms of the enzyme deficiency.

Mental retardation, microcephaly, congenital heart disease, and slow intra-uterine growth are characteristic of genetically normal offspring of mothers with PKU. The fetal damage is due to prenatal exposure to the high concentrations of phenylalanine and its metabolites in the maternal serum. The magnitude of fetal damage appears to be related to the mother's blood phenylalanine level. A level equal to or greater than 20 mg/100 ml (true of most affected individuals) results in a 92-percent incidence of mental retardation, 73-percent incidence of microcephaly, 10-percent incidence of cardiac defects, and 40-percent incidence of intra-uterine growth retardation (Hansen, 1978; Mabry, 1978; Lenke & Levy, 1979).

Affected women have been put on a low-phenylalanine diet before conception or as soon as possible thereafter in an attempt to avoid the maternal PKU syndrome. Thirty-four pregnancies have been so treated, with mixed results (Lenke & Levy, 1979). One problem is that treatment begun after conception may be too late to prevent microcephaly or cardiac defects. Of 11 pregnancies in which treatment began in the first trimester, 3 infants had microcephaly and 4 had lethal cardiac defects. Of 16 pregnancies in which treatment began in the second trimester, 9 infants had microcephaly and 2 had cardiac defects. Even in the 3 pregnancies in which treatment began prior to conception, 1 infant had borderline microcephaly. A second problem is that the effect on the fetus cannot be predicted accurately on the basis of the concentration of phenylalanine in the treated mother. However, it would be premature to draw conclusions from this small series. To provide effective preconception treatment, identification of affected women will be necessary, requiring premarital urine screening to identify asymptomatic affected women.

Homocystinuria

Homocystinuria is caused by an autosomal recessive deficiency of the liver enzyme cystathionine synthase. Manifestations include dislocated lenses, mental retardation, skeletal abnormalities, and vascular occlusions. One form responds to large doses of pyridoxine (vitamin B_6). Thirty-eight pregnancies in 14 homocystinuric women have been reported (Lamon et al., 1981). Among the 20 untreated pregnancies, there were 16 fetal losses, 1 therapeutic abortion, 1 hydrocephalic stillborn, and 2 normal offspring. Of the 17 pregnancies in pyridoxine-responsive women, there were 15 normal offspring, 1 with unrelated brain damage, and 1 with trisomy 21. The 1 pyridoxine-unresponsive woman who was treated with anticoagulants and folic acid had a normal child. Children of men with homocystinuria have had no abnormalities (McKusick, Hall, & Char, 1971; Brenton et al., 1977).

Histidinemia

Histidinemia is an autosomal-recessive deficiency of histidase. There is some question as to whether histidinemia is associated with any symptoms and whether treatment is necessary. Nine children born to 6 women with

histidinemia have been normal (Lamon et al., 1981), but Lyon, Gardner, and Veale (1974) found that 4 of the 5 children of a histidinemic woman had depressed IQs. Additional information will be required before any conclusions on the effect of histidinemia on the fetus can be made.

Galactokinase Deficiency

This condition is caused by an autosomal recessive deficiency. The only manifestation is cataracts. Harley et al. (1974) suggested that maternal partial deficiency of galactokinase may contribute to cataract formation in the fetus. If this observation is verified, maternal milk restriction might be of value in such pregnancies.

Refsum Disease (Phytanic Acid Storage Disease)

This autosomal recessive trait is manifested by night blindness, retinitis pigmentosa, polyneuropathy, ataxia, nerve deafness, EEG abnormalities, and ichthyosis. A drastic worsening of symptomatology has been described during pregnancy (Steinberg, 1978), but there is no evidence that the maternal condition affects the fetus. The usefulness of a low-phytonate diet during pregnancy is undetermined.

The Porphyrias

There are six distinct forms of porphyria, five inherited as autosomal dominant traits and one as an autosomal recessive trait. Acute intermittent porphyria is the most common form and is typical of the acute attack forms. There is a chemical and symptomatic exacerbation of the disease associated with the hormonal changes of pregnancy (Lamon et al., 1981). The birth weights of infants whose mothers experienced an acute attack of porphyria during pregnancy were significantly lower than the weights of infants whose porphyric mothers were asymptomatic during gestation (Brodie et al., 1977).

Hepatolenticular Degeneration

Hepatolenticular degeneration (Wilson disease) is an autosomal recessive trait characterized by neurologic and renal dysfunction, liver disease, and Kayser-Fleisher rings in the cornea. The disease appears to be due to a decrease in the serum copper-carrier ceruloplasmin and to a secondary increase in serum and urine copper concentration. Untreated symptomatic women do not successfully complete pregnancy (Walshe, 1977), and even asymptomatic women are prone to spontaneous abortions (Klee, 1979). The copper deposition in affected organs can be reduced by chelating agents, the drug of choice being penicillamine. In addition to preventing symptoms in asymptomatic women and allowing improvement in symptomatic patients, penicillamine allows pregnancy to proceed normally. Over 50 pregnancies have been reported in which affected women were treated during at least part of the pregnancy with penicillamine (Schienberg & Sternlieb, 1975; Mareck & Graf, 1976; Walshe, 1977). All of the infants were normal. However, two other infants exposed in utero

to penicillamine for maternal cystinuria or rheumatoid arthritis were born with lax skin and unusual facies and subsequently suffered lethal neonatal intraabdominal catastrophes (Mjolnerod et al., 1971; Solomon et al., 1977). It is possible that in hepatolenticular degeneration the excess maternal circulating copper absorbs the penicillamine and thus protects the fetus.

ENDOCRINE DISORDERS

Hypogonadism Syndromes

The Lawrence-Moon-Biedl syndrome is inherited as an autosomal recessive trait characterized by retinitis pigmentosa, mental retardation, obesity, syndactyly, and hypogonadism in three fourths of affected males and one half of affected females (Rimoin & Shimke, 1971). This defect is often categorized under hypothalamic/pituitary disorders because of the obesity, hypogonadism, and occasional diabetes insipidus; however, the etiology of the syndrome is unknown. A number of affected women have had children without incident, and because this is an autosomal recessive trait the offspring are not at an increased risk for the disorder unless the father is heterozygous.

The Prader-Willi syndrome usually occurs sporadically but has been inherited as an autosomal recessive trait characterized by childhood-onset obesity, hypotonia beginning in utero, and mental retardation. Affected males have micropenises and delayed puberty, whereas affected females often have menstrual irregularities. Pregnancy may occur in affected females, but no particular complications are to be anticipated.

The Noonan syndrome is inherited as an autosomal dominant trait. It is characterized by short stature, ptosis, micrognathia, dental malocclusion, hypertelorism, pulmonic stenosis, skeletal abnormalities, mental retardation, and variable hypogonadism. Affected males more often have cryptorchidism, delayed puberty, and hypogonadism, whereas affected females occasionally have primary amenorrhea (Saez et al., 1974). The major complications during pregnancy relate to the presence of cardiac lesions, most often pulmonic stenosis.

Adrenal Disorders

Congenital adrenal hyperplasia is a group of disorders inherited as autosomal recessive traits due to various enzyme deficiencies in the steroid biosynthesis pathways. Masculinization of the female external genitalia may occur, causing female pseudohermaphroditism (see Chapter 10). However, the internal genitalia are normal, and reproduction is usually possible after appropriate replacement therapy is instituted. Treatment with cortisol or its analogs suppresses the synthesis of abnormal steroids and avoids cortisol deficiency. Mineralocorticoids such as DOC or 9-α-fluorohydrocortisone are required in the salt-losing forms of congenital adrenal hyperplasia. There have been many successful pregnancies in patients with treated virilizing congenital adrenal hyperplasia (Bongiovanni, 1978). The neonate should be observed carefully for signs of adrenal insufficiency as result of chronic suppression in utero. The

chance of an affected woman having an affected child is small (approximately 1/300) because the chance of marrying a heterozygote is low (approximately 1/150). Heterozygote identification is not perfect, but may be done in some cases either by measuring serum 17-hydroxyprogesterone levels following ACTH stimulation (Lee & Gareis, 1975) or by HLA-B linkage studies (L. S. Levine et al., 1978).

A syndrome of adrenocorticoid insufficiency, hypoparathyroidism, and moniliasis is inherited as an autosomal recessive trait (Spinner et al., 1969). Premature ovarian failure may occur in affected females (Golonka & Goodman, 1968; Schachner, 1974). Both the adrenal and the parathyroid deficiencies require therapy. Maternal hypoparathyroidism produces hypocalcemia, and decreased calcium produces parathyroid hyperplasia and subsequent mobilization of calcium from the skeleton in the fetus. Treatment of the mother with calcium and vitamin D prevents these fetal effects.

Thyroid and Parathyroid Disorders

Several defects in the biosynthesis of thyroxine are inherited in autosomal recessive fashion. The treatment is thyroid, which both replaces the missing hormone and suppresses the goiter. Adequately treated affected women should have no pregnancy-related complications.

Hyperparathyroidism usually occurs sporadically but has been inherited as an autosomal dominant trait (Cutler, Reiss, & Ackerman, 1964; Graber & Jacobs, 1968). A substantially higher incidence of multiple gland involvement occurs in the familial form than in the sporadic form. During pregnancy there is maternal hypercalcemia and secondary fetal hypercalcemia, which suppresses the fetal parathyroids (Rasmussen, 1974). Johnstone, Kreindler, & Johnstone (1972) reported that fetal parathyroid hypoplasia leads to neonatal tetany and sometimes to death; they also reported an increased incidence of spontaneous abortions and stillbirths. A potential maternal complication is a hyperparathyroid crisis immediately postpartum (Schenker & Kallmer, 1965). The maternal, fetal, and neonatal morbidity and mortality rates indicate that pregnant women should have parathyroid surgery at the time of the diagnosis.

RENAL DISORDERS

Polycystic Kidney Disease

Adult polycystic kidney disease is an autosomal dominant trait that usually manifests itself in the third or fourth decade of life. It is the third most common cause of renal failure in the United States (Advisory Committee to the Renal Transplant Registry, 1973). Increased kidney size is usually noted prior to the development of hypertension, hematuria, and pyelonephritis. Sonography is the best method for diagnosing asymptomatic disease.

In the largest series of pregnant patients with adult polycystic kidney disease, it was demonstrated that the severity of maternal disease predicted the outcome of pregnancy (Landesman & Scherr, 1956). Asymptomatic pregnant

women had no increase in complications; women with renal hypertension but normal renal function had an increase in both maternal morbidity and fetal mortality, and the one woman with renal insufficiency had a stillbirth. This suggests that women at risk of inheriting polycystic kidney disease should become pregnant as early as possible.

Hereditary Nephritis

Hereditary glomerulonephritis is an autosomal dominant trait with variable penetrance. If present in conjunction with bilateral VIII nerve deafness, it is termed Alport syndrome. The nephritis tends to be considerably more severe in males. Pregnancy may exacerbate the nephritis and cause development of either preeclampsia or the nephrotic syndrome.

DISORDERS OF CONNECTIVE TISSUE AND THE SKELETAL SYSTEM

Ehlers-Danlos Syndrome

Ehlers-Danlos syndrome includes at least eight specific entities (Bornstein & Byers, 1980). Classic type I is an autosomal dominant trait characterized by hyperextensible skin, joint hypermobility, fragile tissues, and a bleeding diathesis. During pregnancy, affected women may manifest increased bruisability (Samuel, Schwartz, & Meister, 1953), hernias or varicosities (Beighton, 1970), or rupture of large blood vessels (McKusick, 1972). Delivery may cause separation of the symphysis pubis (Beighton, 1970), and postpartum hemorrhage may be severe (Stoddard & Myers, 1968). Episiotomy and laparotomy (e.g., cesarean section) incisions heal slowly, and perineal hematoma may occur despite episiotomy. It is suggested that retention sutures be used in surgery on these women and that the sutures not be removed for at least 14 days, to avoid wound dehiscence.

Prematurity and precipitous deliveries are common complications, partially due to lax cervical connective tissues. If the fetus is affected, the fetal membranes will be involved; premature rupture of the membranes often occurs at 32 to 36 weeks (Kanof, 1952; Mories, 1954; Barabas, 1966). Affected infants tend to be hyperextensible and may have congenitally dislocated hips. Neonates may even be misdiagnosed as having neurologic problems because of floppiness and bleeding disorders (McKusick, 1972).

Gynecologic problems also are more common in women with Ehlers-Danlos type I. Menorrhagia may be secondary to the bleeding diathesis. Uterine and bladder prolapse or abdominal hernias occur because of abnormal supporting connective tissue.

There is insufficient experience with the effects of other forms of the syndrome on reproduction to draw conclusions. Ehlers-Danlos type IV, which is characterized by greater bleeding tendencies and vascular rupture, is inherited

as an autosomal recessive trait. Menorrhagia has been reported in affected women, and vessel rupture during pregnancy is of particular concern in such women.

Marfan Syndrome

Marfan syndrome is an autosomal dominant disorder characterized by variable degrees of skeletal, eye, and cardiovascular abnormalities (McKusick, 1972). Aortic aneurysm due to cystic medial necrosis is not uncommon, and 50 percent of the aortic aneurysms in affected women under age 40 occur during pregnancy (Mandel, Evans, & Walford, 1954). Aneurysm rupture is most likely to occur in the third trimester and is rare during labor or postpartum (Pedowitz & Perell, 1957). Aortic valve replacement has been performed successfully during pregnancy (Donaldson & de Alvarez, 1965). Affected women with aortic valve changes or aortic dilation prior to pregnancy have a 10 to 20-percent mortality rate during pregnancy, whereas women without aortic involvement have less than a 5-percent mortality rate (Pedowitz & Perell, 1957; Tricomi, 1965; Sutinen & Piiroinen, 1971; McKusick, 1972). Splenic artery rupture also occurs with increased frequency during pregnancy (Schnitker & Bayer, 1944; Sheehan & Falkiner, 1948). In addition, in women with severe scoliosis, respiratory compromise may complicate the pregnancy. There is an increased frequency of hernias in these patients, and retention sutures should be used if surgery is performed.

The use of estrogens to cause premature ephiphyseal fusion and, hence, decreased ultimate height has been suggested for affected girls (Skovby & McKusick, 1977). This has been effective but requires careful monitoring for scoliosis, which may be manifested during the growth spurt. Theoretical questions regarding the relationship of estrogens and cystic medial necrosis must be considered, and long-term use of oral contraceptives may be relatively contraindicated.

Osteogenesis Imperfecta

There are at least four different types of osteogenesis imperfecta (Sillence, Senn, & Danks, 1979), all of which involve osteoporosis and fracture of long bones with minimal trauma. Type I is an autosomal dominant form with blue sclera; type II is a lethal autosomal recessive form; type III is an autosomal recessive form with white sclera and progressive deformities; and type IV is an autosomal dominant variety with white sclera and variable severity of fractures. The expressivity of the autosomal dominant forms may vary greatly among family members.

Pregnancy complications include increasing respiratory compromise, especially in women with short stature and kyphoscoliosis, cephalopelvic disproportion due to previous pelvic fractures, uterine rupture, and separation of the symphysis pubis (Young & Gorstein, 1968; Sengupta et al., 1977). General anesthesia is associated with an increased risk of malignant hyperthermia in affected individuals (Solomons & Myers, 1971), and spinal or epidural anes-

thesia may lead to fracture of osteoporotic vertebrae. A fetogram in the late third trimester is advisable to diagnose an affected fetus with healing fractures, in which case cesarean section to avoid fetal trauma is indicated. Forceps must be avoided, since wormian bones will fracture and cause intracranial bleeding.

Achondroplasia

Achondroplasia, the most common form of short-limbed dwarfism, is inherited as an autosomal dominant trait. As many as 75 to 80 percent of cases represent new mutations (Murdoch et al., 1970). Gynecologic problems, including premature menarche, leiomyomata uteri, enlarged breasts, and premature menopause, are common. Pregnancy causes great reduction in mobility because awkwardness, glucosuria, and cardiorespiratory compromise (due to small chest cavity). All women with achondroplasia should have cesarean section because of a contracted pelvis, and general anesthesia should be used because spinal stenosis makes conduction anesthesia very difficult.

Other Short-Stature Syndromes

A number of other chondrodystrophies are compatible with pregnancy. Asphyxiating thoracic dystrophy may be complicated by cystic renal tubular dysplasia or glomerulosclerosis, and careful monitoring of renal status during pregnancy is required. A few women with diastrophic dysplasia have had children without complications. Chondroectodermal dysplasia (Ellis-van Creveld syndrome) is often associated with cardiac defects, and appropriate monitoring and prophylactic antibiotics are required. Affected women with Kniest dysplasia have delivered without complications. Morquio syndrome (spondyloepiphyseal dysplasia congenita) may be associated with retinal detachments, and this should be considered during pregnancy. Odontoid hypoplasia may make neck manipulation during general anesthesia very dangerous in this disorder. Many women with chondrodysplasia punctata (Conradi-Hunermann syndrome) have borne children without incident. Cartilage-hair hypoplasia syndrome may cause such a short trunk that there is severe cardiac and respiratory compromise; periodic pulmonary function tests during pregnancy may be of value.

A few general statements may be made about pregnancy in women with chondrodystrophies: (1) Cesarean section will usually be required for cephalopelvic disproportion, and a trial of labor is often not advisable. (2) General anesthesia must be done with special care because of odontoid hypoplasia with secondary instability of C1 and C2. (3) Subluxation of C1 on C2 can occur during general anesthesia. Unfortunately, conduction anesthesia also may be problematic because of altered vertebral configurations. (4) Cardiorespiratory compromise may occur secondary to a small chest cavity. Despite these caveats, most women with chondrodystrophies do remarkably well during pregnancy. Contraception can be practiced as in normal women.

NEUROLOGIC DISEASES

Myotonic Dystrophy

Myotonic dystrophy is an autosomal dominant trait characterized by slowly progressive neurologic degeneration with secondary muscle wasting, myotonia, cataracts, early balding, hypogonadism, and mental retardation. Affected males often have testicular atrophy, and affected individuals of both sexes appear to have decreased fertility (Bundey & Carter, 1970; Bundey, 1974). Pregnancy causes increased muscle weakness and myotonia in approximately two thirds of affected women, usually in the last half of pregnancy (Hilliard et al., 1977). Most patients improve immediately postpartum, an observation that has led to speculation that progesterone may be involved in the deterioration during pregnancy. Smooth-muscle involvement in myotonia dystrophy is suggested in that almost one half of affected women have uterine inertia (Hilliard et al., 1977). Polyhydramnios occurs in one half of the pregnancies of affected women, and myotonia dystrophy should be considered as a rare cause of recurrent polyhydramnios (Pearse & Howeler, 1979).

Affected children born of affected mothers may have more severe involvement than those born of affected fathers (Harper & Dyken, 1972). This is thought to be due to a maternal intra-uterine environmental factor. Infants not receiving the mutant gene from affected mothers show no symptoms. Affected infants may have joint limitations, hypotonia, and mental retardation. Approximately one half of affected neonates were stillborn or died in the neonatal period from respiratory insufficiency (Hilliard et al., 1977). Affected women should be cared for in high-risk centers, and a neonatologist should be present at the delivery.

Neurofibromatosis

Neurofibromatosis (von Recklinhausen's syndrome) is an autosomal dominant condition characterized by abnormal cutaneous pigmentation and soft tumors of peripheral nerves. Serious complications include scoliosis, pheochromatosis, optic gliomas, malignant degeneration of neural tumors, seizures, and mental retardation. Progression of both pigmentation and neural tumors has been reported in association with pregnancy, with possible regression after delivery (Swapp & Main, 1973; Ansari & Nagamani, 1976). This suggests that oral contraceptives in affected women should be used only with great care and careful observation. There is some evidence that affected infants born of affected mothers are more likely to have severe complications of neurofibromatosis than are infants either born of affected fathers or who represent new mutations (Miller & Hall, 1978).

CARDIOVASCULAR DISORDERS

Congenital heart disease may be the result of multifactorial inheritance (90 percent), chromosomal anomalies (5 percent), single gene defects (3 percent), or environmental factors such as infections or drugs (2 percent) (Nora

& Nora, 1978). Approximately 1 percent of pregnant women with congenital heart disease will not survive the pregnancy (Kahler, 1975), and approximately 1 percent of all maternal deaths are attributable to congenital cardiac defects (Copeland et al., 1963; Hibbard, 1975). A review of 490 patients with congenital heart disease who underwent 1135 pregnancies revealed a 19 percent fetal mortality rate (Kahler, 1975).

Table 2-2
Mendelian Inherited Syndromes Involving the Cardiovascular System*

Autosomal dominant
Craniofacial dostosis
Ehlers-Danlos syndrome
Holt-Oram syndrome
Idiopathic hypertrophic subaortic stenosis (IHSS)
Marfan syndrome
Multiple lentigines syndrome
Neurofibromatosis
Noonan syndrome
Osteogenesis imperfecta
Treacher-Collins syndrome
Tuberous sclerosis
Ventricular fibrillation with prolonged QT intervals
Waardenburg syndrome
Autosomal recessive
Acrocephalopolysyndactaly (Carpenter syndrome)
Cardioauditory syndrome
Chondrodystrophia calcificans congenita (Conradi syndrome)
Chondroectodermal dysplasia (Ellis-van Creveld syndrome)
Cockayne syndrome
Kartagener syndrome
Lawrence-Moon-Biedl syndrome
Limb reduction-ichthyosis syndrome
Meckel-Gruber syndrome
Mucolipidosis III (psuedo-Hurler syndrome)
Mucopolysaccharidosis IV (Morquio syndrome)
Mucopolysaccharidosis VI (Maroteaux-Lamy syndrome)
Pseudothalidomide syndrome (Roberts syndrome)
Pseudoxanthoma elastica
Refsum syndrome
Smith-Lemli-Opitz syndrome
Thrombocytopenia-absent radius (TAR) syndrome
X-Linked
Aase syndrome
Duchenne muscular dystrophy
Ehlers-Danlos syndrome, type V
Focal dermal hypoplasia (Goltz syndrome)
Mucopolysaccharidosis II (Hunter syndrome)

*See McKusick (1978) for details.

Most single gene mutations that cause a cardiac defect are pleomorphic and cause recognizable syndromes. The Mendelian disorders in which a cardiac defect is part of a syndrome are listed in Table 2-2. Only a few Mendelian disorders limited to the heart are known. Included are both autosomal recessive and autosomal dominant conduction system abnormalities, autosomal recessive hypoplastic left heart syndrome, and X-linked mitral and aortic insufficiency. The most common disorder is idiopathic hypertrophic subaortic stenosis (IHSS), which is autosomal dominant. Although the symptoms of patients with IHSS tend to worsen during pregnancy due to increased blood flow, gestation can be managed successfully (Turner, Oakley, & Dixon, 1968; Kolibash, Ruiz, & Lewis, 1975; Datta et al., 1978). The fetal risks involve not only IHSS but also intra-uterine growth retardation and neonatal bradycardia and hypoglycemia secondary to propranolol, which is often administered to the mother.

Attempts at prenatal diagnosis of congenital heart defects are only now beginning. A few instances of congenital heart block have been diagnosed (Patel & Goldberg, 1976). Attention has turned now to utilizing ultrasonography to visualize fetal cardiac structures in utero (Hobbins et al., 1979). A group at the University of California at San Francisco (Golbus, unpublished data) employed a Toshiba sector scanner to videotape the hearts of eight fetuses at high risk of cardiac defects. Results thus far include prenatal diagnosis of a ventricular septal defect in a trisomy 18 fetus, verified after termination of pregnancy; diagnosis of a ventricular septal defect in a trisomy 9 fetus verified after abortion, although coexisting pulmonary atresia was not detected; one study considered normal although the fetus had a ventricular septal defect and bicuspid pulmonic and aortic valves; one study considered normal with the fetus having a bicuspid aortic valve; and four normal studies that were confirmed after delivery or abortion. These techniques must be considered experimental and, hopefully, will be improved with experience.

RESPIRATORY DISORDERS

Cystic Fibrosis

Cystic fibrosis, an autosomal recessive condition, is the most common lethal genetic disease of Caucasians, with an incidence in the United States of approximately 1 in every 2000 newborns. The basic genetic defect is unknown, although numerous hypotheses have been advanced (Nadler et al., 1978). The major respiratory problems are mucous obstruction of the airways and secondary bacterial infection. Pulmonary function progressively deteriorates, and 60 percent of the affected individuals die before they reach 10 years of age (Bearn & Danes, 1978). Other manifestations include pancreatic insufficiency, neonatal meconium ileus, hepatic cirrhosis, cholelithiasis, salivary gland obstruction, and an elevated concentration of sweat sodium and chloride.

A national survey of cystic fibrosis centers reported the outcome of 70 pregnancies in women with cystic fibrosis (Cystic Fibrosis "GAP" Conference Reports, 1975). Maternal morbidity included worsening pulmonary symptoms in 60 percent, cor pulmonale in 14 percent, and the onset of hyperglycemia in

3 percent. Eight women died. Details of pulmonary function prior to conception were reported for 18 women. Six women became pregnant in the presence of a vital capacity known to be less than 70 percent of normal ("severely impaired"). Of these, 2 women developed pulmonary hypertension, 2 developed diabetes, and 2 had fetuses that died perinatally. Five of these women died less than 28 months after pregnancy. In contrast, 12 gestations occurred in the presence of a vital capacity known to be 70 to 100 percent of normal; of these, 1 woman developed mild pulmonary hypertension and 1 developed diabetes; there were no fetal losses or maternal deaths. Thus, preconceptual vital capacity may be the best indicator of maternal complications during pregnancy.

Alpha-1-Antitrypsin Deficiency

Alpha-1-antitrypsin (AAT) deficiency is inherited as an autosomal recessive trait. This ubiquitous protein, whose synthesis is dictated by a series of codominant alleles, inhibits a wide spectrum of proteolytic enzymes. Deficiency of this protein is associated with 30-fold increased risk of chronic obstructive pulmonary disease as a young adult (Kueppers, 1978) and a 12 percent greater risk of neonatal hepatitis and cirrhosis (Sveger, 1976). Giesler, Buehler, and Depp (1977) reported the delivery of a fetus with retarded intra-uterine growth to a woman with AAT deficiency and obstructive pulmonary disease, but there is insufficient information to make any general statements about the obstetric implications of the disease.

3

Autosomal Chromosome Abnormalities

Approximately 1 of every 160 liveborn infants has a demonstrable chromosomal abnormality (Table 3-1) (Jacobs et al., 1974; Hamerton et al., 1975; Hook & Hamerton, 1977; Hirgurashi et al., 1979). At least half lead to conditions requiring medical intervention. Chromosomal abnormalities are also associated with 50 to 60 percent of first trimester spontaneous abortuses and 5 percent of stillbirths (see Chapter 7). Moreover, frequencies of autosomal aberrations detected by amniocentesis are even higher for any given maternal age group than the frequencies found in newborn studies (Hook, 1978). This difference results from many of these abnormal fetuses being "lost" as late abortions or stillbirths.

In this chapter we shall consider the spectrum of autosomal abnormalities in live-born infants, as well as diagnosis, prognosis, and genetic counseling. Other aspects of cytogenetics are discussed elsewhere in this volume (see Chapters 1, 6, 7, and 10).

GENERAL COMMENTS

Diagnosis of chromosomal syndromes solely on the basis of clinical characteristics is possible but not always reliable. Among the reasons for this are the following: (1) No single anomaly is pathognomonic for a given syndrome. (2) Patterns of anomalies for different chromosomal syndromes share common features. (3) Phenotypic variation exists among individuals with apparently identical abnormal karyotypes. (4) Malformations associated with chromosomal aberrations can also be associated with normal karyotypes (etiologic heterogeneity). Nonetheless, some relatively common chromosomal syndromes can be delineated. Irrespective of whether associated with duplication or deficiency of genetic information, chromosomal syndromes are usually characterized by multiple malformations. Among the deleterious effects consistently associated with duplication or deficiency of genetic material are mental retardation, intrauterine and postnatal growth retardation, and anomalies of many organ systems,

Table 3-1
Frequency of Chromosome Aberrations in Newborns*

Aberration	Incidence	
Numerical		
Sex chromosomes		
47,XYY	1/1,000	MB
47,XXY	1/1,000	MB
Other (males)	1/1,350	MB
45,X	1/10,000	FB
47,XXX	1/1,000	FB
Other (females)	1/2,700	FB
Autosomes		
Trisomies**		
Nos. 13–15 (Group D)	1/20,000	LB
Nos. 16–18 (Group E)	1/8,000	LB
Nos. 21–22 (Group G)	1/800	LB
Other	1/50,000	LB
Structural		
Balanced		
Robertsonian		
t(Dq;Dq)	1/1,500	LB
t(Dq;Gq)	1/5,000	LB
Reciprocol translocations and insertional inversions	1/7,000	LB
Unbalanced		
Robertsonian	1/14,000	LB
Reciprocal and insertional	1/8,000	LB
Inversions	1/50,000	LB
Deletions	1/10,000	LB
Supernumeraries	1/5,000	LB
Other	1/8,000	LB
Total	1/160	LB

LB = Live births, MB = male births, FB = female births.
*Modified from a summary of 6 surveys (Hook & Hamerton, 1977) including 56,952 newborns.
**Because most surveys did not employ banding techniques, individual chromosomes within a group could not always be differentiated. However, Group D trisomies are generally No. 13, group E No. 18, and group G No. 21.

especially the craniofacial, skeletal, cardiac, and genitourinary systems. In particular, abnormal facies, low-set or malformed ears, and certain digital anomalies (e.g., clinodactyly, polydactyly, syndactyly, and single palmar creases) suggest autosomal imbalance, particularly if part of a spectrum of anomalies. There is an increased frequency of antepartum loss, and reduced life expectancy is to be expected among liveborns. However, survival to adulthood is com-

patible with some abnormal chromosomal complements, and medical advances continue to improve life expectancy.

Genetic counseling depends on whether the chromosomal aberration is numerical or structural. Factors that increase the risk of nondisjunction in humans are (1) increased parental ages, (2) parental mosaicism or aneuploidy, and (3) a previous trisomic offspring. All these conditions constitute indications for antenatal chromosomal studies. Sporadic (de novo) structural rearrangements are believed to have a low risk of recurrence. If a familial structural rearrangement exists, however, the risk of having offspring with chromosomal imbalance is increased. The magnitude varies according to the specific rearrangement, but the risk is usually 2 to 10 percent.

Trisomies 13, 18, and 21 were the first autosomal syndromes described. As laboratory techniques became more sophisticated, many other new chromosomal syndromes were confirmed, and doubtless still more will emerge. In this chapter we shall outline the major features of the most common and well-established syndromes, as well as a few of the rare, recently delineated ones. More complete descriptions appear in other publications (Hamerton, 1971; de Grouchy & Turleau, 1977; Yunis, 1977; Bergsma, 1979).

NUMERICAL ABERRATIONS: TRISOMIES

Trisomy 21

Phenotype

The incidence of trisomy 21 (Down Syndrome, mongolism) the most frequent autosomal chromosomal syndrome, is 1 of every 800 liveborn infants (Hook & Hamerton, 1977). The disorder was a recognizable clinical syndrome (Fig 3-1) long before its chromosomal etiology was elucidated. One of the earliest descriptions was provided in 1866 by Langdon Down, who referred to affected individuals as having "mongolism" because associated facial features were evocative of Oriental faces. The use of the term "mongolism" is now discouraged. Lejeune, Gautier, and Turpin (1959) first observed an additional group G chromosome (G = Nos. 21–22) in affected patients, hence the older term "trisomy G" syndrome. The additional chromosome has now been defined as No. 21 by higher-resolution staining techniques. As seen in Figure 3-1, characteristic craniofacial features include brachycephaly, oblique palpebral fissures, epicanthal folds, broad nasal bridge, a protruding tongue, and small, low-set ears with an overlapping helix and a prominent antihelix. At birth, infants are usually hypotonic, but birth weight is not reduced as much as in some autosomal syndromes. Other diagnostically helpful features are iridial Brushfield spots; broad short fingers (brachymesophalangia); clinodactyly (incurving deflections resulting from an abnormality of the middle phalanx); a single flexion crease on the fifth digits; and an unusually wide space between the first two toes. Contrary to widespread opinion, a single palmar crease (Simian line) is not pathognumonic, being present in only 30 percent of individuals

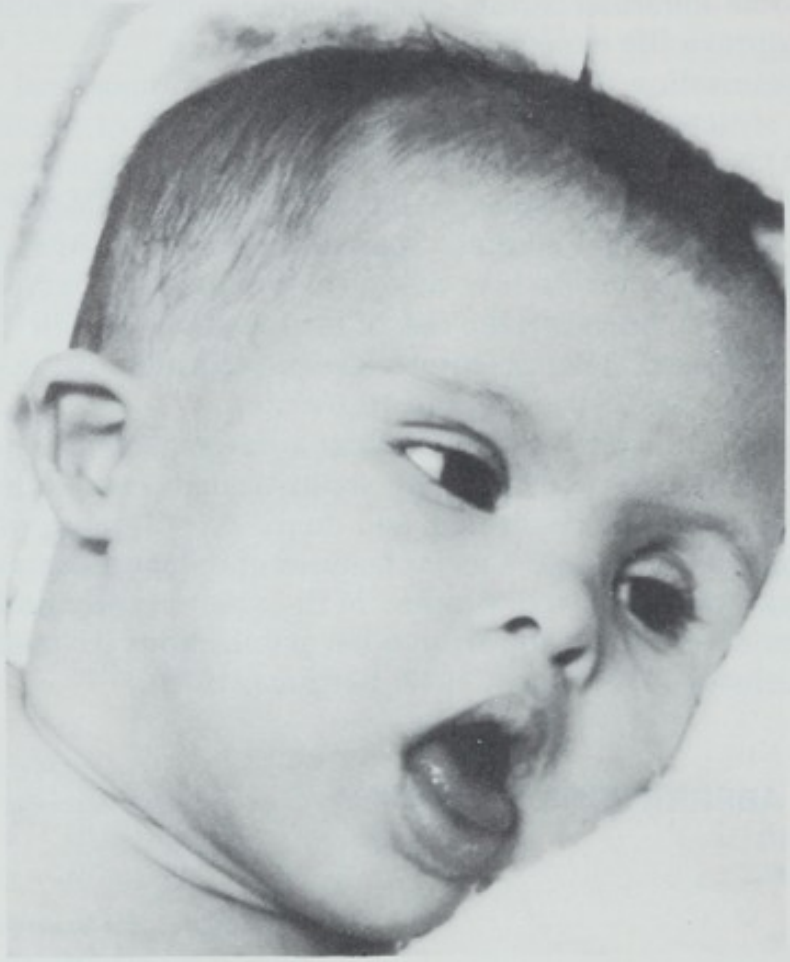


Figure 3-1. An infant with Down syndrome.

with trisomy 21 and in 5 percent of normal individuals. Relatively common internal anomalies include cardiac lesions and duodenal atresia. Cardiac anomalies, increased suceptibility to both respiratory infections, and leukemia all contribute to reduced life expectancy. In the past, approximately 20 to 30 percent of infants with trisomy 21 died during their first year of life, 50 percent were dead by age 5, and less than 3 percent survived past age 50. Survival has increased greatly as result of advances made in the treatment of infections, leukemia, and heart disease. At present, mean survival is approximately 20 years (de Grouchy & Turleau, 1977).

Patients with Down syndrome who survive beyond infancy invariably show mental retardation; however, the degree of retardation is variable and generally not so severe as that of many other chromosomal aberrations. Mean IQ is 50 (range 25–70). Some patients even have been said to have an IQ in the 70–80 range, but in such instances 46/47, + 21 mosaicism should be suspected. Growth retardation and hypotonia persist, and older patients often become obese. Females are fertile but have a high risk of producing chromosomally abnormal offspring. Although few have reproduced, 30 percent of the offspring of trisomic mothers are also trisomic (Scharrer et al., 1975; Simpson, 1981a; Van de Velde-Staquet et al., 1973). Affected males have not yet been proven fertile.

Because Down syndrome has been extensively studied, many anomalies other than those discussed here have been described (Penrose & Smith, 1966; Benda, 1969; Johnson & Abelson, 1969; Smith & Berg, 1976). Our brief description emphasized only those anomalies most useful for clinical diagnosis and important for counseling.

Genetic Counseling

It is essential to obtain cytogenetic data on individuals clinically suspected to have trisomy 21. Cytogenetic studies not only confirm the diagnosis but also provide the basis for genetic counseling. Not only are several different mechanisms possible for triplication of No. 21, but other etiologies may be associated with "clinical Down syndrome," e.g., polysomy X (Carpenter, 1979; Gardner, 1979).

Down syndrome is the result of triplication of a small portion of the No. 21 chromosome, namely band q22 (Niebuhr, 1974a; de Grouchy & Turleau, 1977). This triplication may be caused either by the presence of an entire additional No. 21 (primary or nondisjunctional trisomy) (Fig 3-2) or by the

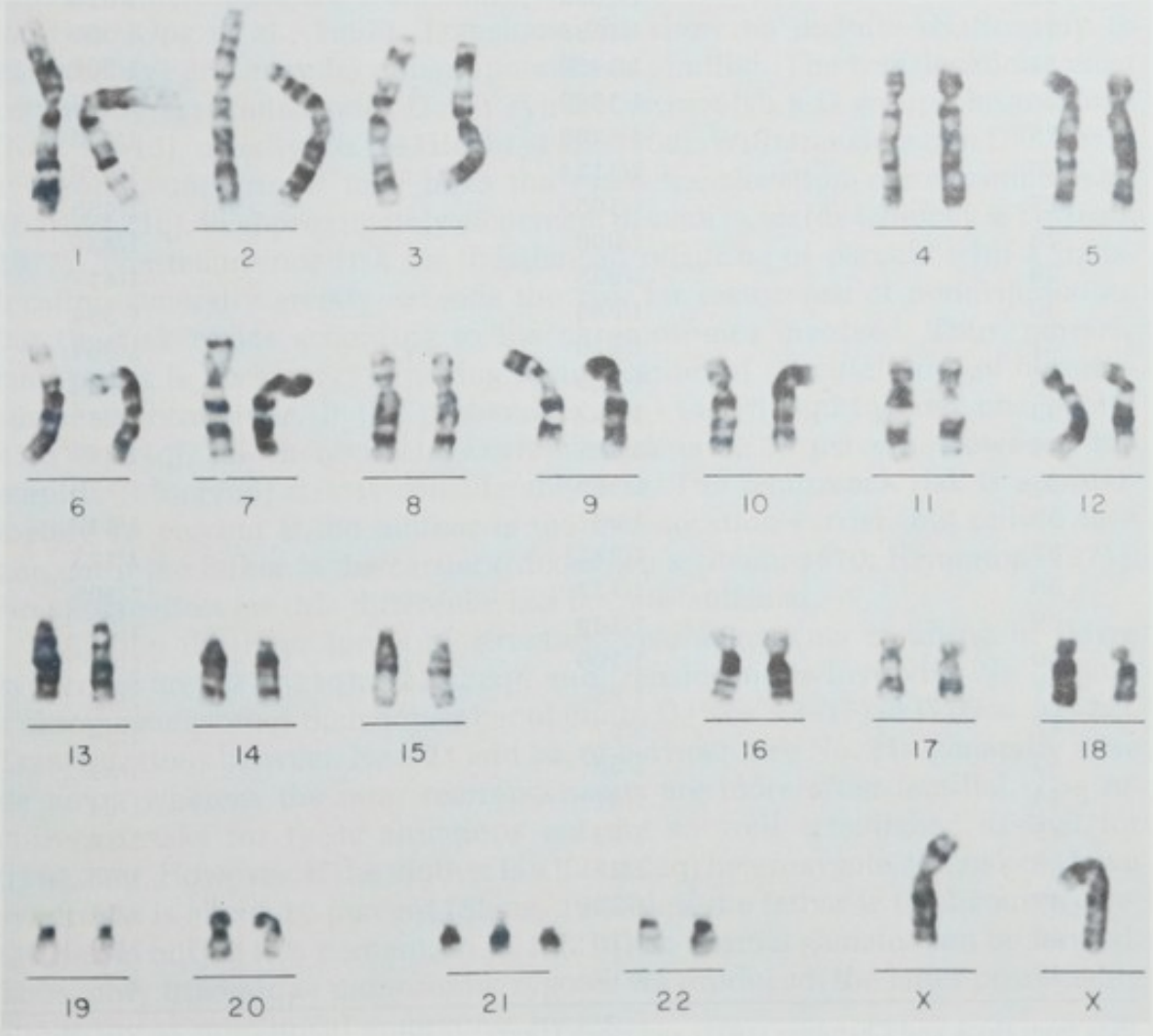


Figure 3-2. A trypsin-Giemsa (GTG)-banded karyotype with the complement 47,XX,+21 (primary, nondisjunctional trisomy 21).

addition of only band q22, which could occur by translocation or other structural rearrangement. Of all cases of Down syndrome, 93 to 95 percent have primary trisomy (47 in lieu of the normal 46 chromosomes). These cases are generally sporadic and show a well-known relationship to maternal age (Table 3-2). In addition, Stene (1970a) and Mikkelson and Stene (1970) estimated an approximate 1 percent recurrence for women under 30 years of age when their trisomic child was born, but for women older when the trisomic child was born no detectable increase over that associated with age per se. This has recently been confirmed with antenatal cytogenetic data (see Simpson, 1980c). The chromosomal complements of parents of affected individuals is almost always normal. However, occasionally parental mosaicism (46/47, +21) (Weinstein & Warkany, 1963; Kaffe, Hsu, & Hirschhorn, 1974; see also Milunsky, 1979) or

Table 3-2
Risk of Having a Live-Born Child with Chromosomal Abnormalities

Maternal Age	Down Syndrome	All Abnormalities Except 47,XXX
20	1/1923	1/526
21	1/1695	1/526
22	1/1538	1/500
23	1/1408	1/500
24	1/1299	1/476
25	1/1205	1/476
26	1/1124	1/478
27	1/1053	1/455
28	1/990	1/435
29	1/935	1/417
30	1/885	1/384
31	1/826	1/384
32	1/725	1/322
33	1/592	1/285
34	1/465	1/243
35	1/365	1/178
36	1/287	1/149
37	1/225	1/123
38	1/177	1/105
39	1/139	1/80
40	1/109	1/63
41	1/85	1/48
42	1/67	1/39
43	1/53	1/31
44	1/41	1/24
45	1/32	1/18
46	1/25	1/15
47	1/20	1/11
48	1/16	1/8
49	1/12	1/7

Data from Hook and Chambers (1977) and Hook (1981).
Because sample size for some intervals is relatively small, 95-percent confidence limits are sometimes relatively large. Nonetheless, these figures are suitable for genetic counseling.

another aberration may be detected. In both instances risk is presumed to be higher than for the appropriate parental age, but precise figures are not available.

Prenatal diagnosis for nonmosaic trisomy is extremely reliable and should be offered all pregnant women aged 35 or older (see Chapter 6) as well as to women who have had a previous trisomic offspring. A relationship between advanced paternal age and nonmosaic, nondisjunctional trisomy 21 has been found by some (Stene et al., 1977) but not by others (Erickson, 1978). Positive association would not be surprising, because in at least 20 percent of cases, two of the three No. 21 chromosomes are paternal in origin (Bott, Sekhon, & Lubs, 1975; Hara & Sasaki, 1975; Mattei, 1979). However, a paternal effect does not occur until the fifth or sixth decade, and even then only doubles the maternal age risk.

Mosaicism (46/47, +21) is detected in 2 to 3 percent of individuals with Down syndrome (Chitham & MacIver, 1965; Richards, 1969, 1974; Sutherland & Weiner, 1972). Mosaicism may confer fewer phenotypic abnormalities than nonmosaic trisomy, but this cannot be predicted in individual cases (Kohn et al., 1970). Approximately 3 to 5 percent of cases of Down syndrome result from translocations. Patients who have Down syndrome due to aneuploidy (47, +21) cannot be distinguished from those who have the disorder as result of translocation (Ong et al., 1967). Translocations show no definite relationship to parental age and may be either sporadic or familial. The translocations most commonly associated with Down syndrome involve a D group chromosome (Nos. 13–15), usually No. 14 (Hecht et al., 1968). With translocation D/G Down syndrome, one parent may have the same translocation chromosome, e.g., 45,t(14q;21q), in approximately 45 percent of such cases (de Grouchy & Turleau, 1977). The recurrence risk for unbalanced offspring of parents with a translocation generally greatly exceeds the risk for recurrence of nondisjunction, but the risk varies according to the chromosomes involved. Thus, empiric counseling is necessary, requiring identification of specific types of chromosomal abnormalities. If the proband is 46, −14, +t(14q;21q) and one parent 45,t(14q;21q), the theoretical recurrence risk is 33.33 percent. However, the empiric (observed) risk is considerably less. The recurrence risk is approximately 10 percent if the mother is the translocation carrier, but only 2 to 3 percent if the father is the carrier (Mikkelsen & Stene, 1970; Hamerton, 1971). No explanation for this difference has been established.

Among the rarer forms of structural rearrangements resulting in Down syndrome are t(21q;21q), t(21q;22q), and translocations involving No. 21 and a chromosome other than a member of group D (Nos. 13–15) or G (Nos. 21–22). Translocations between Nos. 21 and 22, or between two No. 21s, generally arise de novo, whereas the rarer rearrangements are more often familial. The recurrence risks for these situations are not so well established as that for t(14q;21q). However, if the mother is a t(21q;22q) heterozygote, the risk of Down syndrome is about 10 percent (Stene, 1970b). If the father is the heterozygote, the risk is only 2 to 5 percent. In t(21q;21q) no normal gametes can be formed. Thus, only trisomic or monosomic zygotes are produced, the latter presumably appearing as preclinical embryonic losses.

Occasionally, individuals with trisomy 21 will also be trisomic for another chromosome, usually an X (e.g., 48,XXY, +21).

Table 3-3
Major Features of Autosomal Syndromes

Chromosome Aberration	Incidence (Live Births)	Growth Retardation	Mental Retardation	Craniofacial Anomalies
Trisomy 21	1/800	• Mean birth weight - 2900 g* • Adult height below average (154 cm males; 144 cm females)*	Mean IQ = 50 (range 25–70)	• Brachycephaly • Flat occiput • Low-set ears with angular overlapping helix and prominent antihelix • Oblique palpebral fissures • Epicanthal folds • Brushfield spots (irises) • Broad nasal bones • Flattened profile due to hypoplasia of the nasal bones • Open mouth with protruding tongue (macroglossia)
Trisomy 13	1/20,000	• Mean birth weight - 2600 g* • Postnatal retardation	Severe**	• Holoprosencephaly • Microcephaly • Scalp defects, hemangioma • Low-set ears, abnormal (flat, poorly defined) helix • Deafness • Micropthalmia, anopthalmia, coloboma • Cleft lip, palate • Micrognathia
Trisomy 18	1/8000	• Mean birth weight - 2240 g* • Postnatal retardation	Severe**	• Doliocephaly • Prominent occiput • Malformed (low-set, "fawnlike") pinna • Slender, upturned nose • Micrognathia • Small mandible
Trisomy 8	Rare (not detected among live-born surveys)	• Normal birth weight and adult height	Mean IQ = 50	• Elongated facies, nearly normal in appearance • Large, low-set ears • Thick, everted lower lip

*Data from de Grouchy J, Turleau C (1977): Clinical Atlas of Human Chromosomes. New York, Wiley & Sons.
**Because of the early death associated with this syndrome, actual IQ values are rarely obtainable.

Skeletal Anomalies	Internal Anomalies	Other Features	Life Expectancy
• Broad, short fingers (brachymesophalangia) • Clinodactyly of 5th finger	• Cardiac (ventricular septal defect, atrial septal defects, patent ductus arteriosis, endocardial cushion defects)	• Hypotonia • Skin folds on nape of neck • Increased susceptibility to respiratory infections • Axial triradius • Acute leukemia	20–30% die in 1st year, 50% die in 1st five years, 2.6% live beyond age 50*
• Polydactyly • Hypoplastic fingernails • Overlapping, flexed fingers • Hypoplastic or absent ribs • Hypoplastic pelvis, flattened acetabular angle • "Rockerbottom" feet	• Cardiac (ventricular septal defect, patent ductus arteriosis, atrial septal defects) • Genital (cryptorchidism, bicornuate uterus) • Urinary (polycystic kidneys, hydronephrosis, ureteral duplication, renal fusion)	• Single umbilical artery • Apneic spells, seizures • Single transverse palmar crease	45% die in the 1st month, 70% die by the 6th month, less than 5% survive 3 years (Magenis et al., 1968)
• Overlapping fingers, clenched fist • Flexion deformities (ulnar deviation) • Short sternum • Limited hip abduction • Narrow pelvis • Calcaneovalgus • Short, dorsiflexed hallux • "Rockerbottom" feet	• Cardiac (ventricular septal defect, patent ductus arteriosis, atrial septal defects) • Genital (cryptorchidism) • Urinary (ectopic or "horseshoe" kidney, hydronephrosis, double ureter or megaloureter)	• Hypertonia • Females affected more often than males (3:1)	30% die in the 1st month, 50% die in the 2nd month, less than 10% survive 1 year (Gorlin, 1977)
• Short, wide neck • Narrow shoulder • Long trunk • Dorsolumbar kyphoscoliosis • Abnormal or supernumerary vertebrae • Spina bifida • Supernumerary ribs • Hypoplastic and narrow pelvis • Brachydactyly or arachnodactyly • Absent or hypoplastic patellae • Clubfoot • Hallux valgus • Osteoarticular lesions	• Genital (cryptorchidism, testicular hypoplasia)	• Deep palmar and plantar flexion creases • Hypoplastic, convex nails	Normal*

Table 3-3 (continued)

Chromosome Aberration	Incidence (Live Births)	Growth Retardation	Mental Retardation	Craniofacial Anomalies
Trisomy 22	Rare (not detected among live-born surveys)	• Mean birth weight - 2650 g* • Unable to sit up or walk	Mean IQ = 20	• Microcephaly • Large, posteriorly rotated ears • Preauricular tags or sinuses • Antimongoloid slanting of the palpebral fissures • Long beaked nose • Long philtrum • Cleft or high-arched palate • Micrognathia
Trisomy 14	Rare (not detected among live-born surveys)	• Severe	Severe**	• Microcephaly • Low-set ears • Wide, flat nose with bulbous or wide tip • Highly arched or cleft palate • Large mouth with turned-down corners • Micrognathia
Trisomy 9	Rare (not detected among live-born surveys)	• Mean birth weight - 2630 g*	Severe**	• Microcephaly • Doliocephaly • Low-set, round, soft ears • Deep-set eyes • Downward-slanting, narrow, palpebral fissures • Wide, bulbous nose • Overlapping upper lip • Micrognathia
Dup(9p)	Rare (about 60 cases reported)	• Mean birth weight - 2900 g*	Mean IQ = 55	• Microcephaly • Brachycephaly • Large, protruding ears • Small, deep-set eyes • Eccentric pupils • Upward-slanting, oblique, palpebral fissures • Bulbous nose • Everted lip • Downward-turned mouth • Unilateral "grin" • "Worried" look
Triploidy	Frequent in spontaneous abortions, rare in live-borns	• Mean birth weight - 2500g (Wertelecki et al., 1976)	Severe**	• Cranial bone dysplasia • Low-set, malformed ears • Microphthalmia, colobomata • Mild hypertelorism • Cleft lip or palate • Macroglossia • Small mandible
Del(4p) syndrome (Wolf-Hirschhorn syndrome)	Rare (about 50 cases reported)	• Mean birth weight - 2000 g*	Mean IQ = 20	• Microcephaly • Prominent glabella • Low-set, simple ears • Preauricular dimple or sinus • Ocular hypertelorism • Cleft lip or palate • Micrognathia

*Data from de Grouchy J, Turleau C (1977): Clinical Atlas of Human Chromosomes, New York, John Wiley & Sons.
**Because of the early death associated with this syndrome, actual IQ values are rarely obtainable.

Skeletal Anomalies	Internal Anomalies	Other Features	Life Expectancy
• Dislocated hip • Fingerlike, malopposed thumb, long slender fingers	• Cardiac (patent ductus arteriosis, pulmonary stenosis, coarctation aorta, abnormal subclavicular artery) • Cryptorchidism	• Hypotonia • Amyotrophy • Seizures, neonatal	33% die within 1st year. Survival at least until age 12 has been reported (Hsu and Hirschhorn, 1977)
• Short neck • Digital contractions and deviations			Insufficient data
• Hip dislocation or limited abduction • Dislocations of elbows, knees • Spinal column and costal anomalies	• Cardiac (patent ductus arteriosis, ventricular septal defect) • Genital (cryptorchidism, micropenis)		Few cases survive; most die within a few months (Rethoré, 1977)
• Brachymesophalangy • Long palms • Clinodactyly		• Single palmar crease	Normal (Rethoré, 1977)
• Syndactyly	• Cardiac (ventricular septal defects, atrial septal defects) • Omphalocele • Meningomyelocele • Genital (small penis, hypospadias, cryptorchidism) • Hepatosplenomegaly	• Hypotonia • Hydatidiform degeneration of large placenta	Short, but a few older children with mosaicism
• Scoliosis	• Cardiac (atrial septal defect, ventricular septal defect, patent ductus arteriosis) • Genital (cryptorchidism, hypospadias, absent uterus, streak gonads)	• Hypotonia • Seizures	33% die within first 2 years, but survival into 2nd decade reported (Warburton, 1973)

Table 3-3 (continued)

Chromosome Aberration	Incidence (Live Births)	Growth Retardation	Mental Retardation	Craniofacial Anomalies
Del(5p) syndrome (cri-du-chat syndrome)	1/20,000	• Mean birth weight - 2650 g*	Mean IQ = 20	• Microcephaly • Hypertelorism • Epicanthal folds • Wide, flat, nasal bridge • Micrognathia
Del(13q) syndrome	Rare (about 70 cases reported)	• Mean birth weight - 2250 g*	Mean IQ = 50	• Microcephaly (often severe) • Holoprosencephaly • Large ears, deep helix • Retinoblastoma • Micropthalmia • Hypertelorism • Absence of well-defined nasal bridge • Broad, protruberant nose
Del(18p) syndrome	Rare (about 85 cases reported)	• Mean birth weight - 2800 g*	Mean IQ = 50 (range 25–75)	• Holoprosencephaly • Microcephaly • Low-set, large, floppy ears • Hypertelorism • Epicanthal folds • Strabismus • Ptosis
Del(18q) syndrome	Rare (about 95 cases reported)	• Mean birth weight - 2940 g*	Mean IQ = 50	• Microcephaly • Midfacial hypoplasia • Deep-set eyes • Visual defects (glaucoma, strabismus, nystagmus, optic atrophy) • Atretic external auditory canal • "Carp-shaped" mouth • Cleft lip or palate
Del(21q) syndrome	Rare (about 85 cases reported)		Severe**	• Microcephaly • Large, low-set, abnormally formed ears • Downward-slanting palpebral fissures • Blepharocholosis • High-arched or cleft palate, cleft lip • Micrognathia

*Data from de Grouchy J, Turleau C (1977): Clinical Atlas of Human Chromosomes. New York, Wiley & Sons.
**Because of the early death associated with this syndrome, actual IQ values are rarely obtainable.

Trisomy 13

Trisomy 13 was one of the first chromosomal syndromes to be described (Patau et al., 1960; Smith, Patau, & Therman, 1961). Synonyms include D trisomy, D_1 trisomy, and Patau's syndrome. Although rare, trisomy 13 is still among the more common chromosomal abnormalities in liveborns (1 of every 20,000 live births). It is also detected relatively frequently in spontaneous

Skeletal Anomalies	Internal Anomalies	Other Features	Life Expectancy
		• Hypotonia at birth • Hypertonia with increasing age	Normal*
• Thumb aplasia • Agenesis of first metacarpal • Fusion of fourth and fifth metacarpals • Malformed feet • Syndactyly	• Cardiac (ventricular septal defect, atrial septal defect) • Genital (ambiguous, cryptorchidism, hypospadias, epispadias) • Hypoplastic kidney • Anal atresia		20% die prior to 6 months of age (Niebuhr, 1977)
		• Hypotonia	Normal (Miller, 1979)
• Dimples on patellae (lateral epitrochlea), back of hands • Supernumerary ribs • Long, tapered fingers • Talipes equinovarus	• Cardiac anomalies • Hypoplastic external genitalia	• Hypotonia • Seizures	10% die within a few months, but several have reached adolescence*
		• Hypotonia	Insufficient data

abortuses. In this syndrome, intra-uterine and postnatal growth retardation are pronounced, and developmental retardation is severe. Nearly 50 percent of affected children die in the first month, and fewer than 5 percent survive past 3 years of age. (Magenis, Hecht, & Mulham, 1968).

Anomalies characteristic of trisomy 13 include holoprosencephaly, eye anomalies (microphthalmia, anopthalmia, or coloboma), cleft lip and palate, polydactyly, and cardiac defects (Fig. 3-3, Table 3-3). Other relatively common

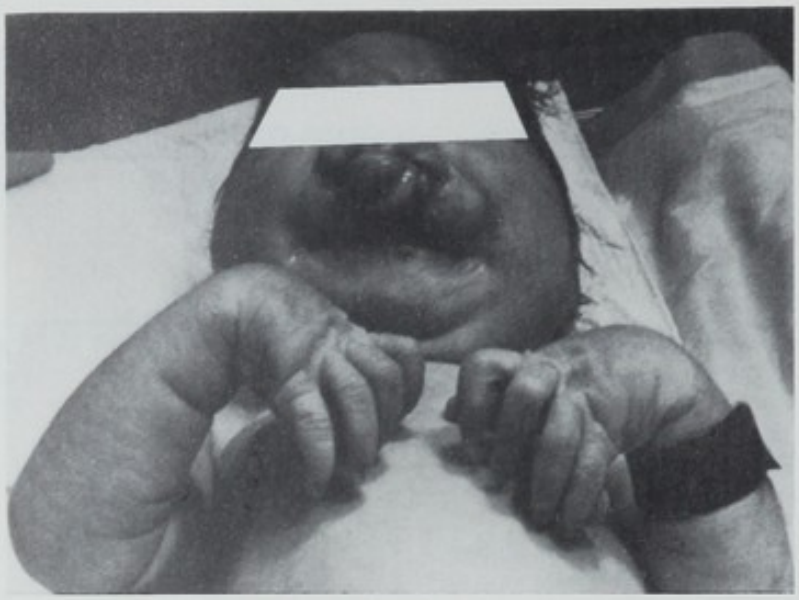


Figure 3-3. An infant with trisomy 13.

features include cutaneous scalp defects, hemangiomata on the face and forehead or neck, low-set ears with abnormal helix, and "rockerbottom" feet (convex soles and protruding heels). No anomaly is unique to trisomy 13, but in aggregate the aforementioned anomalies suggest the diagnosis. However, cytogenetic analysis is necessary to confirm the diagnosis and ensure accurate counseling.

Genetic Counseling

Trisomy 13 is usually (in 80 percent of cases) associated with nondisjunctional (primary) trisomy (47, +13) (Fig 3-4). As in trisomy 21, maternal age and previous offspring are major factors in determining risk. Counseling is similar to that offered following nonmosaic trisomy 21.

Translocation, which occurs in most of the remaining 20 percent of cases, usually involves two group D (Nos. 13–15) chromosomes and is another example of translocation resulting from two acrocentric chromosomes joined at their centromeric regions. Other rearrangements associated with the trisomy 13 syndrome include rings and inversions. In these instances, only a portion of No. 13 may be present in triplicate. Not surprisingly, the phenotypic anomalies may differ from those usually associated with typical trisomy 13, but features are generally evocative of part of the spectrum associated with complete trisomy (Moore & Engel, 1970; Taylor et al., 1970). Life expectancy may be greater then for typical trisomy 13 (Niebuhr, 1977). Only rarely does trisomy 13 syndrome occur as result of familial translocations, but these should nonetheless be excluded. If neither parent has a rearrangement, the recurrence risk for subsequent progeny approaches zero. Nonetheless, it is prudent to offer prenatal diagnosis to families with apparently de novo rearrangements as well as to those few with inherited ones. If either parent has a t(13q;14q), the recurrence risk of an affected offspring increases, although probably less so than for Down syndrome among carriers of t(14q;21q).

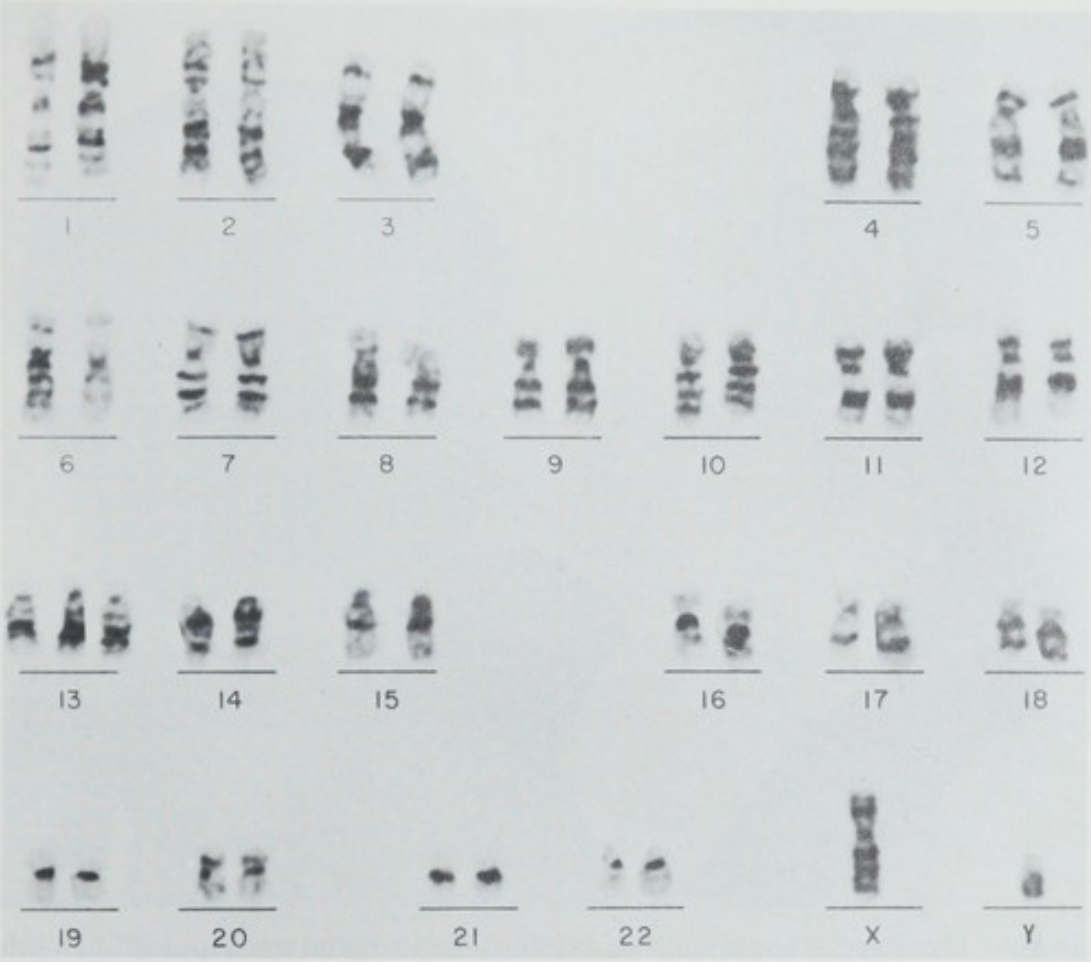


Figure 3-4. A trypsin-Giemsa (GTG) banded karyotype with the complement 47,XY,+13 (primary, nondisjunctional trisomy 13).

Infants with clinical trisomy 13 have been observed to have 46/47+13 mosaicism. A better prognosis has been claimed for mosaic cases (Fryns, Casaer, & Van Den Berghe, 1979), and this indeed appears to be true, particularly if the predominant cell line is normal. However, not only is mosaicism difficult to quantitate, but usually only one tissue is analyzed; thus, attempts to correlate severity of anomalies with presence or degree of mosaicism are hazardous.

Trisomy 18

First detected by Edwards et al. (1960), this disorder occurs with a frequency of 1/8000 live births. Trisomy 18 is also the most frequent chromosomal aberration detected among stillborn, infants, who interestingly usually are not clinically suspected of being trisomic (Bauld, Sutherland, & Bain, 1974; Machin, 1974). Among live-born infants, females are affected more often than males (3:1), but the ratios are more equal among stillborns and abortuses.

Anomalies characteristic of trisomy 18 (Table 3-3, Fig. 3-5) include microcephaly; prominent occiput; low-set, abnormal, somewhat pointed ("fawn-like") ears; and micrognathia. There are also skeletal anomalies, including

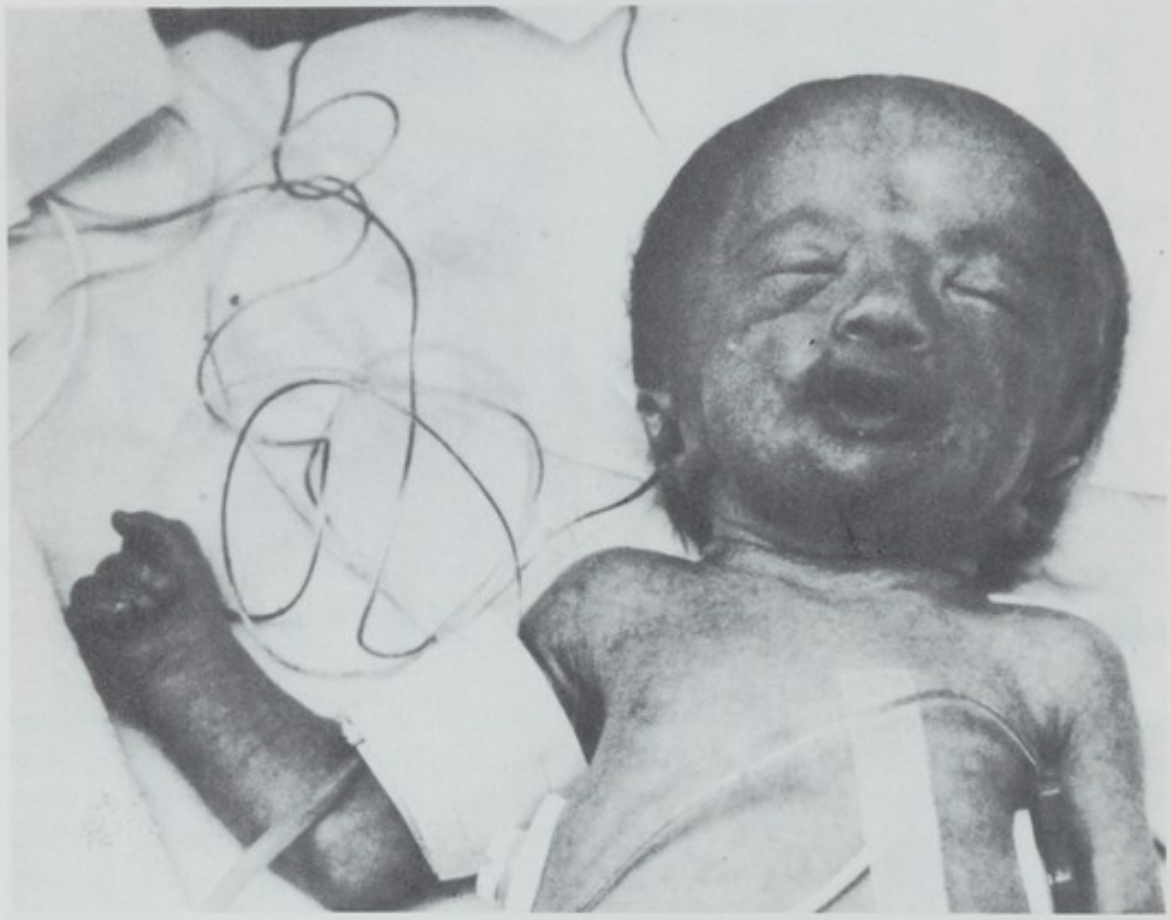


Figure 3-5. Craniofacial appearance and characteristic digital overlapping in an infant with trisomy 18.

overlapping fingers (V over IV, II over III), short sternum, shield chest, narrow pelvis, limited thigh abduction or congenital hip dislocation, and "rocker-bottom" feet with protrusion of the calcaneum. In addition, the great hallux is often short and dorsiflexed ("hammer toe"). Cardiac and renal anomalies are also common.

Although these infants vary considerably in appearance, the skull shape, facies, and skeletal anomalies of feet and hands generally are similar (Fig. 3-5). As in many other autosomal imbalance syndromes, birth weight is below average. Postmaturity is not rare. At birth, fetal movement is feeble, and the mean survival time is only a few months (Weber, 1967). Those surviving show pronounced developmental and growth retardation (Smith, 1978). Fewer than 10 percent survive long enough for IQ evaluation, but neurologic findings suggest severe retardation.

Genetic Counseling

Approximately 80 percent of cases of trisomy 18 syndrome are caused by primary nondisjunction (47,XX,+18 or 47,XY,+18) (Fig. 3-6) (Gorlin, 1977). In such cases, genetic counseling should be analogous to that offered following trisomy 21. The recurrence risk is about 1 percent due to previous trisomy,

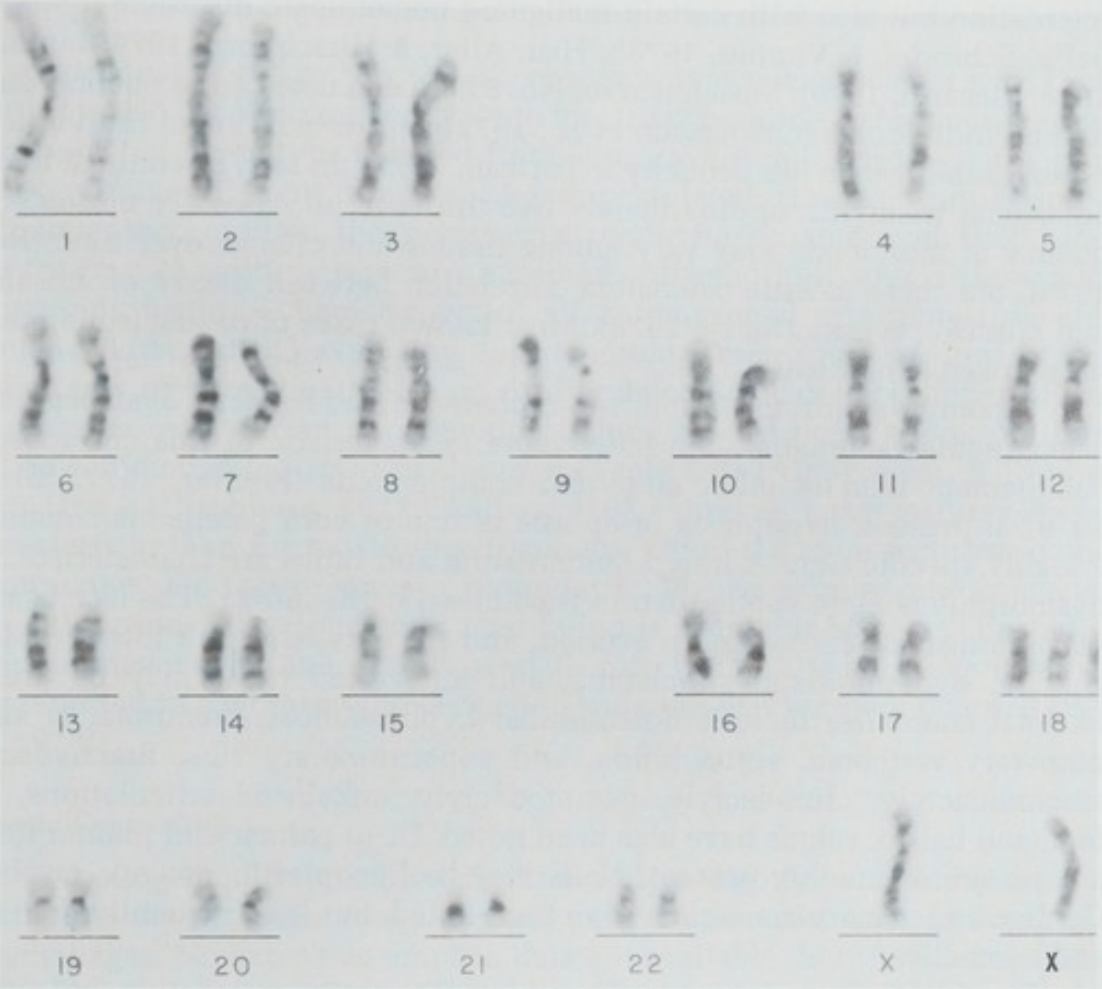


Figure 3-6. A trypsin-Giemsa (GTG)-banded karyotype with the complement 47,XX, +18 (primary, nondisjunctional trisomy 18).

with consideration of additional risk depending upon the age of the mother. Mosaicism has been detected in at least 10 percent of trisomy 18 cases. Such individuals are said to be less severely affected and survive longer than cases without a normal cell line (Shih et al., 1974; Eaton et al., 1975). Most of the remaining cases result from translocations, usually sporadic. Unless a familial chromosomal aberration also coexists, the recurrence risk does not increase.

Trisomy 8

Prior to the use of banding techniques (Caspersson et al., 1970), chromosomes were grouped mainly by relative size and position of the centromere. Chromosomes of the C group (Nos. 6–12,X) generally could not be distinguished from one another, which probably explains why trisomy 8 was not verified until the advent of banding techniques (Bijlsma, Wijfels, & Tegelaers, 1972; Caspersson et al., 1972). Approximately 70 cases have since been described. Trisomy 8 is not only associated with congenital malformations and mental

retardation but also with certain malignant hematologic disorders (de la Chapelle, Schroder, & Vuopio, 1973b; Hsu, Alter, & Hirschhorn, 1974; Lindquist, 1978; Riccardi, 1979). Mosaicism for No. 8 has been detected in phenotypically normal individuals (Caspersson et al., 1972) and in malformed individuals of normal intelligence (de Grouchy & Turleau, 1977). In fact, in contrast to other autosomal trisomics, approximately two-thirds of all cases are mosaics. Frequency of mosaicism may vary among tissues and change over time (Reyes, 1978), but there is little ostensible correlation between degree of mosaicism and clinical severity (Berry, 1978). Most known cases of mosaicism appear to have arisen sporadically.

Trisomy 8 syndrome (Table 3-3) is characterized by facial dysmorphia and osteoarticular anomalies, the latter more characteristic of this chromosomal complement than of other abnormal complements (Pfeiffer, 1977; Silengo, 1979). If present, hypoplasia or aplasia of one or both patellae is considered a highly specific sign. A long, slender trunk and limbs are characteristic, and dysmorphia is more subtle than in trisomies 13, 18, and 21. The face is somewhat elongated, the lower lip everted, and the ears large and low-set. Micrognathia, hypertelorism, strabismus, and anteverted nares may be present. Skeletal anomalies include dorsolumbar kyphoscoliosis, abnormal or supernumerary vertebrae, spina bifida, and supernumerary ribs. Brachydactyly, arachnodactyly, clinodactyly, camptodactyly, ankylosed articulations, club foot, and hallux valgus have also been noted. Deep palmar and plantar flexion creases are commonly present. Nails may be hypoplastic, convex, or absent. Cardiac and urinary anomalies have been noted, but less frequently than skeletal anomalies.

Mental development ranges from normal to severe retardation, but average IQ is 50. Most cases of trisomy 8 show some degree of mental retardation, but this could reflect bias of ascertainment, because some individuals with trisomy 8 mosaicism show a normal IQ and no anomalies suggestive of a chromosomal syndrome. Moreover, life expectancy does not appear to be decreased. Many cases of trisomy 8 may thus pass unrecognized, although no cases were detected in prospective surveys of over 50,000 neonates (Table 3-1).

Genetic Counseling

Most cases of trisomy 8 syndrome occur sporadically, as the result of nondisjunction in parental meiosis (nonmosaics), mitosis during embryogenesis (mosaics), or both. There is a suggestion of elevated maternal and paternal ages. Irrespective of the presence or absence of mosaicism, recurrence risk for the same or other trisomies is not greatly increased; thus, counseling is analogous to that following the occurrence of trisomy 21. Triplication of only a portion of No. 8 has also been reported, and there have been attempts to define these syndromes: short arm [dup(8p)] or long arm [dup(8q)] triplication. There are too few cases for accurate generalizations, but many of the anomalies are similar to those characteristic of trisomy 8. Dup(8p) and dup(8qter) often result from familial structural rearrangements, in which case the recurrence risk would be expected to be appreciably increased over the general population risk and over that following primary trisomy 8.

Trisomy 22

Additional G group (Nos. 21–22) chromosomes not associated with Down syndrome had been recognized prior to the use of banding techniques. Because the associated phenotype differed from Down syndrome, trisomy for a group G chromosome different from No. 21 was postulated. However, until banding techniques were applied, the precise origin could not be confirmed. Delineation of a trisomy 22 syndrome is now accepted. Affected individuals show many nonspecific features characteristic of all chromosomal syndromes: developmental retardation (IQ averaging 20), hypotonia, microcephaly, low-set malformed ears, cleft palate, strabismus, cardiac defects, and cryptorchidism. Facial dysmorphia results from micrognathia, a long beaked nose, a long philtrum, and an antimongoloid slant of the eyes (Table 3-3) (Alfi, Sanger, & Donnell, 1975; Hsu & Hirschhorn, 1977; Iselius, 1978). However, facial features are less characteristic than for most other trisomies. Other common features include preauricular skin tags or sinuses, hypoplastic, low-set nipples, and congenital hip dislocations. Developmental retardation is pronounced, most affected individuals never being able to walk or sit up. Survival to 20 years of age, however, was reported in a woman who learned to walk but was severely retarded (Welter, 1978).

Genetic Counseling

Couseling should probably reflect not only a slightly increased (1 percent) risk due to the occurrence of a trisomic offspring but also risk appropriate to parental ages. No extensive empiric data are available, but the mean parental age is slightly increased (Hsu & Hirschhorn, 1977; Shokeir, 1978b). In addition, 46/47, + 22 mosaicism has been reported in parents of some of the cases (Hsu et al., 1971; Uchida et al., 1968). Presence of parental mosaicism presumably increases the recurrence risk, but the magnitude of the increase is unknown. Although rare, cytogenetic analyses of parents should be performed to exclude mosaicism. Occasionally a structural rearrangement produces the phenotype characteristic of trisomy 22. In this context, a clinical condition worth mentioning is the "cat-eye" syndrome. This appellation was originally applied to children showing coloboma, anal atresia, and usually developmental retardation. The etiology was unknown, although often an additional small acrocentric chromosome was present. It is now recognized that individuals with this syndrome also may show other features suggestive of trisomy 22: preauricular skin tags, antimongoloid obliquity, and congenital heart disease. Unlike most cases of trisomy 22, however, familial aggregates of cat-eye syndrome are not rare, possibly reflecting familial translocations. Indeed, some of these translocations have involved No. 22, perhaps explaining occasional similarities in anomalies between trisomy 22 and cat-eye syndrome (Cervenka et al., 1977).

Trisomy 14

Very few cases of this syndrome have been reported, and only one was claimed to have nonmosaic primary trisomy (Murken et al., 1970). However, other cases are said to be mosaic or trisomic for the proximal part of No. 14,

enabling characterization of a syndrome produced by triplication for 14q (Martin et al., 1977; Rethoré, 1977; Wyandt, Magenis, & Hecht, 1977; Johnson, Aceto, & Likness, 1979). Most individuals reported to have trisomy 14 have a wide flat nose with bulbous tip, large mouth with down-turned corners, protruding lips, retrognathia, low-set ears, and developmental retardation. Digital anomalies (e.g., contractures), palatal anomalies, and cryptorchidism have also been reported. Like trisomy 22, these features suggest a chromosomal aberration but are relatively nonspecific, except possibly for the shape of the mouth.

Trisomy 14 seems much rarer than trisomy 13. This could reflect increased prenatal lethality of trisomy 14, a concept supported by the observation that the complement is the most common of the D (Nos. 13–15) trisomies among spontanteous abortuses (Kajii et al., 1973; Therkelsen et al., 1974). Trisomy 14 also might occur in live births more frequently than is generally appreciated, producing so few phenotypic abnormalities that affected individuals are not necessarily referred for genetic consultation. Although unlikely, this possibly would be analogous to trisomy 8, a disorder detected among older children who show few of the anomalies that usually initiate chromosomal analysis (Caspersson et al., 1972).

Chromosome 14 has also frequently been involved with rearrangements observed in certain malignancies (e.g., Burkitt lymphoma), and in some chromosomal breakage syndromes, (e.g., ataxia-telangiectasia) (McGaw et al., 1975).

Genetic Counseling

Too few cases have been identified to determine the relationship between maternal age and nondisjunction of No. 14, but offering counseling similar to that used for mosaic or nonmosaic trisomy 21 seems reasonable because of increased maternal age associated with trisomies for acrocentric chromosomes (groups D and G). Structural rearrangements leading to triplication of No. 14 should be excluded because familial translocations would increase the recurrence risks. Indeed, most structural rearrangements detected leading to excess No. 14 material have been familial. Prenatal diagnosis obviously should be offered such families.

Trisomy 9

Trisomy 9 is another syndrome for which very few cases have been reported in live births. Most reported individuals have shown mosaicism or triplication for only a portion of No. 9. The malformations observed reflect the nonspecific features generally associated with many other chromosomal aberrations (Table 3-3): microcephaly, low-set malformed ears, narrow and upward-slanting palpebral fissures, anophthalmos or microphthalmos, broad nose with overlapping upper lip, high arched palate, and micrognathia. Cardiac, skeletal, and urogenital anomalies have been reported, and hypotonia and developmental retardation are usually present. Of interest is that sufficient cases of triplication of the short arm (9p) have been reported to characterize an associated syndrome (Rethoré, 1977). These individuals show severe retardation but have no reduction in life expectancy. Interestingly, adults with various hematologic disorders, including malignancies, have also been reported to have trisomy 9

mosaicism. Moreover, translocations involving No. 9 are associated with some leukemias, (e.g., the "Philadelphia" chromosome [t(9q;22q)] and chronic myelogenous leukemias [Rowley, 1977]).

Genetic Counseling

Too few cases exist to determine specific risk figures. Counseling similar to that offered following the occurrence of any trisomy seems appropriate. Similarly, familial structural rearrangements should be sought.

TRIPLOIDY AND TETRAPLOIDY

The presence of additional haploid sets of chromosomes (polyploidy) is rarely compatible with survival in humans; however, both tetraploidy (4n = 92) and (especially) triploidy (3n = 69) (Fig. 3-7) are not uncommon among spontaneous abortuses (see Chapter 7). Triploidy may arise from various cytologic mechanisms (Beatty, 1978), including (1) mitotic irregularities in germ cell precursors, (2) lack of normal division in either the first or second meiotic

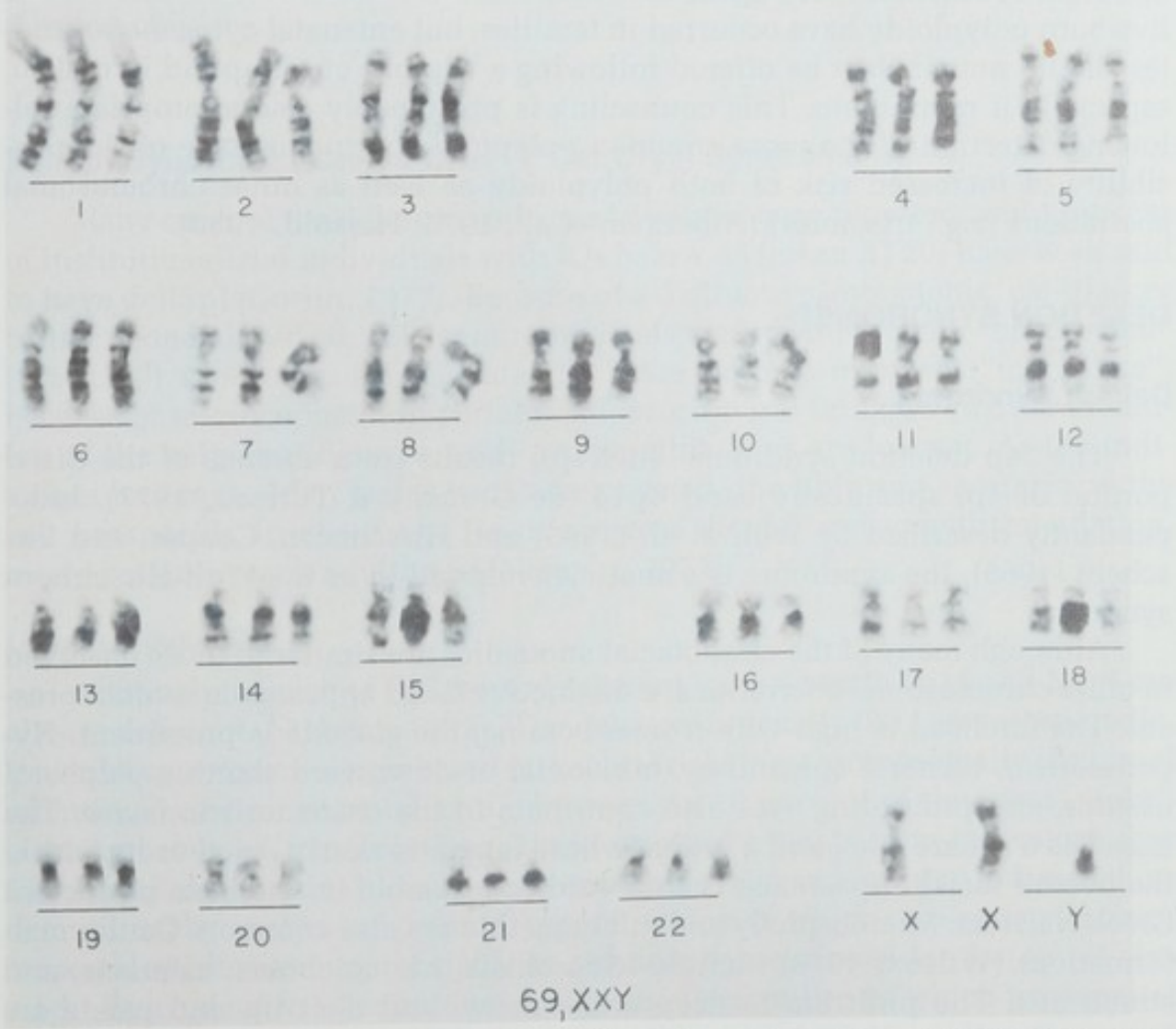


Figure 3-7. A trypsin-Giemsa (GTG)-banded karyotype illustrating a triploid (3N = 69) complement.

division of either parent, or (3) dispermy. The last appears to be the most common mechanism for triploidy in humans (Kajii & Nikowa, 1977). Delayed fertilization can cause triploidy in animals, (Yamamoto & Inglass, 1972) but is not a proved cause of triploidy in humans (Simpson, 1978d).

Live-borns with a triploid cell line usually have a coexisting normal diploid line (46/69 mosaicism), although sometimes only a triploid cell line has been detected (Simpson et al., 1972; Niebuhr, 1974b). Live-born cases usually show severe retardation and multiple anomalies, including hypertelorism, holoprosencephaly, microphthalmia, colobomata of the iris and choroid, low-set malformed ears, cleft lip and/or palate, retrognathia, hydrocephalus with hypoplasia or aplasia of the falx cerebri and corpus collosum, myelomeningocele, ambiguous external genitalia in males, cystic degeneration of the kidneys, aplasia of the adrenals, and syndactyly. An association between triploidy and hydatidiform degeneration of the placenta also exists (see Chapter 7); thus, coexistence of an anomalous infant and hydatidiform changes in an enlarged placenta suggests triploidy (Wertelcki, Graham, & Sergovich, 1976).

One case of nonmosaic tetraploidy survived past 1 year of age (Golbus et al., 1976), and diploid/tetraploid mosaicism has been reported (Kelly & Rary, 1974; Kohn et al., 1967). Multiple congenital anomalies characteristic of chromosomal syndromes were again noted in these few cases. No recurrences of live-born polyploidy have occurred in families, but antenatal cytogenetic studies should nonetheless be offered following a triploid or tetraploid live-birth, especially if nonmosaic. This counseling is presumably also appropriate following detection of the more common polyploid abortus because of the possibility of increased risk of both polyploidy as well as other chromosomal aberrations (e.g., trisomies) (Alberman et al., 1975; Hassold, 1980).

DELETION SYNDROMES

Del(4p) Syndrome

The "4p deletion syndrome" [del(4p)] results from absence of the distal portion of 4p, specifically band 4p16 (de Grouchy & Turleau, 1977). Independently described by Wolf et al. (1965) and Hirschhorn, Cooper, and Firschein (1965), the syndrome is sometimes referred to as the Wolf-Hirschhorn syndrome.

Although many of the craniofacial anomalies are similar to those observed in other chromosomal aberrations, a distinctive facial appearance is often present. The forehead is high with frontal bossing; the glabella is prominent. Hypertelorism, bilateral epicanthus, horizontal or downward slanting palpebral fissures, and protruding eyes also contribute to the characteristic facies. The nose has a square apex and a bridge whose length is nearly equal to its width; the overall facial appearance is thus said to resemble the helmets of ancient Greek warriors. Microcephaly and micrognathia are also common. Ocular malformations (Wilcox, 1978) include iridoschisis, iris coloboma, cataracts, and strabismus. The philtrum is deep and narrow, and cleft lip and palate are frequently observed. Other malformations include cardiac defects, hypospadias, umbilical hernias, skin dimpling (sacral region, shoulder, elbow, knuc-

kles) and various skeletal anomalies. Hypotonia and especially seizures are regular features in neonates. In addition to the facial features, the combination of low birth weight and midline fusion defects (e.g., cleft palate, cardiac defect, hypospadias, umbilical hernias) suggests del(4p). This deletion produces very severe developmental retardation; IQ is usually below 20. Life expectancy varies; A few individuals have survived to adolescence, but most die in the first few years of life (Warburton, 1973). This syndrome is summarized in Table 3-3 and described in more detail by Leao et al. (1967), Miller et al. (1970), and Guthrie et al. (1971).

Genetic Counseling

Most cases (90 percent) have been de novo; thus, the risk of chromosome aberrations in subsequent offspring is no greater than that expected on the basis of parental age. Specifically, risk of trisomic offspring is greater than risk for subsequent del(4p) offspring. However, if parental mosaicism or translocation is present (10 percent of cases), the recurrence risk is presumably higher. In these circumstances, antenatal diagnosis certainly should be offered, despite lack of empiric data. In fact, because small structural rearrangements (notably inversions) and low-frequency mosaicism in parents are difficult to exclude, it is reasonable to offer antenatal diagnosis to all families with a previously affected child.

Del(5p) Syndrome "Cri-Du-Chat" ("Cat Cry") Syndrome

Many cases of the del(5p) syndrome have now been reported, and 1 percent of institutionalized individuals with IQs below 35 (mean IQ 20) have been said to have del(5p) (Gorlin, 1977). Because of a distinctive, monotonic, cat-like cry in the neonatal period, this syndrome is also referred to as the "cri-du-chat" ("cat cry") syndrome. During infancy, facies become rounded ("moon-like") because of microcephaly, hypertelorism, broad nasal bridge, downward slanting of the palpebral fissures, and micrognathia. Ears are low-set. As the individual becomes older, facies become elongated, the philtrum shorter, and the cry nonspecific in character. Despite severe mental and growth retardation, many patients reach adulthood (Niebuhr, 1971).

Genetic Counseling

Although the size of the deleted segment varies, bands 5p14 and 5p15 are always deficient (Niebuhr, 1972). The deletion is usually (90 percent) sporadic, in which case counseling is based on general risks of a chromosomally abnormal offspring based on parental ages. However, in 10 percent of the cases, mosaicism, unbalanced translocations, rings, or recombinants from inversions (see Chapter 1) are present. In some instances these rearrangements are familial, as determined by analysis of parental chromosomes, in which case antenatal chromosomal studies are appropriate. As previously mentioned for del(4p), it also might be prudent to offer antenatal diagnosis to families in which no parental aberration has been detected because small rearrangements or low-frequency mosaicism cannot categorically be excluded.

Del(13q) Syndrome

Although a number of individuals have been reported with deletion of chromosome No. 13, not all show the same phenotype. This could reflect either differences in the length of the deleted segment, erroneous identification of the chromosomes involved, or unrecognized complex rearrangements causing imbalance of chromosomes other than No. 13.

Anomalies usually include ambiguous external genitalia and thumb aplasia (unilateral or bilateral). In other cases microcephaly, trigonocephaly or holoprosencephaly, obliterated sutures, closed fontanels, large abnormally shaped ears, coloboma, hypertelorism, and midface hyperplasia are present (Niebuhr, 1977) (Fig. 3-8). Retinoblastoma also may be associated with deletion of 14q (Yunis & Ramsay, 1978). Although developmental heterogeneity is considerable, depending on the size and position of the deleted segment, the mean IQ is about 50. Twenty percent of affected infants die before reaching 6 months of age (Niebuhr, 1977).

Genetic Counseling

At least half the cases of del(13q) syndrome are associated with ring formation (Niebuhr, 1977), which invariably causes deficiencies. Moreover, rings are often unstable and lead to different-sized deletions in different cell lines. Other cases result from a simple deletion of 13q (Fig. 3-9). Parental karyotypes are usually normal, with the recurrence risk thus no greater than that expected on the basis of parental age. Familial translocations and inversions have occasionally been detected, for which reason parental karyotypes should be ob-

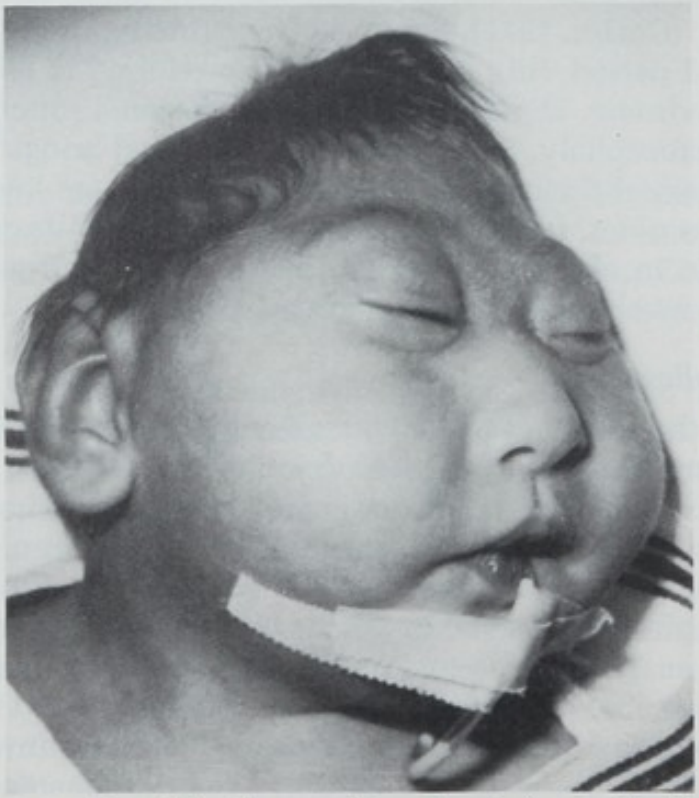


Figure 3-8. Craniofacial anomalies of an infant with del(13q).

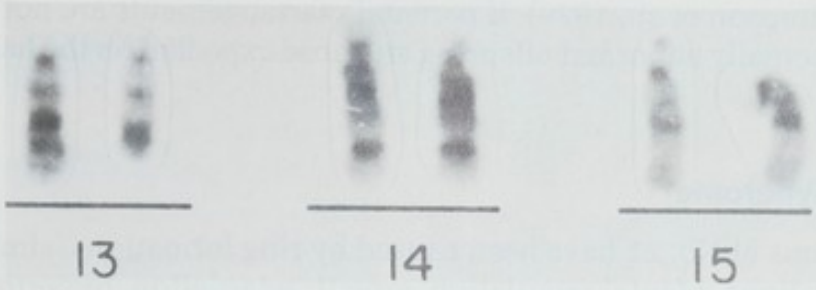


Figure 3-9. Partial karyotypes (D group chromosomes 13-15) from the 46,XX,del(13q) patient shown in Figure 3-8.

tained. Prenatal diagnosis should be offered to all families with a previously affected offspring because of the possibility of undetected parental mosaicism or a small structural rearrangement.

Del(18p) Syndrome

Although at least 85 cases have been reported, no distinctive features exist (Gorlin, 1977). Mental retardation is usually severe but varies (IQ range 25–75). Facies are rounded, with a wide mouth and large ears. Severer craniofacial anomalies (e.g., holoprosencephaly) occur less frequently. Many affected older patients are small and reportedly stand with widespread legs, leaning slightly forward (Table 3-3) (de Grouchy & Turleau, 1977). Life expectancy is generally normal (Miller, 1973). Some investigators have reported features reminiscent of the Turner syndrome. Almost all cases are sporadic, with the recurrence risk believed to be no greater than that expected on the basis of parental ages. However, mean maternal and paternal ages are elevated (Miller, 1979).

Del(18q) Syndrome

At least 95 cases have been reported (Schinzel, Hayashi, & Schmid, 1975; de Grouchy & Turleau, 1977; Wilson et al., 1979). Nonspecific craniofacial anomalies similar to those present in other chromosomal syndromes have been reported: microcephaly, midface hypoplasia, cleft lip or palate, and a mouth that turns inferiorly at the lateral margins ("carp-like" mouth). The nose tends to be short and the eyes deeply set. Ocular defects include coloboma, optic atrophy, and corneal anomalies. The ears show deep sulci and atresia of the external auditory meatus, an unusual and thus diagnostically helpful feature. Osteoarticular malformations are frequently present, including supernumerary or hypoplastic ribs and spina bifida occulta. Dimples occur over the subacromial and epitrochlear areas, lateral to the knees, and on the hands. External genitalia are often hypoplastic, and cardiac anomalies occur in most cases.

Failure to thrive, hypotonia, and seizures are characteristic of surviving infants. Mental retardation is generally severe (mean IQ 50), but a few cases show IQ levels of 70 or greater. About 10 percent die in the neonatal period, but several cases have survived until the second decade (Table 3-3) (de Grouchy & Turleau, 1977). Most cases are sporadic, and several have exhibited mosaicism. Translocations and pericentric inversions have also been reported, some

familial (Simpson et al., 1979). If parental rearrangements are not present, risks of chromosomally abnormal offspring are those expected on the basis of parental age.

Del(21q) Syndrome

Deletions of No. 21 have been caused by ring formations, simple deletions, or translocations. Mental retardation, growth retardation, hypertonia, and skeletal, genital, and craniofacial anomalies are frequently present in affected individuals. Because of the presence of downward-slanting palpebral fissures, micrognathia, large ears, and prominent nasal bridge, it has been suggested that the del(q21) phenotype is opposite to that of trisomy 21 (so-called contretype). Irrespective of the validity of this concept, malformations are severe and life expectancy short (de Grouchy & Turleau, 1977; Yamamoto et al., 1979). Many cases are associated with familial structural rearrangements, obviously having a relatively high recurrence risk. For cases not associated with such rearrangements, the risk of a chromosomally abnormal offspring is probably not greater than that expected on the basis of parental age.

4

Single Anatomical Malformations Usually Inherited in Polygenic/Multifactorial Fashion

Many disorders clearly show heritable tendencies but do not manifest distinctive modes of transmission. That is, the chromosomal complements are normal and the recurrence risks for relatives are much lower than they would be if the disorder resulted from mutation at a single locus. Most of these disorders involve only one organ system. They are presumed to result from the cumulative effects of many genes (polygenic) or (perhaps more likely) from interaction of environmental and genetic factors (multifactorial). Almost none of the disorders discussed in this chapter has been *proved* to be polygenic or multifactorial, but these mechanisms provide the most likely explanation for the observed recurrence risks.

In this chapter we shall discuss common polygenic/multifactorial disorders, specifically those most likely to be encountered by obstetrician–gynecologists. The only clinical details provided are those necessary for orientation; other texts should be consulted for complete descriptions and therapeutic recommendations. These disorders are not only the most common birth defects, but they are also among those most amenable to counseling by physicians who lack special training in genetics. Counseling requires confirmation of the diagnosis, knowledge of the recurrence risks, and awareness of the principles of multifactorial inheritance. One must, however, exclude chromosomal, Mendelian, and teratogenic factors that produce different risks and, perhaps, a different potential for antenatal diagnosis. For example, cleft palate as the sole anomaly carries a recurrence risk for sibs of 4 percent, whereas the disorder encompassing both cleft palate and lip pits is inherited in autosomal dominant fashion.

NEURAL TUBE DEFECTS

The neural tube defects include anencephaly, encephalocele, and spina bifida and are the most frequently encountered malformations of the central nervous system. Anencephaly is the partial or complete absence of the cranial

vault with a rudimentary brain, and is lethal. Encephalocele is the protrusion of brain and its covering membranes through the skull, most commonly in the occipital region. Encephalocele may not be lethal, but serious neurologic deficits, including mental retardation, occur. Spina bifida is failure of the neural arches to close and most often involves the lumbar region. Spina bifida cystica (myelomeningocele) results in the protrusion of meninges, usually with spinal cord and nerves in a cystic sac. This form accounts for over 90 percent of the cases of spina bifida cystica. Less commonly, the cord remains exposed as a flat structure (a myelocele). Spina bifida cystica varies in extent and level. Small, well-covered, inferior lesions may allow normal development with little physical handicap. However, 90 percent of lesions lead to seriously handicapping neurologic impairment of the lower extremities and rectal and urinary sphincters. Hydrocephalus is a frequent complication, and is seen in 70 percent of patients with myelomeningocele. Twenty-five percent of individuals with spina bifida are mentally retarded.

The neural tube defects are the result of failure of the neural tube to close. This occurs in the first four weeks after conception (Lemire et al, 1975). Closure normally begins in the cervical region of the cord and proceeds cephalad and caudad, carrying along the anterior and posterior neuropore until the closure is complete and the surface ectoderm becomes a continuous sheet on the surface of the embryo. The anterior neuropore and posterior neuropore close 24 and 26 days after conception, respectively.

The true incidence of neural tube defects is difficult to ascertain, as approximately 50 percent of anencephaly is spontaneously aborted (Nishimura et al., 1966). Incidence figures are also greatly influenced by geography: Wales and Ireland have the highest incidence, almost 1 percent of newborns being affected. In the United States, incidence generally decreases from north to south and from east to west. (For example, the incidence is about 2/1000 newborns in New York and 1/1000 in Los Angeles.) Neural tube defects are more common in Caucasians than in blacks, and more common in firstborn than later-born children (Janerich, 1972). The male/female ratio is 0.7:1 for spina bifida and 0.45:1 for anencephaly (Leck, 1974).

Recurrence risk is directly associated with incidence of a disorder, since both reflect the frequency of genes for that disorder in the total gene pool. The often-quoted recurrence risk of 5 percent after one affected sibling and 12 percent after two affected siblings is derived from the British Isles and is not applicable to other populations. The recurrence risk in the United States appears to be 1.5 to 2 percent after one affected sib and 4 to 6 percent with two affected sibs. It does not matter if a proband had anencephaly or spina bifida, for the defects appear to be etiologically identical.

The Meckel syndrome deserves special mention. It consists of an occipital encephalocele, polycystic kidneys, polydactaly, cleft palate, and congenital heart defects, although not all features are invariably present. The syndrome is inherited as an autosomal recessive trait and therefore has a 25 percent recurrence risk. Accurate genetic counseling requires accurate diagnosis. Therefore, a stillborn infant with encephalocele requires a post-mortem examination to determine the presence or absence of other anomalies. This syndrome is especially important to obstetricians because antenatal diagnosis is so readily available.

HYDROCEPHALY

Hydrocephaly is characterized by an increase of free fluid in the cranial cavity. The most common form, internal hydrocephaly, is an accumulation of excessive cerebrospinal fluid within the ventricles of the brain that may be caused by aqueductal stenosis, atresia of the foramina of Luschka and Magendie, an Arnold-Chiari malformation, or fluid overproduction. Clinically, an enlarged head, bulging fontanelles, and frontal bossing are common. Diagnosis is usually by a CT scan or pneumoencephalogram. The incidence is approximately 1/1000 newborns, with a predominance of males.

There has been much debate about recurrence risk for isolated hydrocephalus. Hydrocephalus secondary to spina bifida cystica or part of a syndrome must be considered separately, and the appropriate recurrence risk is that of the primary diagnosis. There is a well-established X-linked form of hydrocephalus secondary to aqueductal stenosis (Bickers and Adams, 1949). This has been estimated to represent 2 percent of all cases of uncomplicated hydrocephalus. The recurrence risk after birth of a male with an aqueductal stenosis caused by hydrocephalus is 6 percent (0 percent females, 12 percent males). This suggests aqueductal stenosis represents an admixture of multifactorial cases (with a very low recurrence risk) and a few X-linked cases (Burton, 1979). Families who have had offspring with communicating hydrocephalus, a Dandy-Walker malformation, or hydrocephalus of unknown etiology have a recurrence risk of no more than 2 percent (Bay, Kerzin, & Hall, 1979; Burton, 1979). There are also suggestions that the risk of neural tube defects may be 1 percent following the birth of a proband with hydrocephaly.

FACIAL CLEFTS

Cleft lip and cleft palate are the single most common defects affecting the oral facial structures of humans. They have been the subject of much investigation regarding etiology, and have served as a model for teratogen-induced defects. Many syndromes include cleft lip and/or palate as one feature. Gorlin, Cervenka, & Prozonksy (1971) found 30 percent of these syndromes to be due to a single mutant gene. However, less than 3 percent of all cases of cleft lip and/or cleft palate fall into this category.

Cleft Lip with or without Cleft Palate

Cleft lip may be unilateral or bilateral and may extend a variable distance posteriorly to include all or part of the maxillary alveolar process. The cleft of the palate may involve only the uvula or may extend through the hard palate and alveolar ridge. The prevalence varies with race from 1.7/1000 in Japanese newborns to 0.4/1000 in black infants. The sex ratio also varies, with males predominating among Caucasians and females predominating among blacks. Approximately 80 percent of clefts are unilateral and occur more often on the left than the right. At birth 7 to 13 percent of patients with isolated cleft lip have associated anomalies, and 11 to 14 percent with cleft lip and palate have

associated defects. Associated defects are more common in infants with bilateral clefts than in those with unilateral clefts.

The recurrence risk is 4 percent with a prior affected sib or parent, 9 percent with two prior affected sibs, and 17 percent with an affected parent and sib. The risk is slightly higher if the propositus is female and slighity lower if male, as expected from the sex ratio of affected individuals. The severity of the lesion in the affected proband also alters the recurrence risk. If the original defect is bilateral clefts of the palate and lip, the recurrence risk is 5.7 percent; if unilateral clefts of both palate and lip the risk is 4.2 percent; if a unilateral cleft lip, the risk is 2.5 percent (Poole, 1975).

In counseling the parents of an affected newborn it is important to recognize that their first concerns will be care of the child at home, the age for surgical intervention, embarrassment, and the possibility of other existing anomalies. (Winter & Tal, 1974). Only later will their attention turn to how the cleft was caused and recurrence risks. Parents who are told of the child's deformity immediately and see the child within a short time adapt better; withholding the infant will only cause parental resentment (Dar, Winter, & Tal, 1974).

Cleft Palate

A cleft palate may extend through the hard palate, the soft palate, or both, or may be submucosal with a failure of fusion of the underlying mesodermal elements. Isolated cleft palate is etiologically different from cleft lip with or without cleft palate. The prevalence of isolated cleft palate extends from 80/1000 newborn American Indians to 0.2/1000 newborn blacks, and in Caucasians is 0.4/1000 neonates. There is a slight female predominance. As many as 35 to 50 percent of affected individuals have associated defects.

The recurrence risk is 2 percent with a prior affected sib, 6 percent with a sib with other affected relatives, 7 percent with an affected parent, and 15 percent with an affected parent and sib, at least in Caucasians.

Cleft Lip or Palate and Lip Pits

Lip depressions or pits are usually paramedian on the lower lip. This entity is inherited as an autosomal dominant trait with a 50 percent recurrence risk (assuming the proband is not a new mutation). Prior to counseling families regarding the recurrence risk of cleft, one must be certain the cleft is not part of one of the more than 30 syndromes that include clefting and that are inherited in a Mendelian fashion.

CARDIAC ANOMALIES

Congenital heart disease occurs in approximately 0.1 to 0.5 percent of liveborns, depending upon the number of years children are followed before being termed normal. Numerous different cardiac anomalies have been reported, but 10 to 15 of these account for most cases. About 20 percent of children with congenital heart disease have a ventricular septal defect, 10 percent have an atrial septal defect, 10 percent tetralogy of Fallot, 10 percent patent ductus arteriosus, and 10 percent pulmonic stenosis. Forty percent have other an-

Table 4-1
Recurrence Risks in Sibs of Probands with Congenital Heart Lesions

Anomaly	Proband (No.)	Affected Sibs	
		No.	%
Ventricular septal defect	212	24/543	4.4
Patent ductus arteriosus	204	17/505	3.4
Tetralogy of Fallot	157	9/338	2.7
Atrial septal defect	152	11/342	3.2
Pulmonic stenosis	146	10/345	2.9
Aortic stenosis	135	7/317	2.2
Aortic coarctation	128	5/272	1.8
Transposition of the great vessels	103	4/209	1.9
Atrioventricular canal anomaly	73	4/151	2.6
Tricuspid atresia	51	1/96	1.0
Ebstein anomaly	42	1/96	1.1
Truncus arteriosus	41	1/86	1.2
Pulmonic atresia	34	1/77	1.3
Total	1478	95/3376	

Adapted from Nora JJ (1971): Etiologic factors in congenital heart disease. Pediar Clin North Am 18:1059.

omalies, e.g., aortic stenosis, coarctation of the aorta, transposition of great vessels, tricuspid atresia, the Ebstein anomaly, and truncus arteriosus.

Congenital heart disease usually results from polygenic/multifactorial causes, most lesions carrying a recurrence risk for first-degree relatives of 2 to 5 percent (Table 4-1). About 4 percent of children with congenital heart disease have a demonstrable chromosomal abnormality. Perhaps 4 percent result from a Mendelian mutation and 2 percent from known teratogenic factors.

Briefly defined below are the cardiac anomalies obstetrician–gynecologists are most likely to encounter. Readers desiring more details should consult standard pediatric and cardiology texts.

Ventricular Septal Defect

Ventricular septal defect (VSD) is one of the most common cardiac anomalies, accounting for approximately 20 percent of all cases of congenital heart disease. The defect may be the only anomaly or it may be one of several coexisting cardiac anomalies (e.g., tetralogy of Fallot). Presumably resulting from failure of closure of the ventricular septum during embryogenesis, ventricular septal defects may be located at various sites. The most common site is the membranous portion beneath the aortic valve. Symptoms depend upon the size of the defect. A small VSD may be asymtomatic, but larger defects produce (1) left-to-right shunts because right ventricular pressure and resistance are less than left ventricular pressure and resistance, hence causing (2) increased pulmonary blood flow, (3) pulmonary hypertension, and (4) ultimately, congestive heart failure. The diagnosis is usually not evident at birth, because

the increased pulmonary resistance present in all neonates causes a high right ventricular pressure and prevents left-to-right shunting. Diagnosis is made by auscultory, electrocardiographic and angiographic studies familiar to pediatric cardiologists. Surgery is required in only 20 percent of cases, the remaining 80 percent closing spontaneously. Surgical repair is relatively successful and mortality 1 to 2 percent.

Tetralogy of Fallot

This disorder consists of ventricular septal defect, overriding aorta, infundibular pulmonic stenosis or atresia, and resulting right ventricular hypertrophy. Cyanosis develops as result of right-to-left shunting and becomes accentuated as the right ventricle hypertrophies. Tetralogy of Fallot accounts for 10 percent of all cases of congenital heart disease; males are affected more often (3:2) than females. Surgery is essential, for persisting cyanosis carries a very poor prognosis.

Atrial Septal Defect

An atrial septal defect (ASD) may be of the ostium secundum type or the ostium primum type. Ostium secundum defects result from failure of closure of the embryologically patent septal ostia, usually the fossa ovalis. By contrast, the rarer ostium primum defects are caused by abnormalities of the endocardial cushion. Ostium secundum ASD may be relatively asymptomatic, but a left-to-right atrial shunt will lead to increased pulmonary flow if the ASD is sufficiently large. Congestive heart failure rarely occurs, and cyanosis is not present. Surgical correction is quite successful, with an operative mortality of 1 percent or less. Females are more likely to be affected than males.

Endocardial Cushion Defects

Endocardial cushion defects (ECDS) result from anomalous development of the atrioventricular cushions, embryonic structures crucial for normal development of septa and valves. The defects range from isolated ostium primum atrial defects to a common atrioventricular canal with incompetencies of several valves. Mitral incompetence and pulmonary stenosis are especially common. Clinical features and prognosis depend upon the specific anomalies. ECD is one of the most common cardiac anomalies in trisomy 21.

Patent Ductus Arteriosus (PDA)

This anomaly results from persistence of the duct that, during embryogenesis, directs blood from the pulmonary artery to the descending aorta. PDA is essentially physiologic, especially in premature infants, but it is also a relatively frequent cardiac anomaly in full-term infants, and is commonly associated with rubella embryopathy.

Hemodynamic changes are similar to those associated with VSD. Left-to-right shunting increases pulmonary flow, leading to pulmonary hypertension. Congestive heart failure is unusual, and cyanosis is absent unless pulmonary hypertension is so great that compensatory right-to-left shunting occurs across the ductus. Females are more likely than males (2:1) to be affected. Ligation or division of the PDA is relatively simple, and prostaglandin inhibitors also facilitate closure.

Pulmonic Stenosis and Atresia

Pulmonic stenosis (PS) may be subvalvular, valvular, or supravalvular. Valvular defects are most common, and occasionally the valve may be almost completely atretic. Clinical findings reflect the severity of the obstruction and the concomitant presence or absence of septal defects. Some patients are asymptomatic and develop normally, but those with significant obstruction develop symptoms by 2 to 3 years of age. Right heart failure may develop and cyanosis occur as result of right-to-left shunting through ASD or VSD, or as result of decreased cardiac output (peripheral cyanosis). Surgical correction is usually necessary. Pulmonic stenosis occurs commonly in rubella embryopathy and in Noonan syndrome (see Chapter 4), an autosomal dominant condition in which external features are reminiscent of Turner stigmata (Chapter 10).

Aortic Stenosis (AS)

Aortic stenosis may be subvalvular, valvular or supravalvular. Valvular AS is likely to be the type responsible for infants with cardiac problems. Usually the valve is bicuspid. Valvular AS is often asymptomatic, but obstruction may ultimately lead to left ventricular hypertrophy and left heart failure. Death may occur suddenly, probably because of myocardiac ischemia and conduction abnormalities. Aortic value replacement is usually indicated, especially if the valve is bicuspid.

Supravalvular Aortic Stenosis

In supravalvular AS narrowing of the ascending aorta, focal or diffuse, occurs distal to the sinuses of Valsalva. Because the coronary arteries arise proximal to the site of narrowing, they are subjected to increased pressure and prone to development of arteriosclerosis. Features are otherwise similar to those observed in valvular AS. Males and females are affected in equal numbers. A form of supravalvular AS associated with coarse facial features and dental anomalies apparently results from hypercalcemia and possibly hypervitaminosis D during either pregnancy or early infancy.

Subvalvular Aortic Stenosis

In this disorder a fibrous ring encircles the left ventricular outflow tract. The disorder is often asymptomatic. Many cardiologists consider subaortic AS to be relatively common, based upon cardiac changes in asymptomatic relatives of individuals with overt subvalvular AS. Subvalvular AS occurs twice as often in males as in females. The fibrous ring can usually be excised with relative ease. The condition may be autosomal dominant.

Hypoplastic Left Heart Syndrome

This term is applied to individuals who show hypoplasia of the left ventricle, which may coexist with aortic valvular atresia, aortic valvular stenosis, mitral atresia, mitral stenosis, or atresia of the aortic arch. This syndrome is probably the most common cause of heart failure in the first week of life. Symptoms usually become evident 48 to 72 hours after birth. The defect is not amenable to surgical repair, and prognosis is poor.

Transposition of the Great Vessels

In this anomaly the aorta arises from the right ventricle and the pulmonary trunk arises from the left ventricle. Mixing of the two circulations is obviously a prerequisite for survival; thus, if the neonate is surviving, VSD, ASD, or a patent foramen ovale must coexist. Despite shunting, severe cyanosis develops by the second or third day of life. Although transposition used to be considered inoperable, surgical repair may now be possible in some cases by the Mustard procedure, which redirects blood by means of several surgical steps. Operative mortality is 10 to 20 percent, however, which is considerably higher than that of most other cardiac anomalies.

Truncus Arteriosus

In this defect, which affects twice as many males as females, a single vascular trunk gives rise to aortic, pulmonary, and coronary circulations. A ventricular septal defect is always present, and the common truncal valves are usually incompetent. Oxygen saturation is equal in all three circulations. Cyanosis and congestive heart failure occur, and only about 30 percent of affected individuals survive beyond infancy. This condition was formerly considered inoperable, but corrective techniques now offer a more encouraging prognosis.

Coarctation of the Aorta

In coarctation the aorta is constricted, usually near the ligamentum arteriosum. Most patients have a bicuspid aortic valve. Patent ductus arteriosus and ventricular septal defects often coexist. Arterial pressure is decreased distal to the obstruction, resulting in such compensatory mechanisms as peripheral vasoconstriction and collateral circulation that bypasses the obstruction. Nonetheless, left ventricular overload may lead to congestive failure. Aortic coarctation is said to be the most common cause of congestive failure in the second, third, and fourth weeks of life, and affects more males than females. Infants surviving beyond four weeks may do relatively well. Surgical end-to-end anastomosis is best delayed until 4 to 5 years of age. Coarctation and VSD are the most common cardiac defects of Turner stigmata (Chapter 10).

Ebstein Anomaly

In Ebstein anomaly an enlarged tricuspid valve is not only ectopically inserted but is also adherent to the wall of the right ventricle. Blood flow across the valve is obstructed, resulting in shunting through the foramen ovale to the left atrium. Cyanosis thus occurs because of the presence of deoxygenated blood in the circulation. Prognosis depends upon the degree of obstruction, severe obstruction usually leading to death during infancy or early adult life.

Genetic Counseling for Cardiac Anomalies

Genetic counseling for any cardiac anomaly should be undertaken only after establishing the diagnosis and considering the potential etiologies. Most investigators estimate that approximately 3 to 4 percent of cardiac anomalies result from a chromosomal abnormality, 4 percent from a single mutant gene,

and 1 percent from teratogenic factors. The remainder are presumably polygenic or multifactorial in origin.

Confirmation of polygenic/multifactorial inheritance is difficult, because derivation of empiric risk data is subject to more pitfalls than usual for such studies; specifically, confirmation of diagnosis is often uncertain. Chromosomal studies are often not performed and known Mendelian traits have not always been excluded. If a relative was stillborn, an anomaly may have gone undetected. Nonetheless, several studies show consistent results. Moreover, the theoretical recurrence risk predicted for first-degree relatives of probands with a polygenic trait (see Chapter 1) agrees quite well with empiric data (Table 4-1) (Nora, McGill, & McNamara, 1970a; Nora, 1971; Nora & Nora, 1976, Nora & Nora, 1978).

The largest series are those reported by Nora and colleagues (1970a; 1976) and by Zetterqvist (1972), who surveyed hospitalized samples of children and adults with many types of cardiac anomalies. Data are also available for coarctation of the aorta (Boon & Roberts, 1976; Campbell & Polani, 1961), tetralogy of Fallot (Boon, Farmer, & Roberts, 1972), atrial septal defect (Williamson, 1969; Sanchez-Cascos, 1972), patent ductus arteriosus (Wilkins, 1969), and hypoplastic left heart syndrome (Brownell & Shokeir, 1976).

Recurrence risk for liveborn first-degree relatives is 1 to 4 percent, depending upon the trait in question. In about half the cases the same anomaly recurs in relatives (Fraser & Hunter, 1975), whereas a different cardiac anomaly is involved in the rest. Data are usually not subdivided according to sex of the proband or relatives in question, although some disorders are more likely to affect members of a given sex. For such disorders recurrence risks should ideally be derived as a function of sex. Recurrence risks for second- and third-degree relatives are predictably lower, usually 1 percent or less. However, these figures apply only if cytogenetic studies are normal, if no extracardiac anomalies are present, and if Mendelian disorders are excluded. (See Elias and Yanagi (1981) for tables of Mendelian disorders.)

Common Mendelian disorders characterized by cardiac anomalies include Noonan syndrome with pulmonic stenosis, autosomal dominant subaortic stenosis, and the Holt-Oram syndrome. Many rarer syndromes are also characterized by cardiac anomalies. Occasionally a cardiac anomaly in a given family may appear to result from a single gene (Zetterqvist, 1972), but usually the data in Table 4.1 are appropriate. Again, these risk figures should be applied only after diagnostic confirmation and exclusion of chromosomal and Mendelian disorders.

TRACHEOESOPHAGEAL FISTULA AND ESOPHAGEAL ATRESIA

These two anomalies usually coexist, although esophageal atresia or tracheal anomalies may occur separately. In 90 percent of cases in which both occur together, the proximal esophagus terminates blindly near the thorax (T-2 level) and the distal esophagus communicates with the trachea. Newborns manifest excessive secretions. After feeding, the infant coughs, gags, regurgi-

tates, and often becomes cyanotic; aspiration pneumonia is common. A nasogastric tube cannot be passed. Various roetgenographic procedures readily confirm the diagnosis. Surgical repair is usually successful, but multiple procedures may be necessary if the infant is severely ill or if more complicated anomalies are present. Esophageal atresia may also occur as an isolated defect, and the esophagus may also be stenotic rather than completely atretic. Tracheoesophogeal fistula may be one component of the VACTERL (Vertebral, Anal, Cardiac, TracheoEsophageal, Renal, Limb) syndrome, which is claimed to be related to progestin teratogenicity (see Chapter 15).

Few genetic studies concerning these anomalies have been conducted, but available data suggest recurrence risks similar to those for other disorders presumed inherited in polygenic fashion. Among 35 probands with esophageal atresia with or without tracheoesophogeal fistula, Schimke, Leape, and Holder (1972) noted one kindred in which 2 sibs, 2 third cousins, and 1 second cousin once removed had both. Other familial aggregates were later reported (Dennis, Nicholas, & Kova, 1973; Chen, Goei, & Hertzlon, 1979). Recurrence risks of 1 to 2 percent for first-degree relatives are probably appropriate (Chen, Goei, & Hertzlon, 1979).

DUODENAL AND INTESTINAL ATRESIA

Atresia or partial stenosis of the duodenum, jejunum, or ileum leads to bile-stained emesis, distention proximal to the obstruction, and eventually dehydration and electrolyte imbalance. If a fetus has one of these anomalies, hydramnios is often present. Diagnosis depends upon appropriate roentgenograms, especially upright films. Prompt surgical repair is essential. Duodenal atresia is especially common in trisomy 21, and may be associated with an annular pancreas or malrotation of the colon.

Several of these anomalies may be inherited in Mendelian fashion. Familial aggregates of duodenal or intestinal atresia are especially common, and affected sibs usually have atresia in similar locations. Based upon reports of affected sibs whose parents were consanguineous, McKusick (1978) believes that separate autosomal recessive genes cause (1) duodenal atresia (Mishalany, Der Kaloustian, & Ghandour, 1970; Der Kaloustian, Slim, & Mishalany, 1974), (2) multiple intestinal atresias characterized by obstructions at several sites from the stomach to anus (Dallaire & Perreault, 1974), and (3) jejunal atresia (Mishalany & Najjar, 1968; Blyth & Dickson, 1969). However, most cases of intestinal atresia do not result from an abnormality in one of these genes. For purposes of genetic counseling it is perhaps best to suggest that the risk to subsequent sibs is increased, probably by only 2 to 5 percent but possibly as much as 25 percent. If 2 sibs are affected, autosomal recessive factors should be assumed to be present in that particular family.

PYLORIC STENOSIS

Muscular hypertrophy at the pylorus produces pyloric stenosis, one of the most common congenital anomalies. Affected infants are normal at birth, but around 2 to 3 weeks of age begin vomiting in a projectile-like manner. The

vomit is not bile-stained. Dehydration, hypokalemic alkalosis, weight loss, and stool changes develop. The presence of visible gastric peristalsis or palpable pyloric hypertrophy suggest the diagnosis, and roentgenographic studies confirm it. Longitudinal division of the hypertrophied muscle mass (Ramsted procedure) is relatively simple, and the mortality is very low.

Genetic studies of pyloric stenosis have helped elucidate the principles of polygenic inheritance, specifically for traits in which one sex is more likely to be affected than another. A relatively common trait (2/1000 live births), pyloric stenosis is five times more likely to occur in males than females. Various studies (Carter, 1976; Siebers, 1978) have shown that the recurrence risk depends upon whether the proband is male or female. If male, the risk for subsequent male sibs is 5 percent, whereas the risk for subsequent female sibs is only 2.5 percent. If the proband is female, the risks for subsequent male sibs may be as high as 20 percent, whereas the risk for subsequent female sibs is only 7 percent. Sons of female patients have a 20-percent risk, whereas daughters have only a 7 percent risk. Sons of male patients have a 5-percent risk, whereas daughters have only a 2- to 5-percent risk. Similar sex differences apply to second- and third-degree relatives. Irrespective of sex of the proband, nephews are more likely to be affected than nieces, and male first cousins are more likely to be affected than female first cousins. These sex differences are predictable if one assumes that the threshold on the curve of genetic liability is closer to the mean for males than for females (Chapter 1).

HIRSCHSPRUNG DISEASE (COLONIC AGANGLIONOSIS)

This disorder results from absence or deficiency of intramural myenteric ganglionic plexuses of the distal colon and rectum. The colonic segment proximal to the defect becomes dilated and hypertrophied; the portion lacking ganglia maintains normal caliber. Aganglionosis is distal to the splenic flexture in 84 percent of cases and confined to the rectosigmoid in 70 percent of cases. Constipation is the usual presenting complaint, but other gastrointestinal changes occur secondarily. Following roentgenographic diagnosis, the aganglionic segment may be excised, followed by reanastomoses of normal segments. Affected individuals who survive infancy have a normal life expectancy. Males are affected about twice as often as females.

Hirschsprung disease may occur in isolation or it may be associated with other defects. Risks depend not only upon sex but also upon the length of the aganglionic segment (Passarge, 1967). If the proband is male, the risk is 5 percent for subsequent male sibs, and 2 percent for subsequent female sibs. If the proband is female, the risks for subsequent male and female sibs are 11 percent and 14 percent respectively. If the aganglionic segment extends above the splenic flexure, the risk is even higher.

MECKEL DIVERTICULUM

The yolk stalk, a structure that during embryonic life connects the umbilicus to the intestine partially persists in 1 to 2 percent of live births, and is termed Meckel diverticulum. It arises 18 to 36 inches proximal to the ileocecal

junction. Most individuals are asymptomatic, but symptoms may arise because of torsion, perforation, infection (mimicking appendicitis), intussusception, or ulceration due to ectopic gastric or pancreatic tissue. Complete resection can be performed easily. Familial aggregates have occasionally been reported, but it is difficult to determine whether the prevalence of this common disorder is higher in relatives than expected for the general population.

INTUSSUSCEPTION

In intussusception a proximal portion of intestine "prolapses" into an adjacent segment. It usually occurs between 4 and 14 months of age and is manifested by vomiting, abdominal cramps, bloody, mucoid jelly-like stools, and shock. The blood supply may become interrupted, leading to gangrene, which requires bowel resection. After roentgenographic diagnosis and correction of fluid and electrolyte imbalance, intussusception can be reduced. MacMahon (1955) estimated that the recurrence risk for sibs is about 2 to 3 percent.

DIAPHRAGMATIC HERNIA

Defects in diaphragmatic musculature most often occur in the left posteriolateral portion (90 percent), but other portions may also be defective. As result of the defect, abdominal viscera are displaced to the thorax. The amount of displacement depends upon the size and location of the defect. Severe life-threating symptoms—tachypnea, dyspnea, even cyanosis—usually occur shortly after birth. Respiratory or circulatory collapse may occur. Males are affected more often than females. This condition may be suspected on the basis of decreased breath sounds, percussion dullness, and displacement of thoracic structures. Chest roentgenograms confirm the diagnosis. If the defect is large enough to warrant surgery, it must be performed immediately, and is successful about half the time.

Relatively few familial aggregates of diaphragmatic hernia characterized by absence or deficiency of only part of the diaphragm have been reported (Phillipp & Skelton, 1952; Crane, 1979; Wolff, 1980). However, unilateral agenesis of the diaphragm, a rarer condition producing similar symptoms, may be autosomal recessive (Daentl & Passarge, 1972).

OMPHALOCELE

Failure of the anterior abdominal wall to close permits evisceration of abdominal organs. The viscera are usually covered by a thin membrane, which often ruptures during vaginal delivery. Intestinal malrotation is invariably present. The defect may or may not be amenable to primary closure; if not, silastic prostheses are required. Few genetic studies have been performed, but familial aggregates have been reported (Rott & Truckenbredt, 1974; Osuna & Lindham,

1976). A recurrence risk for first-degree relatives of about 1 to 2 percent seems appropriate (Jorgenson & Salinas, 1979). An omphalocele may also be one component of Beckwith-Wiedemann syndrome, which is probably autosomal dominant.

UMBILICAL HERNIA

Failure of the fascia of the embryonic umbilical ring to close completely produces umbilical protuberance. This relatively common defect most often occurs in blacks and premature infants. Umbilical hernia may also be associated with hypothyroidism, Down syndrome, and some Mendelian disorders. No treatment is necessary prior to age 5, at which time surgical repair should be considered. No formal genetic studies have been reported, but a recurrence risk of about 5 percent seems reasonable.

INGUINAL HERNIA

Inguinal hernias in children are usually indirect and either bilateral or right-sided. Hydrocele may be present in males, who constitute 90 percent of cases.

About 50 percent of testicular feminization (androgen insensitivity) patients have inguinal hernias; however, relatively few females with hernias have testicular feminization (German et al., 1973). Nonetheless, females with inguinal hernias should be screened by cytogenetic studies to exclude testicular feminization. Although affected individuals are often asymptomatic, intestinal incarceration can lead to pain and possible gangrene. Surgical repair is therefore eventually necessary.

Genetic aspects of otherwise normal individuals with inguinal hernias have received relatively little attention. However, affected males in successive generations were reported by Weimer (1949) and Edwards (1974). Simpson, Morillo-Cucci, and German (1974) reported two families in which inguinal hernias occurred only in females. Recurrence risks are probably no greater than 2 to 5 percent.

RENAL ANOMALIES

The most common renal anomalies appear to be renal agenesis and "horseshoe" kidney. Bilateral renal agenesis is obviously fatal, but unilateral renal agenesis may be asymptomatic and is often associated with Müllerian aplasia, incomplete Müllerian fusion, or other malformation patterns. Males are more likely to have bilateral renal agenesis than females. As expected, the recurrence risk is higher for sibs if the proband is female; Pescia and Evans (1977) estimate that the recurrence risk is 10 percent if the proband is female but only 3 percent

if male. Bois et al. (1975) estimated the recurrence risk for unilateral renal agenesis to be 6.3 percent.

If the metanephrosis fails to ascend, fusion of the paired metanephric structures leads to a horseshoe-shaped kidney, which remains in the pelvis. It is usually asymptomatic, and may be mistaken for a pelvic tumor. Few empiric data are available, but Bois et al. (1975) estimated the recurrence risk for first-degree relatives to be 5.1 percent.

URETERAL AND BLADDER ANOMALIES

Obstruction of urinary outflow produces dilation of the organs proximal to the obstruction and predisposes to infection and pyelonephritis. Children with urologic obstruction may be asymptomatic or show growth retardation, but they rarely complain of pain or dysuria. The obstruction may be located at the ureteropelvic junction, ureterovesical junction (leading to ureterocele), bladder neck, or posterior urethral valves. Ureteral duplication is sometimes associated with obstructive uropathies. Diagnosis requires a high index of suspicion, and can be confirmed with various roentgenographic procedures. Surgical correction is usually possible, and prognosis depends upon the extent of pre-existing renal damage.

Familial aggregates of the various anomalies responsible for urologic obstruction have been reported by several groups (Simpson & German, 1970; Bois et al., 1975; Bredin et al., 1975). Accurate derivation of specific recurrence risks is difficult, because different anomalies may occur in the same kindred. Bois et al. (1975) estimated the recurrence risk for sibs to be 4.2 percent for ureteropelvic junction obstruction and 5.3 percent for ureteral duplication. Bredin et al. (1975) noted that only 2.2 percent of sibs of probands with vesicoureteral reflux had complaints similar to those of the proband; however, roentgenographic studies in a small series revealed 8 of 60 sibs (16 percent) to have reflux. DeVargas and Evans (1977) reported that 10 percent of sibs with vesicoureteral reflux had a renal or urologic disorder confirmed by roentgenography. Recurrence risks of between 5 and 10 percent seem appropriate for first-degree relatives of probands with ureteropelvic junction obstruction, ureteral duplication, vesicoureteral reflex, and bladder neck obstruction.

BLADDER EXSTROPHY

In this anomaly a defect in the lower abdominal wall exposes the interior of the bladder. Affected males usually show epispadias and affected females a bifid clitoris. The pubic rami are widely separated. The clitoris may be bifid, but usually the child's true sex is not in doubt. Urinary incontinence is almost invariably present. Complete surgical correction is not always possible, for sometimes the ureters must be transposed to the rectum or the rectosigmoid. If reimplantation is performed, pyelonephritis occurs frequently. Although familial aggregates have been reported (Glaser & Lewis, 1961), they are apparently rare (Ives, 1978). A recurrence risk of about 1 percent seems appropriate.

ISOLATED CRYPTORCHIDISM

Testes failing to descend as expected during embryogenesis may be located along the normal path of descent or may be displaced ectopically. Cryptorchidism is common in premature infants, but only 1 percent of full-term liveborn males are cryptorchid. In most cases, descent subsequently occurs. Retractile testes are much more common. Failure of descent may occur because testes are morphologically abnormal. After age 5, descent does not diminish the likelihood (0.3 percent) of testicular neoplasia associated with persistent intraabdominal position. Chorionic gonadotropin has traditionally been administered to facilitate descent, but surgery is often required.

Familial aggregates of cryptorchid but otherwise normal males have been reported (Perrett & O'Rourke, 1969). Before concluding that an individual has uncomplicated cryptorchidism, one should be certain the external genitalia are normal and that none of the sex differentiation disorders are present (see Chapter 10). If such disorders are excluded, a low but definite recurrence risk may be offered, perhaps 2 to 5 percent.

IMPERFORATE ANUS

Imperforate anus refers to one of several anorectal anomalies involving the terminal rectum. The anus may be stenotic, ectopic, or imperforate. If imperforate, a dimple may be identified. Intestinal obstruction develops shortly after birth, requiring immediate surgical intervention. Ease of repair is inversely related to the length of the absent segment.

Reported familial aggregates of imperforate anus have included multiple affected sibs (Van Gelder & Kloepfer, 1961; Winkler & Weinstein, 1970), affected mother and daughter (Cozzi & Wilkinson, 1968), and males affected in X-linked recessive fashion (Weinstein, 1965). Although imperforate anus may occasionally result from a single gene, the disorder appears to be polygenic; thus a recurrence risk of 2 to 5 percent for sibs or offspring seems appropriate. Anal stenosis, without complete obliteration, may occasionally result from an autosomal dominant gene (Cozzi and Wilkinson, 1968).

Imperforate anus may also occur as part of the VACTERL syndrome or as one end of the spectrum of caudal regression. The VACTERL syndrome may be related to progestin teratogenicity (see Chapter 15), and the caudal regression syndrome is definitely related to maternal diabetes mellitus (Simpson, 1978c).

CONGENITAL DISLOCATION OF THE HIP

Dislocation of the hip is the most common congenital skeletal deformity. A typical affected neonate displays dysplasia of the hip: a flat acetabular roof and an underdeveloped proximal end of the femur, which predisposes to dislocation. At least 80 percent of dislocated hips diagnosed at birth will resolve with adequate hip abduction and thigh flexion, allowing the acetabulum to deepen and the ligaments to achieve normal tension (Clarren & Smith, 1977).

In the United States the condition is rare in blacks but frequent in Indians (Record & Edwards, 1958). An incidence of 1/1000 newborns can be assumed for Caucasian populations (Woolf, Koehn, & Coleman, 1968). Seasonal variation in incidence has been noted, with the highest incidence in winter, probably reflecting the tendency to swaddle infants with their hips extended and adducted. Fetal breech position enhances the likelihood of hip dislocation about 14-fold for the full-term infant; that the fetal position and not delivery causes this predisposition follows from observations that neither external version nor Caesarian section markedly influences predisposition for hip dislocation (Dunn, 1976). There is a 5:1 ratio in favor of females (Woolf & Turner, 1969), presumably due to a hormonally induced joint laxity in the female in the fetal and early neonatal periods. Approximately one third of the children are affected bilaterally, and in the remainder the left hip is involved much more frequently than the right (Finlay, Mautsley, & Busfield, 1967).

The overall recurrence risk is approximately 6 percent: 4 percent for brothers and 8 percent for sisters. If the proband is male, recurrence risks are higher for both males and females. If one parent and one sib are affected, the recurrence risk is 10 to 15 percent.

CLUBFOOT

Talipes equinovarus is the most common form of clubfoot; the heel is inverted, the forefoot supinated, and the ankle plantar flexed. The second most common form is talipes calcaneovalgus, in which the foot is everted and the ankle dorsiflexed. This deformity is bilateral in approximately one half of cases. Incidence is about 1 to 2/1000 newborns, and the disorder affects twice as many males as females. Some 20 percent of infants with clubfoot die because of severe associated congenital malformations, particularly spina bifida cystica. An additional 10 to 20 percent have associated malformations compatible with life. Isolated clubfoot has a recurrence risk in sibs of 2 percent (McIntosh et al., 1954).

5

Mental Retardation and Multiple Malformation Patterns

Parents who have had one mentally subnormal child, a child or stillborn infant with multiple anomalies, or a child with both mental retardation and anomalies often seek advice regarding the risk of having another similarly affected offspring. Unfortunately, a specific diagnosis frequently cannot be established.

GRADATIONS OF MENTAL RETARDATION

Intelligence shows a continuous gradation following a gaussian distribution that is skewed to the lower range. Such a distribution presumably results from the additive effect of many factors, environmental and genetic, each exerting a small influence. This is consistent with polygenic/multifactorial inheritance. There is no sharp distinction between mental retardation and normality. However, the following guidelines are generally used: mild retardation, IQ 75 to 50; moderate, IQ 49 to 35; severe, IQ 34 to 20; and profound, IQ 19 to 0. One percent of newborns are severely or profoundly retarded. However, many of these die in the early years of life, so that by age 7 years only 0.3 to 0.4 percent have an IQ less than 50 (Kirman, 1975; Laxova et al., 1977).

A different spectrum of etiologies may be responsible for mild and moderate retardation than for severe retardation. Many individuals with mild or moderate retardation may simply be those people who fall at the lower end of the gaussian curve. This concept is supported by observations that the intelligence quotients of the sibs of persons with mild to moderate retardation often fall between those of the affected sibs and that of the population mean. In addition, there is a strong familial and presumably genetic tendency for mild mental retardation. On the other hand, at the extreme lower end of the curve there are more individuals than would be expected on the basis of gaussian distribution (Penrose, 1963). This excess of individuals with severe mental retardation is presumably due to gene mutations, chromosome abnormalities,

and such major environmental etiologies as birth injuries and teratogens. In a significant number of retarded individuals, perhaps 40 percent, there is no readily apparent explanation.

MENTAL RETARDATION WITH OR WITHOUT MULTIPLE ANOMALIES

The relative frequencies of various causes for mental retardation have usually been deduced from surveys of mentally retarded individuals residing in institutions (Berg, 1963; Kaveggia et al., 1975; Laxova & Ridler, 1975; Penrose, 1938), attending outpatient clinics (Turner, 1975), or both (Laxova et al., 1977). Moreover, the diagnoses reflect the skills of the examiner and the sophistication of biochemical and chromosomal studies. Because of the lack of uniform criteria and ascertainment, the proportions cited below only represent estimates.

Chromosomal Abnormalities

Between 10 percent (Opitz, 1977) and 32 percent (Laxova et al., 1977) of severe mental retardation is due to chromosomal abnormalities. The difference in surveys can usually be explained by the method of ascertainment. Opitz (1977) studied Central Wisconsin Colony, an institution where the patients require medical and nursing care and have severe or profound mental retardation. Laxova et al. (1977) studied institutionalized mentally retarded children and children living in the community.

Of the 145 cases known to have chromosome abnormalities reported by Opitz (1977), 107 (74 percent) had Down syndrome, including one 46/47, +21 and three 46,t(D;G) translocations. Two individuals had trisomy 18, two had sex chromosomal aneuploidy, eight had 46,del(5p), and 26 had various structural chromosomal abnormalities. By contrast, Laxova et al. (1977) observed that 32 percent (47/146) of their sample had Down syndrome. In a survey of mentally retarded children attending special schools in New South Wales, Down syndrome was detected in 18 percent of all students (Turner, 1975). Although autosomal abnormalities like trisomy 21 and trisomy 18 cause severe or profound retardation, some of the more recently recognized deletion or duplication syndromes are often associated with moderate or even mild retardation. The latter are also characteristic of certain sex chromosome polysomies (e.g., 48,XXXY).

Thus, chromosomal abnormalities are frequently associated with mental retardation. Down syndrome is by far the most common, but other complements are detected not infrequently. In 8 to 10 percent of individuals with idiopathic mental retardation (IQ 75 or lower), three developmental anomalies, no known genetic explanation, and no history of external insult (e.g., encephalitis) a chromosome abnormality is likely the etiology (Summitt & Patau, 1964; Summitt, 1969; Daly, 1970; Doyle, 1976; Magnelli, 1976).

Table 5-1
Diagnostic Categories in Three Groups of Severely Retarded Children: Madison, Wisconsin (CWC), Hertfordshire (Herts), and Sydney, Australia (Aust).

Etiologic Group	CWC (%)	Herts (%)	Aust (%)
Chromosome			
Down syndrome	7.7	32.2	—
Other autosome	2.6	0.7	1.3
Sex chromosome	0.3	—	—
Total	10.6	32.9	1.0
Single gene			
Autosomal dominant	2.3	4.8	5.0
Autosomal recessive	7.5	4.8	11.0
X-linked recessive	2.5	4.8	9.0
Total	12.3	14.4	25.0
Other MCA/MR syndromes*	13.2	13.0	14.0
CNS defects	14.7	7.6	5.0
Cerebral palsy	20.9	4.1	14.0
Seizures	9.7	10.3	14.0
Mental retardation alone	4.0	12.3	0.0†
Environmental factors	13.0	4.1	18.0
Grand Total	98.4	98.7	65.0

Modified from data in Laxova et al. (1977).
*MCA/MR = Multiple congenital anomalies/mental retardation.
†Included in X-linked recessive.

Mendelian Mutations

Some 12 to 14 percent of cases of mental retardation are due to a single-gene mutation (Table 5-1) (Turner, 1975; Laxova et al., 1977; Opitz, 1977). Turner (1975) estimated that the incidence of Mendelian mutation may be as high as 25 percent: 5 percent autosomal dominant, 11 percent autosomal recessive, and 9 percent X-linked recessive.

Autosomal Dominant Disorders

Autosomal dominant disorders account for about 5 percent of severely mentally retarded individuals. Tuberous sclerosis, acrocephalosyndactyly (Apert syndrome), neurofibromatosis, and Waardenberg syndrome are among the more common traits. Most affected individuals—4 of 7 cases of tuberous sclerosis reported by Laxova et al. (1977) and 13 of 14 cases reported by Turner (1975)—represent new mutations. It is important in terms of counseling to identify new mutations, for the recurrence risk would be essentially zero in such cases. To determine if the condition is a new mutation, the affected person's sibs and parents must be carefully examined to exclude a minimally expressed form of the disease. De novo gene mutations are associated with older paternal age; thus, parental ages should be noted at the time of evaluation.

Autosomal Recessive Disorders

At least 5 percent of mentally retarded patients, and possibly more (Turner, 1975), have an autosomal recessive condition. Most inborn errors of metabolism are inherited in this fashion. Children with metabolic disorders often appear normal at birth, only to deteriorate thereafter (e.g., phenylketonuria, galactosemia, maple syrup urine disease, mucopolysaccharidoses, leucodystrophies, and lipidoses). In a few metabolic disorders, dietary treatment can alter outcome substantially. Other syndromes of unknown cause are considered autosomal recessive on the basis of multiple affected sibs or parental consanguinity. Therefore, it is important to obtain a pedigree and to examine all sibs in cases in which the cause for mental retardation is "unknown." Turner (1975) believes that the proportion of retarded patients with autosomal recessive conditions approximates 11 percent even though a definite diagnosis can be made in only 6 percent. Unless a specific diagnosis directs otherwise, however, chromosomal studies of parents and offspring are also indicated to exclude parental rearrangements (e.g., translocations) that could lead to multiple affected sibs.

X-Linked Conditions

X-linked recessive conditions possibly account for at least 3 to 5 percent of severely retarded children (Turner, 1975). Examples of X-linked conditions causing mental retardation include the Lesch-Nyhan syndrome, X-linked hydrocephalus (aqueductal stenosis), and so-called X-linked mental retardation. The last was formerly considered to be without associated malformations, and was termed Renpenning syndrome. However, the situation is now recognized to be more complicated. The importance of X-linked recessive mental retardation is emphasized by observations that there is an excess of males in almost all series of mentally retarded individuals, which is presumably accounted for by this disorder (Turner, 1975). In X-linked recessive mental retardation no biochemical error(s) has been elucidated; however, about 30 percent of cases are associated with a tendency for chromosomal fragility at band Xq27 (X fragile site) (Gerald, 1980; Sutherland et al., 1979). Affected males are distinguished from retarded males lacking the fragile site on the basis of coexisting normofunctional testicular hyperplasia and prominence of their ears and jaw. Some heterozygous females also show mental retardation (Turner et al., 1980), probably reflecting inactivation of the normal X in a high proportion of cells. It is helpful for counseling purposes to identify those mentally retarded males who possess the fragile X. The phenomenon can be reproducibly expressed in fibroblasts, making prenatal diagnosis possible.

In many X-linked disorders, affected male fetuses cannot be distinguished from unaffected ones. If such an X-linked recessive disorder is suspected, one should attempt to determine the likelihood that the mother is a heterozygote and, if so, offer antenatal chromosomal diagnosis to determine fetal sex.

Polygenic/Multifactorial Etiology

Among polygenic/multifactorial conditions characterized by mental retardation are abnormalities limited to the central nervous system—neural tube defects, hydrocephaly, and idiopathic epilepsy. Six to 15 percent of severely

mentally retarded children are presumed to have a primary CNS developmental defect (Laxova et al., 1977; Opitz, 1977) (see Chapter 4). In addition, several other recognizable syndromes—de Lange syndrome, Prader-Willi syndrome, Rubenstein-Taybi syndrome—have recurrence risks (2 to 5 percent) consistent with polygenic/multifactorial etiology; however, in each, subtle chromosomal abnormalities are suspected.

Teratogenic Etiology

Known environmental causes account for 4 to 13 percent of mentally retarded individuals. These causes include birth trauma, chemical teratogens, infectious teratogens (rubella, toxoplasmosis, cytomegalovirus), postnatal infections (meningitis, encephalitis), birth injuries, and postnatal nutritional or stimulatory deprivation. The recurrence risk should be negligible. The proportion of cases due to birth trauma and prenatal infections should decrease with improved prenatal and intrapartum care.

Genetic Counseling

The most important single prerequisite for genetic counseling is an accurate diagnosis. To accomplish this one must consider prenatal and intrapartum records, developmental history of the proband, and a thorough physical examination with particular attention to minor developmental defects. Cytogenetic studies are obligatory, and other studies are often necessary. In particular, an attempt should be made to exclude prenatal teratogens. Selected biochemical studies may be appropriate.

If a specific diagnosis is made, counseling is based upon genetic etiology or empiric data, if applicable. If no diagnosis is possible, the birth of a child with mental retardation, no physical anomalies, no neurologic symptomatology, and no inborn error of metabolism (pure mental retardation) carries a high recurrence risk for sibs—14 percent (Becker et al., 1977) to 19 percent (Bartley & Hall, 1978). Such a high recurrence risk suggests a relatively high proportion of recessive disorders. As noted previously, in X-linked recessive mental retardation cytogenetic studies should be undertaken to identify the "X-fragile site" that could provide the basis for genetic counseling. If the proband has both mental retardation and multiple congenital anomalies, the recurrence risk for sibs is lower, perhaps 4 percent (Bartley & Hall, 1978). If microcephaly or abnormal facial contours coexist with mental retardation, the recurrence risk for sibs is 6 to 11 percent (Bartley & Hall, 1978).

MULTIPLE ANOMALIES WITHOUT MENTAL RETARDATION

Some individuals show unusual facial features and multiple anomalies but no mental retardation. Such a pattern could result from a mutant gene, but is not likely from a chromosomal aberration. For the clinician the significance is that parents of an infant with multiple malformations should not be counseled

to expect their child to show mental retardation. Thorough diagnostic evaluation is thus always obligatory. In a small series of 27 probands with multiple anomalies but normal intelligence, Bartley and Hall (1978) recorded no similarly affected sibs. Thus, empiric recurrence risk is low. However, dysmorphic syndromes not associated with mental retardation can result from mutant genes.

GENETIC COUNSELING FOLLOWING STILLBORN INFANTS WITH MULTIPLE MALFORMATIONS

Not infrequently parents seek genetic counseling following stillbirth or neonatal death. Malformations may or may not have been obvious. Unfortunately, a diagnosis is often impossible because no adequate description of the proband exists. If an autopsy was not performed, the counselor must rely on the memory of the couple and their physician concerning the infant's appearance. In our opinion, almost the only reliable diagnosis obtained in this fashion is anencephaly. If an accurate diagnosis cannot be obtained, counseling is obviously hazardous. To avoid such a dilemma, photography, full-body X-rays, autopsy, and chromosomal studies should ideally be performed on all stillborn infants. Such results would greatly facilitate counseling prior to the next pregnancy.

6

Prenatal Genetic Diagnosis

Recent advances in prenatal and neonatal care have improved perinatal mortality rates, and consequently birth defects represent a larger component of the residual mortality rate. Simultaneously, our ability to obtain information about the fetus has increased dramatically. This combination has produced great interest in the prenatal diagnosis of genetic defects. These techniques have made it possible, in many instances, to convert genetic counseling from an essentially passive endeavor to one in which the counselors and parents, in concert, can take steps to alter the genetic risks to which the offspring of the prospective parents are exposed (Epstein, 1974). As the name implies, the objective of prenatal diagnosis is to determine whether a fetus at risk for some genetic disease is or is not actually affected.

The test results are negative in more than 95 percent of cases (see Table 6-1), and in these cases some pregnancies that might have been terminated in the past because the fetus was at risk of being abnormal may now be completed. On the other hand, a positive diagnosis allows the expectant parents to choose their subsequent course of action: aborting the fetus, treatment of the fetus (in the few cases where this is possible), continuing the pregnancy and treating the neonate, or continuing the pregnancy and having the neonate adopted or institutionalized. This decision ultimately is the right and responsibility of the prospective parents and will be influenced by their social and ethical concepts. Although the ultimate goal of prenatal diagnosis is the treatment and care of the genetically ill fetus, this goal is still distant. In the interim, difficult decisions will have to be made by couples who find they have conceived a genetically abnormal fetus.

Prenatal diagnosis usually relies upon the cytogenetic or biochemical analysis of cultured amniotic fluid cells. Amniocentesis was introduced initially in the 1930s, and by 1950 it was being utilized for the management of erythroblastosis fetalis (Bevis, 1950). The technique gained widespread acceptance for studying Rh isoimmunization, since it caused minimal maternal or fetal morbidity. Fuchs and Riis (1956) demonstrated the prenatal diagnosis of fetal

Table 6-1
Cumulative Experience of Prenatal Detection Centers

Indication	Pregnancies Monitored (No.)	"Affected" Fetuses (No.)	"Abnormal" Fetuses (%)
Chromosomal abnormalities			
Translocation carriers	370	37	10
Mother 35–39 yr*	5301	79	1.5
Mother 35 yr or more*	3012	79	2.6
Mother 40 yr or more*	2796	116	4.2
Previous trisomy 21	2583	30	1.2
Miscellaneous	2446	44	1.8
X-linked diseases	527	235†	44.6
Biochemical defects	1032	255	24.7
Neural tube defects (NTD)	4344	233‡	4.0§
Total	22,411	1098	4.9

*The pregnancies are divided into these three groups because of the methods of reporting employed by different centers.
†Includes one trisomy 21 fetus.
‡Includes 14 fetuses with NTD found at routine screening of fluid drawn for another genetic indication, 3 fetuses with NTD when the indication was family history of NTD, and 66 fetuses with NTD when the indication was an elevated maternal serum AFP level.
§Indication was a first-degree relative with a NTD.

sex by examination of X-chromatin bodies in amniotic fluid cells. The ability to culture amniotic fluid cells in tissue culture and acquire sufficient viable cells for karyotype analysis and biochemical studies was demonstrated in 1966 (Steele & Breg).

Amniocentesis must be preceded by the careful recording of a family pedigree and appropriate genetic counseling. The counselor should be able to verify the diagnosis of previously affected relatives, determine the applicable genetic facts, and be able to recognize and deal with the psychosocial implications of the material with which he or she is dealing. The genetic counseling should include discussion of the risks of having a genetically defective fetus, the dangers of amniocentesis, and any reservations about the results that may be obtained. The father should be urged to be present for the counseling session and to take part in the decision of whether or not to have amniocentesis performed.

INDICATIONS FOR AND RESULTS OF PRENATAL DIAGNOSIS

Chromosome Disorders

The incidence of chromosome aneuploidy varies with the population selected for study. A number of surveys have been performed on unselected neonates, with the cumulative finding of 326 major chromosomal abnormalities

among 54,749 newborns (0.59 percent) (Jacobs, 1977) (see Table 3-1). The incidence rises to 2.2 percent if the population considered is full-term, growth-retarded infants (Chen & Falek, 1974), to 5.8 percent in cases of perinatal death (Machin, 1974; Sutherland, Bauld, & Bain, 1974), and to 32.2 percent of children studied because of a suspected cytogenetic abnormality (Mulcahy & Jenkyn, 1972). Obviously, the size of this last figure will depend upon the degree of suspicion required to order a karyotype at different institutions.

The most common indication for prenatal diagnosis is advanced maternal age. The relationship between the incidence of the Down syndrome and maternal age (see Table 3-2) was noted long before the chromosomal etiology of the syndrome was known (Penrose, 1933). The risk of having a newborn with other chromosomal trisomies (trisomy 13, trisomy 18, 47,XXY, 47,XXX, or 47,XYY) also rises with increasing maternal age, but to a lesser degree (Smith, Patau, & Therman, 1961; Smith, 1964). These increases are all relative, however, and the risk never becomes great in absolute terms. There also is no dividing line above which maternal age should be considered "advanced," but most centers use 35 or 37 years as their criterion of "advanced." Over 11,000 pregnancies have been monitored on account of maternal age as tabulated by the authors; 2.47 percent of the fetuses were found to be aneuploid. Golbus et al. (1979a) found the age-specific risk of fetal trisomy rose from 0.9 percent for mothers aged 35–36 to 7.8 percent for mothers aged 43–44 (Fig. 6-1). These figures are approximately twice the reported maternal age-specific incidence of neonatal trisomy (Hook & Chambers, 1977). The difference appears to be due to late abortions and stillbirths of aneuploid fetuses (Hook, 1978a, 1978b).

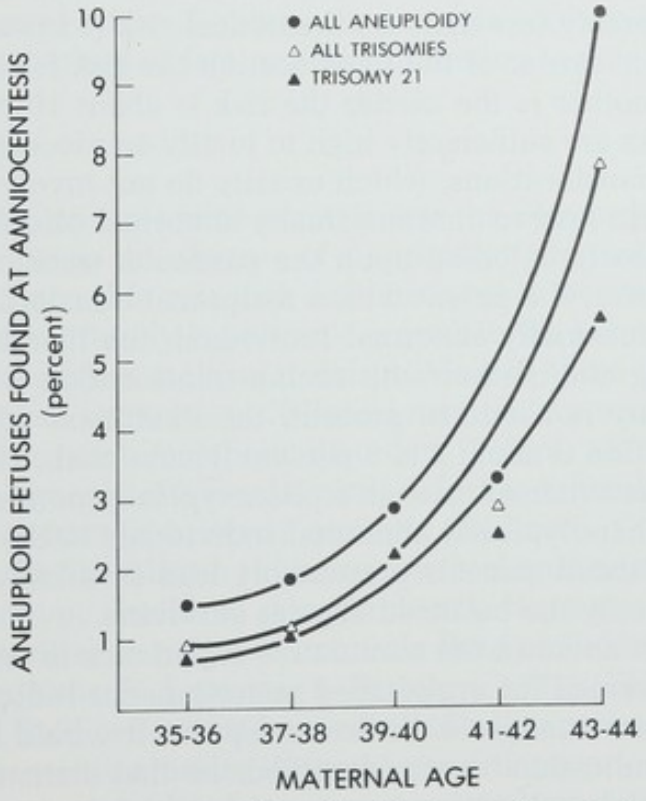


Figure 6-1. Age-specific risk that amniocentesis will demonstrate an aneuploid fetus.

Women who previously have borne a trisomic child may be at an increased risk for having another. Mikkelson and Stene (1970) found a 1- to 2-percent recurrence risk, irrespective of maternal age. The prenatal detection program data on recurrent trisomy 21 usually are not reported according to maternal age, but available data suggest the recurrence risk is approximately 0.5 percent for women ≤35 years old and is equal to the age-specific risk for women >35 years old. Parents who have had one trisomic child manifest great anxiety about fetal well-being, and in these cases it has been considered appropriate to provide prenatal diagnosis in subsequent pregnancies. The cumulative experience has been that 1.2 percent of these at-risk fetuses are chromosomally abnormal (Table 6-1). This, of course, includes the abnormalities expected in any random population.

The greatest risk of producing a chromosomally abnormal fetus exists for those individuals who are carriers of a balanced translocation. The magnitude of the risk is different for each specific translocation. While there is presumably an equal chance of a monosomic or trisomic fetus, monosomy does not have the same clinical significance, because a monosomic fetus is extremely unlikely to be carried to the stage of viability. The risk of producing an abnormal child is greater if the female rather than the male is the translocation carrier (Hamerton, 1970). In this heterogeneous group as a whole, 37 of the 370 fetuses studied showed chromosomal imbalance.

Specific empiric risk data may not be available for a specific translocation. Figures are, however, available for the most common translocations requiring antenatal studies. About 25 to 45 percent of individuals who have the Down syndrome as result of a 14/21 translocation have a parent with the same translocation chromosome. The actual (empiric) risk that a parent carrying a 14/21 (or 13/21, 15/21, or 21/22) translocation will have a child with the Down syndrome is considerably less than the theoretical risk (33 percent). Specifically, if the father is the carrier of the translocation the risk is only 2 to 3 percent, whereas if the mother is the carrier the risk is about 10 percent (Hamerton, 1971). These risks are sufficiently high to justify amniocentesis.

Reciprocal translocations, which usually do not involve acrocentric chromosomes, can also lead to chromosomally abnormal offspring. Genetic counseling should ideally be based upon the particular translocation present. To generalize, however, if a parent with a reciprocal translocation is ascertained through a chromosomally abnormal individual, the likelihood that any subsequent offspring of a mother with such a translocation will have a chromosomal abnormality is about 10 percent; the likelihood when a father has a similar translocation is about 2 to 3 percent (Jacobs et al., 1975). If a reciprocal translocation is ascertained through a phenotypically normal individual, there are usually no phenotypically abnormal individuals in the kindred (Jacobs et al., 1975); unbalanced gametes presumably lead invariably to spontaneously aborted fetuses, only the balanced zygotes surviving.

One problem noted in the cumulative experience is that of the many amniocenteses performed for unspecified miscellaneous indications, 1.8 percent of the fetuses were aneuploid. In future reports, it would be beneficial if the miscellaneous indications were identified, so that statistics could be accumulated as to which indications increase the risk of an abnormal fetus. Golbus

et al. (1979a) reported two trisomy 18 fetuses among pregnancies tested because of severe intrauterine growth retardation in the second trimester, one triploid fetus when the indication was a prior child with Turner syndrome, and one trisomy 21 fetus when the indication was hydramnios. Simpson (1980c) has reviewed additional possible indications for antenatal chromosomal studies.

X-Linked Disorders

Another chromosomal indication for prenatal diagnosis is determination of fetal sex for a woman known or suspected to be a heterozygote for a deleterious X-linked gene. Currently, an affected male fetus can be distinguished from an unaffected one by study of the amniotic fluid cells only for the Fabry syndrome, the Hunter syndrome, the Menke syndrome, and the Lesch-Nyhan syndrome, all of which are enzymatically defined. For all other X-linked disorders the parents should understand that there is, at most, a 50 percent chance that any male fetus identified prenatally actually will have the disorder in question.

For X-linked disorders in which the affected males do not reproduce, one third of the affected individuals represent new mutations, and the mother is not a heterozygote for the trait. Therefore, she is not at increased risk having future sons with the disorder. In some instances the mother's carrier status is demonstrable by pedigree analysis (e.g., her father is affected, she has an affected son and an affected brother, or she has two affected sons). If the pedigree is not conclusive, an attempt should be made to clarify whether or not the mother is a carrier of the deleterious trait.

The results in Table 6-1 indicate that close to the expected 50 percent of fetuses monitored for X-linked disorders were male. One error in fetal sex determination occurred when X-chromatin analysis was used for fetal sexing (Fuchs, 1966). This method of fetal sex determination is now discouraged.

Metabolic Disorders

A significant step in molecular genetics was the demonstration that a disease state could be due to the absence of a normally functioning enzymatic or structural protein. Since this was first demonstrated to be true for humans in 1952, there has been a virtually logarithmic growth in the number of conditions found to result from such a molecular defect. Most of these disorders are inherited in an autosomal recessive or X-linked manner. Approximately 0.8 percent of newborns have such metabolic disorders (Polani, 1973).

The prenatal diagnosis of a biochemical defect is generally performed by assay of enzyme activities in cultured amniotic fluid cells. Cultured amniotic fluid cells are usually fibroblastic in their growth characteristics and express a biochemical phenotype similar to skin fibroblasts. However, the quantitative enzyme specific activities in cultured amniotic fluid and skin fibroblasts may differ considerably (Kaback, Leonard, & Parmley, 1971). Therefore, enzyme studies of amniotic fluid cells must be controlled using normal amniotic fluid cells grown at the same time under the same conditions.

Prenatal diagnoses also have been performed utilizing amniotic fluid per

se or uncultured amniotic fluid cells. Amniotic fluid may be used to diagnose defects in transport or metabolism of amino acids (Goodman et al., 1973) defects in which deficient enzyme is normally present in the fluid, such as Tay-Sachs disease (Friedland et al., 1971) or defects in which unused metabolites accumulate in the fluid, such as the adrenogenital syndrome (Milunsky & Tulchinsky, 1977). However, testing amniotic fluid involves difficulties because maternal blood contamination may alter results, the changing protein content of the fluid makes interpretation difficult, and the unknown qualitative and quantitative contribution of fetal urine to the fluid further obscures the results. Uncultured amniotic fluid cells possess detectable levels of most enzymes found in cultured cells (Nadler & Gerbie, 1969). However, variation in amniotic fluid cell number and viability makes the use of uncultured cells unreliable and led to the abortion of a normal fetus with the misdiagnosis of Tay-Sachs disease (Rattazzi & Davidson, 1970).

The Appendix at the end of this chapter (p 118) lists approximately 40 hereditary biochemical disorders that have been detected in utero and another 35 that can probably be diagnosed in utero. Over 1000 pregnancies have been monitored, at centers throughout the world, for an inborn error of metabolism, with close to the expected 25 percent yielding positive results. More than half the fetuses tested were at risk for a hemoglobinopathy; Tay-Sachs disease, Pompe disease, and the mucopolysaccharidoses were the next most common indications. The programs of mass screening for heterozygote detection now being conducted will increase the demand for prenatal diagnosis of certain rare biochemical defects.

A limiting factor in the diagnosis of hereditary enzyme defects by conventional techniques is the length of time (up to 4 to 8 weeks) required to grow sufficient cells for enzyme analysis (Galjaard et al., 1972). More sensitive techniques for enzyme analysis, developed by Galjaard and collaborators (1974) and by Hösli (1974), are based on the measurements of enzyme activity in small numbers of cells using quantitative microspectrophotometry or microfluorometry. Assays of virtually all the clinically important lysosomal enzymes now are feasible with these techniques.

A more recent development in biochemical prenatal diagnosis is based on the tenet that a hereditary defect identified at a DNA level can be recognized in any cells of the affected individual. This has been demonstrated for α-thalassemia, a disorder produced by absence of all four α-chain genes (alleles). Using molecular hybridization techniques, it has thus been possible to detect α-thalassemia-1 (two genes deleted), hemoglobin H disease (three genes deleted), and α-thalassemia (four genes deleted) by analysis of DNA derived from amniotic fluid cells (Kan, et al., 1975a; Kan, Golbus, & Dozy, 1976). This approach should be applicable to any genetic disorder in which the defect results from the deletion of a gene for which the specific messenger RNA can be obtained or synthesized. Although these requirements have been met thus far for only hemoglobin-related disorders, the recent explosion of molecular genetic research suggests that other disorders soon will be diagnosable by these techniques. The newest development in this area has come from the utilization of restriction endonucleases. These bacterial enzymes recognize specific sequences of four to six nucleic acids and cleave the DNA at these specific sites.

There is an ever-increasing repertoire of restriction endonucleases, each specific for a particular short DNA sequence. The DNA fragments can be separated by agarose gel electrophoresis, and the number and size of the fragments allows genome mapping. Kan and Dozy (1978) found that close to 70 percent of American blacks heterozygous for the β^S globin gene have a second mutant approximately 5000 nucleotides further along the DNA. This second mutant, recognizable utilizing the endonuclease technique, signals the presence of the β^S gene. The DNA of a fetus homozygous for β^S demonstrates a specific pattern of DNA fragments after endonuclease treatment. Since the DNA is identical in all cells, amniotic fluid cells can be used for prenatal diagnosis in approximately 80 percent of couples with the sickle cell trait. Prenatal diagnosis utilizing this technique has recognized AA (homozygous normal), AS (sickle trait), and SS (sickle cell anemia) fetuses correctly (Kan & Golbus, unpublished data). In addition, a new restriction endonuclease (DdeI) cuts the DNA at the site of the β^S mutation and makes all cases at risk for sickle cell anemia diagnosable using amniotic fluid cells (Chang & Kan, 1981).

Neural Tube Defects

The technique of prenatal diagnosis of neural tube defects has relied upon measurement of alpha-fetoprotein (AFP) in the amniotic fluid. AFP, the major protein of fetal serum, is a glycoprotein that resembles albumin in molecular weight, amino acid sequence, and immunologic characteristics (Ruoslahti & Terry, 1976). The AFP concentration in fetal serum reaches a peak between the 13th and 15th menstrual weeks, when it measures 3 mg/ml (Gitlin & Boesman, 1966). AFP concentrations in amniotic fluid follow a curve similar to that of fetal serum, but at a 100-fold dilution. Brock and Sutcliffe (1972) demonstrated that the amniotic fluid AFP level could be used to identify fetuses with neural tube defects. Amniotic fluid AFP also has been found to be elevated in some cases of congenital Finnish nephrosis, omphalocele, Turner syndrome, gastrointestinal tract obstruction, sacrococcygeal teratoma, and missed abortion. In addition, the presence of fetal blood in the amniotic fluid may elevate AFP levels. An elevated amniotic fluid AFP is, therefore, not specific for neural tube defects; however, it usually indicates fetal abnormalities unless there is fetal blood contamination.

The cumulative experience of prenatal diagnoses for neural tube defects consists of 150 (4.02 percent) affected fetuses among the 3735 pregnancies monitored because of a prior affected offspring, 66 (21.1 percent) affected fetuses among the 313 pregnancies tested because of a raised maternal serum AFP level, and 3 (1.0 percent) affected fetuses among the 296 pregnancies monitored because of a family history of a neural tube defect. The figures vary in different geographic locations because the incidence of neural tube defects varies by locale.

It has been the practice at both the University of California at San Francisco (UCSF) and Northwestern University to obtain both a karyotype and an amniotic fluid AFP level regardless of the genetic indication for the amniocentesis. At UCSF five pregnancies tested for advanced maternal age (0.15 percent) had abnormally high AFP concentrations, which were found to be due to neural

tube defects. It is very important that, prior to performing the test, patients understand that not all neural tube defects are diagnosable, and that elevated AFP levels may occur on a statistical basis (false positives) or represent an anomaly other than a neural tube defect. One suggested routine for keeping false positives to a minimum after finding an elevated AFP level is to perform a second amniocentesis and a detailed sonographic examination of the fetus. Amniography and fetoscopy have been recommended as additional investigative methods, but their utility will depend upon the level of expertise available locally. A better mechanism for investigating positive tests would be a second independent variable. It appears likely that amniotic fluid acetylcholinesterase may serve this purpose, confirming "true" positive amniotic fluid AFP elevations. Another approach utilizes observations of an increased concentration of rapidly adhering cells in the amniotic fluid of fetuses with a neural tube defect (Gosden & Brock, 1977). However, this method of verification is cumbersome and cannot be applied to samples transported even a short distance; thus, it has only limited applicability.

Of individuals with a neural tube defect, 90 percent are the first affected individual in the family; therefore, even if amniocentesis were employed by all families with a previous child with a neural tube defect, the disease incidence would be lowered by only 10 percent. Thus, there is great interest in a diagnostic test based on the maternal serum AFP concentration because all pregnancies could be screened. After it was demonstrated that neural tube defects could be identified from maternal serum screening, a collaborative study was initiated in the United Kingdom (1977). Over 18,000 singleton and 150 twin pregnancies were monitored at 16 to 18 menstrual weeks, and maternal serum AFP levels 2.5 times the median were considered abnormal. The overall detection rate of approximately 80 percent suggests that the number of neonates with neural tube defects could be more than halved with widespread application of maternal serum screening. Similar data are available from an American study (Macri, 1978). Nearly 4.5 percent of the sera tested had levels at or greater than the cut-off and required a repeat serum assay and an ultrasound examination. The second serum level was normal or the sonogram identified twins or a missed abortion in somewhat over one half of these cases, so that approximately 2 percent of the women required amniocentesis for assay of amniotic fluid AFP. In the United States, 5 to 10 percent of these amniocenteses will identify neural tube defects.

Some problems anticipated in serum screening programs include (1) anxiety in women who have an elevated AFP level for a physiologic reason, e.g., twins or misdating; (2) trauma of infants with neural tube defects born to late registrants or women electing not to have the serum test; (3) trauma of infants with neural tube defects whose mothers had false-negative serum AFP values; (4) trauma of infants with chromosome abnormalities whose mothers have had amniocentesis (if karyotypes are not performed for logistic reasons); and (5) spontaneous abortion following amniocentesis. With regard to this last issue it is noteworthy that an increased abortion rate following amniocentesis for an increased maternal serum AFP level has been reported (Bennett et al., 1978). It would appear appropriate to initiate a public and physician education program to deal with some of the above anticipated problems.

PROBLEMS ASSOCIATED WITH AMNIOCENTESIS

Somewhat more than 1 percent of pregnancies presenting for prenatal diagnosis will be multiple gestations. If an assay is performed in the case of unidentified multiple gestation, fluid will be withdrawn from only one amniotic sac and the status of a fraternal twin will not be ascertained. Fluid samples almost always can be obtained from both sacs of a twin gestation (Golbus et al., 1979a; Elias et al., 1980b). It may be possible to cause the death of an affected twin while avoiding abortion of the unaffected cotwin (Aberg et al., 1978). In view of this, routine ultrasonography prior to amniocentesis for genetic diagnosis appears advisable. Ultrasonography also will allow recognition of missed abortions and identify misdated pregnancies. Unfortunately, even in experienced hands ultrasonography does not lower the incidence of multiple needle insertions or influence the frequency with which blood is detected in these specimens (Karp et al., 1977; Hohler et al., 1978; Levine et al., 1978).

The amniocentesis may need to be repeated because of failure to obtain sufficient amniotic fluid or because of failure of cell growth. Large collaborative studies have reported that approximately 10 percent of the cases required repeat amniocentesis (NICHD, 1976; N. Simpson et al., 1976). The ability to obtain amniotic fluid is related to the experience of the operator and the gestational week in which amniocentesis is done; if it is performed at 16 weeks, failure to obtain amniotic fluid can be reduced to less than 1 percent, and culture failure can be reduced to 2 percent or less (Golbus et al., 1979a).

One pitfall in the interpretation of chromosomal results is mosaicism. Many cases of reported mosaicism have involved hypo- or hyperdiploidy in only a few cultured amniotic fluid cells and probably represent random loss or gain of chromosomes. However, several well-documented cases of prenatally diagnosed mosaicism do exist (Bloom et al., 1974; Sutherland, Bowser-Riley, & Bain, 1975; Milunsky & Atkins, 1977; Golbus et al., 1979a). The best techniques for making such a diagnosis are in vitro karyotyping of cultured clones (Cox et al., 1974) and harvesting parallel cultures. A single trisomic cell is not unusual, but trisomy for the same chromosome in more than one culture flask suggests true mosaicism.

Probably the most important technical problem is obtaining and communicating correct results. Parents must be counseled that prenatal diagnosis is not infallible and that the results obtained may not reflect the actual fetal status. A number of inconsequential errors due to culturing maternal cells or clerical mistakes in communicating results have been reported, but more serious false-positive and false-negative karyotyping and biochemical errors have also been made (Golbus, 1976). Most of these errors occurred early in the cumulative experience and are avoidable with current methodology. Golbus et al. (1979a) noted the benefit of experience in that their error rate halved after their first 1000 cases. Even if the karyotype is correct, its clinical significance for the fetus is not always known. Two common examples are 47,XYY and 47,XXX karyotypes, which are neither "normal" nor necessarily associated with dysmorphic features and severe mental retardation. No good measure of the risk of a 47,XYY male being socially deviant or of a 47,XXX female having gamete nondisjunction or mental retardation exists.

The cumulative experience with spontaneous abortion following early amniocentesis is disparate. The National Institute of Child Health and Human Development collaborative study (NICHD, 1976) found an age-adjusted fetal loss rate of 3.3 percent in women who had undergone amniocentesis and 3.4 percent in controls. The Canadian collaborative study also showed no increased risk of spontaneous abortion following amniocentesis (N. E. Simpson et al., 1976). However, the British collaborative study indicated a 1.5 percent increase in the rate of fetal loss (Medical Research Council Working Party on Amniocentesis, 1978). The problem with this study is that "a few matched controls had aborted early and had been replaced," and "matched controls were not selected until at a later gestation than the subjects at the time of amniocentesis, so that some potentially acceptable controls might have aborted before they had the opportunity to be selected." A second problem is that the indications were different from those of other studies. Of all amniocenteses, 10 percent were performed because of previous offspring with neural tube defect, and 30 percent were performed after detection of elevated maternal serum AFP. These biases would produce a relative deficit of abortions among the controls and an apparent excess of abortions in the subjects, which possibly explains why this study found a significant increase in the number of abortions following amniocentesis and suggests that this conclusion is suspect. The overall increased abortion rate following amniocentesis is considered to be about 0.5 percent by most US investigators.

One of the greatest risks of prenatal diagnosis is that the fetus may be found to be abnormal. The parents should consider this in order to evaluate their options, and this possibility should be discussed during the counseling session. Selective abortion for a genetic indication induces feelings of guilt and depression that must be resolved (Blumberg, Golbus, & Hanson, 1975). Despite the emotional trauma of the process, most families said they would repeat their course of action and consider abortion preferable to the birth of a defective child.

FETAL VISUALIZATION

Many Mendelian disorders and most multifactorially inherited congenital malformations do not demonstrate biochemical or chromosomal abnormalities. Prenatal diagnosis of such entities has been attempted by either direct fetal visualization or indirect visualization methods, including sonography and radiography.

With the introduction of gray-scale and real-time sonography, a number of congenital anomalies have already been prenatally diagnosed (Hobbins et al., 1979) (Fig. 6-2), including anencephaly (Campbell et al., 1972), myelomeningocele (Campbell et al., 1975), omphalocele (Roberts, 1978), polycystic kidneys (Bartley et al., 1977), hydrocephalus (Lee & Warren, 1977), and duodenal atresia (Loveday, Barr, & Aitken, 1975) (Appendix, p 118). Hydrocephalus may be diagnosed by measuring ventricular size, which may be abnormally large even in the presence of a normal biparietal diameter (Filly & Golbus, unpublished data). It also is possible to determine fetal femur and humerus length with real-time ultrasound. This technique has been used to follow the

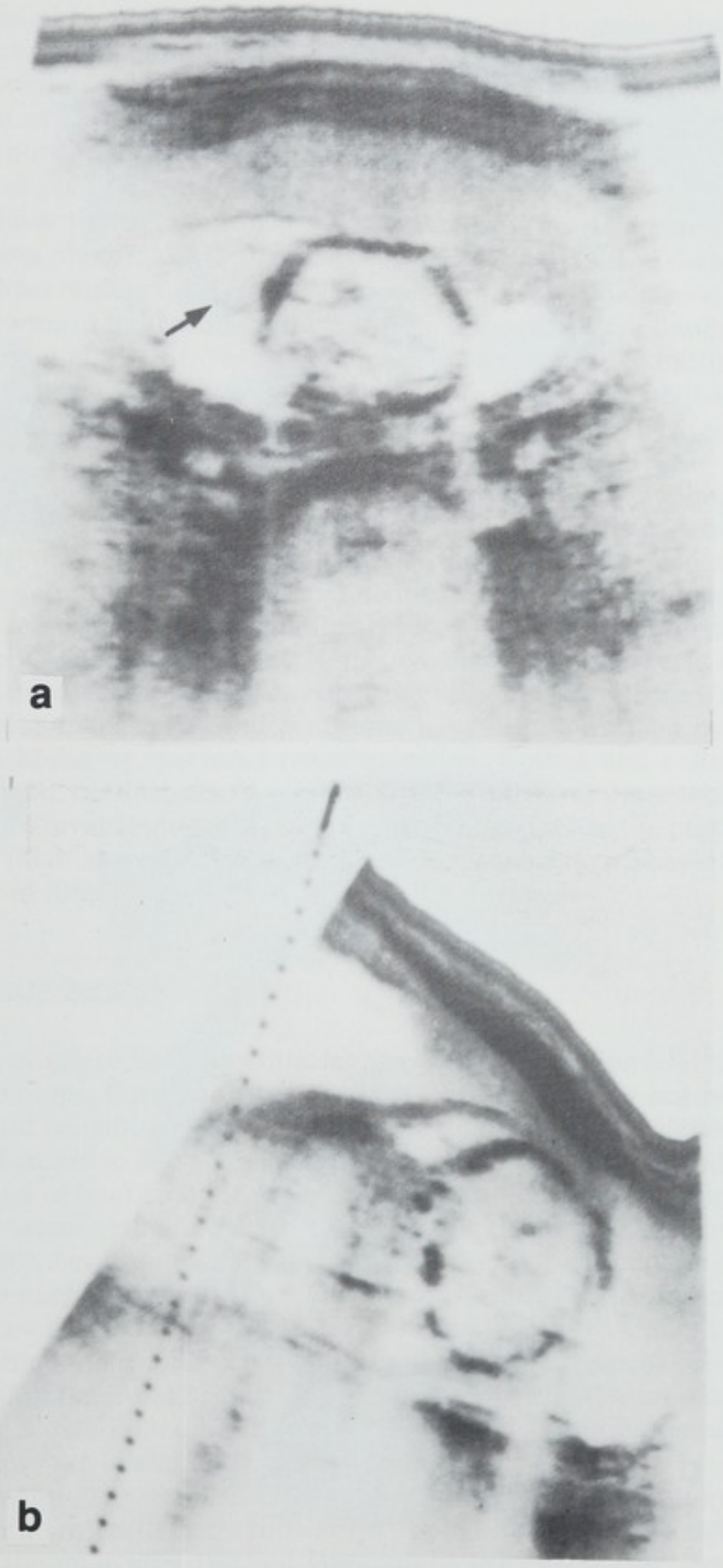
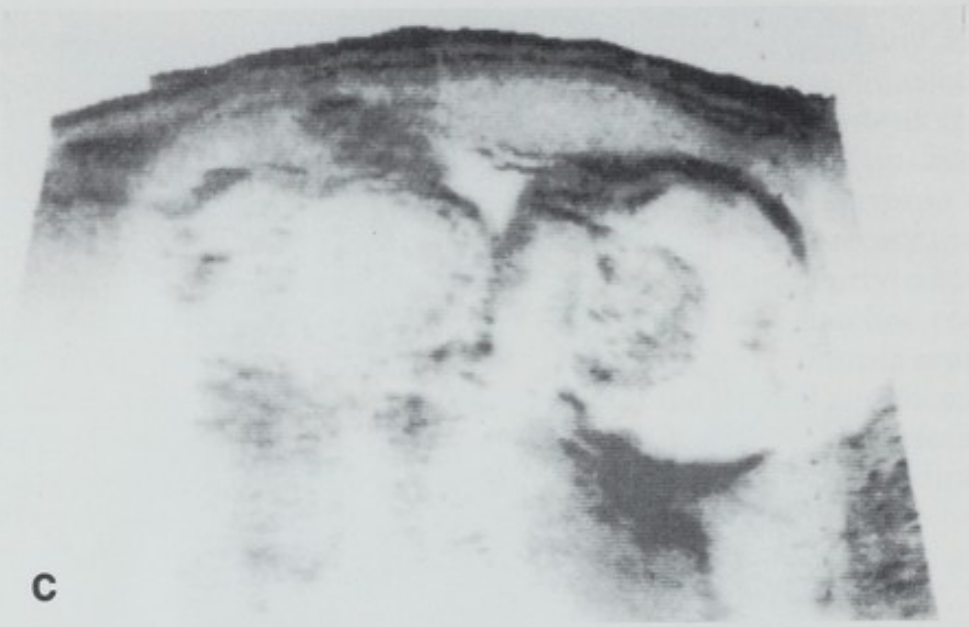


Figure 6-2. See legend p 112.



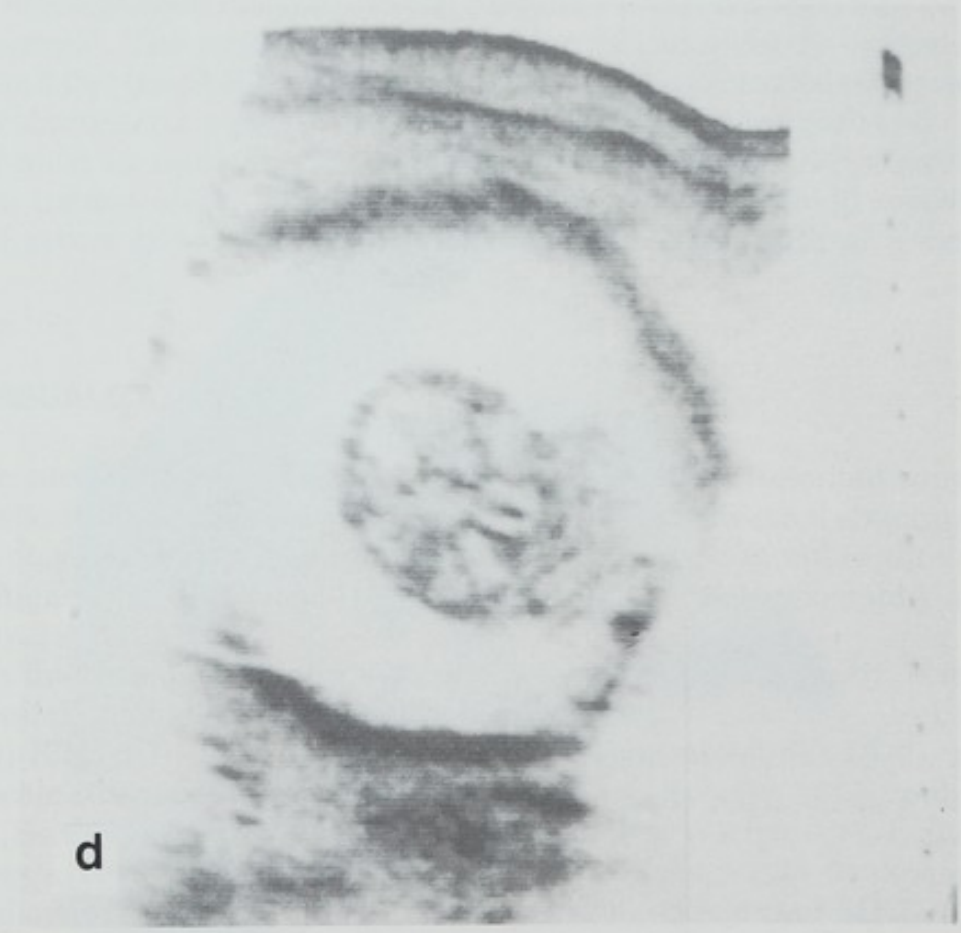


Figure 6-2. Sonographically demonstrated anomalies. (a) Occipital encephalocele. (b) Cystic hygroma. (c,d) Hydranencephaly.

development of two fetuses with achondroplasia and to prenatally diagnose a recessive form of metatropic dwarfism (Filly et al., 1974).

Direct radiography has been used to diagnose Saldino-Noonan dwarfism (Richardson et al., 1977) and to demonstrate fetal radii to establish that the fetus was not affected with the thrombocytopenia-absent radii syndrome (Omenn et al., 1973). However, at 20 weeks of gestation, x-rays of a number of achondroplastic fetuses and of a fetus with infantile osteopetrosis failed to show existing abnormalities (Golbus & Hall, 1974; Golbus, Koerper, & Hall, 1976). Contrast radiography may use a water-soluble dye to demonstrate either fetal swallowing or its absence in the presence of esophageal or duodenal atresia (Duenhoelter et al., 1976). This technique also can be used to outline the fetal silhouette to detect an abnormal mass or short-limbed dwarfism (Golbus et al., 1977). The use of both direct and contrast radiography will probably be superseded by the development of better sonographic techniques.

Direct fetal visualization utilizes a small-bore fiberoptic endoscope under local anaesthesia. The endoscope, which contains a solid lens surrounded by fiber bundles transmitting the light, is 1.7 mm in diameter and fits into an introducing cannula with an external diameter of 2.2 mm. Although isolated parts of the fetal anatomy are sporadically identified, visualization of the entire fetus is rarely accomplished, because the focal length is only 1 to 2 cm. Nevertheless, six pregnancies at risk for either the Ellis-van Creveld syndrome or an autosomal dominant split-hand syndrome have been examined by fetoscopy (Hobbins & Mahoney, personal communication; Golbus and colleagues, unpublished data). On the basis of polydactyly, one fetus was diagnosed as having the Ellis-van Creveld syndrome, and this was verified when the pregnancy was terminated (Mahoney & Hobbins, 1977). There probably will be a continued limited use of direct visualization for prenatal diagnosis.

FETAL TISSUE BIOPSY

The major use of fetoscopy thus far has been for sampling fetal tissue other than the amniotic fluid constituents. Many genetic defects not reflected in amniotic fluid constituents are demonstrable using other fetal tissues or cells, and most attempts to sample fetal tissues have involved fetal blood. The preferred method of fetal blood sampling is by aspiration of an umbilical cord or a placental vessel visualized with the fetoscope (Hobbins & Mahoney, 1975; Rodeck, 1980). For the rare case in which the fetoscope cannot be employed, the placenta may be sampled by sonographically directed aspiration through a 20-gauge needle (Kan et al., 1974b; Golbus, Kan, & Naglich-Craig, 1976). Both of these methods may produce a mixture of fetal and maternal blood. Since fetal red cell volume is greater than that of adult cells, the presence of fetal erythrocytes is determined by analysis of cell volume by a Coulter particle cell analyzer (Kazazian et al., 1972).

The principle abnormalities that have come under investigation are those of hemoglobin structure and synthesis. Although the major hemoglobin synthesized by the fetus after the first few months in utero is HbF, composed of α and γ chains, the fetus also synthesizes β chains from 9 weeks on. Therefore,

it is possible to detect defects of hemoglobin synthesis (β-thalassemia) and hemoglobin structure (sickle cell anemia) that would affect the fetus after birth. Since the first reports of prenatal diagnosis of β-thalassemia (Kan et al., 1975b; Kan, Golbus, & Dozy, 1976b) and sickle cell anemia (Kan, Golbus, & Trecartin, 1976a), over 1500 pregnancies have been monitored by fetal blood sampling because of a fetal risk for a hemoglobinopathy. For these diagnoses only 10 μl of fetal blood is required and the presence of maternal cells is not an insurmountable problem. The overall globin synthetic rate of fetal cells is more than 500 times greater than that of maternal cells; thus, even though β-chain synthesis is approximately 50 percent of the total in maternal cells and only 5 percent of the total in fetal cells, the maternal cells do not make a large contribution to the β-chain radioactivity of the sample (Nathan, Alter, & Frigoletto, 1975). Placental samples containing a low proportion of fetal cells can be enriched for fetal cells by separation by differential agglutination using the antibody anti-i. As the i antigen is present only on fetal cells, concentration by differential agglutination will yield samples containing 75 to 100 percent fetal cells with no selective loss of fetal cells containing HbA (Kan et al., 1974a). The moderate loss of fetal cells that occurs during this procedure is well compensated by the enrichment. Umbilical cord sampling, which usually produces a pure fetal sample, makes these maneuvers unnecessary.

In the initial attempts significant fetal mortality was associated with the attempts to obtain fetal blood. However, as with many new methods, the earliest trials were associated with unforeseen difficulties, which have been prevented by changes in technique, and the safety of the procedure has improved with experience. Currently, the risk of fetal death and spontaneous abortion with fetoscopy and fetal blood sampling is approximately 5 percent in experienced hands (Mahoney & Hobbins, 1979). Fetal blood sampling should be performed after 18 weeks gestation, because, with increasing gestation length, a given blood loss represents a smaller proportion of the total fetal blood volume.

It is likely that fetal red cells will be useful for more than the analysis of hemoglobin. Several hereditary defects of erythrocyte enzymes or structural components result in hemolytic anemias. Such defects can, in theory, be diagnosed prenatally if fetal erythrocytes can be demonstrated to be similar to adult erythrocytes with regard to these constituents. Similarly, fetal erythrocytes may contain enzymes that are not present in amniotic fluid or amniotic fluid cells but that are representative of enzymes present in other tissues. An example of this is arginase, deficiency of which causes hyperargininemia associated with progressive neurologic deterioration. Although arginase deficiency in the liver is presumed to be the cause of the disorder, the enzyme can be assayed in fetal erythrocytes, thereby making possible the prenatal diagnosis of this disorder (Spector, Cederbaum, & Bernard, 1977).

If fetal red cells are obtained by placental sampling, it follows that the other constituents of fetal blood are being obtained simultaneously. The first attempt to capitalize on this was the measurement of fetal plasma creatine phosphokinase (CPK) as a marker for Duchenne muscular dystrophy. This X-linked condition is associated postnatally with very high levels of serum CPK, presumably because of leakage of the enzyme from diseased muscle (Zundel & Tyler, 1965). The possibility that the pathologic process began early enough

in gestation to cause a raised fetal plasma CPK level led to attempts to prenatally diagnose this condition (Mahoney et al., 1977). Twenty-four male fetuses at risk for Duchenne muscular dystrophy were studied; there were three false-negative diagnoses and one probably correct positive diagnosis (Golbus et al., 1979b). This indicates that not all affected fetuses are prenatally diagnosable using this technique; thus, fetal CPK levels should not influence a decision whether or not to continue a given pregnancy.

A number of genetically important serum proteins are synthesized early in development (Gitlin & Biasucci, 1969). Of particular note, since prenatal diagnosis is frequently requested, is factor VIII, the clotting factor that is defective in hemophilia A. This factor can be detected both immunologically and by an activity assay in fetuses as young as 14 to 16 gestational weeks (Holmberg et al., 1974). Prenatal diagnosis of a number of fetuses affected with hemophilia A has been accomplished, and if the reliability of this diagnosis remains high, there will be an increased demand for this procedure (Firshein et al., 1979). Other proteins of genetic note that occur in fetal serum include immunoglobulins, C'1 esterase inhibitor (which is defective or missing in hereditary angioneurotic edema), ceruloplasmin (decreased in Wilson disease), α_1-antitrypsin (defective or absent in certain hereditary forms of chronic emphysema and hepatic cirrhosis), fibrinogen (abnormal in various dysfibrinogenemias and absent in afibrinogenemia), and complement. Prenatal diagnosis of conditions involving these proteins may be feasible via fetoscopy and fetal blood sampling.

The formed elements present in fetal blood (other than red cells) also may be used for prenatal diagnosis of genetic defects.

- Severe combined immunodeficiency disease is a heterogeneous disorder characterized by a lack of functional T and B cells, with a resultant defect in both cell-mediated and humoral immunity. Fetal blood lymphocytes respond to phytohemagglutinin (PHA) stimulation after 15 weeks of gestation (Stites, Car, & Fudenberg, 1974). A "mini"-PHA stimulation test, which requires only 2500 lymphocytes, has been developed and the optimum conditions for testing fetal lymphocytes are being determined. This test could make possible the in utero diagnosis of fetal cellular immunologic competence.
- The X-linked Wiscott-Aldrich syndrome consists of eczema, thrombocytopenia, and recurrent infections with death resulting from hemorrhage, infection, or lymphoreticular malignancies (Rosen, 1974). Platelets of affected males are approximately one-third smaller than those of normal controls (Grottum et al., 1969). It may be feasible to diagnose the syndrome prenatally by studying the number and size of fetal platelets.
- Glycogen storage disease I (von Gierke disease), an autosomal recessively inherited glycogen storage disease, is characterized by hypoglycemia, hyperlipidemia, hyperlacticacidemia, and hepatomegaly. This disorder has been demonstrated to be due to a deficiency of glucose-6-phosphatase (G-6-Pase) activity (Cori & Cori, 1952). It has recently been argued that platelets contain G-6-Pase, and that they accurately reflect the deficiency state (Soyama et al., 1973; Negishi et al., 1974). The miniaturization of this assay and

its application to fetal platelets is now being accomplished, which may make possible the prenatal diagnosis of glycogen storage disease I (von Gierke disease).

The only fetal tissue other than blood which has been biopsied in utero thus far is skin (Valenti, 1972). Golbus et al. (1980) obtained fetal skin to diagnose autosomal dominant ichthyosis, Elias et al. (1980b) diagnosed autosomal recessive harlequin ichthyosis, and Rodeck, Eady, and Gosden (1980) detected epidermolysis bullosa. Although it may be possible to obtain sufficient fibroblasts but not karyotype analysis or biochemical studies somewhat more rapidly from skin explants than from amniotic fluid, the time saved is not worth the risk to the fetus. Very few metabolic disorders are demonstrable in skin fibroblasts but not demonstrable in amniotic fluid cells, so there probably will be no advantage of fetal skin biopsies in this area. Muscle biopsies, however, might be of considerable aid in the prenatal diagnosis of myopathies.

FUTURE PROSPECTS

A totally different approach to the problem of obtaining fetal cells is to obtain fetal blood cells that have crossed the placental barrier and are present in the maternal circulation. This is accomplished by a fluorescence-activated cell sorter, which sorts droplets containing single cells according to the cell size and the amount of fluorescent dye bound to the cell. Although this technique has been useful to enrich a population of cells for fetal cells, neither T-cell not B-cell mitogens have been successful in stimulating the fetal cells to divide (Schroeder et al., 1977). Without mitosis it is impossible to study the cells' chromosome complement. An additional problem is that white blood cells from a previous pregnancy have been found circulating in maternal blood more than one year later (Schroeder, Tiilikainen, & de la Chappelle, 1974). Whether or not these difficulties can be overcome and this methodology successfully applied to prenatal diagnosis remains to be seen.

It is likely that many of the autosomal dominant diseases will not be identifiable by an abnormal gene product. Some of them, as well as some of the X-linked diseases in males, may be detected in the fetus by using linked gene markers. With increasing frequency, genes for readily detectable biochemical or immunologic markers that are closely linked to genes for inherited disorders are being discovered (McKusick & Ruddle, 1977). The approach is to determine whether a marker gene, known to be closely linked to the mutant disease-causing gene, is present in the fetus. In potentially informative situations, the carrier of a deleterious gene is also heterozygous for such a closely linked marker, and the mate is homozygous for the recessive gene at the marker locus. It may be possible to deduce indirectly whether the deleterious recessive gene was transmitted to a fetus by studying the transmission of the marker. The proximity of the marker locus and the disorder locus on the chromosomes is of great importance because crossing-over is proportional to the distance between loci and can lead to error. The crossing-over rate between the two loci

must be known, and the magnitude of uncertainty this introduces should be explained to the couple being counseled.

The first application of such linkage analysis was for the autosomal dominant disorder myotonic dystrophy (Schrott, Karp, & Omenn, 1973). The gene for this disease is closely linked to the gene for ABH-secretor status (Renwick et al., 1971). As the amniotic fluid reflects the secretor status of the fetus (Harper et al., 1971), this linkage was employed to make a positive prenatal diagnosis (Insley et al., 1976). Other potentially useful linkages for prenatal diagnoses include glucose-6-phosphate dehydrogenase (G-6-PD) and hemophilia A (Edgell et al., 1978), HLA and the various complement loci, HLA and adrenal 21-hydroxylase deficiency (DuPont et al., 1977), and HLA and dominantly inherited spinocerebellar ataxia (Boyer & Graham, 1965; Jackson et al., 1977). This last linkage, which is reasonably tight, may make possible the prenatal diagnosis of a condition that does not ordinarily become manifest until adulthood.

A number of recognized inherited metabolic defects are manifested only in one or two tissues that are not represented in amniotic fluid or in other fetal cells liable to biopsy. As the entire genome is present in every fetal cell, the problem is to activate unexpressed genes in that cell. The approach envisioned is to fuse easily obtained fetal amniotic fluid cells with "activator" cell lines, which normally express the enzyme in question. A precedent for this approach exists in somatic cell hybridization experiments in which nuclei from fibroblasts or leucocytes have been observed to initiate synthesis of albumin, a liver cell protein, after fusion with hepatoma cells (Peterson & Weiss, 1972; Darlington, Bernhard, & Ruddle, 1974). The gene for albumin is normally only "on" in liver cells, but it can be activated in fibroblasts and leucocytes by the hepatoma cells. Successful development of methods for the activation of unexpressed genes would be particularly useful for the prenatal diagnosis of conditions such as urea cycle defects, certain mucopolysaccharidoses, muscle abnormalities, and phenylketonuria.

It has been suggested that the ultimate goal of prenatal diagnosis should be treatment of the affected fetus and correction of the defect. For disorders involving physical abnormalities, such as those caused by chromosomal aneuploidy, it is very unlikely that effective corrective or preventive therapy will be developed. Furthermore, in at least some metabolic disorders the fetus may be irreversibly damaged by the time the prenatal diagnosis is made. An editorial by Fox and Littlefield (1971) has appraised the potential and liabilities of gene therapy, with emphasis on its limitation and hazards. Even if fundamental genetic alterations should become feasible, the fetus is not likely to be one of the first subjects for such experimental efforts.

Exciting advances have been made in prenatal diagnosis, and the future holds great promise. The number of disorders diagnosable in utero undoubtedly will increase, and amniocentesis will become routine for women at increased risk of producing a genetically defective offspring. Routine testing may be done in tandem with computerized metaphase chromosome scanning and microanalysis of cells for biochemical defects. A significant part of specialty training is the education of obstetrician–gynecologists to take a central role in genetic history-taking and initial counseling.

Appendix
Potentially Detectable Biochemical Disorders that May Be Prenatally Detectable

Disease	Defect	Prenatal Diagnosis*
Acatalasia	Catalase	Possible
Adenosine deaminase deficiency	Adenosine deaminase	Yes
Adrenogenital syndromes	Metabolites of 11-, 17-, or 21-steroid hydroxylase	Yes
Arginosuccinic aciduria	Arguinosuccinase	Yes
Aspartylglucosaminuria	β-Aspartylglucosamidase	Possible
Cerebellar ataxia, juvenile	Hexosaminidase A and B	Possible (in some cases)
Chédiak-Higashi syndrome	Unknown (intracellular inclusion body)	Possible
Cholesterol ester storage disease	Acid lipase	Possible
Chronic granulomatous disease	Granulocyte NBT reduction	Yes
Citrullinemia	Arginosuccinic acid synthetase	Yes
Cystathioninuria	Cystathionase	Probable
Cystic fibrosis	MUGB reative, proteases	Possible (in some cases)
Cystinosis	Unknown (cystine accumulation)	Yes
Ehlers-Danlos syndrome, type IV	Unknown (lack of type III collagen)	Possible
Ehlers-Danlos syndrome, type V†	Lysyl oxidase	Possible
Fabry disease†	Ceramidetrihexoside galactosidase	Yes
Farber disease	Ceramidase	Yes
Finnish nephrosis	Unknown (α-fetoprotein level)	Yes
Fucosidosis	α-Fucosidase	Yes
Galactokinase deficiency	Galactokinase	Yes
Galactosemia	Galactose-1-phosphate uridyl transferase	Yes
Generalized gangliosidosis (G_{M1} gangliosidosis, type I)	β-Galactosidase	Yes
Juvenile gangliosidosis (G_{M1} gangliosidosis, type II)	β-Galactosidase	Yes
Juvenile G_{M2} gangliosidosis	Partial deficiency of hexosaminidase A	Possible
Gaucher disease	Glucocerebrosidase	Yes
Glycogen storage disease, type II (Pompe disease)	α-1,4-Glucosidase	Yes
Glycogen storage disease, type III	Amylo-1,6-glucosidase	Probable
Glycogen storage disease, type IV	Branching enzyme amylo-1:4, 1:6 transglucosidase	Probable
Hemoglobinopathies	Synthesis of abnormal hemoglobin	Yes

Disease	Defect	Prenatal Diagnosis*
Hemolytic anemia VIII	Triose phosphate isomerase	Probable
Hemophilia A	Factor VIII deficiency	Yes
Hemophilia B	Factor IX deficiency	Yes
Hereditary coproporphyria	Coproporphyrinogen oxidase	Possible
Histidinemia	Histidase	Probable
Homocystinuria	Cystathionine synthase	Probable
Hunter syndrome†	α-L-Iduronic acid-2 sulfatase	Yes
Hurler syndrome	α-L-Iduronidase	Yes
Hyperammonemia, type II	Ornithine carbamyltransferase	Probable
Hyperargininemia	Arginase	Probable
Hypercholesterolemia‡ (type II)	HMG-CoA reductase	Yes
Hyperlysinemia	Lysine-ketoglutarase reductase	Possible
Hyperthyroidism	Unknown (reverse tri-iodothyronine level)	Possible
Hypervalinemia	Valine transaminase	Possible
Hypophosphatasia	Alkaline phosphatase	Yes (some types)
Hypothyroidism	Multiple (reverse tri-iodothyronine level)	Possible
Isovaleric acidemia	Isovaleryl-CoA dehydrogenase	Yes
Ketotic hyperglycinemia	Propionyl-CoA carboxylase	Yes
Krabbe disease	Galactocerebroside β-galactosidase	Yes
Lactosyl ceramidosis	Lactosyl ceramidase	Possible
Lesch-Nyhan syndrome†	Hypoxanthine-guanine phosphoribosyltransferase	Yes
Lysosomal acid phosphotase deficiency	Lysosomal acid phosphatase	Yes
Mannosidosis	α-Mannosidase	Probable
Maple syrup urine disease	Branched-chain ketoacid decarboxylase	Yes
Maroteaux-Lamy syndrome	Arylsulfatase B	Yes
Meckel syndrome	Unknown (α-fetoprotein level)	Yes
Menkes disease	Unknown (copper incorporation)	Probable (in some cases)
Metachromatic leukodystrophy	Arylsulfatase A	Yes
Methylmalonic aciduria	Methylmalonic CoA mutase	Yes
Methyltetrahydrofolate methyltransferase deficiency	Methyltetrahydrosulfate methyltransferase	Possible
Methyltetrahydrofolate reductase deficiency	Methyltetrahydrofolate reductase	Possible
Morquio syndrome	Chrondroitin sulfate *N*-acetylhexosamine sulfate sulfatase	Possible
Mucolipidosis II (I cell disease)	Nonspecific lysosomal enzymes	Yes
Mucolipidosis III	Multiple lysosomal enzymes	Possible

Appendix (continued)

Disease	Defect	Prenatal Diagnosis*
Mucolipidosis IV	Unknown (electron microscopy)	Yes
Mucopolysaccharidosis VII	β-glucuronidase	Possible
Myotonic dystrophy	Linkage analysis (some families)	Yes
Niemann-Pick disease	Sphingomyelinase	Yes
Nucleoside phosphorylase deficiency (with immunodeficiency)	Nucleoside phosphorylase	Probable
Ornithine α-ketoacid transaminase deficiency	Ornithine α-ketoacid transaminase	Probable
Orotic aciduria	Orotidylic pyrophosphorylase and decarboxylase	Possible
Phosphohexose isomerase deficiency	Phosphohexose isomerase	Possible
Placental sulfatase deficiency†	Placental sulfatase	Yes
Porphyria (acute intermittent)‡	Uroporphyrinogen I synthetase	Yes
Porphyria (congenital erythropoietic)	Uroporphyrinogen III cosynthetase	Yes
Pyruvate decarboxylase deficiency	Pyruvate decarboxylase	Yes
Pyruvate dehydrogenase deficiency	Pyruvate dehydrogenase	Possible
Refsum disease	Phytanic acid α-hydroxylase	Probable
Sandhoff disease	Hexosaminadase A and B	Yes
Sanfilippo syndrome, type A	Heparin sulfatase	Yes
Sanfilippo syndrome, type B	N-acetyl-α-D-glucosaminidase	Yes
Scheie syndrome	α-L-iduronidase	Possible
Severe combined immunodeficiency disease†	T-cell incompetence	Possible
Sulfite oxidase deficiency	Sulfite oxidase	Probable
Tay-Sachs disease	Hexosaminidase A	Yes
α-Thalassemia	Decreased synthesis of α-chain hemoglobin	Yes
β-Thalassemia	Decreased synthesis of β-chain hemoglobin	Yes
Vitamin B_{12} metabolic defect	Vitamin B_{12} coenzyme	Possible
Wiskott-Aldrich syndrome†	Microthrombocytes	Possible
Wolman disease	Acid lipase	Yes
Xeroderma pigmentosum	Ultraviolet endonuclease	Yes

*Yes = prenatal diagnosis accomplished. Probable = enzyme activity present in normal amniotic fluid cells. Possible = enzyme activity present in normal skin fibroblasts.
†X-linked
‡Autosomal dominant

7

Spontaneous Abortion and Fetal Wastage

Many causes for spontaneous abortions have been postulated. In the past, hormonal imbalance, maternal overexertion, and exposure to exogenous factors (e.g., viruses) were considered the principle causes. However, chromosomal aberrations are now known to be associated with at least 50 percent of first trimester spontaneous abortions (Boué & Boué, 1974; Boué et al., 1975a; Lauritsen, 1976). Nongenetic factors are doubtless responsible for some abortions (Elias & Simpson, 1980a; Glass & Golbus, 1978; Simpson, 1981b). In this chapter we shall limit our discussion to cytogenetic factors, the role of which is best appreciated by considering the losses in the different stages of embryonic development.

PRECLINICAL STAGES

Although few studies have considered the extent to which embryos are lost prior to clinical recognition of pregnancy, available data suggest that the frequency of morphologic abnormalities is very high among preimplanted and early-implanted embryos. Abnormalities affecting preimplantation and early-implantation stages (less than three weeks of embryonic life) usually escape clinical detection.

The etiology of early losses is usually abnormal embryonic development. Indeed, Hertig and colleagues have shown that morphologic abnormalities exist in very young embryos recovered from the Fallopian tubes, uterine cavities, and endometria of women undergoing elective hysterectomy (Hertig et al., 1959; Hertig, 1975). Dates of ovulation were deduced on the basis of basal body temperature elevation, and coital times were recorded. All women were of proven fertility, and mean age was 33 years. Four of eight preimplanted embryos recovered were morphologically abnormal. In the investigators' opinion, the

four would either not have implanted or would not have survived long after implantation. They detected necrotic blastomeres, multinucleate blastomeres, and decreased numbers of blastomeres compared to normally developing zygotes of comparable age (Fig. 7-1). Similarly, 9 of 26 implanted ova were so morphologically abnormal that Hertig et al. (1959) considered further embryonic development unlikely (Fig. 7-2). Often normal organization patterns were not observed, sometimes only syncytiotrophoblasts were present, and sometimes chorionic cells showed hydropic swelling. In other cases no embryo per se was recovered.

Abnormalities of early embryonic development are thus relatively common, certainly more so than generally appreciated. The etiologies of these

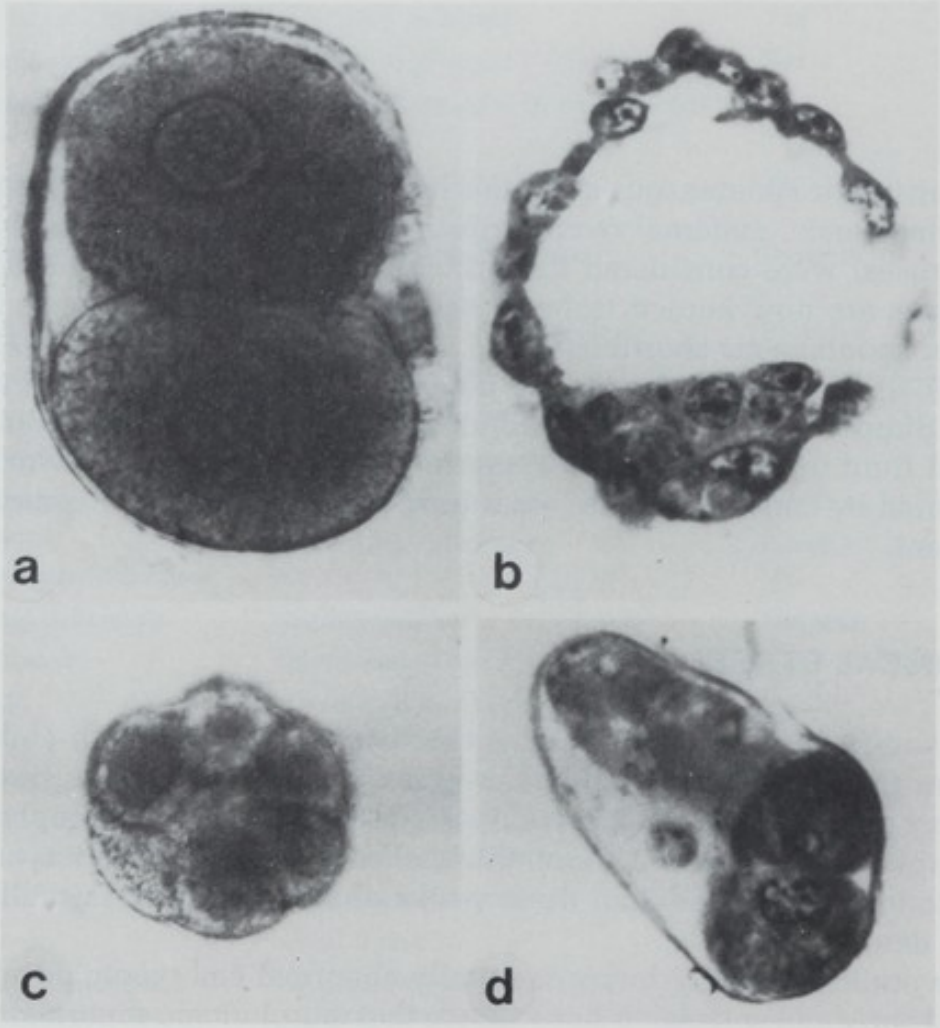


Figure 7-1. Four views of three preimplanted embryos. (a) Normal two-cell embryo about 30 hours old. (b) Section of 107-cell normal blastocyst about 5 days old, showing inner (embryonic) and outer (abembryonic) masses. (c) Defective, necrotic blastomeres. (d) Another view of c showing necrotic blastomeres at left. (Parts (a), (b), and (d) from Hertig A, Rock J, Adams EC, Mulligan WJ: Contrib Embryol 35:199, 1954. Part (c) from Am J Obstet Gynecol 58:968, 1949. Reprinted with permission from the publishers, authors, and Carnegie Laboratories.)

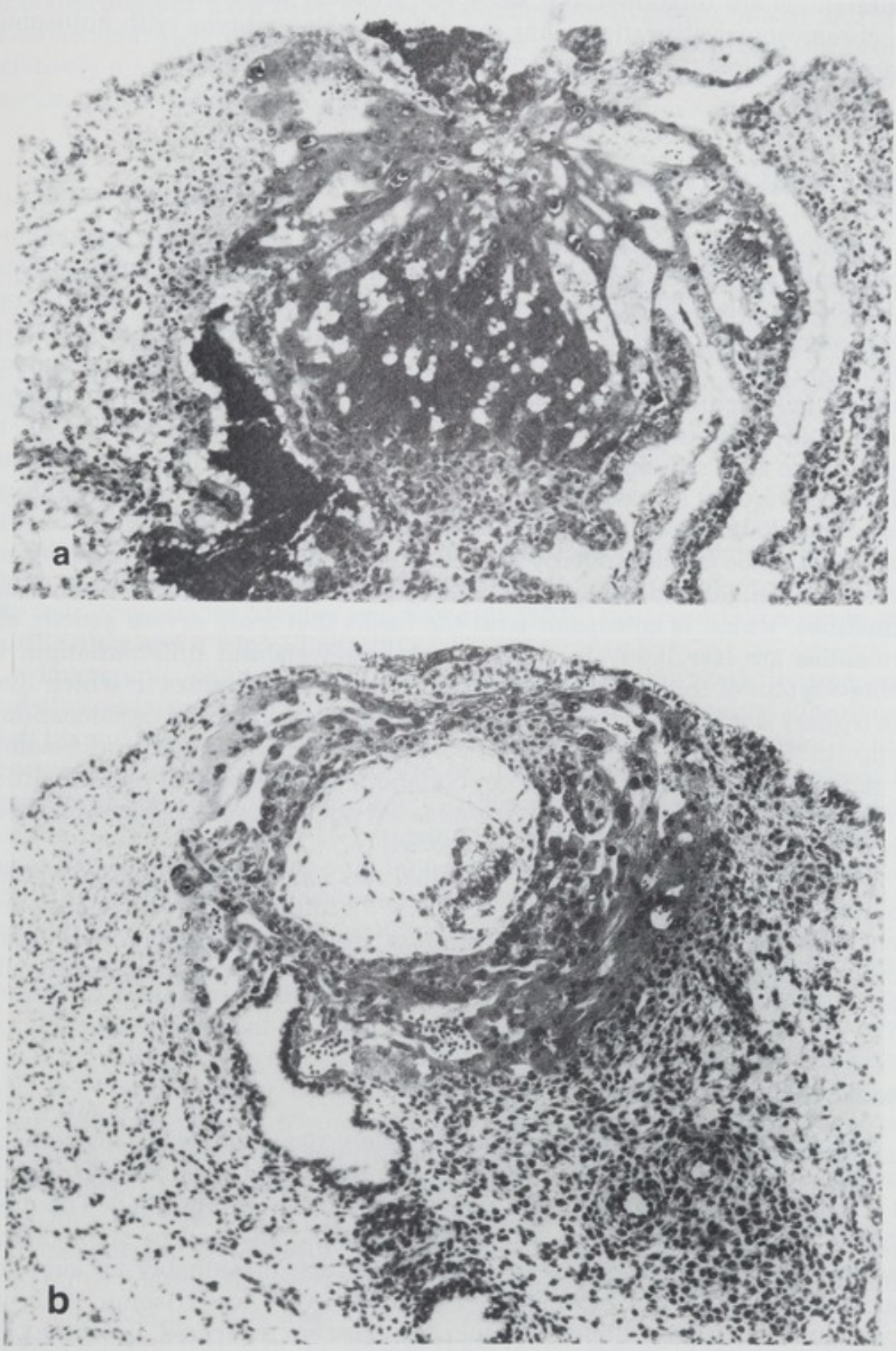


Figure 7-2. Cross section of endometrium containing an abnormal 14-day-old embryo (a), compared to a normal 11-day-old embryo (b). In the abnormal embryo no embryonic disc is present and only syncytiotrophoblasts are identifiable. (Reprinted from Hertig A, Rock J: Am J Obstet Gynecol 47:149, 1944, and Am J Obstet Gynecol 58:968, 1949. With permission of the authors, CV Mosby, and Carnegie Laboratories.)

abnormalities are unknown, but most are probably abnormal cell divisions, e.g., chromosomal aberrations. For example, mouse embryos with autosomal monosomy are lost prior to implantation (Gropp, 1975a, 1975b).

FIRST TRIMESTER ABORTIONS

Approximately 10 to 15 percent of all clinically recognizable pregnancies terminate in spontaneous abortion, usually during the first trimester. At least 50 to 60 percent of first trimester abortuses are now known to result from chromosomal abnormalities (Boué & Boué, 1974; Boué, Boué, & Lazar, 1975a, 1975b; Lauritsen, 1976). The frequency may be even higher because (1) many abortuses fail to grow in tissue culture sufficiently well to permit chromosomal studies, and chromosomally abnormal abortuses seem more likely to fail to grow in vitro than chromosomally normal abortuses, (2) chromosome banding techniques were not utilized in most studies, limiting ability to detect structural chromosomal abnormalities, and (3) induced abortions, most of which would be expected to be chromosomally normal, are inevitably included in all series. The earlier the gestational age, the higher the frequency of chromosomal abnormalities, which is consistent with the thesis that more severe genetic abnormalities are less likely to survive embryogenesis and differentiation. In further support of this hypothesis are studies in mice, a species in which live-born trisomy is very rare. Using a mouse carrying seven balanced translocations, Gropp (1975a, 1975b) recovered embryos effectively monosomic and trisomic for various chromosomes. Before implantation, both classes of abnormalities were detected; after implantation, trisomies but not monosomies were detected; at birth, only normal fetuses were recovered.

Many different chromosomal abnormalities have been identified among spontaneous abortuses (Therkelsen et al., 1973). Interestingly, the relative frequencies of the various abnormalities differ from those encountered among live-born infants. Six general types of chromosomal abnormalities are considered below.

Autosomal Trisomy

Autosomal trisomy comprises approximately 50 percent of chromosomally abnormal abortuses. Trisomies for Nos. 13, 16, 18, 21, and 22 are most common, but trisomy for every chromosome except No. 1 has been observed. The most frequent trisomy involves No. 16. Many trisomies observed in spontaneous abortions have never been observed in live births, presumably because the associated phenotype is incompatible with life; very few trisomic embryos survive until birth. Occasionally double trisomies are recovered, in which case one of the additional chromosomes is usually compatible with life.

Specific phenotypes are associated with autosomal trisomies in abortuses. However, many specimens are macerated or consist merely of empty sacs; thus, relatively few data are available, although for a given trisomy the same types

of malformations have been observed among both live-born infants and spontaneous abortuses. Aberrations known to be compatible with live births occur relatively more frequently during embryonic weeks 9 and 10 than during earlier weeks. By contrast, tetraploidy is relatively more common during earlier weeks.

Autosomy Monosomy

Autosomal monosomy is very rare among abortuses and is probably not compatible with life. Only a few examples of autosomal monosomy have been reported, and most of these were not verified by banding studies to exclude small translocations. Breeding experiments in mice indicated that absence of an entire chromosome is associated with preimplantation loss (Gropp, 1975a, 1975b).

Polyploidy

Polyploidy accounts for 20 percent of chromosomally abnormal abortuses. Triploidy is most common, but tetraploidy also occurs. Interestingly, one of the four pregnancies Steptoe and Edwards (1979) observed following in vitro fertilization was a triploid abortus.

Phenotypic features of triploids vary. Most cases abort early in pregnancy, and often only an empty sac is recovered. In later abortions, neural tube defects, cleft lip and palate, omphalocele, and syndactyly have been observed. Triploid abortuses also often show hydatidiform placental degeneration. However, hydatidiform moles are usually diploid, although they are occasionally triploid. According to Carr (1971), 75 percent of abortuses showing hydatidiform degeneration are triploid, but only 50 percent of triploids exhibit this characteristic. Lawler et al. (1979) estimated that 1 to 3 percent of triploid conceptions develop as a molar pregnancy. Recent investigations have distinguished two forms of moles: (1) A "complete" mole generally is detected in the first or second trimester, exhibits no discernible fetal parts, has a 46,XX karyotype usually totally paternal in origin (Kajii & Ohama, 1977; Lawler et al., 1979), and is more likely to be associated with subsequent malignancy. (2) The second form is a "partial" mole: hydatidiform swelling with some fetal development, often with triploidy or trisomy 16. The relationship of this type of mole to malignancies is less well established (Vassilakos, Riotton, & Kajii, 1977; Szulman & Surti, 1978; Lawler et al., 1979).

There is no evidence that increased maternal age is related to triploidy. In animals, delayed fertilization increases the risk of triploidy, but it has proved difficult to confirm this relationship in humans (Simpson, 1978d). Triploidy may be caused by (1) inclusion of a polar body because of failure of maternal meiosis I; (2) inclusion of the second polar body because of failure of maternal meiosis II; (3) abnormality during paternal meiosis, causing diploid sperm; or (4) dispermy, which is the most common mechanism in humans (Beatty, 1978).

Tetraploid embryos usually cease to develop very early (2 to 3 weeks), and recognizable embryonic differentiation has not been observed in recovered

specimens. Tetraploidy has very rarely been observed among live-born infants (Golbus et al., 1976). Approximately one half of tetraploid abortuses are 92,XXXX and the rest are 92,XXYY (Carr, 1971).

Monosomy X

Monosomy X (45,X) occurs in about 25 percent of chromosomally abnormal abortuses. 45,X is thus the most common single complement among abortuses, an intriguing observation because live-born 45,X females manifest relatively few life-threatening anomalies. Simpson and LeBeau (1981) found cell generation times to be prolonged in 45,X cells, an observation probably relevant to 45,X embryonic lethality. Two thirds of 45,X abortuses cease embryonic development at 6 weeks. They are usually characterized by closed sacs, the umbilical cord ending in a mass of macerated embryonic cells and subchorionic hemorrhage (Breus' mole) (Boué & Boué, 1974). One third appear normal for the stage at which development was arrested; however, in these specimens horseshoe kidneys and lymphangiomas have been observed, and the chorion is often hemorrhagic. Interestingly, germ cells are present in the ovaries (Singh & Carr, 1966), indicating that the pathogenesis of streak gonads in 45,X embryos is not failure of germ cell formation but increased rate of attrition. 45,X fetuses aborted in the second trimester are often characterized by edema, hygroma of the neck, and horseshoe kidney.

Structural Abnormalities

Structural chromosomal rearrangements account for approximately 5 percent of all chromosomally abnormal abortuses. Half are balanced Robertsonian translocations, a common structural abnormality in which duplication or deficiency of genetic material apparently does not occur. Carriers (heterozygotes) of balanced structural rearrangements are usually phenotypically normal. However, subtle and undetected genetic imbalances may have occurred, which would explain the abortions.

Sex Chromosome Polysomy

Among live-born infants, 47,XXY and 47,XYY polysomies occur more frequently than does monosomy X, but among abortuses sex chromosome polysomies are very rare.

SECOND TRIMESTER ABORTIONS

Second trimester abortions have traditionally been considered more likely to result from maternal than fetal (cytogenetic) causes. Cervical incompetence, uterine anomalies, and systemic maternal diseases are well-known causes of abortions between gestational weeks 12 and 24 (Elias & Simpson, 1980; Simpson, 1981b). Nonetheless, Ruzicska and Cziezel (1971) detected chromosomal abnormalities in 20 of 55 (34 percent) second trimester abortions. Although the

frequency of chromosomal aberrations is not so high as in first trimester abortions (50 to 60 percent), it is much higher than that for stillborn infants (5 percent) (Bauld, Sutherland, & Bain, 1974; Machin, 1974; Machin & Crolla, 1974) or live-born infants (0.5 percent) (Hook & Hamerton, 1977).

Interestingly, the spectrum of chromosomal abnormalities detected among second trimester abortuses differs somewhat from those detected among first trimester abortuses. Autosomal trisomies, monosomy X, and sex chromosome mosaicism are common among second trimester abortuses, but polyploidy is rare. Also trisomies considered lethal (e.g., No. 16) are detected less often than those more likely to be encountered in live-born infants (e.g., Nos. 13, 18, and 21).

STILLBIRTHS

Several studies have shown that chromosomal abnormalities are more frequent in antepartum or intrapartum stillborns than in liveborns (Bauld, Sutherland, & Bain; 1974, Machin, 1974; Kulesov, 1976). Approximately 5 to 10 percent of antepartum and intrapartum stillborns have chromosomal abnormalities, with trisomy 18 the most common. Chromosomal abnormalities have been detected both in stillborn infants with no overt anomalies as well as those with multiple anomalies. Stillborns with only CNS anomalies usually show no chromosomal abnormalities, although triploidy is sometimes detected. CNS anomalies are thought to be of primarily polygenic/multifactorial etiology; thus, the absence of chromosomal abnormalities is not unexpected. It makes sense that the frequency of chromosomal abnormalities is higher among stillborn than liveborn infants if selection against genetically abnormal embryos and fetuses occurs throughout gestation, the highest frequencies of abortions occurring earliest.

RECURRENT SPONTANEOUS ABORTIONS

Recurrence Risks

Until the mid-1960's most obstetricians believed that three spontaneous abortions conferred upon a woman the designation "habitual aborter" (Simpson, 1980d). The likelihood of her having another abortion was believed to be as high as 80 to 90 percent. By contrast, a woman who had had one or two spontaneous abortions was believed to be at only slightly increased risk of recurrence than a woman who had never had a spontaneous abortion.

The risks are now known to be much lower following repetitive abortions. In 1964, Warburton and Fraser studied a sample of women who had given birth to at least one live infant and had had at least one abortion. They estimated the likelihood of subsequent abortion to be 25 to 30 percent, irrespective of the number of previous abortions (Table 7-1). Thus, the dichotomy of categorizing women as habitual or nonhabitual aborters was questioned. Subsequent studies suggested, however, that the likelihood of abortion was influenced to a limited

Table 7-1
Empiric Data on the Risks of Spontaneous Abortion Following a Given Number of Abortions*

Previous Abortions (No.)	Risk of Abortions (%)
0	12.3
1	23.7
2	26.2
3	32.2
4	25.9

Data from Warburton D, Fraser FC: Spontaneous abortion risks in man: Data from reproductive histories collected in a medical genetics unit. Hum Genet 16:1, 1964.
*Sample includes couples with at least one liveborn offspring and at least one abortion. These figures are averages estimated from heterogeneous biased samples. Therefore, they are to be considered as approximate population expectations and may not fit specific classes of women.

extent by both previous obstetric history (Boué et al., 1973) and the chromosomal complement of the abortus. Poland, Miller, and Jones (1977) calculated that the likelihood of abortion was 46 percent if a woman had no living children and had experienced at least one spontaneous abortion, stillbirth, or early neonatal death; women with a liveborn infant were at lower risk of abortion. Because their sample was limited to women with one live-born child, Warburton and Fraser would not have been able to identify this subclass. Boué et al. (1973) found that subsequent reproductive history was not a function of the karyotype of the proband abortus, but later studies showed that the risk of prior abortion was slightly less if the karyotype of the previous abortus was abnormal (Alberman et al., 1975, Boué et al, 1975a). In fact, Lauritsen (1976) allegedly identified infectious and other maternal factors in 15 percent of women whose abortuses were chromosomally normal. Further studies to quantitate the relationship of previous obstetric history to recurrence risks are desirable.

Other investigations noted that the complements of two successive abortuses in a given family were more likely to be either both normal or both abnormal, i.e., nonrandom with respect to chromosomal complements (Boué et al., 1973; Alberman et al., 1975; Kajii & Ferrier, 1978; Hassold, 1980) (Table

Table 7-2
Relationship Between Karyotypes of Successive Abortuses

Complement of First Abortus	Complement of Second Abortus				
	Normal	Trisomy	45,X	Polyploidy	Other
Normal	(55)	6	2	5	0
Trisomy	8	(24)	1	1	0
Monosomy X	5	4	1	1	0
Polyploidy	4	4	1	1	0
Other	0	0	1	0	1

Data collected by Hassold (1980) from several reported series. Circled numbers suggest nonrandom losses.

7-2). If the complement of the first abortus was normal about 80 percent of the second abortuses had a normal complement. If the complement of the first abortus was abnormal, about 70 percent of second abortuses were also abnormal. Of special significance are observations that if the first fetus is trisomic, the second is also likely to be trisomic. Recurrence for trisomy does not necessarily indicate familial predisposition to nondisjunction, for it might reflect the increased frequencies of trisomic abortuses and live-born infants associated with advanced maternal age. Nonetheless, if a tendency for recurrence of chromosomal aberrations (especially trisomies) exists, women whose abortuses had an abnormal karotype should be offered antenatal diagnosis to detect fetal chromosomal anomalies in subsequent pregnancies.

The following conclusions seem valid: (1) The likelihood of another spontaneous abortion is increased to at least 25 percent following an abortion. (2) The risk of abortion is slightly greater if a couple has had no live-born offspring. (3) The risk is slightly greater if the abortus had a normal chromosomal complement than if it had an abnormal complement. (4) The chromosomal complement of successive abortuses in a given family will usually show successive chromosomal abnormalities or successive normal complements.

Taken in aggregate, these data suggest that at least three subclasses of couples who have had repeated abortions can be identified. In one subclass the abortus is chromosomally abnormal and recurrence may result from repetitive meiotic abnormalities (e.g., nondisjunction). In a second subclass the abortus is chromosomally normal and recurrence may result from maternal factors (e.g., hormone imbalance, uterine infection or viruses). In a third subclass discussed below, parental chromosomal rearrangements may predispose to gametes with duplications or deficiencies.

Parental Age

The prevalence of chromosomal aberrations increases with maternal age, leading to autosomal trisomies in abortuses, stillbirths, or live births. Trisomies involving acrocentric chromosomes (Nos. 13, 14, 15, 21, and 22) are primarily responsible for the relationship of trisomy and maternal age. In fact, the correlation between risk of abortion and advanced maternal age may result from increased production of acrocentric trisomies by these mothers, not from other biological or physiologic age effects. Because the maternal age distribution for trisomy 21 is similar for both aborted and live-born series, the likelihood of a trisomy 21 fetus being aborted does not appear to depend on the age of the mother. Lauritsen (1976) found the paternal age associated with trisomy 16 to be significantly elevated. However, there is no increase in monosomy X with advancing age, and in fact perhaps the converse (Kajii & Ohama, 1979; Warburton, Kline, & Stein, 1980).

Oral Contraceptive Use

Carr (1971) observed an increase in triploidy among abortuses of women who became pregnant within 6 months after discontinuing oral contraceptives, as compared to previously obtained control frequencies, suggesting an association between triploidy and oral contraceptives. However, oral contraceptives

have not been implicated in the etiology of spontaneous abortions or chromosomal aberrations in more recent studies (Boué et al., 1973, 1975b; Lauritsen, 1976; Klinger & Glasser, 1977). Carr's results may have been due to chance variation in a small sample ($N = 54$) or to the higher levels of estrogens in oral contraceptives in the past. Pregnancies following induced ovulation show an increased rate of spontaneous abortion, which may be due not only to multiple gestations but also to cytogenetically abnormal embryos. When conception occurred during induced ovulation or in the first spontaneous cycle thereafter, Boué, Boué, and Lazar (1975a) observed that 84 percent of abortuses showed chromosomal abnormalities compared to 61 percent when ovulation was not induced.

Heritable Structural Rearrangements

Most chromosomal aberrations occurring in spontaneous abortuses are not heritable. However, sometimes parents may be carriers for structural rearrangements that, by meiotic segregation, lead to unbalanced gametes and abnormal fetuses. The frequency of balanced structural rearrangements (translocations and inversions) in one parent varies according to sample and according to whether a couple has experienced abortion alone or both abortion and malformed infants. Among parents who have experienced abortions alone, pooled data indicate that the frequency of translocations is 3.4 percent in females and 1.6 percent in males. Among parents who have experienced both abortions and either a stillborn or an anomalous live-born infant, the frequency is 16.4 percent in females and 4.2 percent in males (Simpson, Elias, & Martin, 1981). The likelihood of detecting a translocation is not necessarily related to the number of prior abortions (Simpson, Elias, & Martin, 1981). If a structural rearrangement is detected in either parent, subsequent pregnancies should be monitored. Except in rare instances, it is unclear whether carriers of structural rearrangements

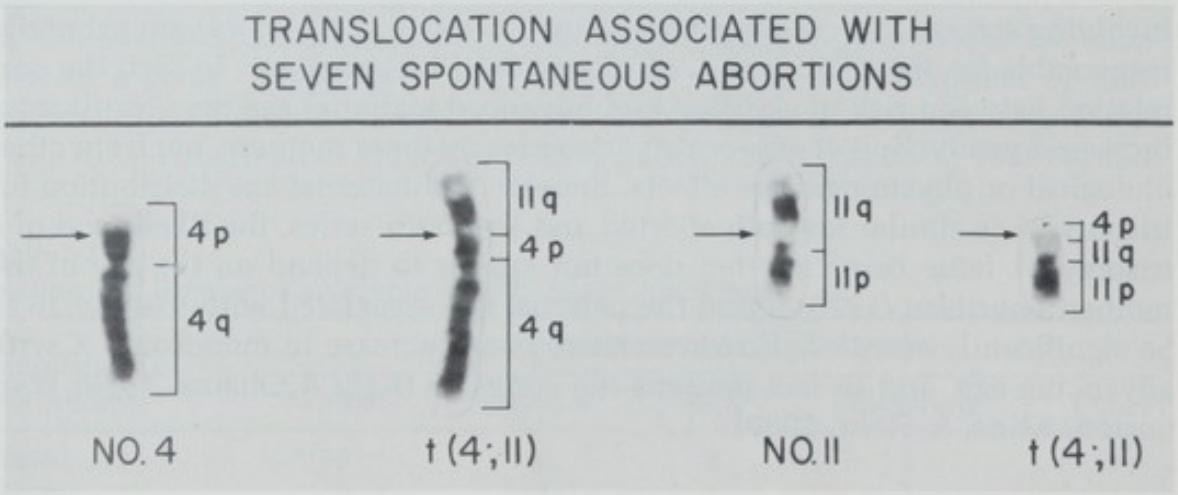


Figure 7-3. Partial karyotype of chromosomes from a woman who had multiple spontaneous abortions, illustrating translocation between chromosome Nos. 4 and 11. (From Simpson JL (1980d): Genes, chromosomes, and reproductive failure. Fertil Steril 33:107, 1980. Reprinted with permission.)

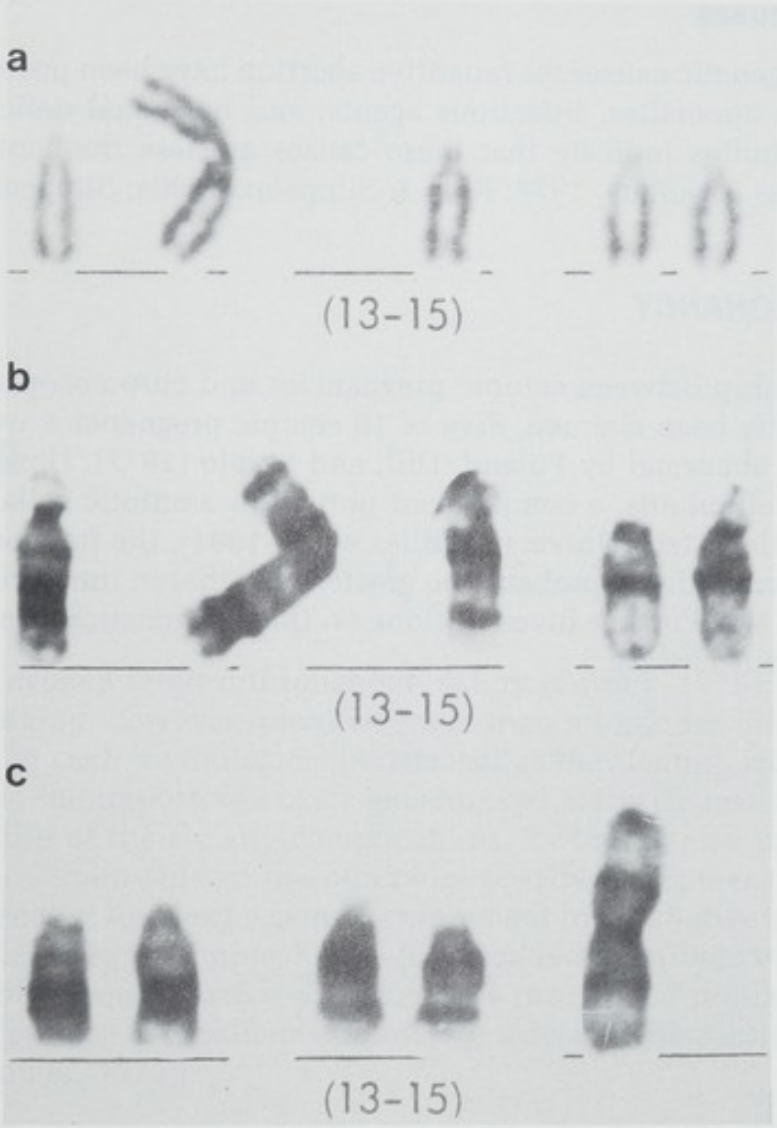


Figure 7-4. Partial karyotype of chromosomes from two women who had many spontaneous abortions but no live-born offspring. (a) Unbanded chromosomes, which do not permit specific chromosomes involved to be delineated and thus constitute inadequate evaluation. (b) t(13q14q), which can lead to normal live-born infants, as well as to abortions and to anamalous live-born infants. (c) t(15q15q). No gametes produced as result of t(15q15q) are viable. (From Simpson JL, Martin AO: Cytogenetic nomenclature. Am J Obstet Gynecol 128:167, 1977. Reprinted with permission.)

have an increased risk of spontaneous abortions; however, data suggest reduction in reproductive fitness may be associated with some rearrangements (Jacobs et al., 1975).

Most structural rearrangements are compatible with the production of normal gametes (Fig. 7-3). However, this is not always true. For example, Figure 7-4c shows a partial karyotype of a patient with a t(15q15q), who would not be expected to produce normal gametes, as the only zygotes produced would show either trisomy No. 15 or monosomy 15. Women with such translocations should be advised to practice rigorous birth control or consider sterilization. If the male has such a translocation, artificial insemination should be discussed.

Nongenetic Causes

Many nongenetic causes for repetitive abortion have been postulated, principally uterine anomalies, infectious agents, and hormonal deficiencies, but most formal studies indicate that these causes are less frequent than often supposed (Glass & Golbus, 1978; Elias & Simpson, 1980a; Simpson, 1981b).

ECTOPIC PREGNANCY

A relationship between ectopic pregnancies and chromosomal abnormalities has recently been claimed. Five of 16 ectopic pregnancies were believed karyotypically abnormal by Poland, Dill, and Styblo (1977). However, two of the five were tetraploids, a complement normal in amniotic cells. In our laboratory at Northwestern University (Elias et al., 1981), the frequency of chromosomal abnormalities is probably no greater than that in intra-uterine fetuses of comparable age. Further investigations on the cytogenetics of ectopic pregnancies will be necessary.

8

Twinning

Twins have evoked interest throughout history (Corney, 1975a). The phenomenon of twinning, however, provides more than a topic for idle conversation or for deriving such mythological personages as the Gemini. Obstetricians are well aware of the importance of diagnosing and correctly managing twin gestations because of their many complications. Twinning also provides an opportunity for certain studies not otherwise possible in humans. Twin studies have been used (1) to detect a genetic component in qualitative traits (e.g., cleft palate) that fail to show simple, Mendelian modes of inheritance, (2) to estimate the heritability of quantitative characteristics (e.g., blood pressure), and (3) to estimate penetrance of dominant alleles. (For a detailed discussion, see Cavalli-Sforza & Bodmer, 1971.)

TYPES AND ORIGIN OF TWINS

There are two types of twins: monozygotic (MZ) and dizygotic (DZ). The MZ twins originate from division of a zygote (a single fertilized ovum) or early embryo. The DZ twins originate from two separate fertilized ova, which develop simultaneously.

If monozygotic twinning occurs early in development, it is believed that two amnions and two chorions will be present (diamnionic, dichorionic) (Benirschke & Driscoll, 1967; Reid, Ryan, & Benirschke, 1972) (Fig. 8-1). After approximately day 3, only one chorion can form. Consequently, monozygotic twins that originate after this time will always be monochorionic and usually but not necessarily always diamnionic; 67 percent of MZ twins are monochorionic (Bulmer, 1970). The amnion becomes established about day 8; therefore, monozygotic twins formed between days 8 and 14 can only be monoamnionic, which is a very rare occurrence. Dizygotic twinning results from double ovulation and fertilization, so two amnions and two chorions will always be present. However, if the implantation sites are contiguous, a single fused placental disc may be formed.

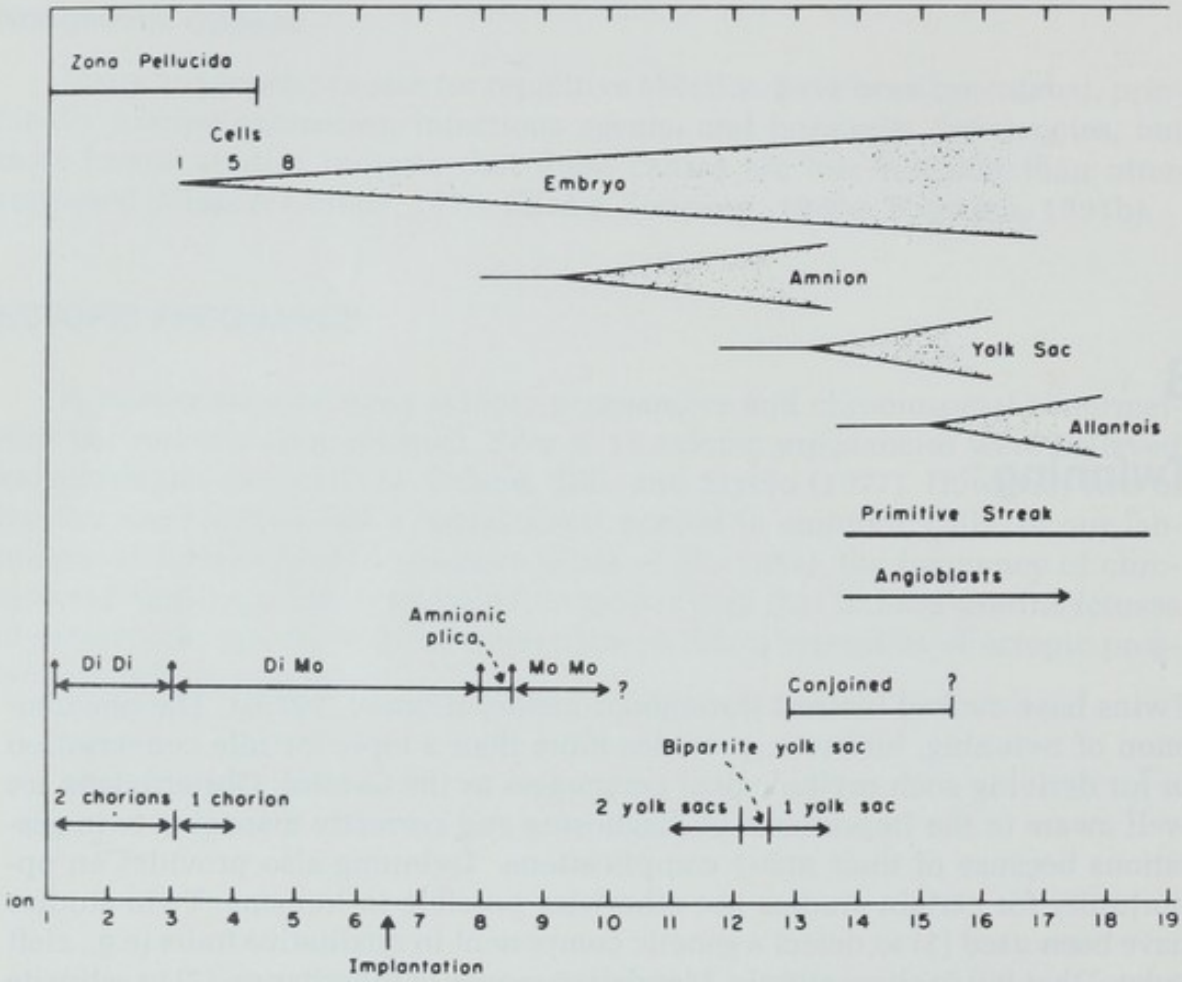


Figure 8-1. Timing of early embryonic developmental events and projected type of placentation in MZ twinning. Before the third day of development two chorions (DiDi) are formed (diamnionic, dichorionic). From day 3 to 8 a diamnionic, monochorionic placenta is expected (Di Mo); thereafter, a single amnionic cavity (Mo Mo) is expected, because the amnion has now formed. (From Benirschke K, Driscoll SG: The Pathology of the Human Placenta. New York, Springer Verlag, 1967, p. 264. Reprinted with permission.)

INCIDENCE OF MULTIPLE BIRTHS

The overall frequency of twin births is approximately 1/70 to 1/100 (i.e., one twin birth for every 70 to 100 single births; thus, one neonate in every 35 to 50 is a twin). This frequency varies among different populations and at different times, and this estimate also refers to twin births in which at least one twin was born alive. Because twinning rates are usually derived from birth records (vital statistics), the frequencies of multiple births underestimate conception frequencies because they do not reflect the increased prenatal and perinatal loss associated with multiple gestations. This may be especially true in view of recent ultrasound studies that suggest that live-born twinning rates at birth are only 30 percent of rates observed in the first 10 weeks of menstrual age (Donald & Levi, 1975; Levi, 1976). Also, survival is decreased for twins as neonates.

An approximation of the frequency of triplets is the square of the twinning frequency (Hellin's rule). Although the biological basis for this oft-quoted aphrodism is obscure, it appears to be a reasonable approximation of the observed rate. Quadruplets are even rarer, their frequency being approximately the cube of the twinning rate.

The frequency of MZ twinning is relatively constant between populations, but that of DZ twinning is not (Bulmer, 1970). Approximately 1 in 250 births in various populations is a set of MZ twins, irrespective of race. By contrast, the frequency of DZ twinning in black populations is higher in certain African groups (1/20 births) (Nylander, 1971, 1975a) than in Caucasians in the United States (1/80 births) (Myrianthapoulos, 1970); both frequencies are higher than in some Oriental populations (1/150 births) (Bulmer, 1970; Nylander, 1975a). The rate of DZ twinning also increases until about age 40 (Bulmer, 1958; see Nylander, 1975b), but thereafter decreases sharply. The reason for the variation in DZ twinning rates is not established. It has been suggested that the rate of DZ twinning is decreasing (James, 1972). Monozygotic twinning is presumably a chance event, both because of the apparent lack of variation in its rate among births in different populations and the lack of a maternal age effect. Most variation in rates of twinning is therefore apparently due to DZ twinning rates.

Because MZ twinning rates are constant between populations, the relative proportions of MZ and DZ twin pairs can be established from population figures by a simple formula devised by Weinberg (1902), which states that the proportion of DZ twins is twice the proportion of all twin pairs of *unlike* sex. (This method assumes a sex ratio of 50 percent; however, the calculations can be modified according to the actual population sex ratio, for example, 1.06 males: 1.0 females.) A survey of surviving twins born in Denmark during 1870 to 1901 (Harvald & Hauge, 1965) indicated the proportion of unlike-sexed twins to be 0.367; thus, twice 0.367, or 0.734 (73.4 percent) would be estimated as dizygotic, and 1 minus 0.734, or 0.266 (26.6 percent) would be monozygotic. Such estimates agreed with proportions of zygosity in the same population determined by methods described later (Determination of Zygosity) in this chapter.

HERITABILITY OF TWINNING

Most studies suggest a heritable component to DZ twinning (see Nylander, 1975b; Wyshak & White, 1965). Because of observed familial aggregates, specifically an increased rate of twinning in relatives of mothers of twins, Weinberg (1909) also suggested that DZ twinning was influenced by genetic factors. Greulick (1934) reported an increased rate of twinning among both male and female relatives of DZ twins, but it has been suggested that there was a bias toward underreporting of singleton births on the paternal side. Studies of Mormons, a population for which extensive geneologic data are available, indicate an increased rate of DZ twins among children of twins and children of the sibs of twins. Female children of the female member of the pair of unlike-sexed twins had significantly greater frequencies of twins (twice as many) than the children of the male members, but the absolute difference was not impressive. These women also had significantly higher twinning rates (1/60 births) than

the general population. A mode of inheritance could not be conclusively established. Nylander (1975b) summarized frequencies of twins from large samples in Western Nigeria, an area with high twinning rates, and concluded that (1) fathers do not contribute to the twinning tendency, (2) women who have had twins previously have a twinning rate almost twice that of women who have not had twins (this could be due to either genetic or environmental factors), and (3) women who are themselves twins, or whose mothers are twins, do not have an increased twinning rate. Genetic factors offer an explanation for the variations in frequencies of DZ twinning among different racial groups. Furthermore, the frequency of DZ twinning among the offspring of interracial matings tends to more closely resemble that characteristic of the maternal rather than that of the paternal race. If the mother was the product of an interracial mating, her frequency of DZ twinning was similar to the lower twinning frequency of her parents. These data were interpreted as suggesting recessive factors (Morton, Chung, & Mi, 1967).

Formerly, MZ twinning was not considered heritable, but recently it has been suggested that a familial predisposition exists for this phenomenon (Harvey, Huntley, & Smith, 1977; Michels & Riccardi, 1978; Shapiro, Zemek, & Shulman, 1978). However, available data are not conclusive because of certain biases inevitable in studies of this type. Specifically, there is a bias towards ascertainment of families with multiple sets of twins. Although many pedigrees documenting multiple sets of twins exist, it is difficult to confirm that these events occur with a frequency greater than expected by chance. To wit, recall that twinning is not an infrequent event, occurring in approximately 1/70 to 1/100 pregnancies (1/250 for MZ twin sets alone). Therefore, the chance occurrence of two consecutive sets of twins is $(1/70) \times (1/70) = 1/4900$, assuming the two events are independent. Familial aggregates of the rarer MZ twins are more impressive. Although we have oversimplified the statistical expectations, it is obvious that selecting pedigrees with many twin pairs does not necessarily prove twinning is heritable. Studies purporting to demonstrate that twin frequencies in certain families are higher than frequencies in control families are also plagued by ascertainment biases. For example, families with twins may be more likely to seek information on other twins in the family because of their own fascination with the twinning phenomenon. Bulmer (1970) has illustrated underreporting of single births on the paternal side in several studies, including his own. Attempts to correct for these biases may or may not be valid. Furthermore, families with unusual aggregates of twins are also more likely to be brought to the attention of an investigator. Finally, it is doubtful whether the "controls" used in most studies are selected in the same fashion as are families with twin probands, or investigated as thoroughly.

In summary, heritability of MZ and DZ twinning has not been established, although more evidence has accumulated for the latter. Moreover, even if a genetic component is assumed to be responsible for either type of twinning, the mode of inheritance is not established and risks cannot be based on genetic models. Even if some families have a genetic predisposition to twinning, it is impossible at this time to differentiate which families belong in this category and which represent chance aggregation. Empiric risks would be improved if population figures were available on twinning recurrence in families after cor-

rection for race, maternal age, and family history. Existing reports indicate no higher than a twofold difference in certain families compared to general populations, and this seems within the range of variation in twin frequencies overall.

GENETIC CHARACTERISTICS OF MZ AND DZ TWINS

With rare exceptions, monozygotic twins are genetically identical because they are derived from a single zygote, which is composed of one ovum and one sperm. Dizygotic twins are produced by two ova and two sperm. Therefore, DZ twins are no more genetically alike than any two sibs. Both DZ cotwins and nontwin sibs share half their alleles. One would expect monozygotic twins to be phenotypically alike for all genetically determined traits that are characterized by complete penetrance and not readily modified by the environment. However, monozygotic twins are not necessarily phenotypically identical. For example, one twin may develop a circulatory advantage due to chorionic vascular anastomoses, whereas the cotwin may even fail to develop a heart (acardia) (Benirschke & Kim, 1973). Although concordance is high for congenital malformation among MZ twin pairs, the malformations are not necessarily the same within a pair (Myrianthapoulos, 1978). Conjoined twins, which are thought to represent MZ twins that attempted but failed to separate after 14 days of development, frequently exhibit unilateral maldevelopment, possibly due to postzygotic events. For example, anencephaly in one twin and cleft palate in the cotwin have been reported (Hamon & Dinno, 1978). Although MZ twins would be expected to have identical chromosomal complements, MZ twin pairs occasionally differ in complements because of postzygotic mitotic nondisjunction. For example, loss of an X chromosome in one twin has led to pairs consisting of one male (46,XY) and one phenotypic female (45,X) (see Benirschke & Kim, 1973).

Dizygotic twins may differ in traits determined by a single genetic locus, but they also may be alike by chance. Vascular communications in utero are rare, but mixtures of genotypes in blood and other tissues have occasionally been demonstrated (see Benirschke, 1970). This condition is referred to as chimerism. Thus, analysis of many loci may be necessary to determine zygosity. Continuously distributed or quantitative traits (blood pressure, cholesterol levels) tend to be more similar in MZ than in DZ twins; the closer the similarity, the greater the proportion of genetic causation.

DETERMINATION OF ZYGOSITY

Determination of whether twins are monozygotic or dizygotic is essential if twins are to be used for genetic studies. This information is also useful in determining the feasibility of organ transplantation, inasmuch as success is directly proportional to genetic similarity at histocompatibility loci. Determination of zygosity is sometimes also necessary to satisfy queries of twins and their relatives. Methods to determine zygosity were developed formally in the

Figure 8-2. Probability of zygosity for twin pairs.*

The following blood types occur in a family with a pair of like-sexed twins.

Family Member	Sex	ABO Type	MN Type
Mother	F	A	MN
Father	M	O	MN
Sib	F	O	MN
Twin A	M	O	MN
Twin B	M	O	MN

Inference from the established mode of inheritance of ABO and MN blood types allows determination of parental genotypes. The mother has genotype AO, and the father has genotype OO. Expected frequency in the offspring are .5 AO and .5 OO.

The mother and father both have genotype MN. Expected frequencies in their offspring are .25 MM, .50 MN, and .25 NN.

Using this information, and aware of the relative probabilities (30 percent/70 percent) of MZ/DZ twins in the population (the "a priori" probability), the relative probability of zygosity of the twin pair can be calculated:

Characteristic	MZ	DZ
Population frequency of zygosity	.3	.7
Sex	1	.5
ABO type	1	.5
MN type	1	.5
Combined†	.3	.875 (.7 × .5 × .5 × .5)

The relative probability that the twins are monozygotic is $\frac{.3}{.3 + .875} = .26$

The relative probability that the twins are dizygotic is $\frac{.875}{.3 + .875} = .74$

With analysis for many additional traits, relative probability of monozygosity or dizygosity readily reaches .99 or more.

*Only one of several possible methods greatly simplified, is shown for purposes of illustration.
†Product of the separate probabilities.

early part of this century, and have since become more elaborate (see Vogel & Motulsky, 1979). Twins of unlike sex may be assumed to be dizygotic except in rare abnormal instances, e.g., a 45,X twin and a 46,XY MZ cotwin. In addition, twins are dizygotic if they differ in any trait known both to be determined by a single genetic locus and to exhibit complete penetrance. Thus, extensive zygosity testing is not necessary if members of the twin pair obviously look very different (allowing for environmental modifications such as dyed hair and weight differences). If zygosity is uncertain, blood groups, serum proteins, HLA haplotypes and other polymorphic Mendelian traits may be compared among the twin pairs (Race & Sanger, 1975; Smith & Penrose, 1954). Barring assay errors, a single difference implies dizygosity. If all traits examined are identical, however, it need not necessarily indicate monozygosity because dizygotic twins may be alike by chance. Consequently, the probability that dizygotic twins are coincidentally identical for the traits analyzed should be computed, as illustrated in Figure 8-2. Chromosomal variants may also be analyzed to determine zygosity. MZ twins should be concordant for all heritable variants (Van Dyke et al., 1977). However, scoring of chromsomal variants is not so reliable as some other indicators of zygosity because the success of this technique varies according to technical excellence, experience in scoring variants, and laboratory standardization of criteria defining a variant.

Another method of determining zygosity is analysis based upon placentation (see Corney, 1975b). Because dizygotic twinning results from double ovulation and fertilization, two amnions and two chorions will be present. If the implantation sites are adjacent, however, a single fused placental disc may be present. If monozygotic twinning occurs rather early in development, there also will be two amnions and two chorions (diamnionic, dichorionic); however, after day 3 only one chorion can form in monozygotic twinning. Between about days 9 and 14, MZ twins can only be monoamnionic (Benirschke, 1972a, 1972b). Gross and microscopic examination of the placenta is required to determine zygosity. The tissue separating the two embryonic cavities should be examined, with a section prepared for histologic studies (Benirschke, 1972b). If only one amnion is present, the twins are monozygotic. If there is only one chorion but two amnions, the twins are also monozygotic. If two chorions and two amnions are present, however, either monozygotic or dizygotic twins could exist. Like-sexed, diamnionic, dichorionic twins must have their zygosity determined by tests of the type described in the previous paragraphs. Using placental studies, one study reported 20 percent of 668 twin pairs were monochorionic (monozygotic), 35 percent were dichorionic and of unlike sex (DZ), and 45 percent dichorionic and like-sexed. When dichorionic, like-sexed twins were subjected to analysis at a series of single locus markers, 37 percent were found to be DZ (Cameron, 1968).

USE OF TWINS FOR GENETIC ANALYSIS

The appropriate methods for genetic analysis naturally depend upon the nature of the trait to be analyzed. Several terms are commonly employed in twin studies. Concordance is said to exist if both members of a twin pair either

have or do not have the trait in question. Discordance is said to exist if only one of the twins has the trait. Investigations usually involve comparison of (1) concordant versus discordant MZ twins, (2) concordance rates in MZ versus DZ twins, (3) MZ twins in specific environments, and (4) MZ twins reared apart versus those reared together (Harvald & Hauge, 1965). For discontinuous traits (e.g., cleft palate), a significantly higher concordance in MZ compared to DZ twins is considered evidence for a genetic component. However, a significant association between concordance and MZ twins does not necessarily imply genetic causation. Moreover, zygosity correlations neither indicate the strength of the genetic component relative to the environmental component nor the mode of inheritance. Instead, it is appropriate to say only that MZ twins are more similar than DZ twins with regard to that trait, and that similarity could be due to identical genotypes.

Many biases must be taken into account when interpreting twin data (Allen, 1965; Cavalli-Sforza & Bodmer, 1971). A thorough consideration of all these potential biases is beyond the scope of this book, but a few examples will suffice. To wit, MZ twins not only share genetic factors but tend to share a more common environment than do DZ twins. This bias is particularly worrisome in the analysis of psychological or behavioral traits. Concordance may thus be due to environmental factors, including prenatal factors peculiar to monochorionic MZ twins (e.g., vascular anastomoses). In addition, MZ twins could show high concordance rates for exposure to infectious agents and their sequelae, but the sequelae may not reflect genetic factors. To some extent such bias can be corrected by comparing MZ twins reared in the same postnatal environment with MZ twins separated early in life. Such studies are difficult to perform and almost inevitably involve small sample sizes, but they do permit estimates of the contribution of environment to the trait being analyzed by comparing variation in the expression of the same genotypes in different environments. This is particularly useful for quantitative traits like blood pressure that are known to be influenced by dietary and other factors. Still another source of bias is the possibility that postzygotic events (e.g., mitotic nondisjunction) may lead to discordance among MZ twins. Thus, caution is necessary before equating discordance in MZ twins with lack of genetic control, for MZ twins may have different complements (e.g., 45,X and 46,XY).

Despite these caveats, concordance–discordance analysis has been employed to identify genetic components in many diseases, including infectious processes like tuberculosis. Demonstration of increased concordance in twin pairs over the age of 40 has led to the proposal that genetic factors are more important in maturity-onset diabetes than in diabetes occurring at younger ages (Pyke & Nelson, 1976). In addition, estimates of heritability (Chapter 1) have been made for these discrete, qualitative traits by invoking additional models that imply a continuous underlying cause (e.g., the threshold model discussed in Chapter 1). In addition to the detection of genetic components underlying "all or none" traits, penetrance may be estimated for conditions known to be controlled by a single locus by calculating the concordance rate for MZ twin pairs of which at least one is affected. This provides an estimate of those individuals with the genotype who express the phenotype.

In conclusion, much imagination and effort has been expended on studies of twins as illustrated in a recently edited series by Nance (1978a, 1978b). Many twin registers exist as data bases for current investigations (Christian, 1978). Unfortunately, the information provided by genetic analysis based on twin data is somewhat limited because of the many biases inherent in the interpretation of such data.

9

Common Gynecologic Disorders

The genetics of common gynecologic disorders have not been adequately investigated. Our lack of knowledge is especially striking compared to data available concerning inherited tendencies in most other organ systems, such as the skin or eye. There are several possible explanations for this lack of knowledge. First, gynecologic disorders usually involve internal and, hence, infrequently observed organ systems, making it more difficult to assess the genetic factors than in more readily assessable organ systems. Second, pedigree information, which is essential for establishing inheritance, may be misleading even when a potentially affected organ can be observed. For example, two sibs with a similar morphologic anomaly are less likely to be aware of such a similarity if the anomaly involved the cervix rather than, say, the hand. Third, gynecologic disorders are limited to members of one sex; thus, fewer examples of familial aggregates exist than if both sexes were affected, delaying the recognition of genetic factors. Last, the relative paucity of geneticists interested in gynecologic disorders has delayed progress.

Despite these caveats, several gynecologic disorders are known to result from mutant genes, and others are influenced by genetic factors. In this chapter we shall discuss some common conditions for which genetic data are available.

NORMAL ANATOMIC AND PHYSIOLOGIC VARIATIONS

Few data concerning the heritability of pelvic architecture are available, but there is reason to suspect existence of genetic tendencies. In most animal species, bone mass and shape are heritable, and such a tendency probably also exists in humans. This assumption is supported by observations that anthropoid and android pelvises are more common in blacks. Naylor and Warburton (1974) observed that the lengths of the diagonal conjugate were heritable in whites, although not in blacks.

Studies of inbred strains of mice suggest that heritable tendencies influence ovarian development. Ovarian weight, numbers of follicles per ovary, time at which estrus begins, ovarian response to gonadotropin, and rate of attrition of follicles relative to age differ between strains, implying genetic control. None of these variables have been studied genetically in humans.

One factor that has been studied is the age of menarche. Social and environmental factors complicate human studies, but several investigators have observed that the age of menarche differs less between monozygotic twins than between dizgotic twins (Petri, 1934; Tisserant-Perrier, 1953). The age of menarche also differs less among sisters than between unrelated women. The magnitude of the observed differences are consistent with the existence of polygenic or multifactorial factors. Knowledge of the age of menarche of relatives might permit a clinician to estimate an individual's age of menarche.

Some investigators believe that the amount of blood lost each menstruation may be influenced by genetic factors (Rybo & Hallberg, 1966). Although a reasonable assumption, only this one study has been conducted and objections could be raised to its experimental design. The correlation between mother and daughter is better than that between unrelated individuals for length of cycle, regularity of menstruation, and presence of dysmenorrhea and premenstrual tension (Widholm & Kantero, 1971). In addition, menstrual abnormalities may result from genetically determined hematologic disorders. Menorrhagia or metrorrhagia may be associated with deficiencies of clotting factors II, V, VII, and X, or with von Willebrand disease, an autosomal dominant trait in which the initial symptom may be excessive bleeding at surgery. Thrombopathic thrombocytopenia (thrombasthenia) may also be associated with metrorrhagia or menorrhagia, although hemophilia (factor VIII deficiency) apparently is not. One might also predict that age of menopause would be more similar among sibs or near relatives than among the general population. However, we are aware of no formal studies.

EXTERNAL GENITALIA

In this section we shall discuss two relatively common conditions encountered by gynecologists—imperforate hymen and fusion of the labia minora. Other disorders of sex differentiation associated with abnormal external genitalia are considered in Chapter 10.

Imperforate Hymen

Ordinarily the central portion of the hymen is patent (perforate), thereby allowing outflow of mucus and blood. If the hymen is imperforate, mucus and blood accumulate in the vagina or uterus (hydrocolpos or hydrometrocolpos). An imperforate hymen is not particularly rare. Fortunately, the anomaly is easily corrected by surgical incisions, preferably cruciform. McIlroy and Ward (1930) reported sibs who possibly had the disorder, but no other familial aggregates have been described.

Fusion of the Labia Minora

Fusion of the labia minora results from inflammation more often than from embryonic maldevelopment. However, Sueiro and Piloto (1964) described congenital fusion in four generations of a Portuguese kindred, and Simpson (1972) observed two affected sibs.

VAGINA

Müllerian Aplasia and "Absence of the Vagina"

The vagina is shortened or absent in many females whose external genitalia are ambiguous (pseudohermaphrodites). In the present context, however, "absence of the vagina" refers only to those females whose external genitalia are otherwise normal: (1) those with absence of most of the vagina and all or almost all of the uterus (Müllerian aplasia), and (2) those with absence of a portion of the vagina but presence of a normal uterus (vaginal atresia). The two conditions are embryologically distinct. Of individuals with an absent vagina, 80 to 90 percent have Müllerian aplasia; the remainder have vaginal atresia (Bryan, Nigro, & Counseller, 1949; Turunen & Unnerus, 1967; Cali & Pratt, 1968; Leduc, Van Campenhout, & Simard, 1968; Jones & Wheeless, 1969; Jones & Scott, 1971).

Vaginal Atresia

In vaginal atresia the urogenital sinus fails to contribute the caudal portion of the vagina. The lower fifth to third of the vagina is therefore replaced by 2 to 3 cm of fibrous tissue, above which are a well-differentiated upper vagina, cervix, uterine corpus, and Fallopian tubes. Vaginal atresia accounts for only 10 to 20 percent of patients with absence of the vagina (Bryan, Nigro, & Counseller, 1949; Turunen & Unnerus, 1967; Leduc, Van Campenhout, & Simard, 1968; Jones & Wheeless, 1969). Familial aggregates of vaginal atresia have not been reported, and in general less information is available about this disorder than about Müllerian aplasia. However, individuals with vaginal atresia are usually reported as part of large series of patients with absence of vagina, and analysis of a heterogenous sample of patients might obscure certain findings that might be apparent if the two disorders were analyzed separately.

Winter et al. (1968) described four sibs with a previously unrecognized autosomal recessive syndrome characterized by vaginal atresia, renal hypoplasia or agenesis, and middle ear anomalies (malformed incus, fixation of the malleus and incus). Another malformation syndrome in which vaginal atresia occurs is the Fraser syndrome (Fraser, 1962) (Table 9-1), characterized by cryptopthalamos and resulting blindness.

Transverse Vaginal Septa

Transverse vaginal septum may occur at several locations and may be complete or incomplete. Transverse vaginal septa are usually about 1 cm thick and located near the junction of the upper third and lower two thirds of the vagina (Jones & Scott, 1971; Lodi, 1951); however, they may be present in the middle or lower third of the vagina (Lodi, 1951). Perforations are usually central in location (Bowman & Scott, 1954; Deppisch, 1972) but may be eccentric

Table 9-1
Multiple Malformation Syndromes with Anomalies of the Uterus or Vagina

Condition	Main Somatic Features	Anomaly	Inheritance
Müllerian aplasia, middle ear anomalies, Klippel-Feil anomaly (Park & Jones, 1971; Nager, Chen, & Mussels, 1971)	Malformation of malleus, incus, stapes, Klippel-Feil anomaly	Müllerian aplasia	Unknown
Winter syndrome (Winter et al., 1968)	Middle ear anomalies, renal agenesis	Vaginal atresia	Autosomal recessive
Fraser syndrome (Fraser, 1962)	Cryptophalamos, external ear anomalies	Vaginal atresia, bicornuate uterus	Possible autosomal recessive
Hand–foot–uterus syndrome	Metacarpal and metatarsal anomalies	Bicornuate uterus	Autosomal dominant
(Poznanski et al., 1975)	Malformed thumbs		
Meckel syndrome (Opitz & Howe, 1969)	Polydactyly, eye anomalies, cleft palate, polycystic kidneys, occipital exencephalocele	Bicornuate uterus, genital ambiguity in males	Autosomal recessive
Rudiger syndrome (Rudiger et al., 1971)	Distal limb hypoplasia, agenesis of ear cartilage, brachydactyly, ureterovesical junction obstruction	Bicornuate uterus	Possible autosomal recessive

From Simpson JL (1976): Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York, Academic Press. Reprinted with permission.

(Kanagasuntheram & Dassanayake, 1958; White, 1966). If no perforation is present, mucus and menstrual fluid have no eggress; thus, hydrocolpos or hydrometrocolpos may develop. Other pelvic organs are usually normal, although occasionally the uterus is bicornuate.

Vaginal septa probably result from failure of the urogenital sinus derivatives and the Müllerian duct derivatives to fuse or canalize. This explanation is deduced from (1) the location of the septa, usually at the expected sites of fusion, and (2) the histologic nature of the septa (Simpson, 1976). An autosomal recessive gene is responsible for some cases of transverse vaginal septum, especially in the Amish (McKusick, Weilbaecher, & Gragg, 1968). Dungy, Aptekar, and Cann (1971) described a patient with transverse vaginal septum, polydactyly, and congenital heart disease. The latter cases suggest that the postulated mutant is pleiotropic, a suggestion to which Pinsky (1974) apparently subscribes.

Longitudinal Septa

Vaginal septa may be longitudinal—sagittal or coronal—as well as transverse. Longitudinal septa, which rarely produce clinical problems, result from abnormal mesodermal proliferation or persisting epithelium. Occasionally they impede the second stage of labor. Heritable tendencies do not appear obvious, but Edwards and Gale (1972) reported an autosomal dominant syndrome characterized by a longitudinal vaginal septum, hand anomalies, and urinary incontinence possibly due to a bladder neck anomaly.

CERVIX

Absence or Atresia of Uterine Cervix

Isolated absence or hypoplasia of the cervix, associated with a normal uterine corpus and a normal vagina, is rare. Relatively few cases have been described (Geary & Weed, 1973), and there have been no reports of multiple affected family members. The disorder presumably results either from failure of Müllerian duct canalization or from increased local epithelial proliferation after canalization is completed. Hydrometrocolpos should be anticipated. The cervical canal may also be absent in true hermaphrodites (Van Niekerk, 1974; Simpson, 1978b).

Incompetent Cervix

No heritable tendencies have been demonstrated (Naylor & Warburton, 1974). However, one would suspect that individuals with connective tissue abnormalities might be predisposed to this disorder (see Chapter 2).

UTERUS

The selected disorders described below are described more extensively elsewhere (Simpson, 1976; Sarto & Simpson, 1978).

Müllerian Aplasia

Aplasia of the Müllerian ducts leads to absence of the uterine corpus, the uterine cervix, and the upper portion of the vagina. A vagina measuring 1 to 2 cm in length is derived exclusively from invagination of the urogenital sinus. Individuals with Müllerian aplasia usually consult physicians because of primary amenorrhea. Their secondary sexual development is normal, but no uterine structures are palpable. Some investigators use the term Rokitansky-Kuster-Hauser syndrome if a rudimentary uterus is present.

Renal anomalies are associated with Müllerian aplasia more frequently (38 percent) than expected by chance (Phelan, Counseller, & Greene, 1953; Thompson, Wharton, & Te Linde, 1957; Leduc, Van Campenhout, & Simard, 1968). The most frequent renal anomalies are pelvic kidney, renal ectopia, and unilateral renal aplasia. The frequency of skeletal anomalies, especially vertebral anomalies, is also increased. Excretory urography and vertebral roent-

genograms are thus obligatory in the evaluation of patients with Müllerian aplasia.

Sibs with Müllerian aplasia have been reported on several occasions (Sarto & Simpson, 1978). These isolated observations are probably most consistent with polygenic or multifactorial inheritance, and indeed a formal study by our group showed too few probands with affected relatives to suggest single gene inheritance (Carson et al., 1982). Our data contrasted with those of Shokeir (1978a), who believes that a sex-limited autosomal dominant gene might be responsible for a small portion of individuals with Müllerian aplasia.

The only disorder that ordinarily needs to be considered in the differential diagnosis is complete testicular feminization (androgen insensitivity). Testicular feminization can be excluded on the basis of chromosomal studies and gonadal composition. In addition, puberal patients with Müllerian aplasia show pubic hair, whereas those with testicular feminization usually do not.

Müllerian Aplasia, Middle Ear Anomalies, and Klippel-Feil Anomaly

Two unrelated females with Müllerian aplasia, conductive deafness, and Klippel-Feil syndrome have been described (Nager, Chen, & Mussels, 1971; Park & Jones, 1971). Their vaginas were 1 to 2 cm long, and no uterus was present. Conductive deafness was due to malformations of the malleus, incus and stapes, and to fusion of the malleus to the attic. Other possible examples of this syndrome have been reported (Baird & Lowry, 1974; Mecklenburg & Krueger, 1974). No familial aggregates have been observed.

True Duplication of the Müllerian Ducts

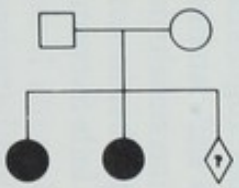
True duplication of the uterus is a rare anomaly that probably results from division of one or both Müllerian ducts early in embryogenesis. Affected individuals have two separate uteri, each of which may have two Fallopian tubes. One or both uteri may be rudimentary or bicornuate. No familial aggregates have been reported. True duplication should be distinguished from incomplete Müllerian fusion, in which each of two hemiuteri is associated with only a single Fallopian tube.

Incomplete Müllerian Fusion

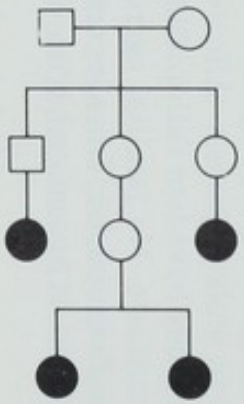
During embryogenesis the Müllerian ducts, originally paired organs, fuse and subsequently canalize to form the upper vagina, uterus, and Fallopian tubes. Müllerian ducts first arise in the 8-mm embryo; fusion and canalization are completed in the 150- to 200-mm embryo. Failure of fusion results in two hemiuteri, each associated with no more than one Fallopian tube, in contrast to true Müllerian duplication. Sometimes one Müllerian duct fails to contribute to the definitive uterus, leading to a rudimentary horn.

Several familial aggregates of incomplete Müllerian fusion have been reported, including multiple affected sibs, and affected mother and daughter (Sarto & Simpson, 1978) (Fig. 9-1). Formal genetic studies have not been undertaken, but the relatively infrequent recurrences among family members suggest polygenic or multifactorial mechanisms.

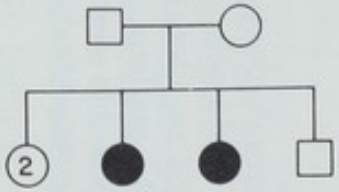
Nikiforuk, 1938



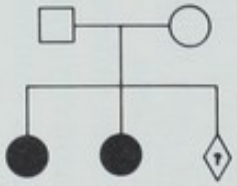
Tyler, 1939



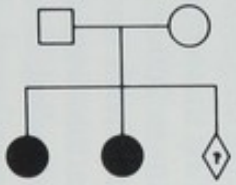
Drescher, 1966



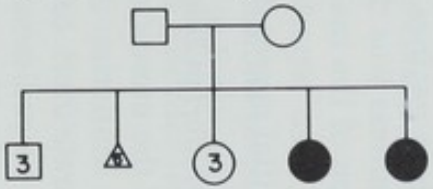
Jones & Wheeless, 1969



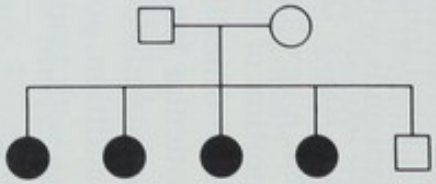
Jones & Wheeless, 1969



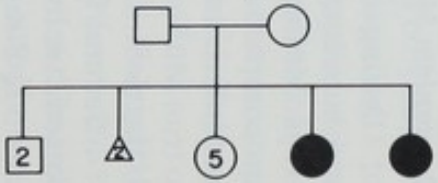
Polishuk and Ron, 1974 (Fam. 1)



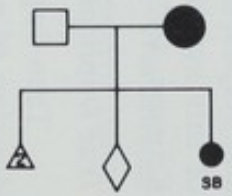
Polishuk and Ron, 1974 (Fam. 2)



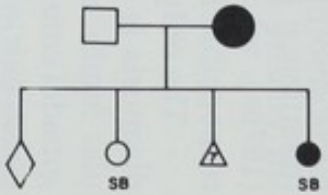
Polishuk and Ron, 1974 (Fam. 3)



Holmes, 1956 (Case 8)



Stevenson et al., 1959



Multiple Malformation Syndromes associated with Incomplete Müllerian Fusion

Incomplete Müllerian fusion may be one component of a genetically determined malformation syndrome, e.g., the Meckel syndrome (Mecke & Passarge, 1971), the Fraser syndrome (Fraser, 1962), and the Rudiger syndrome (Rudiger et al., 1971). One especially interesting syndrome is the hand–foot–uterus syndrome, an autosomal dominant disorder in which affected females have a bicornuate uterus and characteristic malformations of the hands and feet (Poznanski et al., 1975).

Leiomyomas

Differences in racial prevalences suggest that genetic factors are important in the etiology of leiomyomas of the uterus. Several investigators, including ourselves, have observed multiple family members with leiomyomas. In 1938 Winkler and Hoffman compared the prevalence of leiomyomas in relatives of 356 patients with known uterine leiomyomas to the prevalence in relatives of 356 controls. Sixty-two relatives of the patients with leimyomas were said to be affected, compared to only 19 relatives of the controls. Near relatives (defined by Winkler and Hoffman as sibs, mothers, daughters, grandmothers, and granddaughters) were affected four times as often if the proband had leiomyomas; more distant relatives were affected twice as often. These data are consistent with polygenic/multifactorial origin. However, the data were obtained by questionnaires, and systematic studies would be worthwhile. In fact, Naylor and Warburton (1974) failed to observe a strong genetic component.

Leiomyomas of both the uterus and the skin have been described in a mother and her daughter (Knoth & Knoth-Born, 1964). Tyson et al. (1970) also reported that leiomyomas were present in 7 of 33 females with achondroplasia, spondyloepiphyseal dysplasia, or sexual dwarfism.

Endometriosis

Endometriosis has long been suspected of familial tendencies (Gardner, Greene, & Ranney, 1953; Frey, 1957; Ranney, 1971; Simpson, 1972). Recently Simpson et al. (1980) and Malinak et al. (1980) confirmed these impressions in a genetic study of 123 probands with histologically verified endometriosis. Nine of 153 (5.9 percent) female sibs (over age 18) of patients with endometriosis were considered similarly affected; 10 of 123 (8.1 percent) mothers were affected. Only 1 percent of the patients' husband's first-degree relatives had endometriosis. Patients with an affected sib or mother were likely to have severe endometriosis. Eleven of 18 probands (61 percent) who had an affected first-degree relative had severe endometriosis (Malinak et al. 1980), compared to only 23 percent of 105 patients without an affected first-degree relative.

Fig. 9-1. Partial pedigrees of ten families in which multiple members had incomplete Müllerian fusion. ● = affected female, $\overset{\bullet}{SB}$ = stillborn affected female, △ = spontaneous abortion, ◇ = sex unknown, $\overset{\circ}{SB}$ = stillborn. (From Sarto GE, Simpson JL (1978): Abnormalities of the Müllerian and Wolffian duct systems. Birth Defects 14(6c):37, p 47. Reprinted with permission.)

Simpson et al. (1980a) concluded that polygenic/multifactorial factors were the most likely explanations for these observations, although other genetic explanations cannot be excluded.

ISOLATED ABSENCE OF FALLOPIAN TUBES

Absence of a Fallopian tube in an otherwise normal female is very rare (Kent, 1956). In fact, Fallopian tubes usually persist despite regression of all other Müllerian derivatives (uterus, cervix, upper vagina). Unilateral absence of the ovary may also accompany ipsilateral absence of the Fallopian tube (Georgy & Viechnicki, 1974). This suggests the pathogeneis involved a vascular accident following completion of gonadal and ductal differentiation, perhaps analogous to anorchia (Simpson et al., 1971a) (Chapter 10).

OVARIES

Gonadal dysgenesis is clearly the most well known genetically determined ovarian disorder. Other conditions worthy of discussion include ovarian unresponsiveness to gonadotropin, Stein-Leventhal syndrome, and ovarian neoplasia.

Ovarian Unresponsiveness to Gonadotropins

End-organ resistance to various pituitary trophic hormones is well documented (Rimoin & Schimke, 1971). It was thus not completely unexpected when Jones and Moraes-Ruebsen (1969) reported three females with primary amenorrhea, elevated gonadotropin levels, and histologically normal ovaries. All three had normal breasts and pubic hair and were normal in appearance. Other patients with ovarian unresponsiveness have subsequently been reported. The ovaries of these patients may be longer and thinner than normal ("fat streaks") (Jones & Moraes-Ruebsen, 1969).

The mechanism of ovarian resistance is unknown. Possibilities include functionally abnormal FSH or LH, or an autoimmune phenomenon. However, FSH and LH are normal by both immunoassay and bioassay, and at least one study (Glass & Kase, 1970) indicates that autoimmunity is not of primary etiologic importance. No familial aggregates have been reported.

Polycystic Ovary Syndrome (Stein-Leventhal Syndrome)

The Stein-Leventhal syndrome, a common gynecologic disorder, has traditionally been characterized by polycystic ovaries, obesity, hirsutism, and infertility due to anovulation. Detailed clinical considerations and management of this common gynecologic disorder are discussed in standard texts. The syndrome is characterized by varied expressivity, and may actually consist of several distinct entities. Affected individuals usually have hirsutism and either secondary amenorrhea or oligomenorrhea, but an occasional patient has pri-

mary amenorrhea. Perhaps 1 to 2 percent of patients with primary amenorrhea have Stein-Leventhal syndrome. LH secretion appears to be abnormal, but it remains uncertain whether this is a primary defect or merely a secondary effect of hyperandrogenism.

Heritable tendencies exist. Cooper et al. (1968), who studied male and female relatives of 12 probands with the Stein-Leventhal syndrome, concluded that the disorder results from an incompletely penetrant autosomal dominant gene. Cohen et al. (1975) and Wilroy et al. (1975) concluded that the trait is inherited as an X-linked dominant.

PELVIC CANCER

Vulva and Vagina

No heritable tendencies have been identified in human carcinomas of the vagina and vulva; however, no formal studies have been reported. In some strains of mice there is a relatively high frequency of squamous cell carcinoma of the vulva, whereas in other strains a high frequency of carcinoma of the vagina exists (Murphy, 1966). These data suggest that a search for genetic factors in human vulvar and vaginal carcinoma might be worthwhile.

Cervix

Familial aggregates rarely occur among individuals with carcinoma of the cervix. Neither Rotkin (1966) nor Albert and Child (1977) detected an increased prevalence of carcinoma of the "uterus and cervix" or of carcinoma of the ovaries in near relatives. Rotkin also observed several pairs of monozygotic twins discordant for carcinoma of the cervix, although concordantly affected twins have been reported. Inasmuch as carcinoma of the cervix is known to be related to certain socioeconomic and epidemiologic factors, the lack of heritable factors is not surprising.

Uterus

In addition to epidemiologic factors (e.g., estrogens) that are well known to gynecologists, heritable factors are involved in the etiology of carcinoma of the endometrium (Albert & Child, 1977). Lynch et al. (1966) studied 154 probands with endometrial carcinoma, of whom 17 percent had a first-degree relative with adenocarcinoma of the endometrium or colon. The authors concluded that uterine adenocarcinoma results from an autosomal dominant gene of decreased penetrance.

Familial tendencies in uterine carcinoma seem certain, but adenocarcinoma of the endometrium may or may not result from a single gene. In addition, hypertension and obesity, both of which are associated with cancer of the endometrium, are each heritable. Sarcomas or mixed mesodermal carcinomas of the uterus have not been studied genetically.

Ovaries

Ninety percent of ovarian neoplasia arises from germinal epithelium; the remainder arise from germ cells or stromal cells (sex-cord mesenchyme). There is evidence for both genetic factors and environmental factors.

Epidemiology

The epidemiologic aspects of ovarian neoplasia have not been well studied because most investigators fail to distinguish between the various types. Nonetheless, some interesting data are available (Falthalla, 1972; Simpson & Photopulos, 1976a, 1976b). For example, the incidence of ovarian neoplasia is lower in Japan than in most western countries. However, first-generation Japanese living in the United States have approximately the same incidence as American Caucasians; thus, genetic factors cannot be the sole explanation for the ethnic differences. The incidence is higher in Ashkenazi Jews than in Sephardic Jews, higher in South African Bantus than in American whites or blacks, and higher in American Caucasians than in American Indians (Falthalla, 1972; Simpson & Photopulos, 1976b). The frequency is also higher than normal in higher socioeconomic classes in Great Britain. Ovarian neoplasia occurs much more often among the female of an unlike-sex twin pair than among members of a female like-sex pair (Nance, 1975).

Epithelial Neoplasia

Despite data indicating environmental factors, several familial aggregates of ovarian epithelial neoplasia have been reported (Fraumeni et al., 1975; Simpson & Photopulos, 1976b). Although the histology was not always stated, it was usually papillary serous adenocarcinoma. Individuals in two or more generations were usually affected (Fraumeni et al., 1975; Li et al., 1970; Lurain & Piver, 1979), but sometimes only sibs were affected. The ovarian tumors in these kindreds do not appear to differ clinically from sporadically arising tumors; however, in one family (McCrann, Marchant, & Bardawil, 1974) three teenage sibs were affected. Otherwise, the age of onset seems similar in both familial and nonfamilial cases, and the frequency of bilaterality is similar. (Familial cancers are usually characterized by bilaterality and early age of onset [Knudson, Strong, & Anderson, 1973].) The data are consistent with but do not prove postulates that some ovarian adenocarcinomas result from a single dominant gene(s) or from polygenic/multifactorial factors. Our studies in a human genetic isolate also indicate that recessive genes are not of paramount importance in the etiology of ovarian epithelial cancer (Simpson et al., 1981).

Familial aggregates of Brenner tumors or of endometrioid tumors have not been reported. Lynch and Krush (1971) and Fraumeni et al. (1975) have reported kindreds in which multiple family members had either carcinoma of the breast or epithelial carcinoma of the ovary.

Germ Cell Tumors

Ovarian germ cell tumors include benign cystic teratomas, embryonal carcinomas (yolk sac tumor, endodermal sinus tumor, dysgerminoma, gonadob-

lastoma, and choriocarcinoma). No familial aggregates of embryonal carcinoma have been reported, but some genetic data concerning other germ cell tumors are available.

Benign Cystic Teratomas

Teratomas (dermoid tumors) may arise in gonadal or in extragonadal sites. Nongonadal tumors may be related to sequestration of embryonic cells prior to the stage of differentiation in which totipotential capacity is inhibited. By contrast, gonadal teratomas probably arise by parthenogenesis (Linder, McGaw, & Hecht, 1975). That genetic factors are relevant to the etiology of ovarian dermoid tumors are suggested by the relatively high prevalence of bilateral tumors, and the relatively young age of onset. Bilaterality and early age of onset are characteristics of hereditary tumors (Knudson, Strong, & Anderson, 1973). Ovarian teratomas have been reported in sisters, in a mother and her daughters, and in each of triplets (see Simpson & Photopulos, 1976b). If the pathogenesis of ovarian teratomas indeed involves meiotic errors, the mutant gene responsible for teratomas might logically exert its action by interfering with normal cell division.

46,XX, Dysgerminomas

Familial aggregates of dysgerminomas occur not infrequently in individuals with XY gonadal dysgenesis but rarely among 46,XX individuals. However, Jackson et al. (1967) reported 46,XX dysgerminoma in two and perhaps three generations of a Jamaican kindred.

Gonadoblastomas and 46,XY Dysgerminomas

Approximately 25 percent of individuals with XY gonadal dysgenesis and about 15 percent of 45,X/46,XY individuals have either gonadoblastomas or dysgerminomas (Simpson & Photopulos, 1976a). Dysgerminomas may be malignant, albeit usually sensitive to irradiation. Gonadoblastomas are usually benign; however, about 10 percent are associated with malignant germ cell tumors. Dysgerminomas and gonadoblastomas may arise as early as the second decade. Thus, gonadal extirpation should be performed immediately after the diagnosis of XY gonadal dysgenesis, a trait inherited in X-linked recessive fashion, or after 45,X/46,XY mosaicism is established in a phenotypic female.

Sex Cord Mesenchyme Tumors

Sex cord mesenchyme tumors include granulosa cell tumors, theca cell tumors, Sertoli cell tumors, and Leydig cell tumors. Mixed varieties are common.

Granulosa Cell Tumors

Familial aggregates of granulosa cell tumors rarely occur in otherwise normal females. However, ovarian tumors, especially granulosa cell tumors, are frequently associated with the Peutz-Jegher syndrome (colonic polyposis; oral melanosis). In one series 16 of 115 patients with the Peutz-Jegher syndrome had an associated ovarian tumor (Dozois, Kempers, & Dahlin, 1970). Interest-

ingly, granulosa cell neoplasia is a relatively common ovarian neoplasia in many animals. The etiology of granulosa cell tumors in animals appears to be related inversely to ease of elimination of oogonia; if no oogonia are present, granulosa cells apparently proliferate in response to increased pituitary gonadotropin (Falthalla, 1972). In addition, granulosa cell tumors can be induced in rodents by irradiation or administration of 9:10-dimethyl 1:2-benzanthracene or progestational steroids (Krarup, 1970). Neoplastic susceptibility depends upon the genotype of the animal (Marchant, 1959).

Sertoli-Leydig Cell Tumors

These tumors, although rare, have been observed in sibs, in two or more generations, and in cousins (Simpson & Photopulos, 1976b), suggesting genetic influences.

Fibromas and Fibrosarcomas

Ovarian fibrosarcomas were described in each of dizygotic twins; however, no other familial aggregates have been reported (Simpson & Photopulos, 1976b). Ovarian fibromas may also be associated with the basal cell nevus syndrome and with neurofibromatosis (von Recklinghausen syndrome) (Clendenning, Herdit, & Block, 1963).

Trophoblastic Disease

The likelihood of recurrence of trophoblastic disease is usually estimated to be 1 to 2 percent. The risk is considerably higher than the 1/2000 incidence in Caucasians, suggesting genetic tendencies. In addition, the well-known geographic variation in frequencies of trophoblastic disease might also indicate underlying genetic factors. If genetic factors do exist, however, the manner in which they act is uncertain. Moreover, the observed relationship between nutrition and trophoblastic disease necessitates caution, for recurrence could merely indicate persistence of deleterious environmental factors. If genetic factors are important in the etiology of trophoblastic disease, they probably involve the immunologic system. Although there is indirect evidence implicating immunologic factors in the development of trophoblastic disease, there is no direct evidence that specific paternal histocompatibility (HLA) alleles confer increased or decreased susceptibility toward development of trophoblastic disease. On the other hand, women who develop trophoblastic disease reject skin grafts from their spouses less readily than from controls, indicating the importance of immunologic factors. Immunologic responsiveness is genetically determined.

Cytogenetic studies are yielding exciting data. Hydaditiform moles may show diploid (46,XX; 46,XY) or triploid (69,XXX; 69,XXY) chromosomal complements. (Placentas of triploid abortuses frequently show tendencies toward hydropic degenerations, but the converse is not necessarily true [Carr, 1968].) Vassilakos, Riotton, & Kajii (1977) divide hydatidiform moles into two cytogenetic and pathologic classes: (1) "classical" moles, often malignant, which are usually diploid and usually do not coexist with fetal parts, and (2) non-

classical moles, rarely malignant, which are usually triploid and often associated with fetal parts. Using chromosomal variants to deduce parental chromosomal origins, Kajii and Ohama (1977) and Jacobs et al. (1978) reported that in diploid moles both haploid contributions may be paternal (androgenetic). Androgenetic origin implies either failure of the female pronucleus to participate in syngamy, or expulsion of the maternal haploid complement after syngamy. Such phenomena requires diploid sperm, or fertilization by more than one sperm. If diploid moles are more likely to transform into invasive moles or choriocarcinoma than triploid moles, the observation that diploid moles are androgenetic is surprising, because one might reason that exclusively paternal histocompatibility loci would facilitate rejection and hence decrease the likelihood of malignant transformation. Additional data are clearly required before clinical applicability, but cytogenetic studies should eventually prove helpful in diagnosis and management.

10

Disorders of Sex Chromosomes and Sexual Differentiation

The disorders of sexual differentiation can be delineated in several ways. Prior to recent cytogenetic and endocrinologic advances, the usual way to delineate these disorders was on the basis of their anatomic differences, e.g., male pseudohermaphroditism, female pseudohermaphroditism, or true hermaphroditism. With endocrinologic and genetic advances, it became apparent that not only were there many more disorders of sexual differentiation than previously suspected, but the delineation of these disorders on anatomical grounds alone was inadequate.

Although many ways to delineate disorders of sexual differentiation have been proposed, it seems clinically preferable to place an affected individual initially into one of several broad categories that can be recognized readily on the basis of chromosomal complement and gonadal status. Such categories include gonadal dysgenesis, true hermaphroditism, female pseudohermaphroditism, male pseudohermaphroditism, Klinefelter syndrome, and hypogonadotrophic hypogonadism. The various disorders within each category are probably best delineated on the basis of etiology—chromosomal abnormalities, recessive or dominant genes, or teratogenic factors.

In this chapter we shall delineate only the most common disorders of sex chromosomes and sexual differentiation. These disorders are discussed in greater detail elsewhere (Simpson, 1976; Simpson, 1980b). Etiology and diagnosis will be emphasized; surgical aspects, psychological considerations, and hormonal replacement are considered in other texts. Anomalies limited to the Müllerian derivatives were discussed in Chapter 9.

PRINCIPLES OF REPRODUCTIVE EMBRYOLOGY AND H-Y ANTIGEN

If a normal X-chromosome–bearing ovum is fertilized by a Y-chromosome–bearing sperm, a 46,XY male zygote results. If an X-bearing ovum is fertilized by an X-bearing sperm, a 46,XX female zygote results. The testicular

determinant(s) on the Y are localized to the centromeric region, probably on the short arm (German, Simpson, & Lemore, 1973; Simpson, 1975, 1976). This portion of the Y is nonfluorescent. The manner by which the testicular determinant(s) acts is unknown, but Wachtel and colleagues (Wachtel et al., 1975; Wachtel, 1977) have shown that H-Y antigen, a cell-surface antigen, is integrally involved with testicular differentiation.

Circumstantial and direct evidence suggests that H-Y antigen can direct testicular differentiation. The following circumstantial data (Simpson, 1980a; Wachtel, 1977, 1979) are consistent with the hypothesis:

1. H-Y antigen is evolutionarily conservative, being present in all tested mammals in the sex containing the Y chromosome.
2. At least one locus for H-Y antigen is near or identical to the locus for the testicular determinant(s).
3. A 48,XXYY male had twice the H-Y antigen titer of 46,XX males.
4. H-Y antigen is present in humans and mice with androgen insensitivity, indicating that it is not merely induced by androgens.
5. H-Y antigen on XY cells is expressed after transfer to a female host.
6. H-Y antigen is present in approximately 50 percent of mouse blastocysts (Krco & Goldberg, 1976), a stage prior to organ differentiation and, hence, prior to testicular differentiation.
7. H-Y antigen is present in sex-reversed 46,XX true hermaphrodites.
8. H-Y antigen is present in the testicular but not the ovarian portion of ovotestes. (Winters et al., 1979). In general, H-Y has thus been detected in individuals with testes, but not in those lacking testes (Wachtel, 1977, 1979).

Direct evidence also exists. Neonatal mouse or rat testes disassociated by Moscona-type disruption reaggregate into tubular-like structures (Ohno, Nagain & Ciccarse, 1978; Zenzes, Wolf, & Gunther, 1978). However, H-Y antisera cause dissociated testicular cells to reaggregate into follicle-like (female) structure. The more definitive experiment—H-Y induced conversion of ovarian germ cells to tubular-like structures—has also been performed (Zenzes, Wolf, & Engel, 1978).

Although attractive, the H-Y antigen theory is not perfect. In addition to the problems in assay reproducibility, some confusing data remain to be explained. For example, 45,X individuals have H-Y antigen, albeit low titers. Coupled with other observations, this finding suggests that the X contains loci capable of suppressing H-Y. In addition, the above indicates that the structural locus for H-Y is located not on the Y but on an autosome or the X (Wolf, 1981). Nonetheless, it seems obvious that H-Y antigen is integrally involved in testicular differentiation.

Irrespective of H-Y antigen status, primordial germ cells originate in the endoderm of the yolk sac. By about the eighth week of embryonic life they have migrated to the genital ridge, which originated from thickening of the coelomic epithelium. 46,XY and 46,XX gonads are indistinguishable at this stage. If primordial germ cells are 46,XY, the indifferent gonad develops into a testis. In the absence of a Y chromosome (46,XX or 45,X) or more specifically in the absence of sufficient H-Y antigen, the indifferent gonad develops into an ovary.

If two intact X chromosomes are not present, most ovarian follicles degenerate by the time of birth.

After having differentiated, the developing testes secrete two hormones. Fetal Leydig cells produce an androgen, probably testosterone. Testosterone stabilizes the Wolffian ducts and permits differentiation of vasa differentia, epididymides, and seminal vesicles. After conversion by 5α-reductase to dihydrotestosterone, virilization of external genitalia is achieved (Siiteri & Wilson, 1974). These actions can be mimicked by the administration of testosterone to female or castrated male embryos. Fetal Sertoli cells produce a second hormone, a glycoprotein hormone that diffuses locally to cause regression of Müllerian derivatives (uterus and Fallopian tubes). The action of this hormone cannot be duplicated by any known compound. In the absence of these two hormones the external genitalia develop along female lines, the Müllerian ducts develop into a uterus and Fallopian tubes, and the Wolffian ducts regress. These changes occur in normal 46,XX embryos, or castrated 46,XX embryos, and castrated 46,XY embryos.

FEMALE PSEUDOHERMAPHRODITISM

Female pseudohermaphrodites are 46,XX individuals whose external genitalia fail to develop as expected for normal females. Affected individuals may result from either genetic or teratogenic causes. Most have anomalies limited to the external genitalia, but in a few disorders the genital anomalies may be associated with abnormalities of embryonic hindgut, cloacal membrane, or urogenital membrane.

Genetic Forms

Genetic forms of female pseudohermaphroditism can be divided into those in which an adrenal biosynthetic error exists and those in which no biosynthetic error exists.

Adrenal Hyperplasia (Adrenogenital Syndromes): General Considerations

The syndromes of adrenal hyperplasia result from deficiencies of the various enzymes required for steroid biosynthesis (Fig. 10-1). Specifically, deficiencies of 21-hydroxylase, 11β-hydroxylase, and 3β-ol-dehydrogenase produce female pseudohermaphrodites. In adrenogenital syndromes, the common pathogenesis involves decreased production of adrenal cortisol. Cortisol regulates secretion of ACTH through a negative feedback inhibition mechanism. If cortisol production is decreased, ACTH secretion is not inhibited. Elevated ACTH leads to increased quantities of steroid precursors, from which androgens can be synthesized (Fig. 10-1). Because the fetal adrenal begins to function during the third month of embryogenesis, excessive production of adrenal androgens will virilize external genitalia (Fig. 10-2). Müllerian and gonadal development remain unaffected because neither is androgen-dependent.

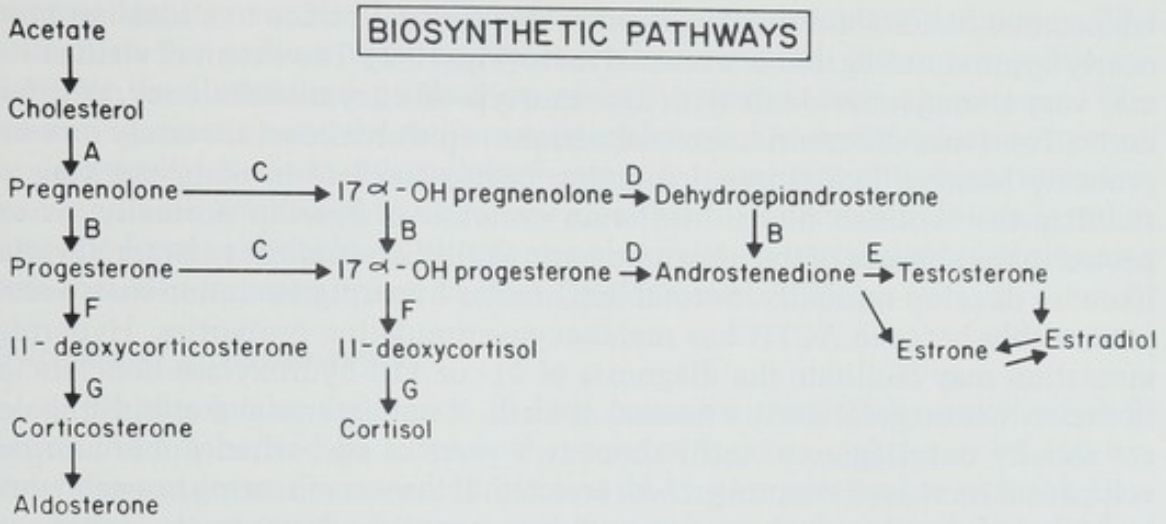


Figure 10-1. Summary of important adrenal and gonadal biosynthetic pathways. Letters designate enzymes required for the appropriate conversions. A = 20α-hydroxylase, 22R-hydroxylase, and 20,22-desmolase. B = 3β-ol-dehydrogenase. C = 17α-hydroxylase. D = 17,20-desmolase. E = 17-ketosteroid reductase. F = 21-hydroxylase. G = 11β-hydroxylase. (From Simpson JL; Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York, Academic Press, 1976, p 158. Modified with permission).

Syndromes of adrenal hyperplasia are the most common causes of genital ambiguity. These disorders must be excluded when assessing an individual with genital ambiguity, because cortisol and corticosterone deficiencies result in sodium wasting. If untreated, these deficiencies can lead to hyponatremia, hyperkalemia, dehydration, and possibly death due to renal insufficiency.

Females deficient for 21- or 11β-hydroxylase show clitoral hypertrophy,

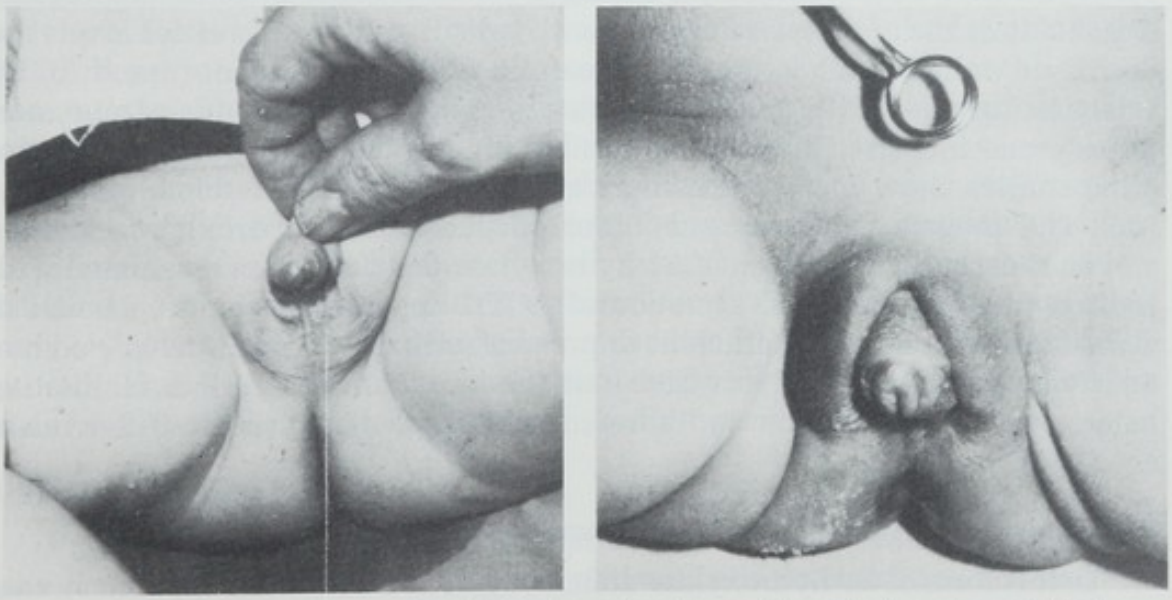


Figure 10-2. External genitalia of two female sibs with 21-hydroxylase deficiency. (From Summit RL: Differential diagnosis of genital ambiguity in the newborn. Clin Obstet Gynecol 15:112 1972. Reprinted with permission.)

labioscrotal fusion, and displacement of the urethral orifice to a location more nearly approximating that of a normal male (Fig. 10-2). The extent of virilization may vary among individuals with the same type of enzyme deficiency. Wolffian derivatives (vasa differentia, seminal vesicles, epididymides) are rarely present, probably because fetal adrenal function begins too late in embryogenesis to stabilize the Wolffian ducts. Müllerian derivatives develop normally, as expected in the absence of the nonandrogenic Müllerian-inhibitory factor. Ovaries likewise develop normally. Scrotal and areolar hyperpigmentation may occur, presumably because ACTH has melanocyte-stimulating properties. Hyperpigmentation may facilitate the diagnosis of 21- or 11β-hydroxylase deficiencies in males, whose genitalia are normal at birth. If not ascertained at birth, males are usually not diagnosed until about two years of age, when pubic hair develops and increased statural growth is noted. If the sex of rearing is established by 18 months and genital reconstruction accomplished at 2 to 4 years of age, the child should be female in psychosexual development.

Immediate attention must be given to the administration of cortisol, correction of hyperkalemia, and restoration of fluid-electrolyte balance. Salt-retaining hormones, e.g., fluorinated hydrocortisone, may be necessary. Cortisol administration must be continued into adulthood, although requirements per unit weight may diminish. Mineralocorticoids are usually not necessary after 5 years of age.

Even if cortisol administration is begun immediately following birth, patients with adrenal hyperplasia usually only attain heights between the third and fifteenth percentiles (Riddick & Hammond, 1975). If cortisol is not administered, affected individuals initially experience increased growth during early childhood; however, premature epiphyseal closure leads to a final adult height below the third percentile.

Deficiency of 21-Hydroxylase without Sodium Wasting

Deficiency of 21-hydroxylase, the enzyme that converts 17α-hydroxyprogesterone to 11-desoxycortisol, is the most common cause of genital ambiguity (Fig. 10-1). If 21-hydroxylase is deficient, cortisol and deoxycortisol levels are decreased unless a compensatory increase in ACTH secretion occurs. If ACTH secretion increases, 17α-hydroxyprogesterone, androstenedione, estrone, and testosterone increase, the last responsible for genital masculinization. Endocrine studies show increased urinary excretion of 17-ketosteroids, pregnanetriol, and pregnanetriolone, and increased plasma 17α-hydroxyprogesterone and testosterone. In the form of 21-hydroxylase deficiency not associated with sodium wasting, increased secretion of ACTH apparently results in levels of aldosterone and cortisol sufficient to prevent sodium wasting. 21-hydroxylase deficiency is an autosomal recessive trait. It is closely linked to HLA, facilitating heterozygote identification and allowing antenatal diagnosis (L. S. Levine et al., 1978).

Deficiency of 21-Hydroxylase with Sodium Wasting

In this form of 21-hydroxylase deficiency, ACTH secretion apparently cannot increase sufficiently to compensate for the enzyme deficiency, presumably because the enzyme deficiency is more severe than in the form discussed above.

External features are similar in both forms of 21-hydroxylase deficiency. The mechanism for sodium wasting is not well understood, but one possible explanation is diminished production of corticosterone and aldosterone. The nonsodium wasting and the sodium wasting forms of 21-hydroxylase deficiency are genetically distinct, although both result from mutant autosomal recessive genes and both are linked to HLA. In a given family affected individuals either have one form or the other. Other forms also exist, in particular an adult-onset form that also appears linked to HLA (Pollack et al., 1981).

Deficiency of 11β-Hydroxylase

11β-hydroxylase deficiency is less common than 21-hydroxylase deficiency. Also inherited in autosomal recessive fashion, deficiency of 11β-hydroxylase results in decreased conversion of 11-deoxycortisol to cortisol. Desoxycortisol, desoxycorticosterone, and testosterone are increased. Tetrahydrocortisol, the major metabolite of 11-deoxycortisol, is increased.

Because desoxycortisol and desoxycorticosterone are potent salt-retaining hormones, increased levels may lead to hypervolemia and hence to vascular hypertension. Patients with 11β-hydroxylase deficiency thus not only manifest the genital virilization characteristic of patients with 21-hydroxylase deficiency, but also often develop hypertension because of hypervolemia. Salt-wasting does not occur. This form is not linked to HLA.

Deficiency of 3β-ol-Dehydrogenase

If 3β-ol-dehydrogenase (Fig. 10-1) is deficient, the only androgen synthesized is dehydroepiandrosterone (DHEA), a relatively weak androgen that cannot be converted to androstenedione and testosterone. Therefore, females with 3β-ol-dehydrogenase deficiency are less virilized than females with 21- or 11β-hydroxylase deficiencies. DHEA is so weak that males with 3β-ol-dehydrogenase deficiency fail to masculinize completely (male pseudohermaphroditism). 3β-ol-dehydrogenase deficiency is the only form of adrenal hyperplasia in which both males and females show genital ambiguity. 3β-ol-dehydrogenase activity is believed to achieve its maximum capacity earlier in embryonic testes (third month) than in embryonic adrenals and ovaries (fourth month). Otherwise, one might expect the external genitalia to be identical in affected males and affected females.

Complete deficiency of 3β-ol-dehydrogenase results in severe salt wasting because of deficiency of salt-retaining hormones. The sodium wasting is often so pronounced as to cause the affected infant to die. However, less severe deficiencies are compatible with long-term survival and, recently, increasing numbers of cases are being detected in older infants (Bongiovanni, 1979). The trait is inherited in autosomal recessive fashion, and is best diagnosed on the basis of elevated urinary *pregnenetriol* (Bongiovanni, 1979).

Adrenal Lipoid Hyperplasia

Prader and colleagues were the first to describe male infants with genital ambiguity, severe sodium wasting, and adrenal cells that contained cholesterol deposits (Prader & Gurtner, 1955). The adrenal cells have a foamy appearance, hence the appellation adrenal lipoid hyperplasia. The cholesterol deposits and

the hormonal profile suggest an inability of the adrenals to convert cholesterol to pregnenolone. This conversion requires three enzymes: 20α-hydroxylase, 22R-hydroxylase, and 20,22R-desmolase. Some investigators believe the desmolase enzyme is more likely to be deficient, whereas others favor a deficiency of 20α-hydroxylase. Sodium wasting is so severe that surviving individuals are probably only partially deficient for the mutant enzyme. Both males and females may be affected; thus, autosomal recessive inheritance seems likely.

Females with adrenal lipoid hyperplasia have normal external genitalia. Therefore, they are technically not female pseudohermaphrodites.

Deficiency of 17α-Hydroxylase

If 17α-hydroxylase is deficient, pregnenolone cannot be converted to 17α-hydroxypregnenolone. Cortisol, androstenedione, testosterone, and estrogens cannot be synthesized if the defect is complete; however, 11-desoxycorticosterone and corticosterone can be synthesized. As ACTH secretion compensatorily increases, 11-deoxycorticosterone and corticosterone increase. This results in hypernatremia, hypokalemia, hypervolemia, and hypertension. Aldosterone levels are decreased, possibly because hypervolemia suppresses the renin-angiotensin system.

Females with 17α-hydroxylase deficiency have normal external genitalia, but at puberty they fail to undergo normal secondary sexual development (primary amenorrhea). Affected males usually have genital ambiguity (male pseudohermaphroditism). 17α-Hydroxylase deficiency is an autosomal recessive trait.

Adrenal Hyperplasia Occurring After Infancy

Late-onset or possibly acquired forms of adrenal hyperplasia probably exist (Riddick & Hammond, 1975). After treatment with prednisone or similar corticosteroids, pregnancy is possible. Adult-onset 21-hydroxylase deficiency is probably genetically distinct from the congenital forms.

Nonadrenal Causes

Jones and Park (1971) and Park, Jones, and Melham (1972) described two sibs with clitoral hypertrophy, a single perineal orifice leading anteriorly to a urethra and posteriorly to a vagina, normal Müllerian derivatives, normal ovaries, and numerous skeletal anomalies (hypoplasia of the mandible and maxilla, brachycephaly, narrow vertebral bodies, relatively long slender bones, dislocation or fusion of the radial heads leading to abnormal-appearing elbows, coxa valga, and phalangeal fusion of several toes). Both sibs developed breasts and pubic hair but failed to menstruate. Their parents were consanguineous; thus, autosomal recessive inheritance seemed probable.

Female pseudohermaphroditism can also be associated with one or more of the following anomalies: absence or duplication of the uterus; absence, duplication, or hydronephrosis of the kidneys; and imperforate anus (Park et al., 1972; Lubinsky, 1980.) Short stature, mental retardation, deafness, ear and nose

malformation, or a blindly ending colon are less often associated. Ovaries are usually normal. The etiology of this trait is unknown.

Marked clitoral enlargement of unexplained origin sometimes results from hemangiomas, neurofibromas, or tumors.

Teratogenic Forms

Hormonal Causes

Female pseudohermaphroditism can result from administration of androgens or certain progestins during pregnancy. These forms of female pseudohermaphroditism are especially important because they are preventable. For decades scientists have known that administration of testosterone and other androgens to pregnant animals could masculinize their female offspring. Phallic enlargement, labioscrotal fusion, displacement of the urogenital sinus invagination, and Wolffian duct development could occur. Androgens do not affect Müllerian differentiation or ovarian differentiation.

Despite earlier reports of androgen-induced female pseudohermaphroditism in humans, the phenomenon was not recognized as clinically important until 1958 and 1959, when it was recognized that women treated during pregnancy with synthetic progestins gave birth to virilized female offspring (Grumbach, Ducharme, & Moloshak, 1959). During those years it was acceptable to administer progestational agents to pregnant women who had previously had spontaneous abortions. Spontaneous abortion was believed to result frequently from inadequate placental synthesis of progesterone; thus, one might logically administer progestins in hopes of favorably influencing the outcome of pregnancy. In retrospect, the prospect of success was small because spontaneous abortion usually results not from endocrine but from genetic causes and because enormous amounts of oral or parenteral progestins are required to increase uterine myometrial levels of progesterone. Administration of progestins during pregnancy is now rarely indicated.

Not every female fetus exposed to a given progestin is masculinized. Teratogenesis depends upon the specific agent, the dosage, the time during gestation when the agent is administered, and the genotype of the mother and fetus. In humans the genital tubercle first becomes evident at about five weeks of gestation (seven weeks from the last menstrual period). By 10 to 12 weeks, male and female embryos are anatomically distinguishable. In males the urinary meatus reaches the top of the glans penis by 14 weeks; in females genital differentiation is completed earlier. In order to influence genital differentiation, a teratogen must be present during the period outlined above. Prior to that time no organ-specific structure can be affected. Thus, labioscrotal fusion occurs only if an appropriate teratogen is administered prior to 12 weeks. After 12 weeks a teratogen may cause clitoral enlargement, but not labioscrotal fusion.

Some hormones are more likely to produce female pseudohermaphroditism than others (Simpson, 1976). Testosterone, ethinyl testosterone, norethindrone acetate, and norethindrone are proved teratogens. Norethynodrel with mestranol medroxyprogesterone and 17α-OH-progesterone caproate have rarely

been implicated. By contrast, all progestins have been claimed capable of causing fetal cardiac anomalies (Heinoen et al., 1977).

Androgen-secreting tumors in pregnant women can masculinize female fetuses, although patients with preexisting androgen-secreting tumors rarely become pregnant. Fetal masculinization has been reported in pregnancies associated with arrhenoblastoma, Leydig cell tumor, luteoma of pregnancy, and certain adenocarcinomas metastatic to the ovary (Krukenberg tumor). Verhoeven et al. (1973) reviewed 45 reported cases in which virilizing tumors were associated with pregnancy. Among the offspring were 18 females whose external genitalia were described; 9 had clitoral or labial hypertrophy, but only 1 had labioscrotal fusion.

Other Causes

Genital abnormalities may also result from maldevelopment of the genital tubercle, cloacal membrane, urogenital membrane, or the entire hind end of the embryo (caudal regression syndrome). In some of these malformations the external genitalia may be so abnormal that the sex of rearing is in doubt; thus, the designation female pseudohermaphroditism is appropriate. These rare disorders, discussed elsewhere in detail (Simpson, 1976), include exstrophy of the bladder, exstrophy of cloaca, and sirenomelia.

MALE PSEUDOHERMAPHRODITISM

Male pseudohermaphrodites are individuals with a Y chromosome whose external genitalia fail to develop as expected for normal males. Some authors apply the appellation only to those whose external genitalia are ambiguous enough to confuse the choice of sex of rearing; however, applying the term more liberally seems more clinically useful.

Cytogenetic Forms

45,X/46,XY Phenotypes

Individuals with both a 45,X cell line and at least one line containing a Y chromosome may manifest a variety of phenotypes, ranging from almost normal males with cryptorchidism or penile hypospadias to females indistinguishable from those with 45,X Turner syndrome. The different phenotypes presumably reflect different tissue distributions of the various cell lines; however, this assumption is unproved. 45,X/46,XY individuals may be grouped into one of three categories, namely individuals with (1) unambiguous female external genitalia; (2) ambiguous external genitalia, or (3) almost normal male external genitalia.

Female external genitalia. Perhaps 5 percent of patients with gonadal dysgenesis and unambiguous external genitalia have a complement consisting of both a 45,X line and a line containing a Y chromosome. These individuals

may have Turner stigmata and thus be clinically indistinguishable from 45,X individuals. These 45,X/46,XY individuals are usually normal in stature and show no somatic anomalies. As in any type of gonadal dysgenesis, the external genitalia, vagina, and Müllerian derivatives remain unstimulated because of the lack of sex steroids. Breasts fail to develop, and little pubic or axillary hair develops. In fact, if breast development occurs in a 45,X/46,XY individual, one should suspect an estrogen-secreting tumor, namely a gonadoblastoma or dysgerminoma.

The streak gonads of 45,X/46,XY individuals are usually histologically indistinguishable from the streak gonads of individuals with 45,X gonadal dysgenesis, but gonadoblastomas or dysgerminomas occur in about 15 to 20 percent of 45,X/46,XY individuals (Scully, 1970; Simpson & Photopulos, 1976a). Neoplasia in 45,X/46,XY individuals may occasionally arise during the first two decades, in contrast to the neoplasia that arises in cryptorchid yet otherwise normal males (46,XY). Gonadoblastomas, which occur almost exclusively in 46,XY or 45,X/46,XY individuals, are probably benign; however, they may be associated with dysgerminomas or other malignant germ cell tumors. Thus, streak gonads should be extirpated from 45,X/46,XY individuals, regardless of their age. Because of the risk of neoplasia, one should distinguish patients with 45,X/46,XY gonadal dysgenesis from those who have complements lacking a Y chromosome. Analysis of buccal epithelial cells for the presence or absence of X-chromatin thus constitutes inadequate evaluation for patients with gonadal dysgenesis because X-chromatin is present in neither 45,X nor 45,X/46,XY individuals.

Ambiguous genitalia. The terms asymmetric gonadal dysgenesis or mixed gonadal dysgenesis are applied to individuals who have one streak gonad and one dysgenetic testis. Individuals with mixed gonadal dysgenesis usually have ambiguous external genitalia and a 45,X/46,XY complement, but occasionally have only 45,X or only 46,XY cells. Many investigators believe that the phenotype is almost always associated with 45,X/46,XY mosaicism, apparent nonmosaic cases merely reflecting the inability to analyze appropriate tissues.

Most 45,X/46,XY individuals with ambiguous external genitalia have Müllerian derivates (e.g., a uterus). Presence of a uterus is diagnostically helpful because it is not present in most genetic forms of male pseudohermaphroditism. If an individual has ambiguous external genitalia, bilateral testes, and a uterus, it is therefore reasonable to infer that such a person has 45,X/46,XY mosaicism, regardless of whether both lines can be demonstrated cytogenetically. Occasionally the uterus is rudimentary, or a Fallopian tube may fail to develop on the side on which a testis is present.

Almost normal male genitalia. Occasionally 45,X/46,XY mosaicism is detected in individuals with almost normal male external genitalia. In these cases a uterus is less likely to be present than in 45,X/46,XY individuals with ambiguous or female external genitalia. 45,X/46,XY individuals with almost normal male genitalia do not appear to develop neoplasia as often as 45,X/46,XY individuals with female or frankly ambiguous genitalia.

45,X/47,XYY: 45,X/46,XY/47,XYY Phenotypes

These complements are rarer than 45,X/46,XY, but they are probably associated with the same phenotypic spectrum. Of particular interest is one family in which two of possibly three sibs had 45,X/46,XY/47,XYY mosaicism (Hsu et al, 1970). The parents were second cousins.

Genetic Forms

Hypospadias without Other Defects

In several disorders the external genitalia are abnormal, yet not to the extent that the sex of rearing is questioned. Some investigators prefer not to designate such patients as male pseudohermaphrodites; however, applying that term facilitates delineation of these individuals from those with related disorders. Such a disorder is hypospadias, in which the external urinary meatus terminates on the ventral aspect of the penis, proximal to its usual site at the tip of the glans penis. Hypospadias can be classified according to the site of the urethral meatus: glans penis, penile shaft, penoscrotal junction, or perineum.

Multiple affected sibs and affected individuals in several generations have been reported to have simple hypospadias. After the birth of one affected child the recurrence risk for subsequent male progeny is about 6 to 10 percent, consistent with multifactorial/polygenic etiology. On the other hand, hypospadias is sometimes only one of several components of a multiple malformation pattern. For example, an X-linked recessive syndrome characterized by hypospadias and hypertelorism has been described. Presence of other anomalies should, therefore, be excluded prior to counseling a family.

Persistence of Müllerian Derivatives in Otherwise Normal Males

Occasionally, the uterus and Fallopian tubes (Müllerian derivatives) persist in otherwise normal males. The external genitalia, Wolffian derivatives, and testes develop as expected; virilization occurs at puberty. The disorder is often detected because the uterus and Fallopian tubes produce an inguinal hernia. Failure of Müllerian duct regression could result either from end-organ (uterus) insensitivity to the Müllerian inhibitory factor (MIF) or from failure of fetal Leydig cells to synthesize or secrete MIF. In at least six families multiple affected sibs have been reported (Brook et al., 1973), and in one family maternal half-sibs were affected (Sloan & Walsh, 1976). Thus, an X-linked recessive gene probably causes the disorder. About 5 percent of reported individuals have had a seminoma or other germ cell tumor.

Disorders with Multiple Malformation Patterns

Genital ambiguity may occur in individuals with multiple malformation patterns, as tabulated elsewhere (Simpson, 1976).

Disorders with Demonstrable Enzyme Deficiency

An enzyme deficiency may be suspected if secretion of testosterone or its metabolites is decreased. Deficiencies of 21- or 11β-hydroxylation, the most common forms of adrenal hyperplasia, do not cause male pseudohermaphroditism because androgen secretion is not decreased. In fact, at birth males affected with these disorders may have a large phallus. During infancy the phallus may continue to enlarge, and pubic hair may develop prematurely. Males with 21-hydroxylase deficiency may lose salt, whereas those with 11β-hydroxylase deficiency may retain salt and develop hypervolemia and hypertension. Thus, all newborn male sibs of individuals with either deficiency should be screened for electrolyte disturbances.

Congenital adrenal lipoid hyperplasia. These male pseudohermaphrodites have ambiguous or female-like external genitalia, severe salt wasting, and adrenals characterized by foamy-appearing cells filled with cholesterol (Prader & Gurtner, 1955). Because cholesterol accumulates, one can assume that it cannot be converted to pregnenolone. As noted previously, the specific enzyme deficient in this disorder is not known.

Deficiency of 3β-ol-dehydrogenase. Deficiency of 3β-ol-dehydrogenase results in decreased synthesis of both androgens and estrogens (Fig. 10-1). The major androgen produced is dehydroepiandrosterone, a weaker androgen than testosterone. 3β-ol-dehydrogenase deficiency is associated with severe salt wasting because of the decreased levels of aldosterone and cortisol, as previously noted.

The incompletely developed external genitalia of affected males are similar to the external genitalia of most other male pseudohermaphrodites: a small phallus, a urethra that opens proximally on the penis, and incomplete fusion of the labioscrotal folds. The testes and Wolffian ducts differentiate normally. This autosomal recessive disorder may occasionally be detected only in later childhood, presumably because a partial deficiency of the enzyme exists. This is the only disorder of steroidogenesis in which both males and females show pseudohermaphroditism.

Deficiency of 17α-hydroxylase. Males with deficiency of 17α-hydroxylase usually show ambiguous external genitalia, normal Wolffian duct development, and normal testicular differentiation. Occasionally, affected males have female external genitalia (Heremans, Moolenaar, & Van Gelderen, 1976). Although females with deficient 17α-hydroxylase usually have hypertension, males usually have normal blood pressure (New, 1970).

Deficiency of 17,20-desmolase. Zachman et al. (1972) reported a family in which three members apparently had 17,20-desmolase deficiency. Two maternal first cousins had genital ambiguity, bilateral testes, and no Müllerian derivatives. A maternal "aunt" was said to have had abnormal external genitalia

and bilateral testes; her breasts were not described. Both cousins had low plasma testosterone and low DHEA but normal urinary excretion of pregnanediol, pregnanetriol, and 17-hydroxycorticoids. Incubation of testicular tissue with appropriate precursors revealed that testosterone could be synthesized from androstenedione or DHEA (excluding 17-ketosteroid reductase); thus, 17,20-desmolase appeared deficient. Additional cases have since been reported (Goebelsmann et al., 1976). The disorder could be inherited in either an autosomal recessive or X-linked recessive fashion.

Deficiency of 17-ketosteroid reductase. Saez et al. (1971) were the first to describe males unable to convert dehydroepiandrosterone to testosterone. This conversion requires 17-ketosteroid reductase (Fig. 10-1). Plasma testosterone is decreased; androstenedione and DHEA are increased. Affected males show ambiguous external genitalia, bilateral testes, and no Müllerian derivatives. Breast development may or may not be present. The disorder is inherited as either an autosomal recessive or X-linked recessive trait.

Complete Testicular Feminization (Androgen Insensitivity)

In testicular feminization, 46,XY individuals have bilateral testes, female external genitalia, a blindly ending vagina, and no Müllerian derivatives. Affected individuals undergo breast development and puberal feminization. Despite feminization, some individuals with testicular feminization have clitoral enlargement and labioscrotal fusion; to these patients the term incomplete testicular feminization (see following section) may be applied. Both complete testicular feminization and incomplete testicular feminization are inherited in X-linked recessive fashion, but the two disorders are genetically distinct.

Individuals with complete testicular feminization are phenotypic females (Fig. 10-3). Many are quite attractive and have excellent breast development; others are similar in appearance to unaffected females. Breasts contain normal ductal and glandular tissue, but often the areolae are pale and poorly developed. Statural growth and body proportions are usually normal. Occasionally the arms and legs are disproportionately long and the hands and feet disproportionately large. Pubic and axillary hair are usually sparse, but scalp hair is normal. These patients show female psychosexual behavior.

The vagina terminates blindly and is shorter than usual, presumably because the Müllerian ducts fail to contribute to formation of the vagina. Occasionally the vagina is only 1 to 2 cm long or is represented merely by a dimple. Neither uterus nor Fallopian tubes are present, although occasionally fibromuscular remnants or rudimentary Fallopian tubes of possible Müllerian origin are detected. The absence of Müllerian derivatives is not unexpected, because the Müllerian inhibitory factor secreted by the fetal testes is not an androgen; therefore, Müllerian regression occurs as in normal males. Testes are usually normal in size, and may be located in the abdomen, inguinal canal, or labia, i.e., anywhere along the path of embryonic testicular descent. Testes located in the inguinal canal may produce inguinal hernias, and half of all individuals with testicular feminization develop inguinal hernias.

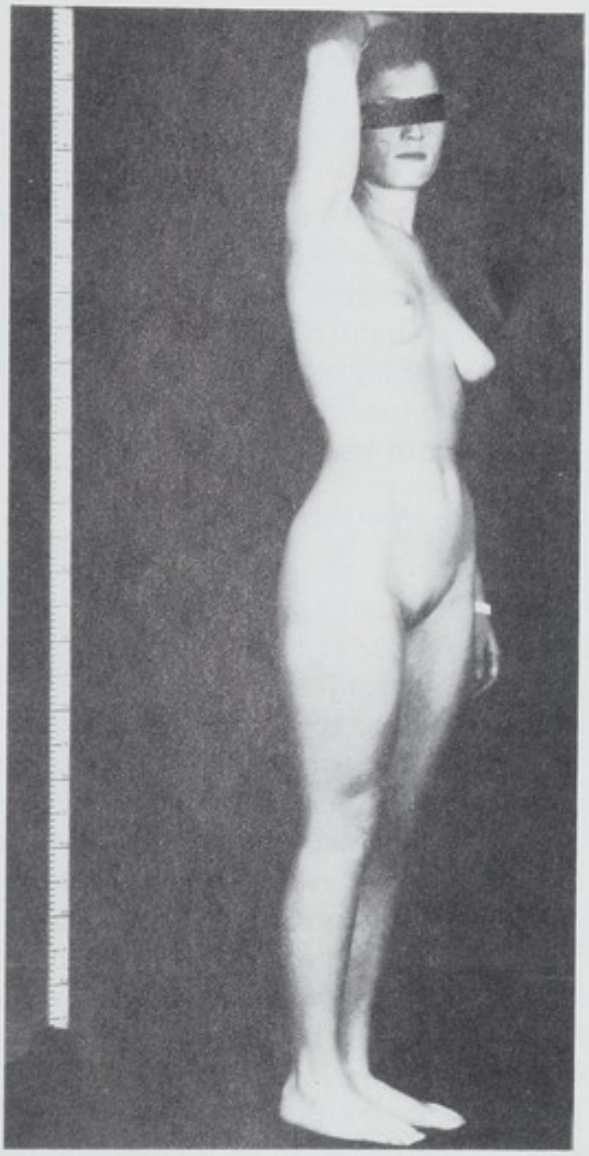


Figure 10-3. Photograph of a patient with complete testicular feminization. (From Simpson JL: Male pseudohermaphroditism. Genetics and clinical delineation. Hum Genet 44:1 1978. Reprinted with permission.)

In prepuberal patients the testes are histologically similar to undescended testes of normal males; in adults the seminiferous tubules are small and consist mostly of Sertoli cells. Few spermatogonia and no spermatozoa are detected, and Leydig cells are hyperplastic. Plasma testosterone is normal for males, and testosterone and androstenedione production rates are either normal or slightly elevated. Plasma FSH is normal, but LH is elevated. The latter observations suggest abnormal gonadal-hypothalamic feedback control.

The frequency of gonadal neoplasia is increased. Most investigators agree that the risk of neoplasia is low prior to age 25 to 30. Thus, many prefer to leave the testes in situ until after pubertal feminization, thereafter performing orchiectomy because the risk of neoplasia increases with age. In postpuberal patients benign tubular adenomas (Pick adenomas) are especially common,

probably as result of increased secretion of LH. The precise risk of carcinomatous change in older patients is unknown. Morris and Mahesh (1963) tabulated that 22 percent of reported patients were affected, but because of biases of reporting the actual risk may be no greater than 5 percent (Simpson & Photopulos, 1976b).

The pathogenesis of testicular feminization involves end-organ insensitivity to androgens. At one time all cases were believed the result of an abnormality in the cytosol receptor for androgens; however, Amrhein et al. (1976) showed that in some cases other mechanisms responsible for androgen insensitivity need to be invoked. For example, androgen insensitivity might result not only from an abnormality of the cytosol recepter but also from abnormalities of the receptor(s) that bind to DNA to initiate transcription.

Incomplete Testicular Feminization and Reifenstein Syndrome

At puberty certain individuals feminize (show breast development) because of androgen insensitivity, yet their external genitalia are characterized by phallic enlargement and partial labioscrotal fusion. Such individuals (Fig. 10-4) are said to have incomplete androgen insensitivity or incomplete testicular feminization. Both incomplete and complete testicular feminization share the following features: bilateral testes with similar histologic features, no Müllerian derivatives, puberal breast development, lack of puberal virilization, normal male plasma testosterone levels, normal response to HCG and ACTH, and failure to retain nitrogen following testosterone administration.

Individuals reported to have incomplete testicular feminization as defined above have differed with respect to the extent of Wolffian differentiation, although an epididymis and one or both vasa differentia are usually present. Incomplete testicular feminization is X-linked recessive, as is complete testicular feminization. Within a given kindred, however, affected individuals show either complete testicular feminization or incomplete testicular feminization. The two disorders are thus nosologically distinct.

Reifenstein syndrome was first reported in 1947 (Reifenstein, 1947), but it was not thoroughly investigated until many years later. Most patients reported to have this X-linked recessive trait do not differ in any important respect from those with incomplete testicular feminization. Traditionally, however, the appellation Reifenstein syndrome was applied to males with relatively small testes, elevated gonadotropin levels, more normal phallic development than in incomplete testicular feminization, no vagina-like perineal orifice, and lack of puberal virilization. Lack of virilization thus appeared to result not from androgen insensitivity, but from inadequate testosterone secretion. Indeed, the proband of Reifenstein's original kindred showed "virilization and libido" after testosterone administration (Bowen et al., 1965).

Recent data indicate that the traditional distinctions between Reifenstein syndrome and incomplete testicular feminization may not be valid. That is, males with small testes and elevated gonadotropin levels may show partial androgen insensitivity (Amrhein et al., 1977). Several investigators believe that

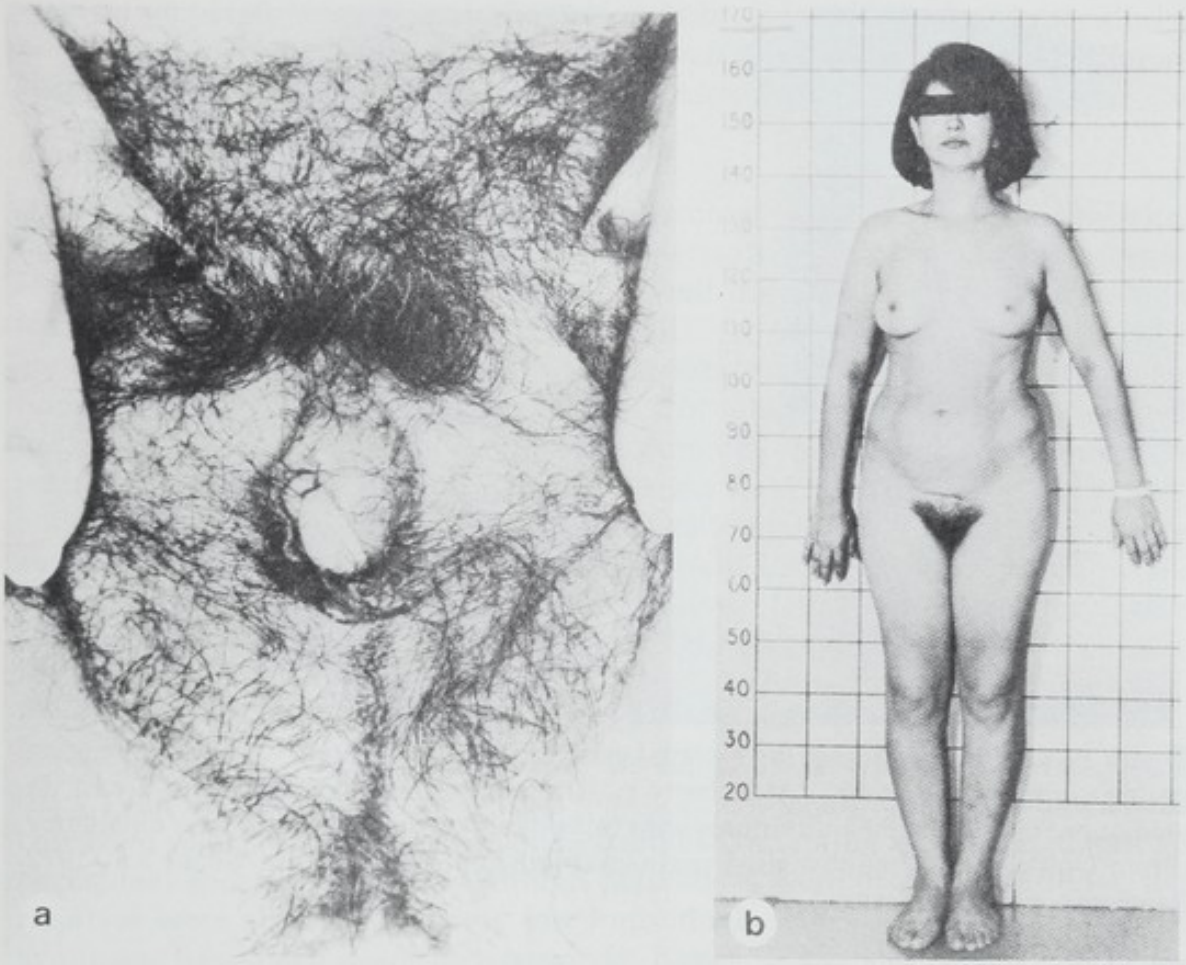


Figure 10-4. Photograph of an individual with incomplete testicular feminization. Despite the enlarged phallus and labioscrotal fusion (a), breast development occurred at puberty (b). (From Park IJ, Jones HW Jr: Familial male hermaphroditism with ambiguous external genitalia. Am J Obstet Gynecol 108:1197, 1970. Reprinted with permission.)

Reifenstein syndrome and incomplete testicular feminization merely represent different spectrums of a single X-linked recessive disorder (Wilson et al., 1974; Wilson, 1977). However, the pathogenesis of Reifenstein syndrome may not necessarily involve an abnormality in the androgen cytosol receptor in all cases. The nosological confusion in this area is discussed elsewhere (Simpson, 1978a). Finally, it should be noted that nomenclature is quite confusing and unstandardized. Of special note is that Wilson and MacDonald (1978) use the term Reifenstein syndrome to refer to patients discussed in this section, and reserve the term "incomplete testicular feminization" for androgen-insensitive patients whose external genitalia are normal (for females) except for posterior labioscrotal fusion. The latter patients may or may not be genetically distinct, for familial cases are rare or nonexistent. We prefer not to use the term "incomplete testicular feminization" in that fashion, since traditionally it has been used otherwise.

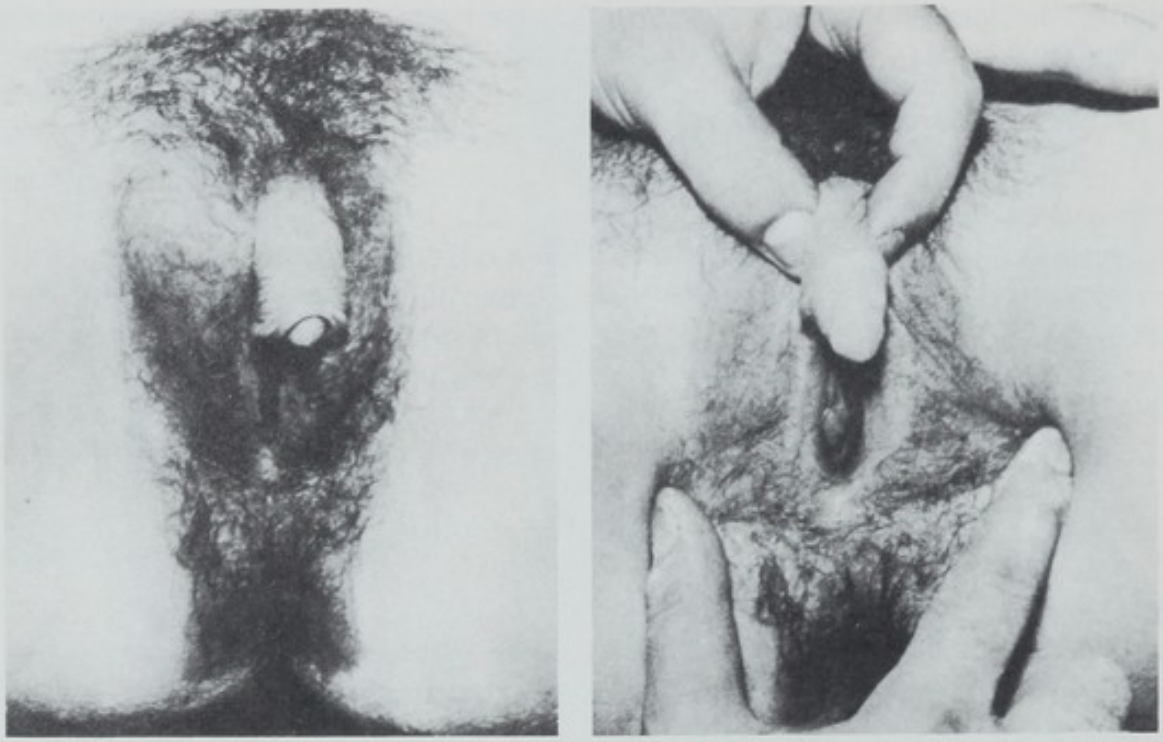


Figure 10-5. Photographs of the external genitalia of an individual with pseudovaginal perineoscrotal hypospadias. At puberty phallic enlargement occurred and breast development did not. Some individuals with this phenotype have 5α-reductase deficiency. (From Opitz et al.: Pseudovaginal perineoscrotal hypospadias. Clin Genet 3:1, 1972. Reprinted with permission.)

Pseudovaginal Perineoscrotal Hypospadias and 5α-Reductase Deficiency

Individuals with pseudovaginal perineoscrotal hypospadias (PPSH), an autosomal recessive trait, have ambiguous external genitalia but otherwise develop like normal males. At puberty they undergo virilization—phallic enlargement, increased facial hair, muscular hypertrophy, voice deepening, and no breast development. In PPSH the external genitalia consist of a phallus that resembles a clitoris more than a penis, a perineal urethral orifice, and usually a separate, blindly ending, perineal orifice that resembles a vagina (pseudovagina) (Fig. 10-5); thus, the designation pseudovaginal perineoscrotal hypospadias was applied (Simpson et al., 1971a; Opitz et al., 1972).

The PPSH phenotype may, but does not always, result from deficiency of 5α-reductase, the enzyme required for conversion of testosterone to dihydrotestosterone, the androgen active within cells (Imperato-McGinley et al., 1974; Walsh et al., 1974; Peterson et al., 1977). If PPSH individuals have deficient 5α-reductase, they should be designated by the enzyme defect. However, individuals with the PPSH phenotype and normal 5α-reductase have been reported. That intracellular 5α-reductase deficiency results in PPSH is consistent with the embryologic findings of Siiteri and Wilson (1974), who showed that

virilization of the external genitalia during embryogenesis requires intracellular conversion of testosterone to dihydrotestosterone, whereas Wolffian differentiation depends only upon the presence of testosterone.

Anorchia

Males (46,XY) with anorchia have unambiguous male external genitalia, normal Wolffian derivatives, no Müllerian derivatives, and no detectable testes. Despite absence of testes, the phallus is well differentiated. Vasa differentia terminate blindly, often in association with the spermatic vessels. Somatic abnormalities are rarely present. Unilateral anorchia is not extraordinarily rare, but bilateral anorchia is extremely rare. The diagnosis is justifiable only if testicular tissue is not detected in the scrotum, the inguinal canal, or the entire path along which the testes descended during embryogenesis. Heritable tendencies exist, but the occurrence of monozygotic twins discordant for anorchia suggests that genetic factors are not paramount in all cases (Simpson et al., 1971b). If a tendency toward in utero torsion of the testicular artery were heritable, familial aggregates of anorchia might be expected.

Syndrome of Rudimentary Testes

Bergada et al. (1962) reported four unrelated males who, despite testes less than 1 cm in greatest diameter, had well-formed, small, penises. Their testes consisted of a few Leydig cells, small tubules containing Sertoli cells, and an occasional spermatogonium. Wolffian derivatives were present; Müllerian derivatives were absent. Relatively few individuals with the rudimentary testes syndrome have been described; however, Najjar, Takla, and Nassar (1974) described five affected sibs. The pathogenesis is unclear, for it seems unlikely that such small testes could be responsible for normal male development. Perhaps the testes were initially normal during embryogenesis, only later decreasing in size. The etiology might thus be analogous to anorchia, yet with retention of some testicular tissue.

Agonadia

In agonadia the gonads are absent, the external genitalia abnormal, and all but rudimentary Müllerian or Wolffian derivatives absent. External genitalia usually consist of a phallus about the size of a clitoris, underdeveloped labia majora, and nearly complete fusion of the labioscrotal folds. A persistent urogenital sinus is often present. By definition, gonads cannot be detected. Likewise, neither normal Müllerian derivatives nor normal Wolffian derivatives are present, although structures resembling a rudimentary Fallopian tube, an epioophoron, or an epididymis may be present along the lateral pelvic wall. Somatic anomalies—craniofacial anomalies, vertebral anomalies, dermatoglyphic anomalies, and possibly mental retardation—are common (Sarto & Opitz, 1973). About 20 cases have been reported (Simpson, 1978a). Any pathogenic explanation for agonadia must explain not only the absence of gonads, but also abnormal external genitalia and lack of normal internal ducts. Either of at least two explanations seems reasonable: (1) The fetal testes functioned long enough to inhibit Müllerian development, yet not long enough to complete male dif-

ferentiation. (2) The entire gonadal, ductal, and genital systems developed abnormally, as result of defective anlage, defective connective tissue, or action of a teratogen. The frequent coexistence of somatic anomalies favors the existence of a teratogen or the existence of defective connective tissue. In one and possibly two kindreds, affected sibs have been reported; thus, a genetic etiology should be considered, possibly one affecting connective tissue. Although agonadia is usually associated with 46,XY individuals, 46,XX individuals may show the same phenotype (Duck et al., 1975).

TRUE HERMAPHRODITISM

True hermaphrodites possess both ovarian and testicular tissue. They may have separate ovaries and testes, or, more often, one or more ovotestes (Fig. 10-6). Most true hermaphrodites have a 46,XX chromosomal complement; however, others have 46,XX/46,XY, 46,XX/47,XXY, 46,XY, or other complements (Simpson, 1978b). Hypospadias and other genital abnormalities almost always exist. About two thirds of true hermaphrodites are raised as males, although their external genitalia may be frankly ambiguous or predominantly female (Jones & Scott, 1971; Van Niekerk, 1974; Simpson, 1978b). However, feminization and breast development usually occur at puberty.

Gonadal tissue may be located in the ovarian region, the inguinal region,

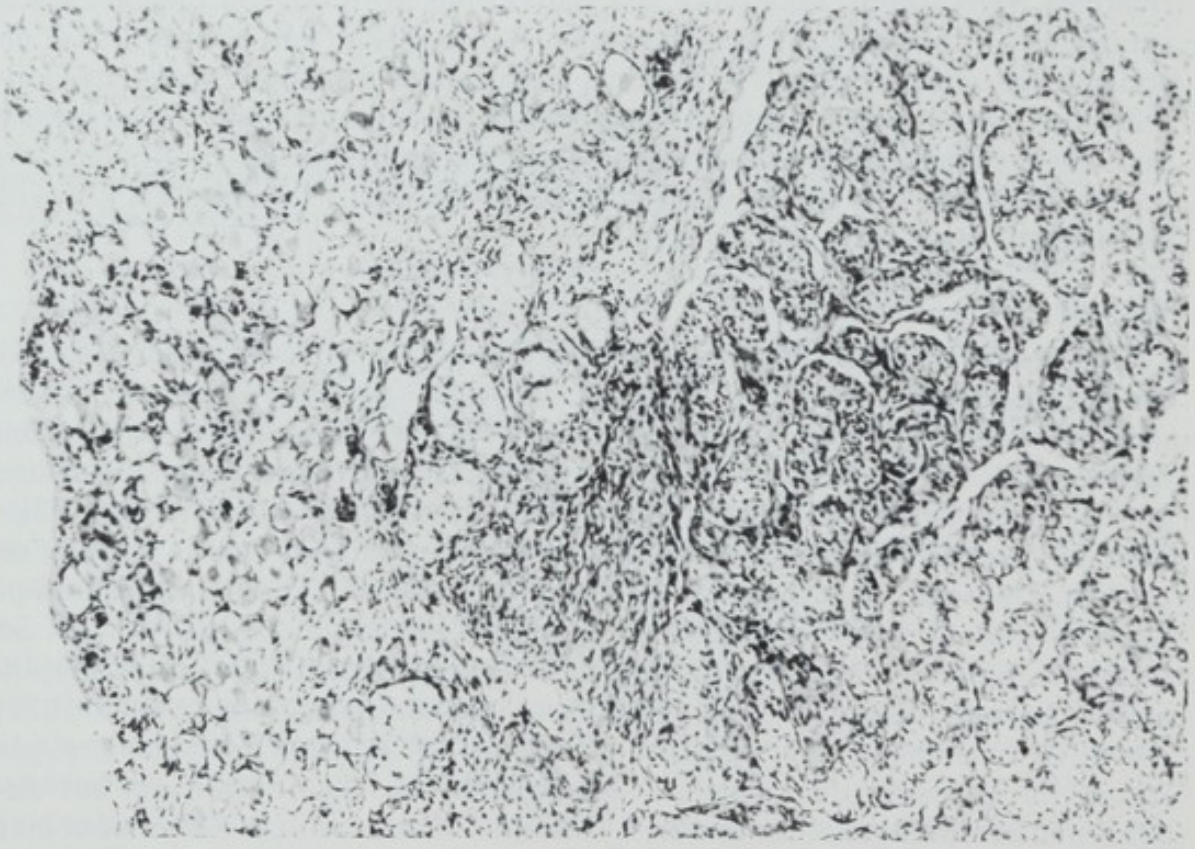


Figure 10-6. Photomicrograph of the left ovotestis of patient No. 2 of Van Niekerk (1974). The patient had a 46,XX complement. Numerous primordial follicles are present in the left portion; infantile testicular tissue is present on the right.

or the labioscrotal region. The greater the proportion of testicular tissue in an ovotestis, the greater the likelihood of gonadal descent. In 80 percent of ovotestes the testicular and ovarian components exist in end-to-end fashion; thus, ovotestes can usually be detected by inspection or possibly by palpation, because testicular tissue is softer and darker than ovarian tissue. A testis or an ovotestis is more likely to be present on the left. Spermatozoa are rarely present; however, apparently normal oocytes are often present, even in ovotestes. Neoplasia, including seminomas and gonadoblastomas, may arise in the testes of true hermaphrodites; however, neoplasia occurs less frequently than in XY gonadal dysgenesis or 45,X/46,XY mosaicism. A uterus is usually present, often bicornuate or unicornuate. The absence of a uterine horn usually indicates an ipsilateral testis or ovotestis. Most true hermaphrodites with a uterus menstruate. The fimbriated end of the Fallopian tube may be occluded on the side of an ovotestis, and squamous metaplasia of the endocervix may occur.

Etiology

The etiology of true hermaphroditism is uncertain but heterogenous. 46,XX/46,XY cases may result from chimerism. Chimerism is the presence of two or more cell lines, each derived from different zygotes in a single individual. In 6 of 28 46,XX/46,XY cases reviewed by Simpson (1978b) this etiology had been verified. However, experimental production of XX/XY mouse chimeras usually does not result in true hermaphroditism, nor do 46,XX/46,XY humans always have true hermaphroditism. 46,XX/47,XXY cases, which may result from either chimerism or mitotic nondisjunction, also have been reported.

46,XX true hermaphrodites are almost certainly heterogenous in etiology. A few doubtless result from undetected chimerism; however, for both phenotypic and genetic reasons (Simpson, 1978b), undetected chimerism cannot easily explain all 46,XX true hermaphrodites. The presence of testicular tissue in 46,XX individuals is ostensibly perplexing because testicular determinants are localized in the Y chromosome, specifically in the short arm. Possible explanations for the presence of testes in individuals who apparently lack a Y chromosome include (1) translocation of the testicular determinant(s) from the Y to an X, (2) translocation of the testicular determinant(s) from the Y to an autosome, (3) undetected mosaicism or chimerism, or (4) sex-reversal genes. Consistent with the first hypothesis is the detection of H-Y antigen in almost all 46,XX true hermaphrodites (Wachtel et al., 1976; Wachtel, 1979).

46,XY cases are also of uncertain etiology. Most are raised as males; half have one ovary and one testis. Phenotypic features suggest undetected chimerism or mosaicism, but this hypothesis (Simpson, 1978b) is unproved.

46,XX MALES (SEX-REVERSAL)

46,XX (sex-reversed) males are phenotypic males with bilateral testes. However, their chromosomal complement is that of a female. They have small testes and signs of androgen deficiency, but otherwise have a normal male appearance.

The penis and scrotum are small but usually well differentiated, and Wolffian derivatives are normal. In one survey 9 percent of 46,XX males had hypospadias, but in none was the sex of rearing in doubt (de La Chapelle, 1972). However, patients with ambiguous genitalia are usually excluded from samples of 46,XX males. If one included such patients, perhaps 15 to 20 percent of 46,XX males have abnormal external genitalia.

The testes of 46,XX males are similar to those of 47,XXY males. In affected adults, seminiferous tubules are decreased in number and in size, peritubular and interstitial fibrosis occurs, Leydig cells are hyperplastic, and spermatogonia usually cannot be detected. Occasionally immature spermatogonia are detected and sometimes the ejaculate contains spermatozoa. 46,XX males fail to undergo normal puberal development. They show decreased facial and body hair, and their pubic hair may be distributed in the pattern characteristic of females. About one third have gynecomastia.

In 46,XX males, as in 46,XX true hermaphrodites, testes develop contrary to the axiom that a Y chromosome is required for testicular differentiation. As noted in the previous section, several explanations have been proposed. Of special note, however, are observations that almost all tested 46,XX males have been H-Y antigen positive (Wachtel et al., 1976; Wachtel, 1979.) This suggests X-Y or Y autosome translocation. On the other hand, familial aggregates of 46,XX males alone and either 46,XX males or 46,XX true hermaphrodites have been reported (Simpson, 1978b), suggesting mutant genes. However, de la Chapelle, Koo, and Wachtel (1978) believe that such kindreds can be explained by Y-X or Y-autosome translocation of portions of H-Y genes; in some families the translocated portion might be too small to confer maleness but large enough to behave in recessive sex-reversal fashion. Supporting the interchange hypothesis are cytologic data showing heteromorphism between the two X chromosomes in 8 of 14 46,XX males (Evans et al., 1979).

GONADAL DYSGENESIS

Individuals with gonadal dysgenesis have streak gonads rather than ovaries. Gonadal dysgenesis is usually associated with monosomy for the X chromosome (45,X) or structural abnormalities of sex chromosomes. Occasionally individuals with apparently normal male (46,XY) or female (46,XX) chromosomal complements have gonadal dysgenesis. Affected individuals lack germ cells, and at puberty show hypogonadism.

Proportions of Various Chromosomal Complements

The complement most frequently associated with gonadal dysgenesis is 45,X. About 80 percent of cases identified by pediatricians will show this complement. However, only about 40 percent of patients with gonadal dysgenesis who are ascertained because of primary amenorrhea have a 45,X complement; 20 percent show an X structural abnormality or mosaicism, and 40 percent show 46,XX or 46,XY complements (Simpson, 1976).

Pathologic Considerations

Gonads and Genital Organs

In adults with gonadal dysgenesis, the normal gonad is replaced by a streak gonad, a white fibrous streak 2 to 3 cm long and about 0.5 cm wide, located in the position ordinarily occupied by the ovary (Fig. 10-7). A streak gonad is characterized histologically by interlacing waves of dense fibrous stroma that are indistinguishable from normal ovarian stroma (Fig. 10-8). In 45,X embryos germ cells develop but apparently degenerate shortly after formation of the primary follicle. Both ovarian rete tubules, which probably originate from either mesonephric tubules or from medullary sex cords, and hilar cells are usually present past the age of expected puberty. In 45,X adults oocytes are usually absent. Dysgerminomas and gonadoblastomas may arise from streak gonads of individuals with a Y chromosome. Neoplasia occurs in 15 to 20 percent of reported 45,X/46,XY patients and in 25 to 30 percent of reported XY gonadal dysgenesis patients (Simpson & Photopulos, 1976b).

The external genitalia of 45,X individuals usually remain infantile because of decreased secretion of sex steroids. The uterus, cervix, Fallopian tubes, upper vagina, and external genitalia are structurally normal, although smaller than

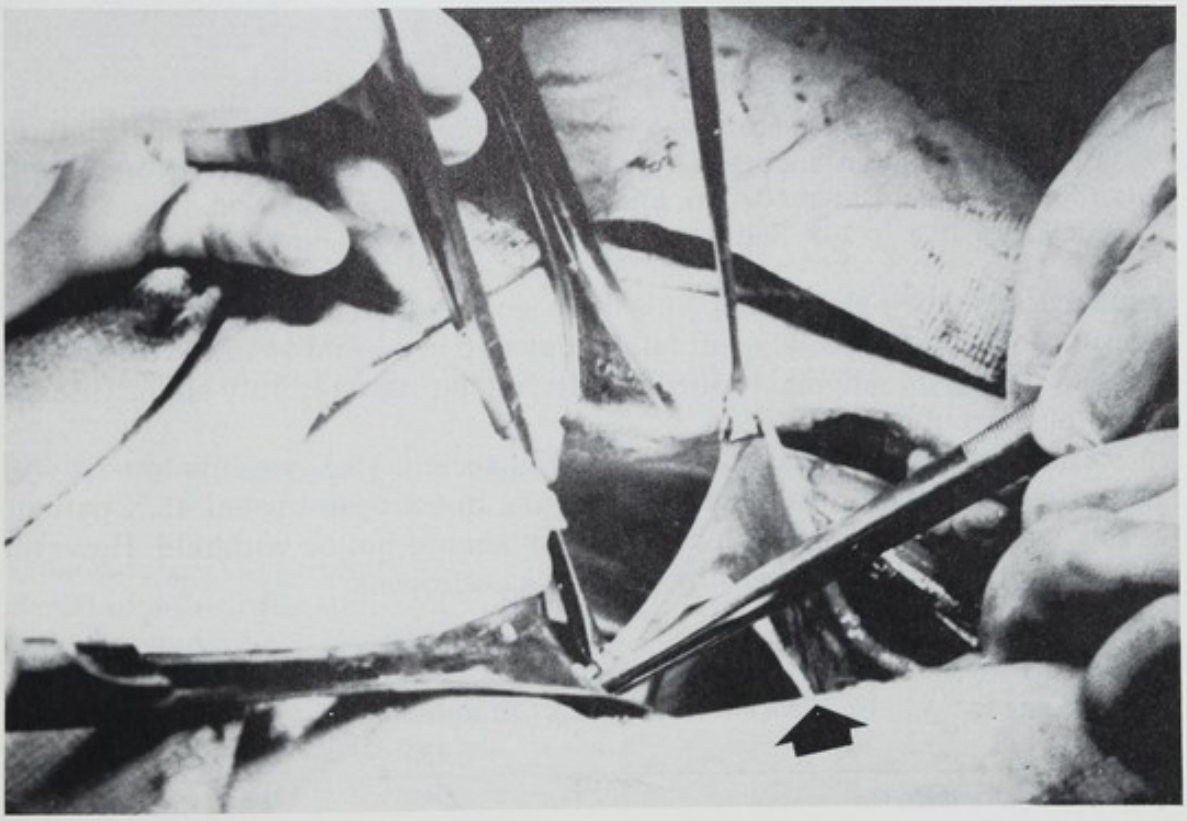


Figure 10-7. Photograph taken at the time of celiotomy (Pfannenstiel incision), demonstrating the usual appearance of a streak gonad (arrow). The clamp is elevating a Fallopian tube. This particular individual had XY gonadal dysgenesis, but the appearance of a streak gonad would have been almost identical in most 45,X individuals. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York, Academic Press, 1976, p 268. Reprinted with permission).

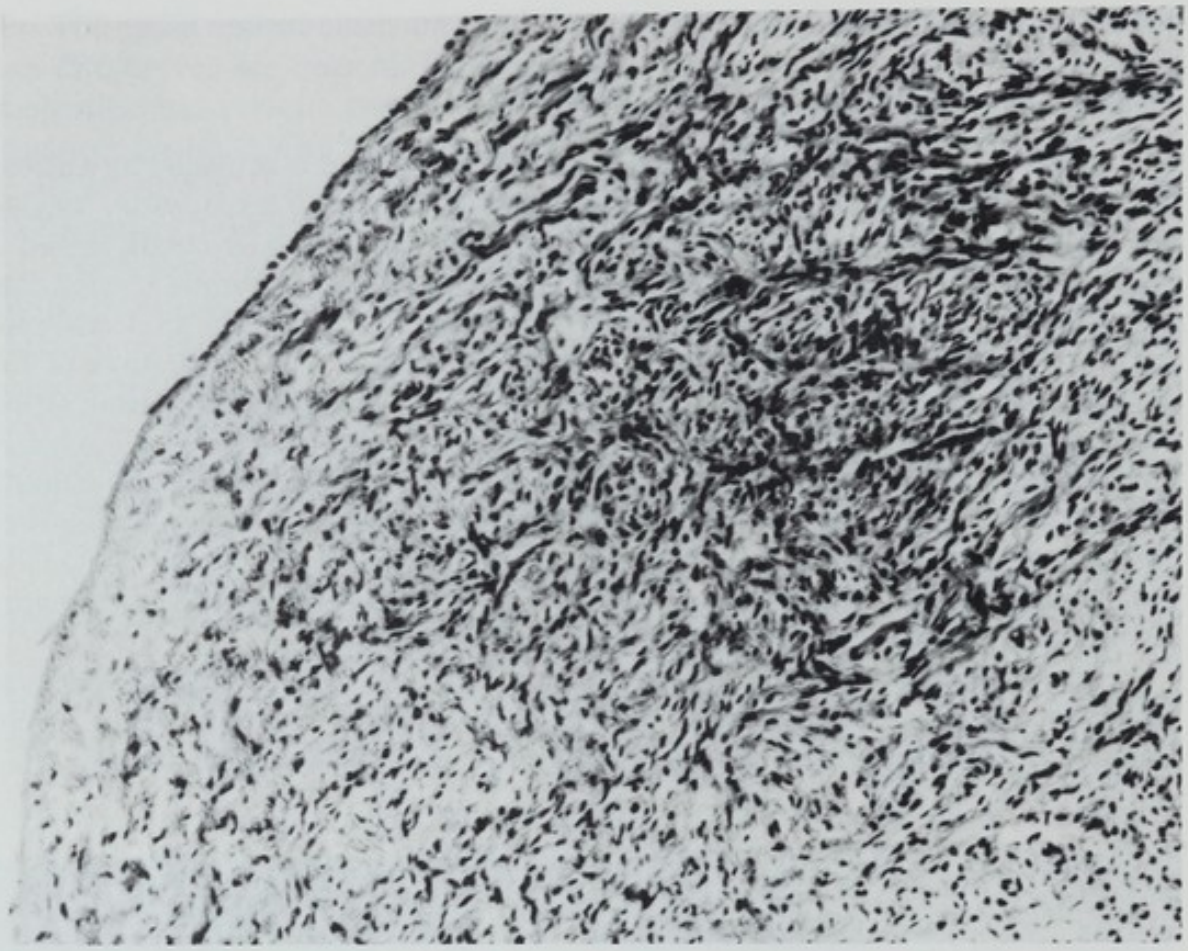


Figure 10-8. Histologic appearance of a streak gonad from a 45,X individual. In the streak gonad no oocytes are present. Hematoxylin and eosin stain. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York, Academic Press, 1976, p 269. Reprinted with permission.)

usual because their growth depends upon sex steroids. Pubic hair is sparse and fine in consistency. Axillary hair fails to develop in normal quantity. The breasts contain little parenchymal tissue, and the areolar tissue is only slightly darker than the surrounding skin.

Endometrial carcinoma has occurred in several 45,X patients treated with DES. However, the prevalence of carcinoma in estrogen-treated 45,X patients is relatively low, and estrogen replacement should not be withheld. However, one should administer progestins as well as estrogens.

Table 10-1
Some Somatic Features Associated with 45,X Chromosomal Complement

Growth Characteristics
Decreased birth weight
Decreased adult height (mean 141 ± 0.62 cm)
Intellectual Function
Verbal IQ > performance IQ
Cognitive deficits (space-form blindness)
Immature personality (probably secondary to short stature)

Craniofacial
- Premature fusion sphenoccipital and other sutures, producing brachycephaly
- Abnormal pinnae
- Retruded mandible
- Epicanthal folds (25 percent)*
- High-arched palate (36 percent)
- Abnormal dentition
- Visual anomalies, usually strabismus (22 percent)
- Auditory deficits: sensorineural or secondary to middle ear infections
- "Woolly hair"

Neck
- Pterygium coli (46 percent)
- Short broad neck (74 percent)
- Low nuchal hair line (71 percent)

Chest
- Rectangular contour (shield chest) (53 percent)
- Apparent, widely-spaced nipples
- Tapered lateral ends of clavicle

Cardiovascular
- Coarctation of aorta or ventricular septal defect (10 to 16 percent)

Renal (38 percent)
- Horseshoe kidneys
- Unilateral renal aplasia
- Duplication ureters

Gastrointestinal
- Telangiectasias
- Inflammatory disease

Skin and Lymphatics
- Pigmented nevi (63 percent)
- Lymphadema (38 percent) due to hypoplasia superficial vessels

Nails
- Hypoplasia or malformation (66 percent)

Skeletal
- Cubitus valgus (54 percent)
- Radial tilt of articular surface of trochlear
- Clinodactyly V
- Short metacarpals, usually IV (48 percent)
- Decreased carpal arch (mean angle 117°)
- Deformities of medial tibial condyle

Dermatoglyphics
- Increased total digital ridge count (mean 166.1 ± 8.62)
- Increased distance between palmar triradii a and b
- Distal axial triradius in position t′

From Simpson (1976): Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York, Academic Press.

*Percentage of 45,X individuals who have this feature.

Turner Stigmata

Certain somatic anomalies associated with monosomy X can be said to represent the Turner stigmata (Table 10-1). Their presence suggests the coexistence of gonadal dysgenesis. Individuals with Turner stigmata form a clinical continuum with respect to the presence or absence of certain anomalies; however, not every anomaly will be present in every 45,X individual, nor is any anomaly pathognomonic. The term "Turner syndrome" should be applied only to individuals with short stature and those somatic anomalies in addition to gonadal dysgenesis.

Metabolic Changes and Acquired Diseases

The endocrine findings are those expected of females who lack gonads: estrogen levels are decreased, whereas FSH and LH are increased as result of lack of feedback inhibition. Diabetes may or may not be more frequent in 45,X patients. Engel and Forbes (1965) detected diabetes in 8 of 42 adults with gonadal dysgenesis, and other reports have demonstrated a slower than normal production of insulin following glucose infusion. On the other hand, the incidence of overt diabetes is not increased in most series of adults with gonadal dysgenesis, nor in children with gonadal dysgenesis. Autoimmune thyroiditis also occurs more often than expected by chance (Doniach, Roitt, & Polani, 1968), and Graves disease may as well (Brooks, Meek, & Schimke, 1977). Long-bone fractures and vertebral compressions have been reported, but osteoporosis is relatively rare in patients with gonadal dysgenesis, even if not treated with estrogens. The osteoporosis that occurs may or may not be the same type that occurs in postmenopausal females. The prevalence of essential hypertension is also increased.

Sex Chromosome Abnormalities

Many chromosomal complements are associated with gonadal dysgenesis. Considerations of the various complements can allow one to deduce possible locations on the X chromosome for gonadal and somatic determinants.

45,X

Almost all 45,X individuals (Fig. 10-9) have short stature and certain of Turner stigmata. No single feature is pathognomonic, but these features (Table 10-1) form a characteristic spectrum that is more likely to occur in individuals with a 45,X complement than in individuals with most other chromosomal abnormalities. Streak gonads are usually present in 45,X patients, but about 3 percent have menstruated at least twice and about 5 percent show some breast development (Table 10-2). Occasionally, the interval between menstrual periods is almost normal, and several fertile patients have been reported (Dewhurst, 1978; Simpson, 1981a). Undetected 46,XX cells should always be suspected in menstruating or fertile 45,X patients; however, it is not unreasonable to expect that a few 45,X individuals could be fertile, because germ cells are present in 45,X embryos but undergo attrition more rapidly than in 46,XX

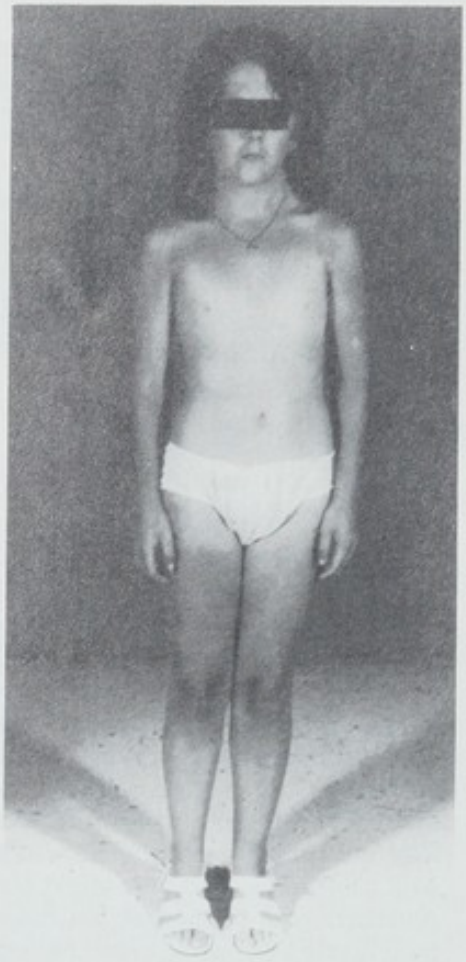


Figure 10-9. Photographs of a 45,X individual. This 140-cm patient showed no secondary sexual development. Somatic anomalies comprising the Turner stigmata included webbing of the neck, low nuchal hair line, and pigmented nevi. (Reprinted from Sutton's *An Introduction to Human Genetics*, Holt, Rhinehart & Winston, 1975.)

embryos (Singh & Carr, 1967). However, menstruation and fertility occur so rarely in 45,X patients that they should be counseled to expect primary amenorrhea and sterility. Antenatal monitoring for chromosomally abnormal offspring should be considered for those who do become pregnant.

Mosaicism without Structural Rearrangements

45,X/46,XX individuals show fewer anomalies than 45,X individuals. Twelve percent of 45,X/46,XX individuals menstruate, compared to only 3 percent of 45,X individuals (Table 10-2). Eighteen percent of 45,X/46,XX individuals undergo breast development, compared to 5 percent of 45,X individuals. The mean adult height is statistically greater in 45,X/46,XX than in 45,X patients, and fewer mosaic patients (75 percent) than nonmosaic patients (95 percent) have adult heights less than 153 cm. Somatic anomalies occur less frequently in 45,X/46,XX than in 45,X.

45,X/47,XXX and 45,X/46,XX/47,XXX complements are rarer than 45,X/46,XX. One would expect fewer anomalies than 45,X individuals, but this is unproved. 45,X/46,XY and 45,X/47,XYY individuals' complements were considered in the section, Male Pseudohermaphroditism, earlier in this chapter.

Table 10-2
Correlation of Endocrine Features Associated with Certain Abnormalities of the X Chromosome.

Abnormality	Primary Amenorrhea	Lack of Breast Development
45,X	173/178 (97%)	169/170 (95%)
45,X/46,XX	56/64 (88%)	50/61 (82%)
Deletion of X Short Arm		
46,X,del(X) (p11)	23/38	23/38
46,X,del (p21 or p22)	1/11	1/11
46,X,i(Xq)	23/23	21/23
Deletion of X Long Arm		
46,X,del(X)(q13)	16/18	13/18
46,X,del(X)(q22 or 24)	10/12	9/12
46,X,i(Xp)	6/6	5/6

Data from sources referenced in Simpson (1979a, 1979b, 1980b) and in Simpson and LeBeau (1981). To minimize biases of ascertainment, estimates for the complements 45,X, 45,X/46,XX and 46,X,i(Xq) were derived from individuals reported as part of surveys for gonadal dysgenesis (with or without Turner stigmata), primary amenorrhea, or sex chromosome abnormalities. Because of their rarity, all reported cases of 46,X,del(Xp); 46,X,del(Xq); and 46,X,i(Xp) were considered. At least two cases previously believed 46,X,i(Xp) have been reinterpreted as 46,X,del(Xq); thus, the 46,X,i(Xp) cases cited here might also be subject to alternative karyotypic interpretation.
Amenorrhea is defined as not more than one episode of spontaneous bleeding per vagina in individuals 14 years or older; includes "slight." Lack of breast development is defined as lack of parenchymal tissue.

Deletions of the X Short Arm

46,X,del(Xp) or 45,X/46,X,del(Xp). A deletion of the X short arm (Fig. 10-10) may result in gonadal dysgenesis and usually results in short stature and some features of Turner stigmata.

Most reported 46,Xdel(Xp) individuals have gonadal dysgenesis. However, menstruation has occurred in about 40 percent of 46,X,del(Xp) patients whose X chromosome appeared telocentric or acrocentric [46,X,del(X)(pll)], and in 10 of 11 reported patients in which the break point involved loss of only Xp distal to the positive G band [46,X,del(X)(p21 or p22)] (Table 10-2) (Simpson & Le Beau, 1981). Breast development is also not rare. These data suggest that functioning ovarian tissue may persist more often in 46,X,del(Xp) individuals than in 45,X individuals. That is, amenorrhea appears more likely to occur if both the proximal and distal (telomeric) portions of Xp are deficient than if only the distal portion is deficient.

Mean heights of 46,X,del(X)p(11) and p(21 or 22) individuals are 152 and 154 cm, respectively. It is worth emphasizing that 46,X,del(X)(p21) patients are short, despite menstruating. Features of Turner stigmata may be present.

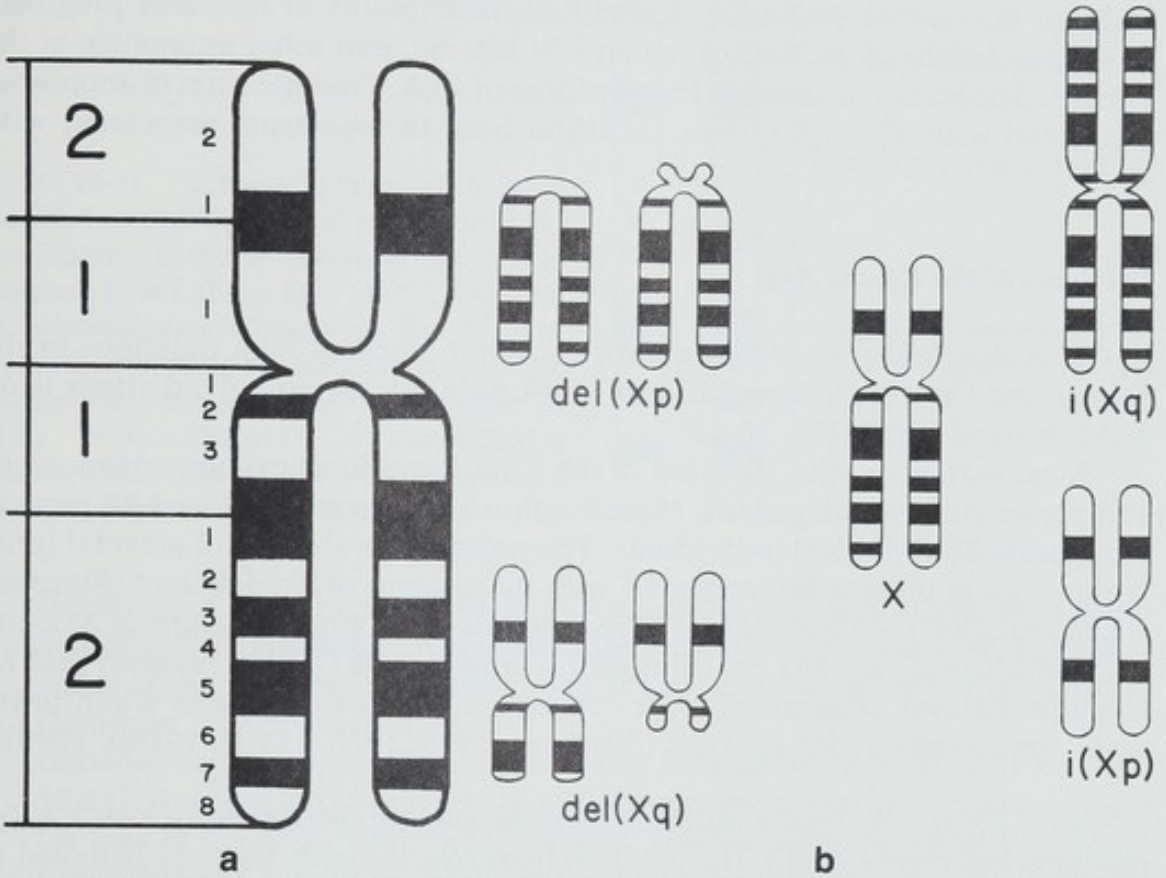


Figure 10-10. Diagrammatic representation of the banding pattern expected. (a) The normal X, and (b) Chromosomes showing del(Xp), i(Xq), del(Xq) and i(Xp). Most del (Xp) chromosomes appear acrocentric or telocentric, but in others the break points occurred at Xp21 or more terminal. In most del(Xq) chromosomes the break points are at Xq13 or Xq22 or 24, but more terminal break points have been reported. (From Simpson JL (1979a): Gonadal dysgenesis and sex chromosome abnormalities. In Vallet H, Porter IH (Eds): Genetic Mechanisms of Sexual Development. New York: Academic, p 365. Reprinted with permission.)

46,X,i(Xq); 45,X/46,X,i(Xq). Division of the centromere in the transverse rather than in the longitudinal plane results in an isochromosome, a metacentric chromosome consisting of isologous arms. Both arms are structurally identical and contain the same genes. An isochromosome for the X long arm [i(Xq)] thus consists of duplication for all of Xq and deficiency for all of Xp. In addition to carrying a duplication of Xq, patients with an i(Xq) chromosome differ from those with a del(Xp) chromosome in that all of Xp is deleted.

Because an isochromosome for the X long arm is the most common X structural abnormality, most investigators would probably not report a single case unless unusual features were present. Thus, care must be taken to base phenotype–karyotype correlations upon as unbiased a sample as possible. Analysis of relatively unbiased samples indicate that 46,X,i(Xq) patients not only have streak gonads but almost invariably have short stature and Turner stigmata. Adult patients thus have primary amenorrhea and lack breast development.

Only an occasional 46,X,i(Xq) individual menstruates or becomes pregnant. The mean height of 46,X,i(Xq) patients is 136 cm, and other anomalies of the Turner stigmata occur about as frequently as in 45,X. The spectrum of anomalies associated with 46,X,i(Xq) does not differ from the spectrum associated with 45,X.

Deletions of the X Long Arm

46,X,del(Xq) or 45,X/46,X,del(Xq). About 50 cases with deletions of the X long arm have been reported. The break point usually involved either band Xq13 or band Xq22 (Fig. 10-10).

Most patients with a deletion of the X long arm have primary amenorrhea and presumably streak gonads. Menstruation has occurred in about 20 percent of reported 46,X,del(Xq) individuals. The presence or absence of gonadal function cannot at present be correlated with the amount of Xq deficient (Simpson & LeBeau, 1981). 46,X,del(X)(q13) individuals have a mean height of 152 cm; 46,X(del)(X)(q22) are 151 cm (Simpson & LeBeau, 1981). This suggests that Xq contains statural determinants, in contrast to conclusions made when fewer cases were available for analysis (Simpson, 1975, 1976, 1979a, 1979b, 1980b).

46,X,i(Xp). At least 8 individuals have been reported to have a 46,X,i(Xp) chromosome complement. However, at least two individuals once believed to be 46,X,i(Xp) were subsequently shown to have a 46,X,del(Xq) complement. In fact, some investigators believe that 46,X,i(Xp) is a lethal complement, all apparent 46,X,i(Xp) individuals actually being 46,X,del(Xq). Nonetheless, the individuals still considered by most investigators to have 46,X,i(Xp) complements have primary amenorrhea, normal stature, and few anomalies of the Turner stigmata.

Phenotype–Karyotype Correlations

Several phenotype–karyotype correlations can be made from the above data. The possibilities of undetected mosaicism and alternate karyotypic interpretations remain.

1. Monosomy X (45,X) is usually associated with streak gonads, short stature, and Turner's stigmata. Phenotypic expression varies more often than is generally appreciated, as evidenced by observations that occasional 45,X individuals menstruate.
2. Deletions of most [del(Xp)] or all [i(Xq)] of the X short arm are usually associated with short stature and Turner stigmata. The somatic anomalies represent the same spectrum of anomalies as in 45,X. Menstruation or breast development occurs in about 40 percent of 46,X,del(Xp) and 45,X/46,X,del(Xp) individuals, but rarely in 46,X,i(Xq) individuals. Deletions of only the distal end of Xp are especially likely to be compatible with ovarian function.

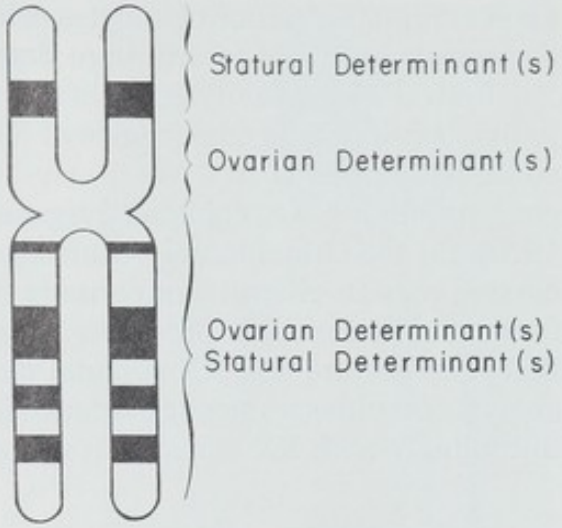


Figure 10-11. Schematic drawing showing relative location of gonadal and statural determinants on the X chromosome. (From Simpson JL & LeBeau MM (1981): Gonadal and statural determinants on the X chromosome and their relationship to in vitro studies showing prolonged cell cycles in 45,X; 46,X,del(X)(p11); 46,X,del(X)(q13) and (q22) fibroblasts. Am J Obstet Gynecol 141:698. Reprinted with permission.

3. Deletions of most [del(Xq)] or all [i(Xp)] of the X long arm are more often associated with gonadal dysgenesis. About 20 percent of adult 46,X,del(Xq) patients menstruate. Nonmosaic del(Xq) patients are shorter than normal (Simpson & LeBeau, 1981).
4. Gonadal determinants thus appear to be located on both Xp and Xq (Fig. 10-11). Duplication of one arm (i.e., an isochromosome) fails to compensate for loss of the other arm; thus, gonadal determinants on Xp and Xq must have different functions, each essential for normal ovarian development. More than a single determinant could exist on each arm.
5. The X short arm contains statural determinants which, if deleted, result in short stature and possibly other anomalies comprising the Turner stigmata. The X long arm now appears to also contain statural determinants.

XX Gonadal Dysgenesis

Gonadal dysgenesis histologically similar to that detected in individuals with an abnormal sex chromosome complement can occur in 46,XX individuals (Simpson et al., 1971c). Over 150 well-documented cases have now been reported (Simpson 1979a, 1979b), including many familial aggregates.

The external genitalia and the streak gonads of patients with XX gonadal dysgenesis are indistinguishable from those of individuals who have gonadal dysgenesis and an abnormal chromosomal complement. Likewise, the endocrine data and the lack of secondary sexual development do not differ from those of other individuals with streak gonads. Most individuals with XX gonadal dysgenesis are normal in stature (mean height 165 cm), although a few are less than 150 cm tall. Somatic features of Turner stigmata are usually absent.

In at least 20 families, more than one sib has had XX gonadal dysgenesis (Simpson, 1979). Parents of patients with XX gonadal dysgenesis are sometimes consanguineous. Available data thus suggest autosomal recessive inheritance. In one family one affected sib had streak gonads, whereas another had primary amenorrhea and extreme ovarian hypoplasia (a few ova were detected). The authors have encountered similar families. Such cases imply that insisting

upon complete absence of oocytes prior to applying the appellation XX gonadal dysgenesis will lead to failure to detect individuals with the mutant gene.

Both XX gonadal dysgenesis and neurosensory deafness have affected sibs in five families. The coexistence of XX gonadal dysgenesis and nerve deafness could be explained in several ways: (1) a pleiotropic gene different from the gene producing XX gonadal dysgenesis without deafness (Pallister & Opitz, 1979), (2) the coincidental occurrence of homozygosity for more than one recessive gene in offspring of consanguineous parents; or (3) varied expressivity for the single gene that produces XX gonadal dysgenesis. Some authors believe that short stature and XX gonadal dysgenesis are distinct conditions, but our analysis provides no strong evidence for bimodal distributions of heights among individuals with XX gonadal dysgenesis (Simpson, 1979a).

XY Gonadal Dysgenesis

Individuals with apparently normal male (46,XY) chromosomal complements may also have XY gonadal dysgenesis (Swyer syndrome). 46,XY females with gonadoblastomas or dysgerminomas can properly be included in this category, even though it cannot always be determined whether the tumors arise from a streak gonad or from a dysgenetic testes. Almost all individuals with XY gonadal dysgenesis are normal in stature, and somatic anomalies are usually absent.

Approximately 20 to 30 percent of reported XY gonadal dysgenesis patients have had a dysgerminoma or gonadoblastoma (Simpson & Photopulos, 1976a). Often the neoplasia arises in the first or second decade. Because of the relatively high probability of their undergoing neoplastic transformation, gonads should be extirpated from any patient with XY gonadal dysgenesis. The uterus and Fallopian tubes need not necessarily be removed.

In many kindreds, multiple family members had XY gonadal dysgenesis (German et al., 1978; Simpson, 1979a; Simpson, Blagowidow, & Martin 1981) and in three families the trait segregated in the manner expected of an X-linked recessive or male-limited autosomal dominant gene. Speculations concerning pathogenesis can be found elsewhere (German et al., 1978). Of note, however, are observations that most affected patients are H-Y antigen positive (Wolf, 1979), leading to suggestions that the disorder involves defective cell receptors. Moreover, the disorder is clearly heterogeneous (Simpson, Blagowidow, & Martin (1981).

KLINEFELTER SYNDROME

Males with at least one Y chromosome and at least two X chromosomes have Klinefelter syndrome (Fig. 10-12). About 1/1000 liveborn males has a 47,XXY complement. The phenotype may also be associated with 46,XY/47,XXY; 48,XXYY; and 49,XXXXY complements. The most characteristic features are seminiferous tubule dysgenesis and androgen deficiency. Somatic anomalies may or may not be associated. The presence of both well-differen-

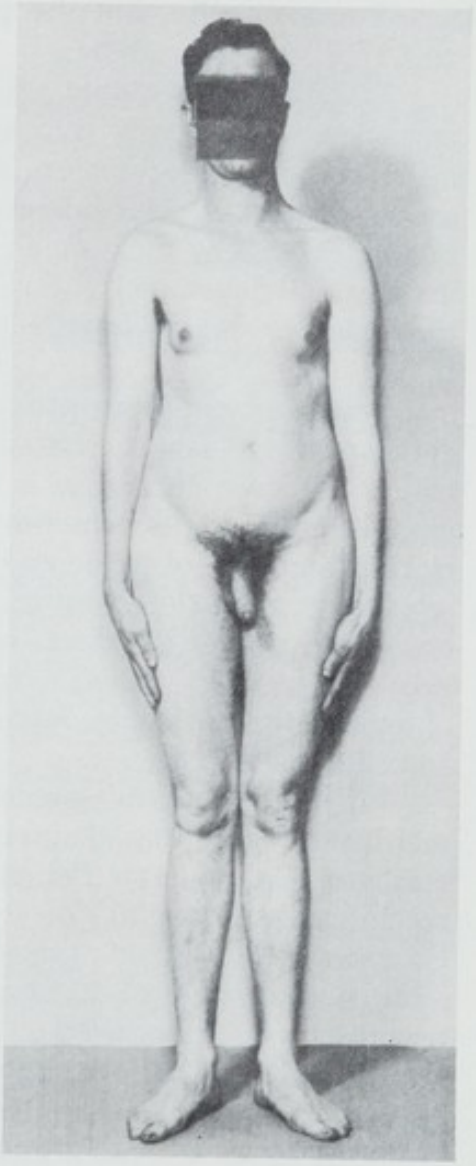


Figure 10-12. Photograph of a 47,XXY male. Gynecomastia is present. (From Ferguson-Smith MA: Testis and intersexuality, in Sorsby A (Ed): Clinical Genetics (ed 2). London: Butterworth, p 517. Reprinted with permission.)

tiated external genitalia and a sex chromosomal aberration differentiates Klinefelter syndrome from forms of male pseudohermaphroditism; presence of both a sex chromosomal aberration and elevated gonadotropin levels differentiates Klinefelter syndrome from hypogonadotropic hypogonadism.

47,XXY

In 47,XXY individuals, Sertoli cells proliferate shortly before the expected time of puberty. Thereafter, seminiferous tubules degenerate, to be replaced with hyaline material. Leydig cells appear hyperplastic because other testicular elements are deficient; however, the absolute volume of Leydig cells in testes is no greater than normal. Leydig cells lack crystalloids of Reinke, and their steroid production is decreased. Testes of affected adults are rarely more than 2 cm at widest diameter. The testes are usually firmer than normal, although occasionally they are softer; differences in consistency probably reflect differences in the extent of seminiferous tubule hyalinization. Spermatozoa are de-

tected occasionally, but the presence of spermatozoa more often indicates 46,XY/47,XXY mosaicism.

The external genitalia are usually well differentiated, although neonates may show a micropenis. Hypospadias probably occurs no more often in 47,XXY males than in normal males. In 80 to 90 percent of 47,XXY patients the penis seems normal in size; however, following administration of androgens the penile length may increase an additional 1 to 3 cm. Similarly, the scrotum is usually well developed, and vasa differentia are normal. The prostate is smaller than usual, presumably reflecting decreased androgen levels. Cryptorchidism is rare.

Decreased androgen production causes lack of normal secondary sexual development. Plasma testosterone is usually approximately half that of normal males; however, the range is so wide that levels overlap those expected of normal males. LH is sometimes elevated even where testosterone is normal. Normal amounts of pubic and axillary hair may be present, but facial hair is usually sparse. Adolescent acne is rare, and temporal hair recession does not occur. Muscle development is poor, voice lowering may not occur, and the skin may appear pale and wrinkled. Fat distribution may be more reminiscent of females than of males. Rarely, individuals appear almost normal in appearance but are infertile.

Fifty to seventy-five percent of 47,XXY patients have increased parenchymal breast tissue, based upon palpation; however, visually obvious gynecomastia is less common. Patients with the Klinefelter syndrome develop breast carcinoma at a rate 20 times greater than normal males (Scheike, Visfield, & Peterson, 1973). In fact, 3 to 4 percent of males with breast cancer have Klinefelter syndrome, leading some investigators to recommend mastectomy if gynecomastia is pronounced.

47,XXY patients are slightly taller than normal males. Their legs are relatively long compared to their trunk and arms, a disproportion manifested clinically by an increased pubis-to-sole length. Scoliosis, kyphosis, pectus excavatum, and clinodactyly V also occur more often in 47,XXY males than in 46,XY males. Dental anomalies, elevations in neurosensory hearing threshold, cardiac anomalies, pulmonary diseases, varicose veins, and hypostatic ulcers occur with increased frequency (Simpson, 1976; Ferguson-Smith, 1969).

47,XXY individuals are more likely to be retarded or socially maladjusted than are normal 46,XY males, but the exact risk is difficult to establish. Perhaps 1 percent of mentally retarded males have a 47,XXY complement. The prevalence of 47,XXY is higher among individuals whose IQ is 50 to 85 than among those with a lower IQ.

Nonretarded patients are often passive, poorly motivated, and unable to reach their goals or complete tasks. They adapt poorly to new situations, and may display inappropriately aggressive behavior when confronted with stressful situations. However, relatively few are overtly sociopathic or psychotic. These characteristics have been explained as secondary to poor self-image, which is probably the consequence of inadequate sexual development at puberty. However, reports of electroencephalographic abnormalities suggest the possibility of an organic defect. These individuals are also at increased risk of incarceration.

46,XY/47,XXY

About 10 percent of individuals with the Klinefelter phenotype are known to have 47,XY/47,XXY mosaicism, and the frequency is probably underestimated. For example, Paulsen et al. (1968) studied six 46,XY/47,XXY individuals in whom mosaicism was present in testicular tissue. In three of the six, mosaicism was detected in lymphocytes; however, in the other three the 47,XXY line would not have been recognized if cytogenetic studies had been confined to lymphocytes.

46,XY/47,XXY individuals are less likely than 47,XXY individuals to have gynecomastia, azoospermia, small testes, or decreased facial or pubic hair. Mean plasma testosterone levels are also higher in 46,XX/47,XXY, and mature spermatozoa are more likely to be detected in testicular biopsies.

48,XXXY

The chromosome complement 48,XXXY has been reported at least 50 times (Simpson et al., 1974; Simpson, 1976). All reported cases have had small testes, androgen deficiency, and mental retardation. Somatic anomalies occur more often in 48,XXXY than in 47,XXY individuals. About half the reported 48,XXXY individuals have some nongonadal developmental anomaly, the most frequent of which are short neck, epicanthal folds, abnormal elbows, and clinodactyly V.

49,XXXXY

49,XXXXY individuals are usually ascertained in surveys of the mentally retarded. All have shown seminiferous tubule dysgenesis, androgen deficiency, and mental retardation, which is often very severe (IQ 20 to 25). Almost all show somatic anomalies, the most frequent of which are hypertelorism, epicanthal folds, prognathism, short neck, clinodactyly V, coxa valga, and pes planus.

48,XXYY and 49,XXXYY

48,XXYY individuals share some features with 47,XXY and other features with 47,XYY. As expected, testicular hypoplasia results in poorly developed secondary sexual characteristics. Many 48,XXYY individuals have been reported to be mentally retarded, but this could merely reflect biases of ascertainment or reporting. However, some 48,XXXY males are inappropriately aggressive, and electroencephalographic abnormalities have been reported. The somatic anomalies present are reminiscent of those occurring in 48,XXXY and 49,XXXXY males.

POLYSOMY X IN FEMALES

47,XXX

47,XXX individuals are more likely to be mentally retarded or mentally ill than are 46,XX individuals. The magnitude of the increased risk is difficult to estimate, however, because most 47,XXX patients have been ascertained in

surveys of the mentally retarded. Prospective studies of the relatively few 47,XXX patients ascertained unbiasly at birth indicate IQ 16 points below those of sibs (Robinson, Lubs, & Bergsma, 1979). One third show at least some mental or behavioral problems. For counseling purposes a minimum estimate is that at least 5 percent of 47,XXX females are retarded, with an IQ between 45 and 70. Some 47,XXX individuals have craniofacial anomalies reminiscent of those detected more consistently in 48,XXXX and 49,XXXXX individuals; however, somatic anomalies are usually not present in 47,XXX.

47,XXX individuals may experience delayed menarche or premature ovarian failure. The basis for ovarian dysfunction is unknown; however, it is tempting to postulate that the increased number of X chromosomes interferes with meiotic segregation and leads to cessation of oogenesis. Most offspring of 47,XXX women have been chromosomally normal, despite theoretical expectations that 50 percent would be chromosomally abnormal. However, chromosomally abnormal offspring of 47,XXX or 45,X/47,XXX women have been reported (Dewhurst, 1978; Simpson, 1981a); thus, antenatal diagnosis should be offered to pregnant 47,XXX females.

48,XXXX

All but one 48,XXXX individual has been subnormal in intelligence. Some patients have had esotropia, electroencephalographic abnormalities, or facies reminiscent of Down syndrome. Others have no somatic anomalies but show ovarian dysfunction.

49,XXXXX

49,XXXXX individuals invariably show mental retardation. The frequency of somatic anomalies is higher than in 47,XXX, and possibly also higher than in 48,XXXX. The anomalies most commonly present in 49,XXXXX include hypertelorism, slanting palpebral fissures, a broad nasal bridge, everted lips, esotropia, small hands and feet, abnormal teeth, clinodactyly of the fifth finger, a short neck, and a decreased total digital ridge count.

POLYSOMY Y IN MALES

Approximately 1 of every 1000 live-born males has the complement 47,XYY. In 1965–1966, several investigators reported data suggesting that 47,XYY individuals were most likely to be detected among tall, mentally retarded, antisocial males (Price et al., 1966; Jacobs, Price, & Whatmore, 1967). Despite many studies since then, definitive answers are still lacking. It is clear that initial studies overestimated the risk of aberrant behavior, but likewise clear that the complement confers some risk.

The first psychological studies (Price et al., 1966; Price & Whatmore, 1967) compared 47,XYY and chromosomally normal inmates at a Scottish maximum security prison, and found that the 47,XYY inmates (1) incurred their first conviction at a younger age than other inmates, (2) less often had a sib who had received a conviction, and (3) committed crimes against property more often than against persons. These observations suggested that 47,XYY inmates

are incarcerated for different reasons than are other inmates. On the other hand, the relatively few prospectively ascertained 47,XYY children have been essentially normal, although some have shown behavioral difficulties. The likelihood of a 47,XYY male becoming incarcerated is perhaps 1 percent, compared to 0.1 percent for 46,XY males (Lancet, 1974b).

47,XYY men usually have grossly normal testes and normal external genitalia. However, tubules in spermatogenic arrest are detected in 50 percent of cases, and tubules consisting solely of Sertoli cells are detected in 30 percent (Shakkebaek et al., 1973). Mean plasma testosterone levels are normal. Most offspring of 47,XYY males are chromosomally normal, in contrast to theoretical predictions that half would be 47,XXY or 47,XYY. Nonetheless, antenatal diagnosis should be considered if a father is 47,XYY.

All reported 48,XYYY individuals have shown multiple somatic anomalies. 49,XXYYY and various types of mosaicism have also been reported. These complements presumably arise by nondisjunction in paternal meiosis, with nondisjunction in the zygote or embryo possibly occuring also in certain cases.

HYPOGONADOTROPIC HYPOGONADISM

Isolated Gonadotropin Deficiency

Deficiency of gonadotropin (FSH and LH) without deficiencies of other pituitary hormones and without associated somatic anomalies is a rare cause of hypogonadism. Affected females show primary amenorrhea and lack of secondary sexual development. Affected males fail to undergo normal secondary sexual development and hence usually consult a physician because of small penis, small testes, high-pitched voice, and scanty beard growth. External genitalia are small yet well differentiated. The testes are characterized by decreased Leydig cells and retarded spermatogenesis. Ovaries show numerous primordial follicles but no oocytes. If somatic anomalies are present, a disorder other than isolated gonadotropin deficiency should, by definition, be considered.

Isolated gonadotropin deficiency is inherited in autosomal recessive fashion. The pathogenesis could theoretically involve abnormalities of either the hypothalamus or the pituitary. In mice a similar disorder results from deficiency of GnRH.

Kallman Syndrome

Individuals with Kallman syndrome have not only isolated gonadotropin deficiency but also decreased or absent olfaction (anosmia or hyposmia) secondary to aplasia of the olfactory bulbs. Unilateral renal agenesis, cleft palate, and other somatic anomalies may also occur. This disorder is the most common cause of hypogonadotropic hypogonadism (Santen & Paulsen, 1973).

Because Kallman syndrome was once believed to occur only in males, it was thought to be inherited in X-linked recessive fashion. It is now apparent that females may also be affected, although they have hyposmia less often than males; thus, the mutant gene is inherited in an autosomal dominant fashion, with olfactory abnormalities more commonly expressed in males. Male-to-male transmission has been observed, formally excluding X-linked inheritance.

Isolated LH Deficiency

Males with normal or nearly normal spermatogenesis who fail to undergo normal sexual development are said to be "fertile eunuchs." LH secretion is deficient. Affected individuals have high-pitched voices, poor muscle development, and scanty beard growth. Their testes have normal numbers of germ cells, but practically no Leydig cells. Sperm counts are usually normal. Administration of hCG and testosterone has resulted in well-documented cases of paternity. Most cases are sporadic, although affected sibs have been described. Affected females have not definitely been reported; however, Park et al. (1976) reported a female believed to have immunologically active (radioimmunoassay) but biologically inactive (bioassay) LH.

Isolated FSH Deficiency

Rabin et al. (1972) and later Bell et al. (1975) reported a 22-year-old woman with primary amenorrhea and no secondary sex characteristics. Her ovaries were small. Numerous primordial follicles were present, but mature oocytes were not. FSH was undetectable (less than 3mIU/ml) on at least eight occasions, whereas LH was markedly elevated (40 to 90 mIU/ml). If only a single gonadotropin-releasing hormone exists, the pathogenesis would seem more likely to involve the pituitary than the hypothalamus. Consistent with pituitary origin was the observation that the β subunit of FSH was impaired.

Hypopituitary Dwarfism

Deficiency of two or more pituitary tropic hormones, including gonadotropin, is a well-known cause of dwarfism. Hypopituitary dwarfs deficient in growth hormone may also be deficient in thyrotopin, ACTH, or gonadotropin. Hypopituitary hypogonadotropic hypogonadism is diagnosed easily on the basis of associated growth retardation, hypothyroidism, and hypoadrenalism. The disorder may result from any of many causes—a mutant autosomal recessive allele, a mutant X-linked recessive gene, an infection, or a neoplasm involving the pituitary or hypothalamus.

OTHER DISORDERS

Germinal Cell Aplasia (Sertoli-Cell Only Syndrome)

In 1947 Del Castillo, Trabucco, and DeLa Balze (1947) described several normally virilized yet sterile males, whose seminiferous tubules lacked spermatogonia and whose testes were slightly smaller than average. However, Leydig cell function was normal; thus, secondary sexual development was normal. FSH is elevated but LH is normal. Tubular hyalinization and sclerosis usually do not occur. Occasionally a few spermatozoa are present, but affected individuals are usually sterile. The infertility cannot be treated, and androgens are unnecessary because secondary sexual development is normal. Histologically similar testes may be detected in mumps orchitis, in irradiation orchitis, in cryptorchid testes of otherwise normal males, and occasionally in Klinefelter syndrome.

Rudimentary Ovary Syndrome and Unilateral Streak Ovary Syndrome

The rudimentary ovary syndrome, a poorly defined entity of unknown etiology, is said to be characterized by ovaries containing decreased numbers of follicles. Anatomically analogous to the rudimentary testes syndrome, it is also probably heterogenous in etiology. Many cases have been associated with chromosomal abnormalities, particularly 45,X/46,XX mosaicism. These same statements apply also to individuals said to have the unilateral streak ovary syndrome.

11

Common Nongynecologic Diseases

The tendency for certain diseases of adults to aggregate in families may result from both genetic and nongenetic factors. The genetic factors are often poorly understood and may play only a part in the overall etiology. If a disorder is particularly common in the population as a whole, occurrence in more than one family member may reflect only the frequent incidence of the disorder. In addition, environmental factors may simulate genetic disease. Nonetheless, genetic factors are important in many conditions. In this chapter we shall consider the genetices of some very common diseases of interest to obstetrician–gynecologists.

HYPERTENSION

Although the study of hypertension is subject to difficulties arising from the variable nature of blood pressure measurements, several investigations have documented familial aggregation of essential hypertension. However, there is no unanimity concerning mode(s) of inheritance. For example, Pickering (1968) showed that sex-corrected blood pressures of adult first-degree relatives aggregated at all levels of blood pressures (regression coefficient 0.2 to 0.3), and theorized that blood pressure is a quantitative trait transmitted in polygenic/multifactorial fashion. On the other hand, Morrison and Morris (1960) and Platt (1963) favored a major gene effect. More likely, essential hypertension is genetically heterogeneous. Perhaps in some individuals a major gene effect is principally responible for hypertension, whereas in others polygenic/multifactorial factors are responsible.

Kass et al. (1975) demonstrated that by 2 years of age, there is already significant familial correlation of blood pressure. Twin studies also support the

theory of genetic influences upon arterial blood pressure and hypertension (Feinleib et al., 1975), monozygotic twins showing a higher correlation than other relatives. In addition, there is a relatively higher correlation for dizygotic twins than for nontwin sibs or for parents and offspring. This phenomenon is consistent with the hypothesis that shared early environmental factors play a role in determining blood pressure levels (Feinleib et al., 1975). Although some studies have shown no significant correlation between the blood pressure of individuals and their nonrelated (e.g., adopted) children (Biron, Mongeau, & Bertrand, 1975; Biron & Mongeau, 1978), small positive correlations have been observed among adoptees living together. These correlations were linked to the length of time that had elapsed since adoption, and to similarity of body weight, which appears to be inherited in a multifactorial manner. [Other important environmental factors, such as salt intake, stress and physical activity, also may play roles.]

In conclusion, familial aggregation of blood pressure appears based in part on genetic factors. The degree of heritability is uncertain, although Feinleib et al. (1975) suggested that approximately 60 percent of the variability of blood pressure may be accredited to the genetic component. However, this estimate may be high because of inherent difficulties relating to twin investigations. Perera, Gearing, and Schweitzer (1972) found approximately a twofold risk of hypertension in sibs of hypertensive individuals, a figure perhaps appropriate for use in genetic counseling.

CORONARY ARTERY DISEASE

Several studies have demonstrated a twofold to threefold greater incidence of coronary heart disease in first-degree relatives of patients than in control subjects (Rose, 1954; Thomas & Cohen, 1955; Slack & Evans, 1966; Phillips et al., 1974; Rissanen & Nikkila, 1977). However, many studies were subject to methodologic problems, principally selective recall and the difficulty of confirming cause of death. Case-control studies are also subject to some of these same problems. Frequently there is no assurance that cases and controls are representative of the same populations. In addition, familial aggregation does not distinguish genetic and environmental contributions.

Nonetheless, many major risk factors for coronary artery disease are polygenically inherited. These risk factors are particularly important among younger people (Rissanen, 1979). Indeed, the younger an individual who develops coronary artery disease, the greater the risk for parents and sibs. If myocardial infarction occurs in a male prior to age 46, the risk that his father will die of coronary artery disease is 50 percent, a threefold to fivefold increase over normal, and the risk to his male sibs is 45 to 50 percent, a 6- to 11-fold increase. The corresponding risk for relatives of patients with middle-age onset myocardial infarction (46 to 50 years) was 32 percent (3.8 times greater) for fathers, 35 to 45 percent (3.8 times greater) for sibs, and 20 to 25 percent (3 to 4 times greater) for sisters (Rissanen, 1979). These data support an additive polygenic/multifactorial etiology for myocardial infarction in younger individ-

uals. Myocardial coronary disease occurring in older patients is relatively more likely to be environmental in origin.

Mendelian disorders can also predispose to myocardial infarction. Approximately 20 percent of men under age 60 who survived a heart attack had a monogenic disorder of lipid metabolism, i.e., hypercholesterolemia, hypertriglyceridemia, or combined hyperlipemia (Motulsky, 1976, 1978). These conditions are probably autosomal dominant. The presence of xanthomas should also suggest a Mendelian disorder.

PEPTIC ULCER DISEASE

Genetic factors are clearly important in peptic ulcer disease, which is actually several different entities (Rotter, 1980). Gastric and duodenal peptic ulcers show familial aggregation as independent entities; that is, in a given family only one type is usually observed. Several genetic studies (Doll & Buch, 1950; Wretmark, 1953; Kuenssberg, 1962; Monson, 1970) have shown that the frequency of peptic ulcer is two to three times greater in first-degree relatives of probands with peptic ulcer than in similar relatives of controls. The risk of recurrence is higher if the proband is young, and the risk for male relatives is higher than for female relatives (Cowan, 1973). Twin studies (Eberhard, 1968; Gotlieb-Jensen, 1972) show higher concordance among monozygotic than dizygotic twins.

Individuals with group O blood have a 30- to 40-percent greater incidence of ulcers than do individuals with other blood groups (McConnell, 1966; Mourant, Kopek, & Domanlewska-Subczak, 1978). An association with blood group O has been documented for duodenal ulcers, combined gastric and duodenal ulcers, and ulcers of the antrum of the stomach, but not with ulcers of the body of the stomach. Hemorrhage and perforation also occur more frequently in ulcer patients with blood group O (Langman, 1973; Langman & Dahl, 1965). Peptic ulcers are also associated with nonsecretor status for ABO substances: nonsecreters are 40 to 50 percent more common among ulcer patients than controls (see Langman, 1973; Mourant, Kopek, & Domanlewska-Subczak, 1978). Nonsecretor blood type O individuals have an approximate 2.5 greater risk for duodenal ulcer. Interestingly, blood group A is associated with an increased risk of gastric cancer (Aird et al., 1954).

Although polygenic/multifactorial inheritance is most often invoked to explain familial aggregations, peptic ulcer is known to be associated with several syndromes inherited in simple Mendelian fashion (Rotter, 1980; Rotter & Rimoin, 1977; Rotter, Rimoin, & Samloff, 1978). This confirms genetic heterogeneity. Monogenic disorders associated with ulcers include type I multiple endocrine adenomas, and a syndrome comprising tremors, congenital nystagmus, duodenal ulcer, and narcolepsy-like sleep disturbance (Neuhauser et al., 1976). Other disorders associated with peptic ulcer include hyperparathyroidism, cystic fibrosis, and alpha-1 antitrypsin deficiency. In addition, Rotter (1980) has presented evidence, based on serum pepsinogen, gastric emptying, and gastrin response to meals, that several types of peptic ulcer disease occur in otherwise normal individuals.

REGIONAL ENTERITIS AND ULCERATIVE COLITIS

There have been several reports of families in which more than one member has had regional enteritis (Crohn disease); however, the prevalence of the disease in families has rarely been compared to that in appropriate controls. Because the risk for first-degree relatives is about 5 percent, the most likely hypothesis is polygenic/multifactorial. There also have been reports in which more than one family member had ulcerative colitis (McConnell, 1966). The frequency with which family members are affected varies, but for first-degree relatives the risk is again about 5 percent. If the index case has regional enteritis, other relatives may have either that disorder or ulcerative colitis. However, if the index case has ulcerative colitis, other relatives usually have the same disorder (Passarge, 1979).

DIABETES MELLITUS

Familial aggregates in diabetes mellitus have been recognized for decades, but the mode or modes of inheritance have proved elusive. Irrespective of age of onset or severity, 4 to 8 percent of relatives of diabetic individuals also have diabetes mellitus, whereas only 2 percent of age-matched controls are similarly affected (See Simpson, 1978c). Four to six percent of sibs of diabetic individuals have diabetes, compared to fewer than 1 percent of controls. About 80 percent of monozygotic twins are concordant for diabetes, whereas fewer than 20 percent of dizygotic twins are concordant. Data showing familial predisposition to diabetes mellitus have led to various hypotheses, including dominant, recessive, and multifactorial modes of inheritance. However, hypotheses based upon the assumption that diabetes mellitus is a single entity are inappropriate because it is actually several different genetic entities. Thus, a single genetic model need not fit.

Several factors provide indirect evidence for genetic heterogeneity for diabetes mellitus. For example, diabetes mellitus is one of several consistent features of some 30 different genetic malformation syndromes, thus indicating that the disorder can result from gene mutations at different loci (Rotter & Rimoin, 1980). Diabetes mellitus also shows variability of expression among different ethnic groups, a phenomenon not always explainable on the basis of different environments. Genetic heterogeneity for diabetes has been demonstrated to exist in mice, a species in which different diabetic genes can be identified. (Coleman & Hummerl, 1967). All these factors suggest that diabetes mellitus in humans can result from mutations at different loci (Rotter & Rimoin, 1981).

Most classifications currently distinguish between at least three major types of diabetes mellitus in otherwise normal individuals: (1) juvenile-onset, insulin-dependent form (JOD), (2) maturity-onset, non–insulin-dependent form (MOD), and (3) maturity-onset diabetes of youth (MODY), in which diabetes is diagnosed relatively early (before 25 years of age) yet behaves clinically like maturity-onset diabetes in that those affected manifest few overt symptoms, show little disease progression, and usually require no insulin.

Juvenile-Onset Diabetes

Most data suggest that maturity-onset and juvenile-onset diabetes are separate conditions. Environmental factors appear to be more important in juvenile-onset diabetes. Although concordance for both disorders among monozygotic twins is much higher than in nontwin sibs, juvenile-onset concordance is less than maturity-onset concordance (Tattersall & Pyke, 1972; Pyke & Nelson, 1976). An even more convincing argument that the disorders are distinct is an association between juvenile-onset diabetes and certain HLA antigens (Rotter & Rimoin, 1980). Similar HLA associations in diabetes—actually HLA alleles in linkage disequilibrium (for detailed explanation see Simpson, 1978c)—have been observed in other disease states frequently suspected of being autoimmune in etiology. Among the explanations proposed to explain these HLA associations are that (1) the disorder and the particular HLA antigen each occur with sufficiently increased frequency in a population so that their simultaneous occurrence is coincidental; (2) a direct causal relationship occurs as result of the presence or absence of the HLA antigen; and (3) the HLA locus is linked to another gene that either causes diabetes or confers susceptibility for its development. Nerup et al. (1976) and Cudworth & Woodrow (1976) were apparently the first to show a significant association of HLA-B8 and HLA-Bw15 with juvenile-onset diabetes. Additional studies have shown that HLA-B18 also occurs with greater frequency among juvenile-onset diabetics. The associations of DRw3 and DRw4 with juvenile-onset diabetes appear to be even stronger. DRw3 is usually associated with B8 and DRw4 with B15 (Rotter & Rimoin, 1980, 1981). The associations have not been found for maturity-onset diabetes.

An individual with either B8,DRw3 or Bw15, DRw4 is two to three times likely to develop juvenile-onset diabetes than an individual lacking these antigens (Rotter & Rimoin, 1980, 1981). Persons who are heterozygous for both HLA-B8, DRw3 and HLA-B15, DRw4 are five to six times more likely to develop diabetes, than persons with either group alone. DRw3 and DRw4 in combination are present in more than 50 percent of offspring of affected sibs with juvenile-onset diabetes, but are present in only 8 percent of nondiabetic sibs in the same families. The relative risk for developing juvenile-onset diabetes is nearly 40 percent for individuals who are heterozygous for both DRw3 and DRw4. In addition, there are indications that B8, DRw3- and B15, DRw4-associated juvenile-onset diabetes are distinct. The B8, DRw3-associated form may be autoimmune in pathogenesis, whereas the B15, DRw4-associated form is more likely to result from deficiency in insulin synthesis or release.

Maturity-Onset Diabetes

The association of specific HLA types with maturity-onset diabetes has not been nearly so striking as with juvenile-onset diabetes. This suggests different etiologies in juvenile-onset and maturity-onset diabetes. However, twin data strongly suggest that genetic factors, albeit different from those operating in juvenile-onset diabetes, are very important in maturity-onset diabetes. Overall concordance among monozygotic twins for diabetes mellitus is about 70 percent. If the diagnosis was established after 45 years of age, concordance among monozygotic twins is nearly 100 percent. Such cotwins usually develop diabetes within three years of each other.

Maturity-Onset Diabetes of Youth

Tattersall and Fajans (1975) identified 26 individuals in whom the age of onset was less than 25 years. None required insulin until at least two years after the initial diagnoses. Twenty-two (85 percent) had a diabetic parent, and 25 of 47 sibs (53 percent) were affected. In 12 instances, there were three generations of affected relatives. These data suggest autosomal dominant inheritance. If so, the likelihood that a first-degree relative (sib, parent, or offspring) of an individual with MODY will inherit the mutant gene is 50 percent, although not all need be affected if penetrance is not 100 percent.

Genetic Counseling

If an individual has maturity-onset diabetes of youth, the likelihood that a parent, sibling, or offspring will inherit the mutant gene is 50 percent. Because specific modes of inheritance have not yet been established for juvenile-onset and maturity-onset diabetes, counseling for these disorders is empiric. The likelihood that a child of an individual with juvenile-onset diabetes will be affected seems to be about 2 percent. The likelihood that subsequent sibs of a child with juvenile-onset diabetes will be similarly affected is about 10 percent. If a proband has maturity-onset diabetes, the likelihood that any first-degree relative will have the same disorder is 5 to 10 percent. The likelihood that they will have juvenile-onset diabetes is only slightly greater than that of the general population. (The above figures are generally applicable for adults showing normal life expectancy.)

RHEUMATOID ARTHRITIS AND ANKYLOSING SPONDYLITIS

Familial clustering of rheumatoid arthritis and ankylosing spondylitis occurs, although the exact risks to relatives of probands with either disease is unknown. In one study, relatives of probands with rheumatoid arthritis were 2.8 times more likely to have the disorder than relatives of controls. Even more impressively, the frequency of ankylosing spondylitis was 22.6 times greater in relatives of affected individuals than in controls (DeBlecourt, Polman, & DeBlecourt-Meinderasma, 1961). Vobecky Lussier, & Munan (1974) showed that rheumatic disease occurred twice as frequently in the parents of persons with ankylosing spondylitis than in parents of individuals with rheumatoid arthritis or controls. Although the genetic mechanism in these disorders is unknown, the association in linkage disequilibrium of ankylosing spondylitis and HLA-B27 suggests a genetic basis. Over 90 percent of patients with ankylosing spondylitis have HLA-B27, compared to only 6 to 8 percent of the normal population (Calin & Fries, 1975).

SCHIZOPHRENIA AND MANIC DEPRESSION

In the general population, the risk of developing schizophrenia is perhaps 1 percent or less (Kety et al., 1978), and the risk of developing a manic-depressive or depressive disorder is 2 to 3 percent (Gershon et al., 1977).

Genetic factors play a major role in the etiology of major psychiatric disorders. The first solid evidence of a genetic component was derived from twin studies. In schizophrenia, concordance is 40 to 50 percent for monozygotic twins, compared to only 9 to 15 percent for dizygotic twins (Kringlen, 1967; Allen, Cohen, & Pollin, 1972; Gottesman & Shields, 1972). For depression and manic-depression, the concordance was 70 percent for monozygotic twins and only 13 percent for dizygotic twins (Gershon et al., 1977). The same degree of concordance was observed in twin pairs reared apart, indicating that the monozygotic concordance was not merely due to a shared environment (Price, 1968). Heston (1966) and Rosenthal et al. (1971) found the frequency (8 to 10 percent) of schizophrenia in children born to schizophrenic mothers but reared by others, to be greater than in children of controls. This suggests that schizophrenia is strongly influenced by genetic factors.

Based upon tbe above studies, it is not surprising that first-degree relatives of individuals with schizophrenia or a disorder characterized by mood swings show increased prevalences of these disorders, compared to the general population. Of these, relatives of probands with manic-depression tend to have an equal tendency to depression and manic-depression. Affected relatives of individuals with depression usually show depression alone (Gershon et al., 1977). Relatives of probands with schizophrenia are more likely to have schizophrenia.

The risk that a parent with schizophrenia will have a similarly affected child is 8 to 10 percent (Heston, 1966; Rosenthal et al., 1971). The risk that schizophrenia will occur in a sib of a schizophrenic individual is reported to be 5 to 12 percent. If both a parent and a sib are affected the risk is even higher. Because these figures are the cumulative risks over a normal lifetime, an adult of 30 who has not yet developed schizophrenia has a risk only half this (2.5 to 6 percent). If such an individual is 45 years of age the risk is very small. Similar figures are appropriate for manic-depressive states, some suggestions of monogenic inheritance notwithstanding.

It should be noted that 20 to 30 percent of patients with postpartum psychosis have had mental illness (Fondeur et al., 1957; Martin, 1958; Targum & Gershon, 1981). The frequency of psychiatric illness in their first-degree relatives is about 10 percent (Herzog & Detre, 1976), a figure similar to that for other major psychiatric disorders.

BREAST CARCINOMA

Heritable tendencies exist for a variety of common cancers, e.g., gastric cancer, colon cancer, and leukemias (Schimke, 1980). A common neoplasia for which existence of genetic factors is unquestioned is breast cancer. Breast carcinoma probably involves several etiologic mechanisms. Genes, viruses, hormones, and other chemical agents have all been implicated. However, much of these data have been gathered in animal studies, and extrapolations to humans are not necessarily appropriate.

Breast cancer risk is believed increased in women whose first full-term pregnancy is delayed until age 35 years or older (MacMahon, Cole, & Brown, 1973). Women who have their first infant before age 18 have only about one

third the risk as do women in the general population. Lactation per se apparently has no significant effect on the risk of breast cancer. Increased risk of breast cancer is also associated with early menarche and late natural menopause. Surgical menopause prior to the age of 35 has been stated to reduce the risk of breast carcinoma.

All the above risk factors notwithstanding, breast cancer has long been demonstrated to aggregate in families. Between 15 and 30 percent of all patients with breast cancer are suspected of having genetically determined disease (Knudson, Strong, & Anderson, 1973). Surveys comparing breast cancer morbidity and mortality rates among relatives of women with breast carcinoma with the rates among controls in both high- and low-incidence countries demonstrate a twofold to threefold increase in breast carcinoma in mothers, sisters, and daughters. The risks are similar in maternal and paternal relatives. First-degree relatives of women with premenopausal bilateral disease have a tenfold increased risk compared to age-matched controls, whereas first-degree relatives of postmenopausal women with unilateral breast carcinoma have a twofold increased risk.

Selected pedigree data substantiate the genetic basis of breast cancer (Anderson, 1974, 1976, 1977). The cumulative risk for a patient whose mother and grandmother were both affected is about 40 percent, compared to only 8 percent in the general population. If a patient's sib but not her mother is affected, the risk to other sibs is 12 to 16 percent. If only a second-degree relative is affected, the risk does not differ significantly from controls. Familial aggregates are more common if onset of disease is premenopausal and the cancer bilateral.

There are several distinct hereditary types of breast carcinomas. These include (1) the syndrome of breast carcinoma, leukemia, soft tissue sarcoma, and brain tumors (Lynch et al., 1973); (2) the syndrome of breast and colon adenocarcinomas (Lynch, Krush, & Guirgis, 1973); (3) the syndrome of breast carcinoma and ovarian epithelial carcinoma (Fraumeni et al., 1975; Lynch & Krush, 1971); and (4) Cowden disease, which is characterized by breast carcinoma, thyroid adenoma, and colonic polyps. In all these conditions the risk of breast carcinoma in first-degree relatives is 30 to 50 percent, suggesting autosomal dominant inheritance. The studies cited above also provide evidence for an autosomal dominant form of premenopausal breast cancer characterized by early onset and bilaterality (Anderson, 1974). In addition, see Simpson et al. (1981) for evidence consistent with possible recessive factors.

Genetic heterogeneity exists for breast cancer; thus, the genetic basis underlying the disease in one family need not be the same as in another. Certainly women in families in which there is early onset and bilateral involvement should be counseled about their high risk of breast cancer and taught self-breast examinations. Whether additional diagnostic procedures are warranted in asymptomatic patients is as yet undetermined. A woman who has developed cancer in one breast should be informed of the possibility of bilaterality, particularly if a relative has had bilateral breast carcinoma.

12

Principles of Human Embryology and Teratogenesis

Basic knowledge of normal human embryonic development is a necessary prelude to understanding the processes leading to abnormal development. In this chapter we shall first review the basic steps of human development and then discuss general principles of teratology. Detailed descriptions of human development are provided elsewhere (Tuchmann-Duplessis, David, & Haegol, 1972; Moore, 1973; Crowley, 1974).

EMBRYOLOGY

Preimplantation

The first three days of development* take place in one of the Fallopian tubes. At the time of fertilization, a pronuclear stage exists (Noyes et al., 1965; Zamboni et al., 1966), during which nuclei from the egg and the sperm retain their integrity within the egg cytoplasm. After the pronuclei fuse (syngamy), the fertilized egg begins a series of mitotic cell divisions (cleavage). The two-cell stage is reached about 30 hours after fertilization (Hertig et al., 1954). Each successive division requires less time, finally reaching a constant rate (Rugh, Shettles, and Einhorn, 1971). With continued division, daughter cells (blastomeres) aggregate into a solid ball of cells. This ball, called a morula, reaches the endometrial cavity about three days after fertilization. Thereafter, a fluid-filled cavity (blastocele) forms within the cell mass, at which time the conceptus is called a blastocyst (see Fig. 7-1). Overall, the number of cells increases from approximately 12 to 32 at the end of the third day to 250 by the sixth day.

Until approximately three days of development, any cell is thought to be totipotential, that is, capable of initiating development of any organ system.

*Unless stated otherwise, the days referred to are following ovulation.

For example, separation of cells during this time period can give rise to monozygotic twins, each normal. At the close of the preimplantation period (blastocyst stage), cells first begin to differentiate. The blastocyst is located in the uterus, where implantation (see Fig. 7-2) occurs 6 to 9 days after conception. At this stage one group of cells forms the inner cell mass (embryonic disc or blastoderm), which will ultimately develop into the fetus. Only two cell layers (ectoderm and endoderm) comprise the embryonic disc at this stage. The third layer, the mesoderm, will form later. Different tissues will ultimately develop from each of the three cell layers. For example, the brain, nerves, and skin will develop from the ectoderm; the lining of the digestive tract will develop from the endoderm. The group of cells forming the periphery of the blastocyst is termed the trophoblast. Fetal membranes will develop from this outer cell layer.

Damage to the developing organism during the preimplantation period will be an "all or none" phenomenon. Because so few cells exist in the early stages, irreparable damage to some may be lethal to the entire organism. If the organism remains viable, however, organ-specific anomalies are not manifested, because either repair or replacement will occur to permit normal development. On the other hand, a similar insult at a later stage could produce organ-specific defects because replacement mechanisms are no longer operative.

Implantation to Third Week

Implantation of the blastocyst into the endometrium marks the initiation of the embryonic period, which lasts until 7 weeks after conception. One major early feature of this period is establishment of the maternal–fetal circulation. Implantation occurs during the luteal phase, when the endometrium is thick, vascularized, and filled with glycogen.

Around the sixth or seventh day of development, the blastocyst implants in the endometrium and collapses. Implantation per se is accomplished through the lytic activity of syncytiotrophoblasts, which dissolve endometrial stroma; thereafter, the overlying endometrial surface is repaired. The amnion and the yolk sac also form at this time. The trophoblast continues to develop, and lacunae form within the previously solid syncytiotrophoblasts, which up to this time were a mass of multinucleated protoplasm without distinct cell boundaries. The lacunae are the precursors of the intervillous spaces. Meanwhile, the cytotrophoblast (the cellular portion of the trophoblast) is forming cell masses that will become chorionic villi (10 to 14 days). All these changes are preparatory to the formation of the placenta. The uteroplacental circulation begins on day 14 or 15. The embryo is now approximately 1.5 mm in length.

From the third to the eighth week, the embryonic disc undergoes major developments that lay the foundation for all organ systems. This period is characterized by cell migration. During the third week the appearance of the primitive streak marks the onset of gastrulation. At the level of the primitive streak, ectodermal cells invaginate to form the third germ layer, the mesoderm. Cells from the mesoderm form the central core of the chorionic villi and begin to differentiate into connective tissue and blood vessels. The primitive streak is also the site at which the vertebral column will eventually develop.

Although other organs are developing concurrently, the period from day

18 to day 28 is traditionally called the *neurula* stage because the main activity during this period is development of the nervous system. Along the length of the primitive streak a neural groove forms and then begins to close into a tube by the third week. Doubling of the cephalic end of the embryo between days 15 and 20 can lead to certain facial anomalies. Various other facial congenital malformations, e.g., median cleft lip, originate during this period. The notochord also forms during days 17 through 20. This ectodermal structure functions as a primary skeleton around which the vertebral bodies later become organized; the notochord eventually undergoes regression. By week 6 the primitive neurons aggregate and permeate the embryo, thus initiating muscular movement. The muscular and skeletal systems thus develop simultaneously from somites.

Thus, by one month after conception the fertilized ovum has progressed from one cell to millions of cells. The maternal–fetal circulatory system is established, the rudiments of all major systems (nervous, muscular, digestive, skeletal and cardiovascular) have differentiated, and the blueprints are set for developmental refinements. The embyro has been transformed during gastrulation into a curved tube approximately 6 mm in length and isolated from the extraembryonic membranes. An incompletely formed heart is beating. The first month of development is obviously crucial, for disruption of any of these processes is apt to be either lethal or to have widespread effects involving many systems.

The Second Month

The second month is also a critical stage with respect to production of congenital anomalies because all systems are engaged in major developmental sequences. All major organ systems are developing simultaneously.

The embryo first begins to assume features evocative of human appearance. The face emerges, with the formation of discernable eyes, nose, and ears. The palate begins to close, and teeth, tongue and taste buds form. Disruptions during the latter part of this period lead to various forms of cleft lip and palate. Limbs emerge from protruding buds; digits, cartilage and muscles develop. The cerebral hemispheres begin to fill the brain area, and the optic stalk becomes apparent. Nerve connections are established between the retina and the brain. The pituitary gland, thyroid, and thymus differentiate.

The body divides by membranes into separate cavities. The heart continues its development, and the digestive tract rotates from its prior tubular structure (esophagus, stomach). The liver starts to produce blood cells and bile, although the latter is not yet utilized. Insulin begins to be produced by the pancreas. Two tubes emerge from the pharynx to become bronchi. The lungs will have lobes and bronchioles by the end of this period. By the end of 8 weeks the heart is almost completely developed. A diaphragm is beginning to separate the heart and lungs from the intestines. The kidneys approach their final form (metanephros) toward the end of this period. The urogenital and rectal passages separate, and an oval membrane appears to rupture in the eighth week, forming the anus. Germ cells migrate toward the genital ridges for future transformation into ovaries or testes, which will begin to descend by the end of the second

month. Differentiation of internal ducts begins, with persistence of either Müllerian or Wolffian ducts. Virilization of external genitalia occurs in males.

Major activities during the second month thus include differentiation and growth. The embryo increases from approximately 6 mm to 33 mm in length and increases 50 times in weight. During this period the embryo is extremely vulnerable to teratogenic factors such as radiation, drugs, and viruses. Disruptions to development during this period have extensive and severe consequences, because all major organ systems are developing simultaneously and rapidly.

The Third Month

Until the third month, the component systems of the embryo are developing simultaneously yet somewhat independently. During the third month various systems begin to interact. Nervous impulses connect the brain and muscles, permitting the initial activity of the fetus. Whole-body reflex action to stimili begins, and eventually this action will become specific for the stimulated area.

During the third month the urogenital system develops. The kidneys begin to function and form urine, excreting and conveying it first to the fetal bladder and later to the amniotic liquor. Parts of the urinary system degenerate, and others become integrated into the reproductive system (e.g., mesonephric remnants contribute to the Wolffian ducts, which play a role in female and male genital development). Toward the end of the third month, the external genitalia have differentiated sufficiently to allow identification of sex. The testes are also formed but not fully functional, and the prostate is forming. The ovaries are present, but the female secondary sex organs will not be completely established until the fourth month.

Structurally the fetus has become straighter, and the tubular neural canal along which the spinal cord develops becomes filled with nerve cells. The eyes are ready to be connected with the optic disc. Ears remain low on the sides of the head. Teeth are forming, and the two bony plates of the palate fuse in the midline. The thyroid gland, pancreas, and lungs are completely formed, and the gallbladder secretes bile. Bones are forming, but blood is still created in the liver.

By the end of the third month, all major organ systems have become established and integrated, with activity being stimulated by nervous impulses. Thereafter, the fetus becomes relatively resistent to teratogens, although it cannot yet survive independently because of its small size (approximately 75 mm long).

Second and Third Trimesters

The most characteristic features of later gestation is rapid growth. Because most organ systems already are developed, fetuses are relatively resistant to teratogens during this period. Damage can occur to certain organ systems still developing, notably the brain, eyes, and gonads. Generally, however, the fetus responds to drugs in similar fashion to the neonate.

TERATOGENESIS

Teratogenesis is the production of defects in the fetus, and a teratogenic agent is responsible for producing such a defect. Teratogens include irradiation, chemicals (drugs), and infectious agents. In addition to anatomic defects, genetic and cytogenetic disorders could be said to result from teratogens, since some environmental factor was responsible for the initial gene mutation or chromosomal error. It is more useful, however, to apply the term *teratogen* only to those agents that deleteriously affect an embryo that was previously differentiating normally. A teratogen may cause a malformation by chromosomal abnormalities, gene mutations, or mechanical means; however the method is usually unknown.

The teratogenicity of an agent depends upon the specificity of the drug or infectious agent, the dosage, the exposure time during embryonic development, the genotype of the mother and the embryo, and the coexistence of other agents.

Specificity of Agent

Some agents are obviously more teratogenic than others. An agent may be teratogenic in certain species only. Within a given species, however, a teratogen can usually affect many organ systems. The pattern of anomalies reflects those organ systems that were differentiating at the time the agent was administered. For example, if administered between days 35 and 37, thalidomide causes ear malformations; if administered between days 41 and 44, it causes amelia or phocomelia (Knapp et al., 1962).

Dosage

At any given time an embryo can respond to a teratogen in one of three ways, depending on the dose level: At a low dose there is no effect; at an intermediate dose a pattern of organ-specific malformations can result; at a high dose the embryo may be killed, causing the organ-specific teratogenic action to go unrecognized. In animals teratogens appear to exert their action within a relatively narrow dosage range, usually one fourth to one half of the average dose (LD 50) that would kill the mother (Wilson, 1973). The effect also depends upon the developmental stage during which the drug is administered. That is, an agent may be teratogenic at a given dose during one state of embryonic development, but teratogenic only at a higher or lower dose at a different stage. Similarly, at one dose level an agent might be lethal yet not teratogenic, whereas at another level it could be either lethal or teratogenic. The route of administration also must be considered, because some agents appear to be teratogenic only if administered in a particular fashion. This is probably related to absorption phenomena. Finally, small doses administered over several days may produce a different effect than an equal total amount administered at one time. Sequential administration of small doses may induce an enzyme system that can degrade the teratogen, thus possibly causing less damage than if the entire

dose were administered at one time. Conversely, a drug administered sequentially might destroy those cells that catabolize the drug, leading to more deleterious consequences than might otherwise be expected.

Stage of Embryonic Development

The time during embryogenesis when the fetus is exposed to a potential teratogen is crucial. Three stages of susceptibility may be identified (Fig. 12-1). Knowledge of human embryonic differentiation can suggest the approximate stages of susceptibility in humans for a given organ system. These times vary from organ system to organ system. In general, however, the embryo is relatively resistant to teratogenic insults during the first few weeks of life, perhaps 2 weeks in humans (Wilson, 1973). A large insult might kill the embryo, but a surviving embryo usually manifests no organ-specific anomalies. Presumably, early embryonic cells have not differentiated irrevocably, and if one cell is destroyed, a surviving cell normally can assume its function unless the insult produced chromosomal or genetic abnormalities that hinder the cell's metabolic processes.

Organogenesis, the process of organ differentiation, occurs in most human organ systems between weeks 3 and 8; however, differentiation in some systems, particularly the brain and gonads, occurs later. During organogenesis

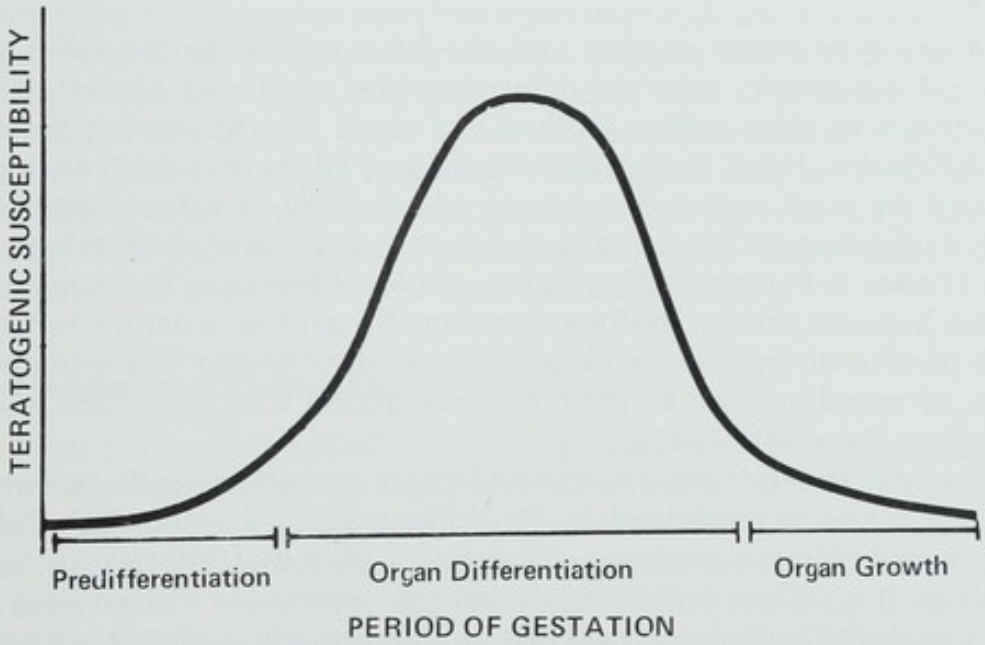


Figure 12-1. Schematic representation of embryonic periods of differential susceptibility to a teratogen. During the first weeks of embryogenesis a teratogen can be lethal, but if the embryo survives it will not necessarily be malformed. Following the period of maximum susceptibility a teratogen can interfere with growth but will not directly affect organogenesis. Thus, secondary effects (e.g., vascular occlusion) could still produce anomalies. (From Simpson JL: Disorders of Sexual Differentiation. Etiology and Clinical Delineation. New York, Academic Press, 1976, p 46. Reprinted with permission.)

susceptibility to teratogens is maximal. Furthermore, teratogens act in an organ-specific fashion; a teratogen may affect one organ system at one stage of development and another system at another stage. The precise time at which the insult occurs determines not only whether a malformation will occur, but also the specific spectrum of anomalies. For example, in the rat, 100 rads of radiation produce no anomalies on days 8 or 11 but cause numerous anomalies on day 9 (eye, brain, spinal cord, heart, aortic arch, and urinary systems) and day 10 (eye, brain, and urinary system) (Wilson, 1954).

Following organogenesis (for most human organ systems, from the fourth month on), embryonic development is characterized primarily by increasing organ size. A teratogen taken at this time can affect the overall growth of the embryo or the size of a specific organ but usually will not produce a visible malformation. (Brain and gonadal tissue, which continue to differentiate, are possible exceptions.) For example, after the twelfth week, administration of androgens to a pregnant woman may produce clitoral enlargement of her female fetus, but will not cause displacement of the urethral orifice or fusion of the labioscrotal folds. Moreover, a drug (e.g., chloramphenical) that adversely affects a neonate will also affect an older fetus in a similar fashion. In addition, anomalies can result from secondary effects. For example, overgrowth of the intima of an artery could lead to vascular occlusion, causing secondary atrophy of a distal organ.

Genotype

The genotype of the mother and the fetus influences the efficacy of a teratogen. For example, genotype determines the prevalence of cleft palate in inbred strains of mice whose mothers are given cortisol during pregnancy (Fraser & Fainstat, 1951). Daily administration of 10 mg of cortisol during days 11 through 14 produced cleft palate in all offspring of A/Jax parents, in 68 percent of offspring of C3H parents, and in only 12 percent of offspring of CBA parents (Fraser & Fainstat, 1951; Kalter, 1965). Differences in frequencies of anomalies between various strains are presumably genetic in nature. In humans, only 18 percent of females developed clitoral hypertrophy following administration of norethindrone to their mothers during a specific time and at a specific dose (Jacobson, 1962).

Differences in teratogenic susceptibility are probably usually polygenic in nature and might be explained by (1) differences in the maternal ability to absorb or metabolize a teratogen, (2) rates of placental transfer, or (3) fetal metabolism. It is informative to recall that the response to a given drug varies between adult individuals (Vessell, 1972). Interestingly, a plot of responses of various individuals might yield a normal distribution, which is consistent with the concept of continuous variation based upon polygenic inheritance. Moreover, adult monozygotic twins handle drugs more similarly than do dizygotic twins (Vessell, 1972). Polygenic or multifactorial inheritance could exist for any of several metabolic or morphologic determinants and could explain genetically determined teratogenic susceptibility. On the other hand, a few individuals may be unusually susceptible or unusually resistant to certain drugs because of a single mutant allele. Such individuals are said to have a phar-

macogenetic disorder, examples of which include pseudocholinesterase deficiency, warfarin resistance, heparin resistance, or inability to catabolize (decarboxylate) drugs like hydralazine or isoniazid. If a mutant allele renders a fetus incapable of inactivating a potential teratogen, administration of that teratogen might adversely affect that fetus, yet not affect a normal fetus. The clinical importance is that certain individuals (fetuses) may be unusually sensitive to certain teratogens.

Drug Interactions

Simultaneous administration of two teratogens may produce a different effect from that when the two are administered separately. In mice, folic acid reduces the frequency of cortisol-induced teratogenesis (Peer et al., 1958), possibly because of induction of enzyme systems that catabolize the teratogen or complete for binding sites. On the other hand, one agent (not necessarily a drug) may enhance the teratogenic potential of another. For example, the food preservative benzoic acid enhances aspirin teratogenicity in rats (Kimmel, Wilson, & Schumaker, 1971), possibly by enzyme inhibition, destruction of enzyme-producing cells, or saturation of binding sites on carrier proteins, which, if available, would decrease levels of the unbound, active teratogen.

Other Factors

Variability in teratogenic response sometimes appears to be associated with other environmental or morphologic factors such as maternal or fetal weight, in utero position of a fetus, proximity to other affected litter mates, uterine vasculature, or diet (Kalter, 1965; Kernis, 1971). However, investigation often reveals that these factors are related to other factors that are correlated with the response. For example, the inverse correlation between maternal weight and susceptibility of the fetus to cortisol-induced cleft palate is related not to weight per se but to dose per unit mass (Kalter, 1965).

Cellular Action of a Teratogen

A teratogen may potentially affect embryogenesis by causing gene mutation, chromosome breakage or nondisjunction, depletion or inhibition of precursors or substrates, depletion of energy sources, inhibition of enzymes, or changes in intracellular osmolarity secondary to changes in membrane integrity (see Wilson, 1973). An embryo may morphologically manifest the action of a teratogen by cell death due to reduced cell division, failure of expected interactions between cells, disruption of cell migration, or mechanical disruption of a cell.

Regardless of the specific mechanism, the result is usually an organ with too few cells. The critical mass necessary for induction or continuation of differentiation is lacking; thus, the particular organ system fails to develop (Saxén, 1970). Some anomalies (e.g., polydactyly and labioscrotal fusion) could result either from increased cell proliferation or from failure of localized cell degeneration.

Proof of Teratogenicity

Teratogenicity is difficult to prove in humans, for several reasons: First, a large sample size is usually necessary for appropriate analysis. Second, often only retrospective data are available. Third, similar congenital anomalies usually occur also in fetuses that were apparently not exposed to a teratogen. Fourth, humans are genetically heterogeneous.

Observations such as those listed below can implicate a particular agent.

1. The agent was associated more often with cases having a particular anomaly than with suitable controls.
2. An anomaly or pattern of anomalies is consistently associated with the suspected teratogen.
3. The agent was present during the stage of organogenesis when the anomaly would have been likely to occur.
4. The anomaly was less common prior to the time the potential teratogen was available (e.g., phocomelia was almost unreported prior to the time thalidomide was introduced).
5. The anomaly can be produced in experimental animals by administration of the agent during a stage of organogenesis comparable to that believed to be involved in causing the anomaly in humans. (However, negative animal data do not prove that a drug is innocuous to humans.)

Surveillance methods and experimental designs to detect teratogenic agents have been discussed elsewhere (Wilson, 1973). Suffice to say that no single method or design is universally reliable. In particular, data from a single species are not conclusive. The rat is most commonly used for teratogenic testing because spontaneous malformations occur less frequently in the rat than in the mouse or rabbit. Unfortunately, rats are more resistent than humans to some important teratogens (e.g., thalidomide); therefore, primates should ideally be tested. If rats and mice are used, several different inbred strains are preferable.

13

Radiation, Ultrasound, and Other Physical Agents

Potentially deleterious physical agents include electromagnetic radiation, ultrasound, hyperthermia, and microwaves. These agents are potentially teratogenic, mutagenic, and carcinogenic. Exposure can occur as a consequence of (1) therapeutic or diagnostic medical and dental procedures, (2) occupation, (3) general environment ("background" level), (4) accidents (often occupational) and (5) war. Subsequent biological damage may occur to the exposed person, to the conceptus if the exposed woman were pregnant during exposure, and to descendents of both. Some effects, e.g., radiation sickness or spontaneous abortion, may be immediate. Other effects, including teratogenic effects exhibited at birth, and temporary or permanent sterility, may not be immediately apparent. Long-term effects, such as predisposition to malignancy, may be initiated by genetic or cytogenetic damage inflicted upon germ cells and somatic (nongerminal) cells.

GENERAL PRINCIPLES IN COUNSELING

All physical agents to be discussed in this chapter are capable of inflicting biological damage under certain circumstances at certain dose levels and exposure rates. To determine whether an agent is actually deleterious in a particular instance, the circumstances under which exposure occurred must be considered. A single, simple answer to patient inquiries is thus inappropriate because biological effects not only vary with dose and exposure rate, but also according to the agent and characteristics of the exposed organism. For example, an adult, a child, and a fetus each respond differently to a comparable amount of an agent. Furthermore, fetal effects vary dramatically according to the stage of gestation during which exposure occurs. Based on experimental data derived from Drosophila (fruit flies), mice, and other nonhuman organisms, response is expected to vary according to age and probably also according to sex, genetic background, and physiologic state. To determine the outcome of exposure to

a physical agent, one should thus ideally know the nature and route of the exposure, dose, dose rate, tissue exposed, and stage of gestation. Unfortunately, in humans the effects of the above variables are poorly understood. Consequently one must generally extrapolate on the basis of general principles obtained from animal experiments, which leads to somewhat tenuous assumptions. Thus, limiting exposure of humans to potentially harmful agents, even in the absence of proof of their deleterious effects, seems wise. If current knowledge does not suggest definite cause for alarm, unconditional reassurance is still unwarranted. For all these reasons genetic counseling is difficult and imprecise. Nonetheless, some useful guidelines can be derived.

TYPES OF RADIATION

Radiation occurs in several forms: electomagnetic (x-rays, ultraviolet radiation), which is characterized by frequencies and wavelengths, and particulate (α-particles, neutrons) (Table 13-1) (Hutchinsen & Pollard, 1961; Lea,

Table 13-1
Types of Radiation

Type	Wavelength	Biological Action
Nonionizing		
Electromagnetic		
Shortwave	10^9 nm*	Produces heat; can penetrate internal organs, fetal tissues.
Microwave	10^5–10^9 nm	Produces heat; low tissue-penetrating power at commonly encountered wavelengths.
Ultraviolet	300–380 nm ("near" UV, in sunlight) 5–300 nm ("far" UV)	Low tissue-penetrating power; effect on specific molecules at specific wavelengths; strongly absorbed by tissue at < 300 nm, thus mutagenic and carcinogenic.
Ionizing		
Electromagnetic		
γ-rays	1 nm	High tissue-penetrating power; effect a function of dose and dose rate; mutagenic, carcinogenic, teratogenic.
X-rays	1 nm	
Particulate		
α particles	Positive charge (helium nucleus)	Extremely low tissue-penetrating power.
β particles	Negative charge (electrons)	Low tissue-penetrating power; commonly emitted from radioisotopes used clinically.
Neutrons	Uncharged	High tissue-penetrating power.

*1 nm = 1μ = 10 Å

1962; Adey, 1981). Electromagnetic radiation can be nonionizing or ionizing. Ionizing radiation can eject electrons from their orbits, effect production of ion pairs, and cause chemical changes that may ultimately lead to biological effects. Nonionizing radiation produces biological effects by other mechanisms.

Ultraviolet Light

The mechanism of ultraviolet waves is called *excitation*, which means that there is sufficient energy to move electrons to other orbits but not to create ion pairs. The biological effects of ultraviolet light are determined by wavelength, because absorption varies according to the molecular structure of the exposed material. For example, chromosomes absorb ultraviolet light according to both distribution of nucleic acids within the chromosomes and the stage in the cell cycle at the time of exposure. In general, wavelengths less than 3000 Å (300 nanometers) are readily absorbed by biological material. (These wavelengths are not characteristic of sunlight, which ranges from 3000 to 3800 Å.) Examination of graphs showing genetic responses to ultraviolet light shows that response reflects the absorption curves of nucleic acids, which peak at 2563 Å (Lea, 1962). One mechanism by which ultraviolet damage occurs is formation of thymine dimers, which can produce crosslinks between DNA strands. DNA repair mechanisms may mitigate this damage (see Jagger, 1973). In experimental organisms, "point" mutations, i.e., single-gene effects, are much more likely to result from ultraviolet exposure than are structural chromosomal aberrations.

Ultraviolet light cannot penetrate tissue well, usually being absorbed within 10 μ. Consequently, it reaches neither the fetus nor the mother's gonads; thus, direct teratogenic effects or production of gene mutations in germ cells is extremely unlikely to occur in humans. However, ultraviolet exposure can be related to human malignancies, particularly skin cancer (Jablon, 1975). This risk is increased in individuals whose genotypes confer increased susceptibility to ultraviolet radiation because of deficiencies in their DNA repair systems, e.g., persons with xeroderma pigmentosa.

Nonionizing Electromagnetic Radiation and Hyperthermia

Wavelengths longer than those associated with ultraviolet light (microwave, or shortwave) are classified based on ranges of wavelengths in cycles per second (Hertz [Hz]) (Dalrymple, 1973; Adey, 1981). These low-energy waves are incapable of producing ionization (Michaelson, 1969). A major mechanism of biological action of nonionizing radiation is hyperthermia.

As for all forms of radiation exposure, the prediction of biological effects must take into account many variables. For microwave and shortwave radiation, it is important to consider frequency and intensity as well as the size of the exposed organism. In general, the higher the frequency, the shorter the wavelength and the lower the penetrating power. Low-frequency waves can penetrate to internal organs and fetuses. For example, 27.5-MHz shortwave radiation can heat mammalian tissues to a depth of 10 to 12 cm and has damaged fetuses in experiments with nonhuman organisms (Brent, 1977). The clinical implication

of this phenomenon is that an exposed individual is not alerted to prevent damage to internal organs or to a developing fetus.

For frequencies below 1000 MHz, tissues deep within the body (including fetuses) absorb the radiation, and skin thermal receptors are generally insensitive to these frequencies. Microwave frequencies above these levels have little penetrating ability and are thus the least damaging biologically. For example, microwayes at 2450 MHz, the frequency of most microwave ovens and diathermy machaines, cannot produce a noticeable thermal effect beyond 3 to 4 cm; therefore, hazards to the developing organism from these sources need not be considered significant.

Intensity of exposure and the source, size, and location of the tissue at risk must also be considered when predicting biologic effects (Sekins & Emery, 1982). Assuming that tissue has been penetrated, response varies directly as a function of water content. Skin and muscles are more sensitive than is bone; bladder, brain, and testes are even more sensitive. The ability of the tissue to dissipate heat is another important modifying factor. Molecules respond to microwave radiations by vibrating, producing heat that may cause local damage if it is not dissipated. In fact, damage incurred by shortwaves and microwaves is probably the result of hyperthermia. It is not conclusively know if nonthermal effects of microwave radiation can produce biological damage.

Investigations of nonmicrowave hyperthermia in experimental organisms have shown (1) embryotoxicity, particularly during the preimplantation stage (Chang, 1957; Fernandez-Cavo, 1958); (2) physical effects similar to those of heat prostration or high fever; and (3) the induction of cataracts by frequencies between 1,000 and 3,000 MHz (Michaelson, 1969). If low-frequency wave lengths are applied for a sufficient length of time to a developing organism, the excess heat cannot be easily dissipated because the developing organism cannot yet use the major heat-exchange organs, skin and lungs. This "thermal isolation" is characteristic of both the embryo and the lens of the eye, which are enclosed in fluid-filled compartments. Therefore, the sensitivity of these two sites to microwave damage is not surprising. In addition, the pregnant woman is relatively insensitive to thermal changes within the uterus and probably would not notice a change in temperature.

There is no definitive proof that either hyperthermia per se (Edwards & Wanner, 1977) or microwave or lower-frequency radiation is deleterious to humans at the commonly encountered exposure ranges (Brent, 1977; Adey, 1981; Michaelson, 1982). However, these agents do show teratogenic characteristics in experimental organisms. Discrepancies between animal data (which suggest effects under certain conditions) and human data (which show less definite effects) could be due either to the paucity of human data or, more likely, to the exposure of humans to higher wavelengths will lower penetrating power. Human exposure to microwaves primarily occurs as result of leakage from microwave ovens, which have wavelengths that can penetrate only 3 to 4 cm. In the past, pelvic hyperthermia was induced to treat certain gynecologic conditions, but the wavelengths that were used (~ 2450 MHz) are thought to have insufficient penetrating power to damage internal organs or fetuses.

Thus, nonionizing electromagnetic radiation is probably not a tetratogenic agent worthy of concern in the wavelengths commonly encountered by humans.

However, in the event of sustained (more than 10 minutes) low-frequency exposure, particularly at occupational levels, there may be some cause for concern. Probability of penetration, length of exposure, and the nature of the exposed tissue must all be considered, although dose estimates are difficult to calculate. Studies of the induction of genetic mutations or chromosomal aberrations by microwaves and shortwaves are as yet inconclusive. Recent interest in the bioeffects of nonionizing radiation has generated research that should provide more explicit risk estimates.

IONIZING RADIATION

Ionizing radiation, which includes alpha, beta, gamma, and x-rays; protons and neutrons (see Hutchinson & Pollard, 1961; Lea, 1962), is defined by its ability to create ion pairs. Simple chemical reactions may be caused by either excitation or ionization. However, excitation is much less effective than ionization in producing reactions of biological importance, e.g., dissolution of large molecular structures or destruction of cells. In addition to primary damage, sequential reactions occur after the initial impact, particularly with ionizing radiation. Sequential damage includes secondary ionizations, breaking of molecular bonds, production of free radicals, molecular vibration, and ultimately heat release.

As previously stated, all forms of ionizing radiation induce biological damage through the creation of ion pairs. Different types of ionizing radiation have different mechanisms to produce ionizations. Clinical implications of the different forms of ionizing radiation are primarily related to varying degrees of tissue penetration. For example, charged particles (e.g., α particles, which are composed of two protons and two neutrons) have a very short penetration range in tissue. Thus, a given dose of α particles will produce denser ionization per area than will the same dose of either uncharged particles or a more penetrating type of radiation, such as x-rays. Charged particles are generally of less biological impact because of their localized effect; however, this is not always true. Beta particles, which are commonly produced by radioactive isotopes, e.g., ^{131}I, can have deleterious effects on the target organ, although their effects will not extend beyond that organ. Gamma rays, usually produced by radium, have a short wavelength and greater penetrating power. At the cellular level, ionizing radiation can potentially cause disruption of cellular movements and interactions, death, mitotic inhibition, chromosomal aberrations, and genetic damage.

Effects of Dose

Dose and dose rate are critical parameters in determining the biological damage of ionizing radiation. Exposure dose is expressed in roentgens (R), a unit defined by a standard number of ionizations produced in air (1 R = 83 ergs/gm air). The term rad refers to absorbed radiation (100 ergs/gm tissue) and

rem refers to roentgen equivalents in humans. For practical purposes, the roentgen, rad, and rem may be considered to be equivalent for human exposures.

All people incur some degree of radiation exposure. Total average radiation exposure in the United States has been estimated at 200 mrad/yr (Brent, 1977, 1979; BEIR, 1980). Approximately 100 to 125 mrad whole body dose comes from backround sources, and medical x-rays are estimated to contribute another 55 to 100 mrad. It has been estimated that by 1990 exposure from medical sources will be two to four times the current background levels (Gaulden, 1973). Dose estimates for different geographical locations, occupations, and medical exposures are tabulated in BEIR (1980). The human fetus receives 60 mrad from background sources.

The amount of radiation delivered to the fetus when the mother is exposed depends on gestational age (size of the fetus) and the portion of the mother's body that was exposed. For example, cholecystography (upper abdominal exposure) has been estimated to deliver 0.6 mrads to the fetus, compared to 350 mrads from a barium enema with direct pelvic exposure. Scatter radiation ensuing from cancer therapy has been estimated by using "phantoms" designed to assess dose to the fetus under conditions that simulate actual therapy (Gaulden & Murry, 1980). For example, 9 rads are delivered to the fetus in the case of therapy for a brain tumor, or a unilateral breast tumor, and 20 rads for lung cancer.

In addition to total dose, rate of exposure can drastically affect biological response. Dose fractionation (dividing administration over time) is generally less harmful than an acute dose of the same magnitude; however, in certain instances, e.g., exposure of male gonads, fractionation may cause greater harm by sequentially affecting more than one sensitive period. Another major factor is the specific tissue, because absorption varies in different tissues. Rapidly dividing tissue is highly radiosensitive. Furthermore, in the case of internal emitters (radioisotopes) designed for specific target organs, concentration and consequently dose may vary dramatically among tissues. For example, oral administration of 5 mCi of ^{131}I may deliver 10,000 rads to the thyroid but only 1 to 2 rads to the gonad (Gaulden, 1973); a dose of 4 mCi of ^{32}P delivers approximately 100 rads to the bone marrow, liver, and spleen, but only 10 rads to the remainder of the body (Witcofski, 1973).

To counsel accurately, it is thus obvious that certain information should ideally be available: in particular, the nature of the radiation, route of entry, dose, and dose rate. A medical physicist can estimate the dose received to the tissues of interest. In the absence of these data, estimates may have to be derived from published exposure tables (Brent, 1977; BEIR, 1980; Gaulden & Murry, 1980).

IMMEDIATE MEDICAL EFFECTS OF RADIATION

Radiation sickness (nausea, vomiting, damage to the bladder or gut) ensues at exposure doses greater than 100 R. At higher doses, destruction of the immune system may lead to overwhelming infection; brain and nervous tissue

is destroyed. At 450 R, 50 percent of exposed persons die. If an exposed individual survives immediate insults, the most serious future effects involve predisposition to malignancy.

TERATOGENIC EFFECTS OF RADIATION ON FETUSES

The developing embryo is particularly sensitive to the effects of ionizing radiation. Recall that the basis for using x-rays to kill cancer cells is that such cells divide more rapidly than normal cells, and consequently are more sensitive to disruption of cell division. Disruption of embryonic differentiation may have widespread effects.

Internal Emitters

Internal emitters are generally isotopes that have specific tissue distributions and may remain in the body, where they continue to emit radioactivity. Radioactive isotopes can not only damage the target tissues, but also damage a developing fetus (Rugh, 1965). Radioactive strontium, plutonium, phosphorus, iodine and tritium are among the isotopes capable of crossing the placental barrier. Radioactive isotopes have variable target distributions and effects on the embryo, reflecting the stage of gestation, ability to cross the placental barrier, affinity for a given tissue, and type of energy emitted. Until recently, a commonly used isotope was radioactive iodine (^{131}I or ^{125}I.) Although a dose as high as 175 mCi prior to conception has not been shown to be associated either with malformations in subsequent offspring or cytogenetic aberrations (Einhorn et al., 1972), administration of a similar dose during pregnancy can destroy the fetal thyroid (Sternberg, 1970). In fact, a 5mCi maternal exposure delivers 6500 rad to the fetal thyroid, thereby destroying it. The dose to the fetus and fetal thyroid relative to the amount of radioisotope administered to the mother is shown in Table 13-2. The fetal thyroid incorporates iodine by the tenth embryonic week and has a greater avidity for circulating iodine than does the maternal thyroid (Sternberg, 1970). Therefore, if ^{131}I must be administered to the pregnant woman for diagnostic purposes it should be done before the fifth to sixth embryonic week, i.e., before the thyroid has differentiated. Small doses of iodine have not been shown to be deleterious to the fetus; however, ^{131}I in any dose is best avoided during pregnancy because of the potential of subsequent thyroid malignancies (BEIR, 1980). If therapeutic doses of ^{131}I of 10 rads or greater have been administered, however, the therapeutic abortion should be considered. A substitute isotope available for diagnostic use is ^{99m}Tc, which has a short half-life and emits gamma rays, thus exposing the patient and the fetus to a low dose. Furthermore, excretion of ^{99m}Tc is so rapid (24 to 48 hours) that its administration does not interfere with breast-feeding.

Other radioisotopes have also been shown to be harmful to experimental animals. High doses of radioactive phosphorus or stronium can lead to fetal death (Sikov and Noonan, 1958; Frolen, 1970). Phosphorus, radium, and stronium will be localized to fetal bones after the fourth month when ossification

Table 13-2
Average Absorbed Dose to Mother and Fetal Thyroid for Various Diagnostic Tests Utilizing Radioactive Isotopes

		Whole Body Dose			Fetal Thyroid Dose
		Mother		Fetus	
Radionuclide	Procedure	mR/μCi Administered	mR per Typical Procedure	mRem to Fetus	mRem per Typical Procedure
[^{131}I]NaI*	Thyroid scan	0.45	27.0	15.0	5000
[^{131}I]RISA*	Plasma volume	1.70	17.0	10.0	5000
[^{131}I]Olcic acid*	Lipid absorption	0.65	32.5	17.0	5000
[^{131}I]Rose bengal*	Liver function scan	0.36	36.0	19.0	5000
[^{131}I]Hippuran	Kidney function scan	0.04	0.6	0.3	100
[^{99m}Tc]Pertechnetate	Brain scan	0.01	100.0	—	—
[^{99m}Tc]Sulfur colloid	Liver scan	0.015	30.0	—	—
[^{131}I]RISA*	Placentography	—	15.0	7.0	4900
Technetium-99m*	Placentography	—	9.0	2.0	10
^{51}Cr (RBC)*	Placentography	—	4–12	3–4	3–4

Modified from Tabuchi A (1964): Fetal disorders due to ionizing radiation. Hiroshima J Med Sci 13:125.
*Compounds currently seldom used, having been replaced by ^{123}I or $^{99m}TcO_4$ (thyroid tests), ^{125}I (plasma volume studies), and ^{99m}Tc glucoheptinate (brain scans).

has begun. Tritium, a nuclear pollutant commonly found as tritiated water, can cause intra-uterine aplasia of the ovaries.

In summary, for any particular exposure to radionuclides, the risk estimates will be a function of type of agent, site and route of administration, dose, radiation characteristics, and gestational stage of the fetus. If it is determined that the fetal dose is 10 rads or greater, serious risk should be assumed.

External Sources

In terms of dose levels, external ionizing radiation may be classified as therapeutic or diagnostic. Human embryos exposed to therapeutic doses show radiation effects similar to those found in studies of experimental organisms, namely, death, growth retardation, or malformations (Goldstein & Murphy, 1929; Murphy, 1929; Dekaban, 1968; Kučerova, 1970). Measurable damage was produced by maternal exposure (in the 10 to 19 rad range) to the atomic bomb in Hiroshima (BEIR, 1980). For low doses (less than 10 rads), the effects are less clearly established (Oppenheim, Griem, & Meier, 1975; Neumeister, 1976; Granroth, 1979; Gaulden & Murry, 1980). In experimental organisms, notably mice, gross malformations can be induced by administering doses of 25 R or greater, particularly during the period of major organogenesis (Russell, 1957), which in humans probably corresponds to weeks 3 through 6. If the fetus is not exposed directly, e.g., if therapeutic radiation could be delivered to, for example, the breast with appropriate shielding, no teratogenic effect per se should ensue, although complete shielding is generally not possible. Either maternal total body-radiation or uterine (fetal) radiation presents hazards to the fetus, the extent of which depends on the type of exposure and dose to the fetus. As already noted, these parameters are often difficult to estimate, particularly in retrospect. Nonetheless, some general guidelines may be presented for counseling. Recall that much of the basis of these guidelines comes from work on experimental organisms (United Nations, 1977; BEIR, 1980).

If the exposure of high doses of ionizing radiation occurs during the preimplantation and early implantation stages of mice or rats, the major effect is embryonic death, possibly due to the induction of lethal cytogenetic aberrations disrupting cell division. However, surviving embryos are generally normal, functions of deceased cells presumably having been taken over by the other cells not irrevocably committed to differentiation of a given organ system. Thus, there is an "all or none" response characteristic of even low doses ($\leq$ 10 rads). In humans the "all or none" response lasts for 10 to 14 days following ovulation.

More than 100 R of radiation received by the fetus at any stage after this period results initially in growth retardation, which in a given organ may lead to malformation. Exposure early in organogenesis produces more pronounced retardation at birth; however, the later in gestation the exposure, the less likely the fetus is to compensate postnatally for intrauterine growth retardation. The central nervous system is particularly sensitive throughout all postimplantation stages, with microcephaly and mental retardation being principal consequences of exposure to more than 25 R (Brent, 1977; BEIR, 1980). The proportion of offspring exhibiting microcephaly and mental retardation depends upon the

dose received. The most notable example in humans of the correlation between maternal ionizing radiation and microcephaly and mental retardation in offspring was observed in the atom bomb survivors in Hiroshima. Twenty-five percent of the offspring of mothers receiving at least 25 R of neutron (0.35 rad fetal exposure) plus gamma radiation (5.3 rad fetal exposure) were affected. Even 10–19 R exposure produced a significant frequency of microcephaly (17 percent), although profound retardation was not associated with maternal exposures of less than 50 R. Exposures only to gamma radiation showed no effects below 150 R (Yamazaki, Wright, & Wright, 1954; RW Miller, 1956, 1969, 1970; Wood, Johnson, & Omori, 1967; Wood et al., 1967a, 1967b). Growth retardation was also observed among survivors of in utero exposure in Japan. Although data are scarce, the possibility of cataracts should also be considered if high doses ($\geq$ 100 rads) were delivered during the early part of the fourth week of gestation (Brent, 1977).

It is puzzling that gross malformations other than those of the central nervous system have not been observed consistently in humans. A practical consequence of this is that radiation-induced teratogenesis can reasonably be excluded if the central nervous system is normal, regardless of what other systems are abnormal. However, the consistent central nervous system involvement in humans contrasts with results on experimental organisms, which relate the induction of specific malformations to radiation exposure during specific stages of gestation (Russell, 1957). The latter responses are highly reproducible. For example, exposure of mouse embryos at day 7, 8, or 9 to 200 R produces cleft palate (see Rugh, 1961).

One possible explanation for the discrepancy between human and animal data is the relatively short sensitive periods during development of specific organ systems in humans compared to the continuous sensitivity of the human central nervous system during pregnancy.

Of clinical significance is that no conclusive evidence of induced malformations of any degree has been obtained in fetuses exposed to 5 R or less (Oppenheim, Griem, & Meier, 1974); therefore, less than 5 rads is considered a "safe" limit. The American Academy of Radiologists (1975) suggests 10 R as the level above which the possiblility of damage to the fetus should be considered. It is impossible, however, to determine a threshold of radiation exposure below which absolute assurance of safety can be given. The scanty evidence available for human populations suggests that fetal doses of 25 R or more are associated with appreciable risk; deleterious effects of exposure to 5 R have not been demonstrated, although effects too subtle for ready detection, such as slight decrease in IQ, or shortened life span, are possible. Another possibility is that deleterious effects indeed occur following exposure to less than 5 R but not frequently enough to be statistically verifiable. The risk associated with doses between 5 and 25 R are even more difficult to assess. The proposed threshold of 10 rad fetal dose seems reasonable, albeit arbitrary; however, this level may change as more data are compiled (Brent, 1979).

Several other major factors must also be considered to provide adequate counseling on the effects of radiation exposure. For example, the later in gestation the exposure, the more resistant the fetus, and the higher the dose required to cause damage (Brent, 1977). In addition, if doses are fractionated over

time, damage is greatly reduced; however, a possible exception is exposed germ cells, for which continuous or fractionated exposure may be more likely than a single dose to produce damage. Finally, genetic susceptibility may also vary between individuals; thus, identical exposure at the identical stage of gestation could be innocuous to some fetuses yet deleterious to others.

INDIRECT AND LONG-TERM EFFECTS OF RADIATION

Gene Mutations

Changes in the genetic material of the adult may also occur as result of radiation. Since Müller's discovery in 1927 that x-rays produce genetic mutations, ionizing radiation has proven mutagenic in every plant and animal species that has been adequately investigated (Wolff, 1961). Single-gene mutations for coat colors were induced in mice (Russell & Major, 1957; Russell, 1978). Selby (1979) demonstrated the induction of dominant skeletal mutations in mice with a fractionated dose of gamma radiation delivered to spermatogonia (100 R + 500 R). Interestingly, recessive lethals and reciprocal translocations also occurred. The general conclusions of many studies include that (1) the dose response is linear, (2) there may not be a threshold dose below which no deleterious effect occurs, (3) fractionation of doses generally reduces biological damage as compared to damage caused by the same total dose delivered in acute fashion, and (4) the induced mutations are similar to those occurring spontaneously. It is reasonable to expect the same genetic consequences in humans, but such information is even more difficult to obtain than information on somatic, teratogenic, and carcinogenic effects of ionizing radiation, because genetic damage must be assayed in generations subsequent to exposure. Moreover, possible mutagenic effects of other environmental factors could obscure the analysis.

Mutations could be induced in the gonads of either adult or embryonic organisms. Furthermore, somatic mutations are one possible mechanism of damage during embryonic development and may also be involved in oncogenesis. Within dose ranges compatible with survival and subsequent fertility, the linear response between single gene changes and dose levels suggests that damage is directly proportional to dose, i.e., one "hit" can produce one unit of damage. This implies that even low doses may produce genetic damage, albeit at a low frequency. No safe threshold may be assumed. In practical terms, however, very low doses may not appreciably increase the spontaneous mutation level. Moreover, most mutations are recessive; thus, they will not usually be expressed in the first generation after exposure. Indeed, they may not be expressed at all, because the usual fate of unique mutations is extinction. To persist, a mutation must (1) be compatible with cell viability, (2) be included in a fertilizing gamete (a probability which approaches zero, particularly in male gametes), and (3) if recessive, be united with a similar allele to allow expression of the phenotype. The fate of a mutation already existing in the population is only slightly different. An increase in homozygosity will occur in the population, but the change in gene frequency will be very slow. Even-

tually a new equilibrium will be reached by selective mechanisms. Furthermore, dose fractionation leads to less damage than an acute dose of the same magnitude, suggesting the existence of DNA repair mechanisms.

Chromosomal Damage

It has been well established by studies on experimental organisms that ionizing radiation causes cytogenetic aberrations (Lea, 1962; Bloom, 1972). In humans, in vivo cytogenetic damage has been demonstrated after exposure to doses as low as 3 R. This produces mosaicism, at least in rat embryos (Soukup, Takacs, & Warkany, 1965) and humans (Lejeune et al., 1964). The extent of phenotypic damage related to mosaicism depends on tissue distributions and the ratio of abnormal to normal cells. Similar types of mosaicism have been found in ostensibly normal individuals, so even induced mosaicism is not invariably harmful.

The frequency of structural aberrations induced by various forms of radiation in adults is predictable enough to be used for radiation dosimetry. In particular, the frequency of dicentric (Fig. 13-1) and ring chromosomes in peripheral lymphocytes is a reliable indicator of exposure dose (Lloyd & Dolphin, 1977). Furthermore, chromosomal aberrations have been demonstrated in a few fetuses exposed only to diagnostic radiation (see Gaulden & Murry, 1980), although most such studies show no effect (Sato, 1966).

Simple chromosomal aberrations, such as deletions, show a linear dose response, similar to that observed for the induction of gene mutations. Thus, again there is probably no safe level. Indeed, chromosomal deletions have been demonstrated for doses as low as 0.25 rads (Gaulden & Read, 1978). However, other aberrations (e.g., translocations, dicentrics) usually require at least two breaks. The frequencies of these types of aberrations thus generally show an exponential dose–response curve, suggesting that two or more "hits" are re-

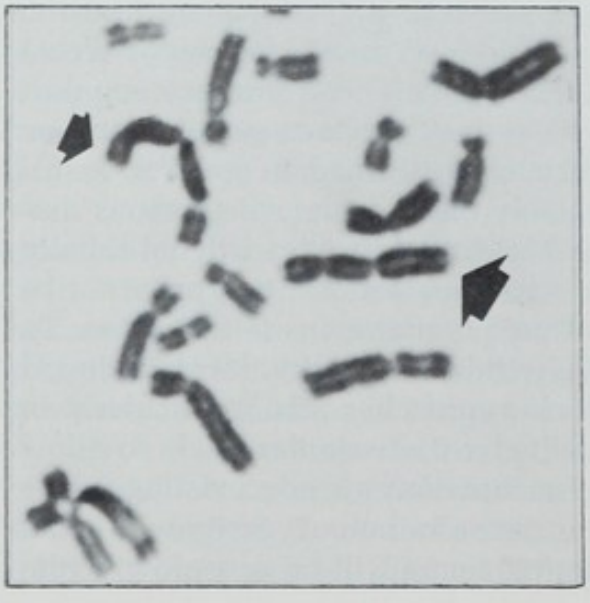


Figure 13-1. Portion of a metaphase showing two dicentric chromosomes arrows (lymphocyte, unbanded Giemsa staining). Quadriradial formation is shown in the lower left corner.

quired for their production. In general, dose fractionation shows less damage than acute doses suggesting the existence of repair mechanisms.

Although frequencies of both numerical (aneuploidy) and structural aberration frequencies are increased with ionizing radiation exposure, precise data suitable for genetic counseling are not available. Many physical and biological factors are known to affect response, including dose, dose rate, type of radiation, stage of cell cycle, temperature, and metabolic parameters such as the presence of oxygen. Furthermore, the biological significance of structural aberrations depends upon whether genetic material has been gained or lost and whether these genetic alterations are compatible with cell survival. Mouse studies indicate that structural aberrations in germ cells can be produced by even 5 R; the frequency of aberrations at less than 1 R is low. Furthermore, for effects to be evident, aberrations must be transmitted to a zygote that survives long enough to be detected clinically. Thus, much damage passes undetected (Ford et al., 1969). Few direct data are available from human studies, but the frequency of reciprocal translocation in human testicular material was found to be 3 to 5 percent after 80 R to spermatogonia (Brewen et al., 1973; Brewen & Preston, 1975). Extrapolation to female gametes is tenuous; however, because female meiotic and postmeiotic stages are relatively short compared to overall gametic history, exposure of oocytes or oogonia may incur a lower biological risk than for spermatogonia or spermatocytes.

Several clinical studies have suggested increased risks of aneuploidy, specifically increased frequency of trisomic offspring born to mothers previously exposed to diagnostic x-rays (Uchida, Holunga, & Lawler, 1968; Alberman et al., 1972). It has been suggested that the doubling dose for this effect (dose required to double the background rate) is as low as 2 R. This increase is not necessarily independent of maternal age effects. Moreover, unforeseen biases could exist in these retrospective studies, despite ostensibly proper controls. In addition, increased aneuploidy was not observed in offspring born to atomic bomb survivors (Neel, 1963). Different results may reflect different background responses. For example, a strain of mice with a high spontaneous frequency of aneuploidy would be expected to respond differently than a strain with a different background frequency. In the absence of conclusive evidence, one should be very cautious about recommending antenatal cytogenetic studies to women exposed to diagnostic radiation. Nonetheless, in certain situations (e.g., prior therapeutic roentgenography for Hodgkin disease), antenatal cytogenetic testing may be appropriate (Simpson, 1980c), albeit accompanied by a lucid discussion of diagnostic limitations.

Effects of Irradiation on Fertility

Exposure to radiation can cause sterilization. However, the dose required varies with species, sex, and life stage. Exposure to 100 R can damage rat testicular tissue directly, causing reduction in testis size, and producing sterility in 3 percent (Brent, 1960); neonatal rats are completely sterilized by 300 R. In pigs, goats, and mice the sterilizing capacity of ionizing radiation is greatest in the fetus. It should be noted that higher doses are required to sterilize a fetus than to cause some of the previously mentioned effects, e.g., microcephaly.

Similarly, rat ovaries irradiated in utero are depleted of oocytes. Female mice are very sensitive, and less than 100 R will cause sterilization. On the other hand, 2000 R are required to sterilize rhesus monkeys in utero.

Little is known about the fertility of humans exposed to ionizing radiation in utero, although no ostensible reduction in fertility was observed among Japanese adults and fetuses in Hiroshima and Nagasaki (Blot & Sawada, 1972). Furthermore, fluoroscopic doses of 1 to 5 R during childhood are not associated with decreased fertility (Mondorf & Faber, 1968). Thus, in practical terms, sterilization appears to be a clinical issue only in cases of high doses of direct gonadal irradiation by accident or for therapeutic reasons. Doses required to cause permanent sterility in adult humans differ, depending on whether acute or fractionated doses are delivered. Contrary to the general principle that fractionated doses are less harmful than acute doses, 2000 rads are required to achieve complete sterility in all males if delivered in a single dose, yet 200 rads achieve the same effect if fractionated (American College of Radiology, 1975). Lower doses may cause temporary sterility, presumably because germ cells and their precursors are only sensitive at certain stages. Heterogeneity of sensitivity of germinal cells is well established in experimental organisms (Clemendson & Nelson, 1961; Rugh, 1961; BEIR, 1980). In humans, type B spermatogonia are most sensitive, being depleted by only a few rads. Spermatids are the most resistant. An acute dose may not damage cells in resistant stages; however, continuous or fractionated doses will eventually deplete all stem cells. The dose required to permanently sterilize all human females is a function of age, being approximately 2000 rads in young women and 200 rads in older, premenopausal women (American College of Radiology, 1975). The minimum tolerance threshold for female gonads is 200 to 300 rads, compared to 100 rads for testes.

Following a sterilizing dose of radiation, the following events occur in males: (1) cessation of production of new sperm, followed by rapid decrease in testicular size and weight; (2) persistance of sperm that were mature at the time of exposure and that may have cytogenetic or genetic abnormalities; and (3) complete azoospermia after 3 to 6 weeks. For females, production of new graafian follicles is eliminated, followed by disappearance of already maturing follicles, and cessation of menses 4 to 8 weeks later.

Little is known about the risk in humans of transmitting genetic mutations or chromosomal aberrations if permanent sterility does not ensue (Searle, 1975). Somewhat arbitrarily, it has been suggested that females receiving direct radiation to the pelvic area "defer conception for at least several months" (Brent, 1979), and that males wait at least 1 year (American College of Radiology, 1975). Because postgonial cells in experimental mammals have shown higher mutation rates than gonial cells, these recommendations could reduce, although not eliminate, mutations and chromosomal aberrations in live-borns conceived by exposed individuals.

Predisposition to Cancer

Carcinogenesis is an effect of even low doses of radiation (United Nations, 1977; BEIR, 1980), and therapeutic doses can be correlated with site-specific subsequent malignancies (Upton, 1973). Long-term studies of Japanese atomic

bomb survivors have shown a significant increase in leukemia. For example, children exposed under the age of 10 showed a sixfold increase (see Brent, 1977). It also has been shown that other forms of cancer are significantly more frequent in atomic bomb survivors than in controls. The increased risk of cancers other than leukemia (notably lung, breast, and stomach) has become apparent as the exposed population reaches the mean ages of onset of various other cancers (Jablon, 1975; Schull, 1980). A dose–response curve is also exhibited, further implicating radiation as a causative agent. Therapeutic radiation in childhood also elevates the leukemia risk (Kitabatake, 1966; C. L. Simpson, Hemplemann, & Fuller, 1955). Increased risk for leukemia has also been noted by some investigators following in utero exposure; 1 to 2 R have been said to increase risk twofold (Stewart, Webb, & Hewett, 1958; MacMahon, 1962; Stewart, 1973; also see Brent, 1979). However, the absolute risk appears to be low, and there is doubt about the purported association because of possible sampling biases, an increased frequency of leukemia among non-irradiated sibs of the experimental subjects (Stewart, 1973), and failure to detect elevated leukemia risk in children who were exposed in utero to the atomic bomb (Burrow, Hamilton, & Hrubec, 1965; Kato, 1971; Schull, 1980). Oppenheim, Griem, and Meier (1975) suggested that the clinical conditions requiring radiation exposure might themselves be predisposing to leukemia. Irrespective, the risk is sufficiently low that it should not deter a woman from obtaining a pelvic series for proper management of labor.

Certain ocupational groups and patients exposed to therapeutic radiation have shown significant increases in cancer mortality attributable to ionizing radiation: Irradiated spondylitis patients showed 10 times the expected number of deaths due to leukemia and cancers of the stomach, pharynx, pancreas, and bronchi; large excesses of thyroid carcinoma and tumors of the salivary glands occurred in children whose thymus glands were irradiated; miners exposed to radon-containing air are at high risk for lung cancer. There are also some well-known examples of the carcinogenic effects of internal emitters, such as the osteosarcomas that developed in radium dial painters and the liver malignancy characteristic of patients who had liver scans using Thoratrast (Jablon, 1975). In summary, there is no doubt of the association of cancer risks and appreciable doses of ionizing radiation, delivered either externally or by internal emitters. Controversy exists, however, about the extent and nature of risks associated with low-level acute or chronic exposure (BEIR, 1980). Estimates of the lifetime mortality risks from a single postnatal whole body absorbed dose of 10 rad of x-rays range from 0.5 to 1.4 percent of background cancer mortality (BEIR, 1980).

ULTRASOUND

Ultrasound may affect biological materials by temperature elevation, cavitation, or direct effects (Baker & Dalrymple, 1978). Thermal effects were discussed earlier in this chapter (in the section Nonionizing Electromagnetic Radiation and Hyperthermia). The thermal response of matter will depend not only on characteristics of the ultrasound but also on the media and tissue state relative to heat transfer and tolerance. Temperature changes as great as 10°C/sec have been produced in small tissue volumes by intense, highly focused

beams (Hill, 1968). Cavitation—creation of mechanical resonance within cells, which can in certain localized areas disrupt subcellular structures—can also result from ultrasound exposure. Direct effects also occur. For example, free radicals may be formed, as in ionizing radiation. More severe effects of cavitation and free radical formation are, however, observed only at ultrasound frequencies much higher than those currently used clinically (900 KHz to 6 MHz).

Ultrasound can thus clearly produce biological damage at certain doses when delivered under certain conditions. However, of clinical interest is the question of whether diagnostic ultrasound, usually a low-amplitude pulsed dose, is detrimental. Fortunately, few deleterious long-term effects of ultrasound have been observed. No serious damage has yet been established as result of diagnostic ultrasound on humans, but conclusive epidemiological data is not yet available (see Baker & Dalrymple, 1978; Scheidt, Staneley, & Bryla, 1978; Elias & Simpson, 1980). Consequently, the possibility should not be precluded. For example, rat spinal cord paraplegia and hemorrhage have been observed after pulsed exposure (0.5 to 6.9 MHz at 25 or 50 W/cm^2). The lowest frequencies produced the most severe effects. Furthermore, the particular system tested showed no ability to repair itself. Therefore, recurrent ultrasound exposure at doses not individually harmful could theoretically lead to an accumulated dose which is harmful. Whether clinical ultrasound affects chromosomes is controversial.

In conclusion, ultrasound in doses utilized diagnostically is currently considered safe (Baker & Dalrymple, 1978; Scheidt, Stanely, & Bryla, 1978; Elias & Simpson, 1980). However, continued caution is appropriate pending results of extensive, prospective surveys of the effects on humans.

CLINICAL PERSPECTIVE

Concern for biological damage induced by radiation and other physical agents is not unwarranted, because under certain circumstances these agents can be harmful. Fortunately, in most clinical situations significant risk requiring major intervention can be excluded. Ionizing radiation is the agent of major concern. Immediate medical effects following adult exposure e.g., radiation sickness, will be obvious and occur only after therapeutic or accidental exposure. Temporary or permanent sterility will become apparent within a few months. If sterility does not occur, two questions persist: Is there risk to offspring produced by exposed gametes? Are there long-term effects to the population or exposed individuals? No exact figures are available for humans, but results on experimental organisms and observations on offspring of atomic bomb survivors suggest the overall clinical risk is low. Even when there is a relative increased risk, the absolute increase is low. Antenatal studies may be offered to detect induced chromosomal aberrations transmitted to the offspring, but most gene mutations cannot be detected in utero. Dominant lethals might be manifested as spontaneous abortions, whereas recessive mutations might not be expressed until future generations, if ever. Delaying conception reduces but does not eliminate the risk of transmitting abnormal gametes. Carcinogenic

effects of ionizing radiation are also possible, but little can be offered the exposed individual except regular medical surveillance.

Fetal exposure to therapeutic or accidental maternal radiation is also of concern. Currently available data suggest that fetal doses of 10 rads warrant consideration of therapeutic abortion, although gestational age must be taken into account. If exposure occurred during preimplantation or early-implantation stages, spontaneous abortion could ensue; however, the probability of the continuing pregnancy resulting in offspring with malformations or retardation is no greater than the background risk. Long-term effects (e.g., malignancy) could occur in fetuses exposed in utero, but exact risks cannot be given; however, available data suggest risks are low. Predictions of risk based on therapeutic radioactive isotope administration must be specificially computed for each case, the most important variables again being the dose absorbed by the fetus, the target organ, and the stage during exposure. If therapeutic exposure to radioactive isotopes occurs, the risks are high. Conversely, risks are minimal following diagnostic isotope exposure.

Based on current information, the doses and conditions under which most nonionizing radiation (e.g., ultraviolet light, microwaves) and other forms of energy (diagnostic ultrasound) are delivered to the fetus or liveborn individual usually indicate no cause for clinical concern. However, each case must be considered to exclude unusual circumstances, and ultraviolet exposure predisposes skin to malignancy. Other effects of many of these physical agents—genetic and chromosomal damage, infertility, spontaneous abortion, stillbirth, long-term morbidity, reduced longevity—remain potential hazards that are difficult to assess until results of larger-scale, well-controlled experiments are available.

14

Viruses and Infectious Agents

Infectious diseases are responsible for an estimated 4 to 9 percent of institutionalizations of mentally retarded children. Although the frequency of significant infection during pregnancy cannot be stated, some estimates can be offered. Among 58,828 pregnant women in the Collaborative Perinatal Project, clinically recognized infections occurred in approximately 8180 (13.9 percent): viral infections accounted for 3401 (5.8 percent), bacterial infections for 4539 (7.7 percent), fungal infections for 102 (0.2 percent), and parasitic infections for 138 (0.2 percent). Influenza-like illness occurred during approximately 1400 (2.4 percent) pregnancies, herpes virus (cold sores) in 900 (1.5 percent), and viral gastroenteritis in 350 (0.6 percent). By contrast, in another prospective study of 23,000 pregnancies, 25 percent of the pregnant women showed significant rises in antibody titers to at least 1 of the 10 viral antigens for which testing was performed (Sever, 1968). The differences between these two studies suggest that maternal infection often goes unrecognized.

Since maternal infection is often unrecognized, the true incidence of intrauterine infections can be determined only with serologic techniques. Using such techniques, the incidence of cytomegalovirus has been estimated as 1 in 400 pregnancies (Alford, 1971) to 1 in 150 pregnancies (Desmonts & Couvreur, 1974). For rubella, the incidence is 1 in 950 (Alford, 1971); for herpes simplex 1 in 4000 (Hanshaw, 1973); for syphilis 1 in 1250 (Alford, 1971) to 1 in 300; and for toxoplasmosis 1 in 700 (Alford, 1971) to 1 in 400 (Desmonts & Couvreur, 1974).

Fetal development of the immune system begins during the first trimester. Infectious challenges during fetal life induce formation of serum IgM globulin in utero. Infected newborns therefore have elevated IgM globulin values, which are maintained throughout, and often beyond, the neonatal period. Cord blood IgM globulin levels, therefore, may be a valuable screening test for rubella,

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cytomegalovirus, herpes simplex, syphilis, and toxoplasmosis. In most laboratories, IgM globulin levels greater than 20 mg/100 cc are suggestive of fetal infection. Specific IgM fluorescent antibody tests are available to make a diagnosis of rubella, herpes simplex, syphilis, and toxoplasmosis. In addition to specific IgM globulin production, infection may be documented by isolation of the organisms from the infant, or by observing a stable or rising IgM antibody titer in the neonatal period.

VIRUSES

Mechanism of Action

Viruses adhere to most of the principles of human teratology. The effect of the virus depends upon (1) its ability to interfere with normal organogenesis, (2) stage of embryologic development, (3) target organ, and (4) duration of exposure to the virus. The actual pathogenesis probably involves cell death.

The goal is self-replication of the virus, not destruction of the host. Viral replication requires attachment to the cell membrane, penetration of the cell membrane, utilization of the host genome, synthesis of individual viral components by transcription and translation, reassembly of viral genome, and then release of the virus—usually, but not always, with concomitant cell death. Viruses also are known to produce chromosomal damage, particularly chromosomal breaks, but the significance of the breaks is yet to be determined. Some viruses, particularly rubella, have been shown to inhibit mitosis as well as to produce chromosomal breaks (Plotkin, Boué & Boué, 1965). Inhibition of mitosis may in fact interfere with growth and maturation, and may be the basis for decreased growth of some of the organ systems. Factors that increase susceptibility of the fetus to certain viruses include diminished fetal antibody production, which allows dissemination of the virus throughout the fetus, and the rapidly dividing cells in the developing fetus, which offer favorable cultivation conditions for viruses. Viral infections known to produce malformations in humans are rubella, cytomegalovirus, and varicella. Other viruses have been implicated, but at present there are insufficient data to establish a clear causal relationship.

Rubella

With the report of Gregg (1941), it became known that rubella infection early in pregnancy is associated with rather consistent congenital malformations in the newborn. The abnormalities then attributed to rubella consisted of ocular, cardiovascular, and auditory anomalies and mental retardation; however, it soon became known that this virus also could cause abortions and stillbirths.

The magnitude of the problem has been variably estimated. The first surveys, which were retrospective, suggested that the frequency of congenital defects among live-born infants exposed during the first trimester in utero to rubella was between 50 percent and 90 percent (Swan et al., 1943; Aycock & Ingalls,

1946; Wesselhoeft, 1947; Swan, 1949); however, as with most retrospective studies, the estimates were unduly high. Prospective studies initiated in the 1940s (Sallomi, 1966) showed that the risk for anomalies was 61 percent in the first 4 weeks of pregnancy, 26 percent in weeks 5 to 8, and 8 percent in weeks 9 to 12. These risk figures, however, are an underestimate, because deafness is not easily detected in small infants and was only later recognized as the most common sequela of congenital rubella infection. A prospective study (Jackson & Fisch, 1958) showed that, of fetuses exposed to rubella in the first 16 weeks in utero, 30 percent were deaf; however, in two thirds of cases the hearing loss was not recognized until the children were 4 years of age. Second trimester infection also has been associated with neonatal deafness (Forrest & Menser, 1975). More recently, it was demonstrated that early-onset diabetes mellitus may be caused by congenital rubella infection (Menser, Forrest, & Bransby, 1978). In addition, approximately 16 to 18 percent of pregnancies complicated by early rubella end in spontaneous abortions or stillbirths (Warkany & Kalter, 1961; Siegel, Fuerst, & Peress, 1966).

Pathophysiology

Rubella, an RNA virus, reaches the embryo by the hematogenous route, through the placenta. Rubella virus acquired in utero persists throughout pregnancy, is present at birth, and can be shed by an infected child for many months (Alford, Neva, & Weller, 1964). Malformations are caused by disturbances in cell division or by cell destruction. Intra-uterine growth retardation has been attributed to inhibition of cell multiplication (Singer et al., 1967). Chromosomal breakage has been found in fetal cells (Nusbacher, Hirschhorn, & Cooper, 1967; Heggie, 1977) and in tissue culture cells (Plotkin, Boué, & Boué, 1965).

Clinical Manifestations

Mother. In the adult, rubella is acquired by direct person-to-person contact. The usual incubation period is 10 to 18 days. Nasopharyngeal shedding of the virus begins on approximately the fourth day following exposure and marks the start of the infective period. The clinical manifestations may be subtle, with onset of posterior auricular and occipital lymphadenopathy followed by malaise, coryza, low-grade fever, and conjunctival irritation. Later a maculopapular rash starts on the face, trunk, and upper extremities. The rash usually begins 10 to 14 days following exposure and lasts 2 to 3 days. More serious manifestations include thrombocytopenia, arthralgia, arthritis, and encephalitis; however, these occur rather infrequently. The ratio of inapparent to apparent rubella infection in adult women is approximately 1:1 (Alford, Stagno & Reynolds, 1974).

Neonate. Although the original description of the rubella syndrome consisted of defective development of the eye, ear, and heart, it soon became obvious that there were additional effects (Sever et al., 1969), including anemia, encephalitis, pneumonitis, myocarditis, skin lesions with associated thrombocytopenia, hepatitis, and bone lesions. The occurrence of thrombocytopenia in the neonatal period carries a poor prognosis for life; such infants have low

birth weights and other complicating diseases, which cause more than one third of them to die within the first year of life (Cooper, Ziring, & Ockerse, 1969).

In 50 percent of rubella-affected infants, the head circumference is below the third percentile. Central nervous system damage resulting in mental retardation is common. Ten percent of rubella infants require institutionalization, and an additional 30 percent have difficulty performing at the first grade level. Encephalitis, which may be transient, is associated with neurologic defects including seizures, poor language and motor development, and poor growth.

Ocular defects occur in more than half of rubella infants and include retinopathy, nuclear cataracts, glaucoma, microphthalmia, and myopia. The retinopathy is benign, nonprogressive, and characterized by disturbances in pigment homogeneity. Cataracts usually develop during the first year of life, despite apparently normal lenses at birth. Both eyes are affected in two thirds of the cases.

Hearing loss, which is difficult to diagnose at birth, occurs in 30 to 50 percent. It may be unilateral or bilateral and may be the only congenital defect, particularly if rubella occured after the first trimester. Degenerative and atrophic changes also occur in the inner ear structures, and additional hearing loss may result from chronic postnatal otitis media.

Defects of the major arteries occur more commonly than intracardiac abnormalities. Although patent ductus arteriosis is considered the most common rubella cardiac lesion, recent studies indicate that pulmonary artery stenosis may approach it in frequency. Pulmonary valvular stenosis and aortic valvular stenosis also occur. Ventricular septal defect is the most common intracardiac lesion.

Disruption of the cartilagneous development of the long bones occurs commonly, involving the distal femur and proximal tibia (Cooper, Ziring, & Ockerse, 1969; Heggie, 1977).

Diagnosis

Mother. When the disease is obvious, an experienced observer can make the diagnosis by clinical examination alone; however, clinical manifestations are often quite subtle and may go unnoticed. If a question exists, a hemagglutination inhibition (HI) antibody titer should be obtained immediately and then repeated 2 to 3 weeks after exposure. A titer of less than 1:8 indicates absence of previous rubella infection. A fourfold or greater rise in antibody titer 2 to 3 weeks after exposure indicates infection. It is advantageous to rerun the original titer at the same time at which the second titer is determined, as interassay variations exist. The HI antibody titer peaks in 6 to 12 days. Thus, if a pregnant woman is first seen at the time of the rash, the HI titer may have begun rising and the seroconversion may be obscured. Complement fixation titers trail those of HI, and serial CF testing may establish the diagnosis. If a question still exists, rubella-specific IgM testing may be helpful.

Neonate. The fetus produces IgM antibodies in response to rubella infection. Thus, prenatal infection can be confirmed by specific IgM antibodies in cord blood or peripheral blood during the first week after birth. Rubella virus

can be recovered from the fetus or placenta in about 50 percent of infected cases. After birth, the newborn sheds virus in virtually all body fluids. Viral excretion from the nasopharynx has occurred up to one year postnatally, and the virus has been noted in the lens up to three years postnatally. A prenatal diagnosis of intra-uterine infection at 12 weeks gestation has been made by amniocentesis and culture of the rubella virus (Levin et al., 1974). However, IgM and HI antibodies in amniotic fluid are both undetectable, even in the presence of fetal infection (Cederqvist et al., 1977).

Treatment and Prevention

There is no specific treatment for rubella syndrome, but the development of rubella vaccine offers hope that rubella will cease to be a significant cause of congenital defects. Mass immunization of preadolescent children theoretically should decrease the possibility of transmission of the virus to susceptible women during childbearing years. Since 1969, more than 55 million doses of rubella vaccine have been administered to children ages 1 through 12. Since such mass immunization programs began, the incidences of rubella infection and congenital rubella have declined substantially in the United States (Cooper, 1975; Modlin et al., 1975). The immunization programs also have answered many questions. For example, it appears that "herd immunity" will only partially protect a community during an epidemic (Horstmann et al., 1970; Klock & Rachelefsky, 1973). Reinfection has been observed among persons with natural as well as vaccination-induced immunity, although it is more common after vaccination (Krugman & Katz, 1974). However, viremia has not been observed during reinfection, so fetal infection is highly unlikely. There is some evidence that vaccination at 12 years of age yields benefits greater than vaccination earlier in life (Schoenbaum et al., 1976). The major unanswered question is whether vaccination-induced immunity will prevent viremia when reexposure takes place many years after the original vaccination.

The rubella vaccine is a live, attenuated virus that is known to cross the placenta. In a few cases in which the vaccine was given inadvertently to pregnant women, the virus has been recovered from the products of conception (Bolognese et al., 1973; Wyll & Hermann, 1973; Ebbin et al., 1973; Fleet et al., 1974). The Center for Disease Control compiled information regarding 343 women inadvertently given rubella vaccine shortly before or after conception. The maximum risks of fetal infection after maternal vaccination are 5 to 10 percent (Modlin et al., 1976). There were no congenital anomalies or viral recovery in the offspring of women electing to continue their pregnancies. Women receiving the vaccine should be instructed to avoid pregnancy for at least three months following vaccination.

Cytomegalovirus

Cytomegalovirus (CMV), a member of the herpes virus group, is frequently responsible for prenatal and perinatal infections. Prior to the capability of virus recovery, congenital CMV infections were recognized retrospectively, primarily on the basis of post-mortem pathology. During 1956 and 1957, CMV was independently isolated in three different laboratories (M. G. Smith, 1956; Weller

et al., 1957). Development of cytologic, virologic, and immunologic procedures opened the way for prospective studies that provided a better estimate of the frequency with which this virus caused intra-uterine infection.

CMV infection is common. CMV antibodies are present in 20 to 30 percent of children less than 10 years and some 50 to 80 percent of adults greater than 25 years (Leinikki, Heinonen, & Pettay, 1972). Most people acquire CMV postnatally. With such high prevalence rates, it is not surprising that many pregnant women either have had CMV before conception or, less often, become infected during pregnancy. Of 198 women reported by Sever, Huebner, & Castellana (1963), 6 percent converted from a negative to a positive serologic test for antibodies to CMV during the course of pregnancy. Cytomegalovirus has been isolated from the urine in from 2 to 6 percent of all pregnant women (Alexander, 1967; Hildebrandt et al., 1967; Shinefield & Eichenwald, 1968; Montgomery, Youngblood, & Medearis, 1972; Reynolds et al., 1973; Stern & Tucker, 1973). A greater proportion—from 2 to 18 percent—of gravid women shed CMV in cervical secretions (Alexander, 1967; Foy et al., 1970; Montgomery, Youngblood, & Medearis, 1972; Reynolds et al., 1973). Cytomegalovirus has also been recovered from the semen of young, apparently healthy, asymptomatic, adult males; the recovery of the virus from the semen of one young, adult male and from the cervix of his sexual partner suggests that the virus may be transmitted venereally (Lang, Dummer, & Hartley, 1974). Congenital CMV infections have occurred in utero in approximately 1 percent of all liveborn infants (Hanshaw, 1971), and at least 10 percent of the infected infants eventually manifest significant neurosensory damage. Higher rates of congenital CMV have been reported among blacks and among persons of low socioeconomic status (Li & Hanshaw, 1967).

Pathophysiology

Cytomegalovirus exhibits species specificity, has the ability to establish latent persistent infections, and shows characteristic cytopathology. Pathologic changes consist of localized interstitial inflammatory processes with a marked tendency to necrosis, fibrosis, and calcification. Infected cells appear swollen and contain intranuclear and intracytoplasmic inclusion bodies. The intranuclear inclusion body is dense, stains reddish-purple with hematoxylin and eosin, and is surrounded by a halo. The intracytoplasmic inclusions are more granular and basophilic staining than the intranuclear inclusion bodies. Infected cells can be found in every organ. Fetal infection can occur at any time and may become chronic. During the first trimester CMV infection may cause a spontaneous abortion.

Though it has been stated that primary infection must occur during the pregnancy if the fetus is to be affected, recent studies show that this may not always be true. Despite substantial levels of preconceptional antibodies, intrauterine CMV infection occurred in the fetuses of 7 of 208 women (3.4 percent) (Stagno et al., 1977), but the rate of congenital cytomegalovirus in their general delivery population was 2.4 percent. All the infants observed in the study were born with subclinical CMV infection. The 7 born after proved recurrent maternal infections showed normal development during follow-up examinations performed between 12 and 51 months (mean 26 months) of age. Those delivered

after primary maternal infection likewise remained normal through 6-, 12-, and 15-month follow-up examinations. From these and other observations (Monif, Souards, & Eitzman, 1972; Stern & Tucker, 1973), it does not seem that primary maternal infection frequently leads to fetal pathology or that maternal humoral immunity is sufficient protection against the spread of infection to the fetus.

Mothers who give birth to congenitally infected infants may have a specific impairment of cell-mediated immunity compared to controls (Rula-Plesczynski et al., 1975). A similar type of immune defect may explain chronic infection in neonates. Gehrz et al. (1977) used an in vitro lymphocyte proliferation assay to show that four young children with active cytomegalovirus infection had a CMV-specific cell-mediated immune defect. Despite antibodies to CMV, these infants were still actively shedding the virus in the urine. Starr et al. (1977) reported on four more infants with active CMV infection and found a defect in the cell-mediated immune response. Thus, the defect in the mother may facilitate transmission of the virus to the fetus, and the immune defect in the fetus may in turn be responsible for the failure to clear the virus from infected organs, resulting in chronic intracellular viral replication and damage to the infected organs. Further studies are needed to clarify the role of the cell-mediated immune response to CMV and its relationship to the pathogenesis of congenital infections.

Clinical Manifestations

Mother. Most cases of maternal CMV infection are asymptomatic, although some primary infections may present with a heterophil-negative infectious mononucleosis syndrome. Thus, it is usually impossible to determine at what stage in fetal development the infection occurred. Nonetheless, cytomegalovirus can be isolated from urine of 2 to 6 percent of all pregnant women, and more women shed the virus in the cervical mucus. The virus also can be excreted in the breast milk. In view of this, it seems rather remarkable that only 1 percent of infants show evidence of intra-uterine cytomegalic infections.

Neonate. Although CMV infection in utero was once considered fatal for the fetus or neonate, there is now evidence that infants with intra-uterine CMV, although excreting virus, may show no discernible effects or only mild symptoms. The mild type of infection occurs much more frequently than the severe. The most common "mild" effects are hearing loss and school difficulties (Reynolds et al., 1974; Hanshaw et al., 1976). Severely affected neonates demonstrate intra-uterine growth retardation (birth weight often less than 2500 g), hepatitis, pneumonitis, and meningoencephalitis. Necrotizing meningoencephalitis causes severe neurologic degeneration and, hence, microcephaly. Obstructive hydrocephalus has been reported, and frequently periventricular calcification can be noted on roentgenologic examination. Transient changes include diffuse purpura secondary to thrombocytopenia, anemia, hepatosplenomegaly, and jaundice. Long-term effects include profound mental retardation, deafness, seizures, varying degrees of spasticity, optic atrophy, and chorioretinitis. Gross structural defects do not occur frequently, except for indirect inguinal hernias in males. Lang (1966) noted that 11 of 14 affected males (79 percent) had indirect inguinal hernias, whereas hernias were not noted in affected females.

Diagnosis

CMV infection should be suspected in the presence of elevated IgM in the newborn infant. On occasion IgA is also elevated (McCraken & Shinefield, 1965). The complement fixation antibody test is most valuable in the first year of life, but the diagnosis also may be made by isolation of the virus or by evidence of inclusion bodies in the urine exfoliative cytology.

Treatment and Prevention

Treatment is primarily supportive. A vaccine has been developed (Elek & Stern, 1974), but clinical studies of efficacy are not available. Repeated fetal infections have been found with sequential pregnancies; therefore, if a woman has one infant infected with CMV inclusion it is still possible for her to have an asymptomatic infected second infant (Embil, Ozere, & Haldane, 1970; Stagno et al., 1973).

Varicella

Varicella-zoster virus is a member of the herpes virus family. Varicella (chickenpox) and an associated viremia can occur at any time during pregnancy, but it is potentially harmful to the fetus only if it occurs during the first 16 weeks or last 4 days of gestation.

Congenital defects due to maternal infection with varicella early in gestation have been reported, but this is uncommon. Williamson (1975) reviewed the literature and found nine cases of congenitally malformed infants. Evidence that the defects in the nine cases may have been due to maternal varicella infection consisted of a similar pattern of neonatal anomalies and all mothers having had varicella in the first trimester. Anomalies included ocular defects (e.g., cataracts, microphthalmia, and optic atrophy), brain damage, cicatricial skin lesions, and hypoplastic limbs. Since no estimate of the total number of in utero exposed infants are available, accurate risk figures cannot be derived. There has been no association of varicella with spontaneous abortions, prematurity, or fetal demise.

Infection late in pregnancy results in a newborn with characteristic pox lesions of the skin or severe pneumonia. Maternal infection 5 to 15 days before delivery may not produce disease in the infant, and, if it does, it will not be lethal. However, maternal infection 4 days or less before delivery leads to neonatal death in approximately 20 percent of cases. In neonates whose onset of the rash is in the first 4 days of life, the maternal illness had occurred long enough prior to delivery to allow her to produce antibodies and have them cross to the fetus. No deaths occurred in this group of infants. In contrast, 4 of 19 (21 percent) neonates in whom the rash began at 5 to 10 days of age died (Meyers, 1974).

The diagnosis of varicella usually can be made clinically. Isolation of the virus is difficult; however, specific antibodies to the virus and elevated infant IgM serum antibody may aid in the diagnosis. There is no effective treatment. Zoster immune globulin will prevent varicella when given to susceptible children within 72 hours of exposure (Brunell et al., 1969). It is not known if zoster immune globulin would be useful in the prevention of neonatal varicella when the mother's disease occurs in the 4 days prior to delivery.

Herpes zoster, which is caused by latent or secondary infection with the varicella virus, has been associated with only two instances of neonatal cataracts (Duehr, 1955). Many later reports have indicated that maternal herpes zoster is associated with virtually no risk of fetal infection.

Herpes Simplex

Neonatal infection with herpes simplex virus (HSV) was first described over 40 years ago (Batignani, 1934). A minimum of 120 cases occur in the United States every year (Nahmias et al., 1971). The incidence of herpetic genital infection during pregnancy is as high as 2 percent in lower socioeconomic groups, an incidence 10 to 50 times greater than that seen in upper socioeconomic groups (Hanshaw, 1973). More than one half of the genital herpes infections are asymptomatic.

Pathophysiology

Herpes simplex virus can be divided into two antigenic types. Infections due to type 1 virus are usually associated with lesions in nongenital sites (e.g., mouth, eyes, central nervous system, and skin about the waist), whereas type 2 infections are usually associated directly or indirectly with genital tract lesions and herpetic infections of the newborn. Serologic techniques differentiate types 1 and 2, thus allowing detection of specific antibodies to these antigens. However, herpes virus does not undergo immune elimination and persists as latent virus despite the presence of specific antibody.

The pathogenesis of recurrent herpes is not well defined. The virus may extend along sensory nerve sheaths during the primary infection, localize in corresponding sensory ganglia, and then persist in an asymptomatic or static state for a period of time. The appropriate stimuli then act to trigger viral replication, which is followed by appearance of lesions (Kibrick & Gooding, 1965; Roizman, 1965). The basic pathology of herpetic disease is localized tissue necrosis. The base and sides of the vesicles contain intranuclear inclusions in multinucleated giant cells. The presence of such giant cells is pathognomonic of infection due to herpes virus.

Clinical Manifestations

The incubation period for the primary herpes infection ranges from 2 to 10 days. The lesions begin with erythema and proceed to vesicles, pustules, and finally crusts. The lesions generally heal completely within several days to two weeks. Systemic reactions with primary infection include fever, headache, malaise, chills, and general lymphadenopathy. The virus may localize in an occult form at varying sites. In spite of the presence of neutralizing antibodies, various stimuli (e.g., fever, sun, infection, emotional stress, irritation) may induce the reappearance of the virus within one or two days. In most hosts, reinfection is generally contained at the initial sites of infection, and progression to viremia is not common. In individuals with severe infection, however, a transitory viremia may occur with spread of the virus to other

susceptible organs. Recently, it has been shown that different strains of herpes simplex virus may cause new episodes of "primary" infection.

Herpesvirus type 2 is commonly harbored in the cervix and vagina; lesions of the vagina may be associated with leukorrhea and pain. Cervical infection may be manifested by nonspecific inflammation rather than by vesicles and ulcers. The presence of multinucleated giant cells or intranuclear inclusion bodies on routine cytologic smears is highly characteristic. Over 90 percent of herpesvirus identified from adult female genitalia is type 2, and serologic studies of infected newborns and their mothers indicate that the major source of herpetic infections in newborns is the maternal infected genital tract. Most infants are infected at the time of birth by direct contact with the virus. If maternal infection occurs after 32 weeks' gestation, the risk of neonatal infection approximates 10 percent (Nahmias et al., 1971). There also is a twofold increased risk of prematurity. If active maternal infection occurs at the time of delivery, the risk of fetal infection is 40 percent. Herpetic infection during the first 20 weeks of pregnancy is associated with a marked increase in abortion rate (Nahmias et al., 1971).

The manifestations of neonatal herpetic infections vary considerably. Cutaneous vesicles may be present at birth or may develop during the first two weeks of life. Other signs of neonatal infections include temperature instability, poor feeding, jaundice, and occasionally severe hemorrhagic manifestations due to thrombocytopenia. The mortality rate is approximately 50 percent in neonates with systemic or CNS disease. Of infants born with only skin lesions, one half develop systemic disease. Death may occur from the second to the fourth week in cases complicated by hemorrhage, vascular collapse, and pneumonia. Autopsy examination has revealed extensive necrosis of the liver and adrenal glands. More than half the survivors will manifest some form of neurologic or ocular sequelae, but malformations per se are not common.

Diagnosis

The diagnosis of neonatal herpetic infections can be accomplished quickly. Herpesvirus can be grown rapidly in tissue culture, usually within 24 to 48 hours. Cells from the herpes vesicle show characteristic eosinophilic intranuclear inclusion bodies. Prenatal infections may be associated with specific IgM antibody in neonatal serum if the infection has occurred more than two weeks prior to the delivery (Nahmias et al., 1969).

Treatment and Prevention

Because most cases of neonatal herpes are acquired from the infected maternal genital tract, the fetus should be delivered by Cesarean section if genital herpetic lesions exist (Nahmias et al., 1971). If the fetal membranes are ruptured in the presence of genital herpes, the key variable appears to be the length of time before delivery. For example, of 16 neonates delivered by Cesarean section within four hours of membrane rupture, none manifested signs of infection, although one died of a later-appearing infection (Nahmias et al., 1975). Of 10 neonates delivered by Cesarean section more than four hours after membrane rupture, 5 died of overwhelming infection, three lived with severe residual defects, one had only a cutaneous infection and only one was uninfected.

Therefore, the current recommendation is that if the membranes have been ruptured four hours or longer, a Cesarean section is not indicated. If a known case of herpes genitalia has cleared more than one month before term, a vaginal delivery should be allowed.

Specific therapy has been attempted with iododeoxyuridine, cytosinearabinoside, and adenyladenoside. The number of cases treated with any of these is too small to draw definite conclusions about their efficacy (Nahmias et al., 1975).

Influenza

Data concerning the relationship of influenza virus infections during pregnancy with congenital malformations have been contradictory. Although abortions and premature labor as complications of influenza were reported to be high during the pandemic in 1918, reports of the occurrence of congenital anomalies due to influenza during pregnancy did not occur after the pandemic of Asian influenza in 1957. However, prospective studies were too limited to provide adequate evidence of a causal relationship between influenza and congenital malformations. Retrospective studies were unreliable because information was often obtained from self-diagnosis, from a clinical diagnosis, or from hospital records.

Clinical Manifestations

Mother. Because of the epidemic nature of influenza, many pregnant women are infected during each epidemic. In some series the attack rates, based on clinical diagnosis, are 25 to 50 percent. During the 1957 Asian influenza epidemic in New York City, there were 216 deaths from influenza, including 22 pregnant women. Nearly one half of all women of childbearing age who died of influenza were pregnant.

Neonate. In a retrospective study in Dublin during 1953, Coffey and Jessop (1955) were the first to note a possible association between influenza and congenital malformations of the central nervous system. During the 1957 Asian influenza epidemic, a prospective investigation showed a significant increase in the incidence of CNS malformations if the maternal illness took place in the first trimester. The sample was small, however, and the incidence of CNS malformations in the control group was higher than expected; therefore, it is difficult to interpret these data. A study in South Wales (Laurence, Carter, & David, 1968) failed to detect an increased incidence of CNS malformations following an influenza epidemic between 1956 and 1962. Therefore, there probably is no direct association between influenza in the pregnant women and subsequent congenital malformations. Influenza may exert an ill-defined, unfavorable effect, however, possibly related to such factors as increased maternal drug ingestion during the illness (Karkinen-Jääskeläinen & Saxen, 1974). A definitive study requires serologic evidence of infection. Such a study should be done during the next pandemic.

Treatment and Prevention

Because influenza may be serious or even fatal for pregnant women, it is considered by some to be advisable to vaccinate all pregnant women. This is quite controversial and depends upon the virus strain causing an epidemic and the quality of the vaccine available. The Center for Disease Control usually makes specific recommendations prior to an expected epidemic.

Mumps

There have been few studies of the effects of mumps infection during pregnancy. The only adverse effect of gestational mumps noted with any consistency is fetal wastage when the infection occurs in the first trimester. A prospective analysis of 501 cases of maternal mumps (Manson, Logan, & Lou, 1960) revealed no excessive malformation rate among the offspring. The possibility of endocardial fibroelastosis in association with mumps was noted by Noren, Adams, & Anderson, (1963), who reported an association of endocardial fibroelastosis and hypersensitivity to the mumps antigen. There have been no other reports confirming this association, however. In another report three cases of aqueductal stenosis and hydrocephalus were noted in association with congenital mumps infection (Johnson, 1972).

Roseola

Roseola is not so common as some other viral infections during pregnancy. The best data on the fetal effects of roseola infection come from a series of ten epidemics in Greenland between 1951 and 1962 (Jesperson, Littauer, & Sagild, 1977). Of 77 pregnancies infected in the first trimester there were 16 spontaneous abortions, 6 stillbirths, and 55 live-borns, of whom 5 had congenital malformations. Of 120 second trimester infections there were three spontaneous abortions, 3 stillbirths, and 114 live-borns, of whom 2 had congenital malformations and 10 died before 1 year of age. Of 133 third trimester infections, there were 2 stillbirths and 131 live-borns, of whom one had a congenital malformation and 16 died before 1 year of age. Roseola infection during pregnancy may thus produce increased rates of spontaneous abortion, stillbirths, and early childhood deaths, with infections in the first trimester possibly causing an increase in congenital malformations among survivors. However, these effects may merely be the result of a serious febrile illness in the mother and not due to fetal infection per se.

OTHER INFECTIOUS AGENTS

Toxoplasmosis

Toxoplasma gondii is a protozoan that occurs naturally in herbivorous, omnivorous, and carnivorous animals, including almost all orders of mammals. It is probably one of the most common infections in humans. Intra-uterine

infection with *T. gondii* was first proved by Wolf, Cowe, and Paige (1939), who isolated the parasite from an infant with chorioretinitis, hydrocephalus, and cerebral calcifications. The rate of acquired infection in pregnant women (and hence in the fetus) depends upon the rate of toxoplasmosis in the general population. There are striking geographic differences between countries and between populations in the same country. In London 22 percent of pregnant women have positive Sabin-Feldman dye tests (Desmonts & Couvreur, 1974). In Palo Alto, California, 442 unselected pregnant women showed a prevalence of positive dye test titers of 27 percent (Remington, Newell, & Cavanaugh, 1964). A collaborative study of approximately 23,000 women of childbearing age in various areas of the United States showed 38 percent to be positive reactors, using a hemagglutination test. Military recruits, of whom 95 percent were less than 23 years of age, had more positive reactors living east of the Mississippi than west. Previous infection rates decreased sharply in the west central area and in the mountain zones of the United States.

The incidence of congenital toxoplasmosis in the United States is 0.25 to 1 of 1000 live births (Sever, 1977). Transmission to a fetus has never been observed when maternal infection was acquired prior to pregnancy, but it occurs in 40 percent of cases when the primary infection takes place during pregnancy (Desmonts & Couvreur, 1974). Desmonts and Couvreur (1974) also showed that if the mother is infected in the first trimester, 20 percent of offspring are infected; if the primary infection occurs in the last trimester, 33 percent are infected. Maternal infection in the first trimester usually produces a severe fetal infection, whereas infection in the last trimester usually produces subclinical neonatal infections.

Pathophysiology

Toxoplasma gondii exists in three forms: trophozoite, tissue cyst, and oocyst. The trophozoite is crescentric, with one end rather rounded, and measures approximately 2 to 4 μ in width and 4 to 7 μ in length. The trophozoite is seen durig the acute stage of the infection and can invade every mammalian cell except perhaps non-nucleated red blood cells. Freeze-thawing or the human digestive enzymes will destroy trophozoites. The second form of the parasite, the tissue cyst, forms within the host cell, measures as much as 100 μ in size, and contains many organisms. This form stains readily with periodic acid-Schiff (PAS) stain, making it readily visible in tissue specimens. Freezing-thawing and heating to 60° C will destroy this form of the parasite. The third form of the organism, the oocyst, has been described only in cats. Oocysts are excreted in the feces of cats and are potentially hazardous to humans and domestic animals. About half of the cats in the United States have antibodies to toxoplasma (Sever, 1977). A possible source of transmission to humans is from the soil contaminated with oocysts from the gastrointestinal tract of the cat; however, cat-to-human transmission has yet to be proven. Raw or undercooked meat is the major source of infection. Worldwide, about 1 percent of cattle, 20 percent of hogs, and 30 percent of sheep have toxoplasmosis, according to estimates based on the isolation of the organism from animal muscle tissue (Sever, 1977).

Pregnant women may become infected and develop a parasitemia that leads to a focal infection in the placenta, from which the organism is disseminated to the fetus. The parasite is an obligate intracellular parasite. Feldman (1963) believes that a well-established placenta is necessary for the placental infection and transfer of the parasite; therefore, infection occurs only after organogenesis, decreasing the likelihood of structural abnormalities. Eichenwald, McCracken, & Kindberg (1967) agree that the fetus is infected only during the primary infection of the mother; however, Remington, Melton, & Jacobs (1960) believe that the toxoplasma can exist in an encysted stage in the uterus; thus the uterus remains chronically infected and is theoretically able to cause fetal infection in subsequent pregnancies. The fact that organisms have been isolated from menstrual blood, lochia, and products of conception of chronically infected women supports this possibility (Langer, 1963).

Clinical Manifestations

Mother. Although it is widespread, acquired toxoplasmosis usually has few clinical symptoms. In rare instances, acquired infections can result in encephalitis, pneumonitis, and rash; however, maternal infections that result in prenatal toxoplasmosis are usually subclinical. Clinical manifestations, when present, are usually similar to those of infectious mononucleosis.

Neonate. Most infected infants are asymptomatic at birth and remain so. Approximately 10 percent of infants born with congenital toxoplasmosis manifest severe disease, half of which have ocular involvement but no other evidence of disease. If the infection is generalized when the child is born, cyanosis, pneumonia, hepatosplenomegaly, jaundice, thrombocytopenic purpura, and edema are presenting signs. The classic triad of congenital toxoplasmosis (chorioretinitis, hydrocephalus, and cerebral calcifications) occurs in only a very few cases. Chorioretinitis is by far the most common abnormality in infants with overt toxoplasmosis, occurring in 75 to 95 percent (Feldman, 1958; Eichenwald, 1960; Couvreur & Desmonts, 1962). Fifty to sixty percent have central nervous system involvement (seizures, microcephaly, hydrocephaly, or brain calcifications). Ten to fifteen percent die. Some recover, but fewer than 10 percent without overt disease avoid residual cerebral or ocular damage.

Since only 10 to 15 percent of infants with proven congenital toxoplasmosis manifest signs and symptoms suggesting acute generalized toxoplasmosis, the remainder of the infected infants would escape detection if laboratory screening aids were not employed. Alford, Stagna, & Reynolds (1974) wrote that, in spite of the lack of overt neurologic findings, abnormalities of the cerebrospinal fluid (e.g., lymphocytosis and elevated protein indication of CNS involvement) are detectable in the asymptomatic infants. They felt that for prognostic purposes initial CSF protein level is the only real monitor. For example, one infant initially had no clinical manifestations of the disease, but did have abnormal CSF protein levels; by 2½ months of age, retarded head growth, generalized intracranial calcification, chorioretinitis, and mild hepatosplenomegaly were evident. At age 4 the child functioned developmentally as a 2-year-old. Des-

monts and Couvreur (1967) studied 22 infants who were infected during fetal life. Sixteen remained asymptomatic during infancy, 5 demonstrated severe cerebral or ocular signs, and 1 died. No follow-up of the 16 asymptomatic infants was reported.

Diagnosis

Mother. The diagnosis of acute toxoplasmosis can be established by demonstration of trophozoites in tissues or body fluids; however, because the disease is often subclinical, diagnosis on this basis may not be practical. Thus, the diagnosis is usually based on increased titers of anti-toxoplasmal antibodies. The serologic tests most commonly performed in the United States are the Sabin-Feldman dye test, complement fixation test, hemagglutination test, and indirect fluorescent antibody test. The Sabin-Feldman dye test is based upon the observation that *T. gondii* incubated with normal serum for 1 hour become swollen and deeply stained when alkaline methylene blue is added to the suspension. The parasites exposed to serum containing antibody under the same conditions appear thin and distorted and are not stained when the dye is added. Complement-fixing antibodies appear later than those demonstrable by the dye test; therefore, active infection is indicated by a negative complement fixation test that becomes positive, or by a fourfold increase in CF titer in conjunction with a stable, high, dye test titer.

The indirect fluorescent antibody test has proved useful because it does not require living organisms. Most workers consider the indirect fluorescent antibody test equal to the dye test in specificity, and it has been successfully used to establish the diagnosis of congenital and acquired infections. An indirect fluorescent antibody titer of 1:512 or greater correlates well with active infection.

Neonate. The specific IgM indirect fluorescent antibody test developed by Remington, Miller, and Brownlee (1968) is a simple and rapid procedure for the diagnosis of congenital toxoplasmosis. The test is positive not only in infants who have signs of congenital toxoplasmosis, but also in infants who are asymptomatic at birth. There has been a small percent of false positives in infants who did not have any evidence of congenital toxoplasmosis later in life. In addition, some newborns who later manifest features of congenital infection have shown no IgM antibody demonstrable by the IgM fluorescent antibody test. Therefore, the absence of IgM fluorescent antibodies in the newborn period or even during the first months of life does not absolutely exclude the possibility of congenital infection.

Treatment and Prevention

Optimally, serologic testing should be performed at various intervals during gestation. As this is not practical, it has been considered advisable to test maternal serum at the time of delivery (Remington, Miller, & Brownlee, 1968). Mothers with high antibody levels demonstrated by the dye tests, with a conventional fluorescent antibody test titer of 1:512 or greater, or with a positive specific IgM fluorescent antibody test are very likely to have recently acquired

an infection. Their infants must be carefully followed for both clinical and serologic evidence of the disorder.

Treatment of women who acquire toxoplasmosis during pregnancy with spiramycin (2 g/day for 3 weeks) decreases but does not eliminate the risk of having a child with CNS damage (Desmonts & Couvreur, 1974). Couvreur feels that early treatment of the neonate with spiramycin or pyrimethamine and sulfonamide compound may prevent neurologic sequela; therefore, he feels that chemotherapy is warranted. Additional studies addressing these questions are necessary. Pyrimethamine is a folic acid antagonist and its use during pregnancy is not recommended.

Syphilis

The incidence of primary and secondary syphilis in the United States has been increasing. Associated with this has been an increase in the number of reported cases of congenital syphilis (Center for Disease Control Annual Report, 1979). Almost all newborns whose mothers acquired the disease during the first several months of pregnancy are affected. If maternal syphilis is untreated, 25 percent of fetuses die in utero and 25 to 35 percent die within a few weeks of birth.

Pathophysiology

Treponema pallidum, the causative agent of syphilis in humans, reaches the fetal circulation by transfer across the placenta. Although there have been two instances in which spirochetes were found in the tissues of fetuses of approximately 3 months' gestation (Harter & Benirschke, 1976), it is rare that overwhelming fetal infection occurs during the first four months of pregnancy. It used to be accepted that the Langhans' layer of the chorion prevented the passage of the *T. pallidum* from the mother to the fetus. This layer disappears after the sixteenth week of gestation, allowing the fetus to be infected. An alternate theory has recently been accepted. This states that the damage of the infection is related to the fetal inflammatory response and that the response is not mounted until the second half of gestation. Thus, the lesions of syphilis do not become apparent until after 16 weeks' gestation, when the fetus develops a functional immunologic apparatus and the ability to mount a chronic inflammatory response (Silverstein, 1962; Harter & Benirschke, 1976). *T. pallidum* thrives in utero, leading to widespread dissemination of the organisms into many fetal tissues.

Clinical Manifestations

Mother. Syphilitic infection is systemic from its onset, and its manifestations are protean. The incubation time is 10 to 90 days; however, most primary lesions appear in about 21 days. The primary stage of syphilis is characterized by the chancre, a firm, nontender ulcer which is the first clinical evidence of the disease. There is usually only one chancre, which is most often present on labia majora, labia minora, or posterior fourchette. This lesion persists for 1 to 6 weeks and heals spontaneously, even without treatment.

Usually about 6 to 8 weeks, but possibly as long as 3 months, after the initial infection the secondary stage of syphilis begins. Secondary syphilis may involve any cutaneous or mucosal surface, as well as any organ. The skin lesions are papulomacular, copper colored, and symmetrical, and characteristically occur on the palms and soles. Moist cutaneous lesions are present on the vulvar, perianal, and perioral areas, and may coalesce to form condyloma lata. Mucous patches occur on the tongue, lips, buccal mucosa, and genital tract. The mucous patches and condyloma lata contain large numbers of spirochetes and are highly infectious. Generalized lymphadenopathy is commonly present. Other manifestations include periostitis, iritis, and meningitis.

Following this is the latent stage, during which there is no apparent clinical evidence of infection, although serologic tests for syphilis are still reactive. The latent phase is arbitrarily divided into early latent (less than 4 years after the initial infection), and late latent syphilis (over four years). During the early latent phase, 25 percent of cases undergo an exacerbation of the mucocutaneous lesions of secondary syphilis, which once more are infectious. Although the late latent phase is not infectious by sexual transmission, pregnant women may transmit the spirochetes across the placenta to their fetus.

Untreated individuals may remain in the latent phase of syphilis for the remainder of their lives. One third of cases of untreated syphilis develop tertiary syphilis, a reflection of a tissue-damaging immunologic response to previously deposited spirochetes. Neurologic, cardiovascular, visceral, osseous, and cutaneous lesions may occur. Since the advent of penicillin, fewer cases of tertiary syphilis are seen.

Neonate. The more recent the untreated maternal infection, the more disastrous the effects of syphilis on the fetus. Untreated syphilis contracted 6 to 12 months prior to pregnancy usually results in midtrimester abortion or intra-uterine fetal death. Pregnancy occurring during the early latent phase of syphilis may lead to intra-uterine fetal death or to a neonate with the stigmata of congenital syphilis infection. A woman who becomes pregnant in the late latent stage may deliver a normal-appearing infant with latent congenital syphilis, which might not become clinically apparent until later in childhood. Spirochettes affect every organ system; however, a predilection exists for the liver, mucus membranes, skin, bones, and central nervous system.

Congenital syphilis is divided into two categories. Early congenital syphilis appears prior to age 2, usually within 2 to 4 weeks after birth. It is characterized by mucocutaneous lesions, hepatosplenomegaly, generalized lymphadenopathy, anemia, osseous lesions such as osteochondritis and periostitis sometimes resulting in Parrot's pseudoparalysis, thrombocytopenia, jaundice, and CNS involvement. The cutaneous lesions may vary considerably in appearance, ranging from maculopapular lesions similar to those seen in adults to a pathognomonic extensive vesicular eruption involving the palms and soles. There is increased lacrimation secondary to iritis, and involvement of the mucosa of the nasal pharynx, resulting in a heavy mucoid nasal discharge known as "snuffles." The cutaneous and mucous lesions contain numerous spirochetes. Involvement of the mucocutaneous junction of the mouth and nose result in ulcerated areas and fissures, which leave residual scars or rhagades. Late con-

genital syphilis appears after age 2. The stigmata of late congenital syphilis include Hutchinson teeth, mulberry molars, interstitial keratitis, eighth cranial nerve deafness, saddle nose, rhagades, and saber shins.

Diagnosis

The diagnosis must be made from the clinical history and the physical examination of the mother, as well as from laboratory evaluation of the infant and the mother. The diagnosis of congenital syphilis in a newborn can be made by darkfield examination of the lesions.

All pregnant women should have a serologic test for syphilis (VDRL, Kahn, or Wasserman) at their first prenatal visit. Most screening tests utilize a non-treponemal antigen (Cardiolipin). These tests become positive one to three weeks after infection and 90 percent become negative within one year after treatment. The VDRL may be negative during primary syphilis; thus, diagnosis best relies on darkfield examination of suspicious lesions. Biologic false-positive VDRL reactions may also occur. Women who react positively to these tests should be evaluated with a test that employs a treponemal antigen. The fluorescent treponema antibody (FTA) test employs fluorescent staining to detect treponemal antibody. This test detects specific antitreponemal antibodies in patients with acquired syphilis. The FTA and the fluorescent treponemal antibody-absorption test (FTA-ABS) become positive within one to three weeks after infection, and remain positive for life. False-positives rarely occur. It has been recommended that serologic examination of the cerebrospinal fluid be included in the evaluation of pregnant women with positive VDRL and FTA-ABS tests (Jones & Harris, 1979). Failure to evaluate pregnant women with serologic evidence of syphilis for CNS involvement could lead to inappropriate therapy and management.

Serologic tests also may be used to detect congenital syphilis in newborns. A significantly higher (fourfold) titer in a newborn than in the mother confirms fetal infection. A rise in the serologic titer in the first few months of life also indicates fetal infection. The FTA-ABS test has been shown to be positive in infants with congenital syphilis (Alford et al., 1969); however, this may be because fetal IgM is directed against maternal IgG.

Treatment and Prevention

Prenatal screening for syphilis and treatment of infected pregnant women should prevent the disease in their offspring. In one study, 23 women were between 21 and 30 weeks' gestation when syphilis was diagnosed and treated (Taber & Huber, 1975). Only one of the infants born to these women weighed less than 2500 g, but eight had bilirubin values greater than 10 mg/dl. Three had abnormalities other than hyperbilirubinemia or low birth weight: two showed periosteal reaction of the long bones, and the third was dysmature. In contrast, of 22 untreated pregnant women in whom the diagnosis of maternal disease was made at the time of birth or soon thereafter, eight of their babies were born with abnormalities, which ranged from a very mild clinical disease to abnormal laboratory values in cerebrospinal fluid. Eleven babies were treated in the immediate newborn period, and the other 11 were discharged without therapy. All 11 untreated babies were later readmitted to the hospital with

overt disease. Treatment should thus be given to newborns born to infected mothers who were not treated with adequate penicillin dosages, regardless of when syphilis occurred in pregnancy.

Screening of all pregnant women should be carried out with serologic tests for syphilis, and in those who are at "increased risk" a second screen should be done late in pregnancy. Pregnant women who have been treated for syphilis should have monthly quantitative nontreponemal serologic tests (VDRL) for the remainder of the pregnancy. All newborns of mothers with a reactive VDRL should have a careful laboratory clinical examination at birth, be treated, and undergo follow-up examinations at regular intervals.

15

Chemical and Environmental Teratogens

In the 1940s reports appeared that introduced the age of modern experimental teratology. First, Gregg (1941) recognized the association between maternal rubella infection and abnormal fetal development. Second, Warkany and Nelson (1940) and Warkany and Schraffenberger (1947) published a series of articles demonstrating the adverse effects of environmental factors on intra-uterine mammalian development. Studies concerning the effects of experimental environmental manipulation on embryogenesis continued to accumulate over the ensuing 15 to 20 years, but the significance of teratology to the practicing obstetrician was recognized only after a drug-induced catastrophe: In 1961 Lenz (1961) and McBride (1961) simultaneously reported that an epidemic of limb-reduction malformations in newborns was due to maternal ingestion of a sedative, thalidomide, early in pregnancy. This finding had obstetric, legal, pharmaceutical, and governmental regulatory reprecussions, which are still ongoing. Every physician should be exquisitely discriminating when using medication during pregnancy.

The scope of drug usage in pregnancy is revealed by the list of 900 different drugs taken by the pregnant women in the NIH Collaborative Perinatal Study (Heinonen, Sloan, & Shapiro, 1977). Pregnant women take an average of almost four drugs (excluding nutritional supplements) during pregnancy, and only 20 percent abstain from all drug usage (Forfar & Nelson, 1973). Perhaps even more significant is that 40 percent of the women took medication during the first trimester, and approximately one half of the total drug consumption during pregnancy occurred during the period of organogenesis (Forfar & Nelson, 1973; Schenkel & Vorherr, 1974). Comparison of drug administration during pregnancy in 1960 (Peckham & King, 1963) and in 1973 (Hill) is not very encouraging. The later study found an increased frequency of use of analgesics, antibiotics, antiemetics, antacids, antihistamines, and diuretics and a decreased use of iron and vitamins.

Wilson (1972) pointed out that 65 to 70 percent of developmental defects are of unknown origin and that only 2 to 3 percent are known to be due to

drugs and environmental chemicals. This is somewhat less than reassuring, in view of the overwhelming size of the "unknown" category. It is difficult to blame specific defects or constellations of defects on specific drugs because of many confounding factors, including that (1) the drug may be administered as therapy for an illness which itself causes the malformation, (2) the fetal malformation may cause maternal symptoms which are treated with a specific drug, (3) the drug may inhibit the abortion of an already malformed infant, and (4) the drug may commonly be employed in combination with a second drug, and the interaction between the two drugs may cause the malformation.

A second problem in assigning etiologies to birth defects is that any congenital malformation series under study may include only structural malformations, all congenital defects, or all disorders with a possible prenatal etiology. As the structural malformation group is progressively diluted, the likelihood of assigning causality decreases (Klemetti, 1977). Additionally, since most clinical teratology studies do not contain appropriate controls, they are of limited usefulness. It is nevertheless possible to categorize many drugs as definitely or possibly teratogenic and to indicate some drugs that involve little or no teratogenic risk under normal conditions of use. (See Tables 15-1 and 15-2.)

Table 15-1
Chemical Teratogens

Definite relationship to fetal abnormalities

- Alcohol
- Diphenylhydantoin
- Folic acid antagonists
- Inorganic iodides
- Lithium
- Organic mercury
- Sex steroids
- Streptomycin
- Tetracyclines
- Thalidomide
- Thiourea compounds
- Trimethadione
- Warfarin

Probable relationship to fetal abnormalities

- Alkylating agents
- Chlorobiphenyls
- Diazepam
- Kanamycin

Questionable relationship to fetal abnormalities

- Amphetamines
- Chlordiazepoxide
- Clomiphene
- Penicillamine
- Diphenhydramine
- Ethionamide
- General anesthesia (chronic exposure)
- Gonadotropins
- Haloperidol
- Lysergic acid diethylamide (LSD)
- Meprobamate
- Metronidazole
- Oral hypoglycemic agents
- Quinine
- Phenothiazines
- Cigarette smoking

No relationship to fetal abnormalities

- Bendectin
- Corticosteroids
- General anesthesia (short-term exposure)
- Heparin
- Isoniazid
- Meclizine
- Penicillin
- Sulfonamides

Table 15-2
Some Drugs with Potential Adverse Effects on the Neonate

Drug	Potential Adverse Effect
Azathioprine	Decreased immunologic competence
Chloramphenicol	Gray syndrome
Hexamethonium	Paralytic ileus
Naphthalene	Hemolysis (G6PD deficiency)
Narcotics addiction	Withdrawal
Nitrofurantoin	Hemolysis (G6PD deficiency)
Oxytocin	Hyperbilirubinemia
Phenobarbital (excess)	Neonatal bleeding
Propranolol	Hypoglycemia and bradycardia
Quinine	Thrombocytopenia
Reserpine	Nasal congestion
Salicylates	Platelet dysfunction
Cigarette smoking	Intra-uterine growth retardation
Sulfonamides	Hyperbilirubinemia
Thiazides	Thrombocytopenia and electrolyte imbalance

ANTINEOPLASTIC AGENTS

Antineoplastic agents kill cells, especially rapidly dividing cells, which are characteristic not only of a neoplasm but also of a fetus. Therefore, it is not surprising that these compounds are among the most potent teratogens known. Antineoplastic agents may be classified as alkylating agents, antimetabolites, or miscellaneous cytotoxic drugs. Hormones and antibiotics that may be used as anti-tumor medications will be discussed in other sections of this chapter.

Alkylating agents replace protein and nucleic acids and form crosslinks with DNA, causing its inactivation. The most commonly used alkylating drugs are busulfan, chlorambucil, cyclophosphamide, nitrogen mustard, triethylene melamine (TEM), and triethylene thiophosphoramide (thio TEPA). Currently, busulfan therapy for chronic granulocytic leukemia probably accounts for the greatest number of exposed fetuses. Thirty-five cases of busulfan use during pregnancy have been reported, with four anomalous fetuses. Diamond, Anderson, and McCreadie (1960) described an infant with growth retardation, cleft palate, micropthalmus, cloudy corneas, hypoplastic ovaries, and poorly developed external genitalia. The mother had been irradiated and received 6-mercaptopurine in addition to busulfan during this pregnancy. de Rezende, Coslovsky, and de Aguiar (1965) reported an abortus with numerous unspecified malformations from a mother who received only busulfan. Boros and Reynolds (1977) described an infant with growth retardation, absence of the right kidney, hydronephrosis of the left kidney, and hepatic subcapsular calcifications born to a patient treated with busulfan and allopurinal. Pyloric stenosis was seen in one infant exposed only after the first trimester. Of the 12 infants exposed to busulfan in utero for whom birth weight and gestational age were reported, 8 manifested severe intra-uterine growth retardation. A recurrent problem with numbers such as these is that the likelihood is greater for a

positive rather than a negative case report to be published. In the absence of a registry of all pregnancies exposed to a rarely used drug, the prevalence of affected neonates is thus probably inflated. Nevertheless, on the basis of the evidence presented thus far, busulfan should be considered a teratogen causing both malformations and intra-uterine growth retardation.

Chlorambucil administration was associated with one case of unilateral renal and ureteral agenesis out of six fetuses exposed to the drug (Shotton & Monie, 1963). Cyclophosphamide used during five pregnancies has been associated with 2 cases of extremity defects (one infant was stillborn) (Greenberg & Tanaka, 1964; Toledo, Harper, & Moses, 1971). Nicholson (1968) reviewed 11 pregnancies during which nitrogen mustard was administered; there were seven normal infants, one induced abortion, and three spontaneous abortions. Since then, two other infants with malformations who were exposed to nitrogen mustard as part of multiple drug therapy have been reported (Garrett, 1974; Mennuti, Shepard, & Mellman, 1975). At least nine fetuses have been exposed in utero to TEM or thio TEPA, with no resulting anomalies (Nishimura & Tanimura, 1976).

The antimetabolites are structural analogs of naturally occurring substances and either cause a deficiency of, or replace, the corresponding compound. From a teratogenic point of view the most infamous antimetabolite is the folic acid antagonist aminopterin, which was used as an abortifacient in the 1950s (Thiersch, 1952, 1956; Goetsch, 1962). Seventy percent of the exposed fetuses aborted and many of the abortuses demonstrated abnormalities. In the pregnancies that continued, one third of the infants showed deformities. The 11 anomalous fetuses and newborns (one was also exposed to thalidomide) demonstrated striking bone maldevelopment, globular heads, abnormal triangular facies, small ears, and markedly retarded growth. Another commonly used folic acid antagonist, methotrexate, also has been linked causally with two infants born with multiple skeletal defects (Milunsky, Graef, & Gaynor, 1968; Powell & Ekert, 1971). These two cases are derived from a sample of approximately 40 infants reported to have been exposed to methotrexate in utero. The recent enthusiasm for using methotrexate to treat psoriasis will probably lead to an increased number of fetal exposures.

Interestingly, significant numbers of pregnancies have been treated with purine antagonists without adverse effects. Of 50 infants exposed to 6-mercaptopurine, the only one with anomalies was also exposed to busulfan. Another purine antagonist of great importance is azathioprine, an immunosuppressant used to treat the increasing number of women with renal transplants who are becoming pregnant. There have been over 125 conceptions in transplant recipients, with a single case of pulmonic stenosis being the only reported structural defect (Penn et al., 1971). One infant whose father was taking azathioprine was born with a myelomeningocele and, secondarily, bilateral dislocated hips and bilateral talipes equinovarus (Tallent, Simmons, & Najarian, 1970). The number of anomalies reported thus did not differ from that expected in a control population. There have been, however, a number of newborns exposed to azathioprine in utero who were born with lymphopenia, adrenal insufficiency, growth retardation, and an increase in chromosome breakage (Nolan et al., 1974). More extensive surveillance of the development, immunocompetence,

and chromosomal complement in neonates exposed to azathioprine is required before the safety of this drug can be verified.

Several infants have been exposed in utero to other cytotoxic agents, with the only anomalies occurring in one newborn who had been exposed to vinblastine, procarbazine, and nitrogen mustard. Very few pregnancies have occurred in women treated with each of these drugs; however, the absence of reported defects should not be interpreted as evidence for a total lack of teratogenicity.

ANTIMICROBIAL AGENTS

The sulfonamides may be a hazard to newborns because they compete with bilirubin for albumin-binding sites; however, they present little danger as teratogens. Although Nelson and Forfar (1971) reported that a higher proportion of mothers of infants with anomalies than control mothers took sulfonamides during pregnancy, this was not confirmed in other studies (Pap & Tarakhovsky, 1967; Richards, 1972). Trimethoprim/sulfamethoxazole has been advocated for therapy of urinary tract infections and is of some concern since both components are teratogenic to rats (Udall, 1969). Williams et al. (1969) reported no abnormalities in ten pregnancies exposed to this combination during the first trimester. Nevertheless, until more animal and human data are available, this drug should be avoided during pregnancy unless there are no other suitable alternatives.

Penicillin has been widely used during pregnancy for the last 20 years without any implication of teratogenicity, and it may be considered a "safe drug" during pregnancy. However, there has been no systematic epidemiologic study of the prenatal effects of the newer drugs chemically related to penicillin. Chloramphenicol is another antibiotic that has been used widely during pregnancy with no demonstrable adverse fetal effects. It is, however, quite toxic to the newborn, causing the so-called "gray syndrome" or "gray ghost syndrome" of abdominal distention, cyanosis, and vascular collapse. Chloramphenicol therapy of the mother during labor may cause toxic blood levels in the neonate; therefore, use of this antibiotic should be restricted during late pregnancy. Erythromycin exposure during pregnancy has been associated with one infant with exencephaly and other defects (Liban & Abramovici, 1972). However, examination of a published photograph indicates that the fetus had the amniotic band syndrome, a known sporadic malformation complex; thus, the anomalies should not have been attributed to the erythromycin exposure.

All tetracyclines form a chelated complex with calcium orthophosphate and become incorporated into bones and teeth if present during the period of calcification. Deciduous (but not permanent) teeth begin calcifying during the fifth month of fetal life and, if exposed to tetracycline, will appear yellow and fluoresce bright yellow, with intensity directly proportional to the total amount of tetracycline taken (Wallman & Hilton, 1962). After years of exposure to light, the yellow color turns to gray or brown. In addition to this cosmetic problem, teeth containing tetracycline are more susceptible to caries and display enamel hypoplasia. Bones begin calcifying at two months of fetal life and also can

incorporate tetracycline if exposed in utero; however, tetracycline-containing bones do not appear to be more liable to fracture (Tötterman & Saxén, 1969). The suspicion that tetracyclines might be implicated in developmental anomalies of the extremities has been raised, but numerous studies of tetracycline administration at the critical time for limb development have not corroborated this concern.

Streptomycin derivitives have been used extensively as antituberculous agents. The ototoxicity known to occur in adults can apparently also occur in the fetus, and there have been more than 30 cases of hearing deficit and eighth cranial nerve damage in infants exposed to streptomycin derivatives in utero. The risk of fetal ototoxicity ranged from 3 percent (Rasmussen, 1969) to 11 percent (Ganguin & Rempt, 1970). Other aminoglycosides (e.g., neomycin, kanamycin, and gentamicin) are also potentially ototoxic: nine of 391 infants exposed in utero to kanamycin had hearing loss (Fujimori, 1967). Aside from their ototoxicity, however, the aminoglycoside antibiotics have shown no other teratologic effect.

The standard antituberculous agents have been isoniazid and para-aminosalicyclic acid. Studies of many pregnant women treated for tuberculosis have revealed no increased incidence of anomalies in their offspring (Marynowski & Sianozecke, 1972; Jentgens, 1973). Potworowska, Sianozecke, and Szufladowicz (1966) reported that 7 of 23 infants of mothers treated with ethionamide had anomalies, with CNS defects being the most common. Although this has not been verified in other studies (Bignall, 1965; Zierski, 1966), it seems advisable to avoid this drug in pregnancy. The newer antituberculous agents, rifampin and ethambutol, have been used in hundreds of pregnant women without evidence of any teratogenic effect (Jentgens, 1973). Data were collected on 229 conceptions during which these drugs were administered; 6 of 202 exposed infants (3 percent) had malformations (Steen & Stainton-Ellis, 1977). No control data were reported, but the malformation rate is not significantly higher than that of the population at large.

Antimalarial agents have been suspected of being teratogenic, but no systematic or epidemiologic surveys are available. Maternal ingestion of quinine was reported to cause neonatal deafness over 100 years ago (Roberts, 1870). Winckel (1948) reviewed 17 cases of ear or eye anomalies following in utero exposure to quinine, but in view of the widespread use of the drug he felt that a causal relationship was unlikely. Large doses of quinine also have been used as an abortifacient. Tanimura (1972) surveyed the literature and found reports of 20 malformed infants who had been exposed in this manner; however, the total sample size was not stated. Chloroquine has now replaced quinine as an antimalarial drug, and it is also employed in the therapy of systemic lupus erythematosus. The only report implying teratogenicity concerns a woman who took chloroquine during four pregnancies; she had one spontaneous abortion and gave birth to three children with congenital defects (one with Wilm's tumor and hemihypertrophy; one with sensorineural deafness; and one with sensorineural deafenss, and mental and physical delayed development); she also had three normal children when not taking chloroquine during pregnancy (Hart & Nauton, 1964). A third antimalarial agent, quinacrine hydrochloride, has been associated with a single newborn with renal agenesis, spina bifida, and

megacolon (Vevera & Zatloukal, 1964). In the absence of any systematic studies it is difficult to arrive at a final conclusion regarding the antimalarial compounds; thus, any pregnant woman taking these drugs should be apprised of this uncertainty.

Metronidazole is commonly used to treat *Trichomonas vaginalis* and *Entamoeba histolytica*, and also may have merit in treating anaerobic infections. However, this agent and its urinary metabolites have been shown to be mutagenic in a bacterial test system (Legator, Connor, & Stoeckel, 1975). Metronidazole also significantly increases chromosome aberrations in treated patients' lymphocytes (Mitelman, Hartley-Asp, & Ursing, 1976). Peterson, Staunsh, and Ryder (1966) studied 206 pregnancies exposed to metronidazole and found no increase in the overall prevalence of congenital anomalies, but did find four infants with malformations among the 55 exposed during the first trimester. The significance of these findings to the individual patient or to the fetus is unclear, but discretion suggests that in pregnancy this drug should be avoided if possible.

Many antibiotics inhibit DNA or RNA synthesis, making them potential antiviral or antineoplastic agents. Those which have been studied (actinomycin D, mitomycin C, adenine arabinoside, fluoro-deoxyuridine, and idoxuridine) are all teratogenic in rodents. Thus far, there have been no published reports associating these drugs with human fetal malformations, but neither have there been series of negative cases attesting to their safety.

HORMONES AND ANTIHORMONES

Adrenal corticosteroids are potent palatal teratogens in rodents. Exhaustive literature surveys of 688 humans exposed *in utero* to corticosteroids or ACTH revealed 20 newborns (3 percent) with congenital anomalies, a frequency not different from that of control data (Bongiovanni & McFadden, 1960; Serment & Ruf, 1968). Although only six cases of acute adrenal insufficiency in the neonate have been reported, this is a risk to which the pediatrician should be alert. The likelihood of stillbirth or placental insufficiency with intra-uterine growth retardation is difficult to evaluate, because no untreated control population exists. It is likely that these complications are associated with the underlying maternal disease requiring corticosteroid therapy rather than with the drug itself.

Androgens, including 19-nortestosterone derivatives found in some birth control pills, can masculinize the female fetus (see Chapter 10). Labioscrotal fusion can occur if exposure occurs prior to the twelfth week of gestation; clitoral and labia majora enlargement without labioscrotal fusion can occur when the exposure occurs only in the second and third trimesters. The incidence of fetal masculinization varies according to the drug and dosage. An 18-percent incidence of masculinization was noted for female infants exposed to norethindrone acetate (Jacobson, 1962), compared to only 1 percent of those exposed to medroxyprogesterone acetate (Burstein & Wasserman, 1964). Male fetuses do not appear to be adversely affected, but may have genital development somewhat advanced for their gestational age. In animals the antiandrogen

cyproterone acetate may induce feminization of the male fetus (Steinbeck & Neumann, 1972).

The possibility of transplacental chemical carcinogenesis emerged in 1971 when Herbst and colleagues reported eight cases of vaginal adenocarcinoma in young women, seven of whom had been exposed to diethylstilbestrol (DES) in utero. Although over 200 cases of vaginal adenocarcinoma associated with prenatal DES exposure have been reported, there were probably 500,000 patients at risk; thus, the chances of developing cancer during the first two to three decades of life is low. In addition, the carcinoma-in-situ prevalence rate among these patients is 1.4 percent (Mattingly & Stafl, 1976). A prevalence rate of 30 to 90 percent for vaginal adenosis has also been reported, but the meaning of this lesion regarding future development of cancer is unknown (Gunning, 1976). Male fetuses exposed to DES have a 25-percent incidence of epididymal cysts, hypotrophic testes, and capsular induration of the testes, and a 32-percent incidence of abnormal spermatozoal analyses (Gill, Schumacher, & Bibbo, 1977).

The only other estrogenic compound implicated teratologically has been clomiphene citrate. There have been approximately a dozen case reports of malformed infants conceived after clomiphene stimulation, with half of these having a neural tube defect. Two surveys totaling 321 similarly treated pregnancies found four infants with major malformations, a rate certainly within expected limits (Hack et al., 1972; Harlap, 1976). A more disturbing question exists regarding the association of clomiphene or gonadotropins with meiotic nondisjunction. Oakley and Flynt (1972) reported twice the expected rate of trisomy 21 among offspring in which conception followed induced ovulation. In addition, spontaneous abortions of pregnancies conceived the month of or month following ovulation induction by clomiphene or menotropin have a significantly higher incidence of aneuploidy (Boué, Boué, & Lazar, 1975b). Careful monitoring of pregnancies conceived following induced ovulation should resolve these questions. Until the question of whether ovulation induction is related to meiotic nondisjunction rates is answered, women who conceive during such therapy should possibly be apprised of the availability of amniocentesis to verify the chromosomal normality of the fetus.

Progestogens or combinations of estrogens and progestogens (birth control pills) have been employed widely in early gestation either as a withdrawal pregnancy test or to "support" a threatened abortion. In addition, some women have continued to take birth control pills after conceiving, unaware of their pregnancy. The teratologic implications of this exposure to progestogens have been debated for years. In 1967, Gal, Kirman, and Steen reported that women who gave birth to infants with meningomyelocele or hydrocephaleus were significantly more likely than control women to have had a withdrawal pregnancy test in the first trimester. However, a similar retrospective study of 271 women revealed no association between hormonal pregnancy tests and births of infants with a neural tube defect, and suggested that Gal, Kirman, & Steen's control population had been geographically different from the index population (Laurence et al., 1971).

In 1973 it was found that 6 of 76 mothers of children with transposition of the great vessels had been given sex steroids for therapy of threatened abor-

tions during the first six weeks of pregnancy (Levy, Cohen, & Fraser, 1973). However, a similar retrospective case control study the following year failed to support the suggested association between transposition complexes and prenatal exposure to progestogens (Mulvihill, Mulvihill, & Neill, 1974). The Jerusalem Perinatal Study of 11,468 newborns found an excessive number of heart and blood vessel developmental defects in infants exposed to progestogens in utero (Harlap, Prywes, & Davies, 1975). The only prospective study contains 100 infants exposed to progestogens in the first trimester; six had major malformations, of which four were congenital heart anomalies (Nora et al., 1976). A matched case control study of 70 newborns with specified cardiac defects indicated that in utero exposure to exogenous sex steroids was 6.5 times more common than among controls (Janerich et al., 1977). The Collaborative Perinatal Project report on 50,282 pregnancies noted cardiovascular defect rates of 18.2/1000 for children exposed to progestogens during the first four months of pregnancy, 21.5/1000 for children whose mothers specifically used combination oral contraceptives during early pregnancies, and 7.8/1000 for non-exposed children (Heinonen et al., 1977). The rate of cardiovascular malformations was not increased when hormone exposure occurred only after the fourth month of gestation. Medroxyprogesterone was the only specific hormone for which a statistically significant association with cardiac malformation could be demonstrated with the available data. No anomalies other than cardiovascular were associated with early fetal exposure to progestogens. The conclusion from these studies is that progestogen exposure in the first trimester is associated with an increased prevalence of cardiovascular anomalies.

A somewhat different variation on this theme was the claim that progestogen exposure was related to the occurrence of the VACTERL syndrome (vertebral, anal, cardiac, tracheo-esophageal, renal, and limb anomalies) (Nora & Nora, 1973, 1975). This association has not been supported by other investigators (David & O'Callaghan, 1974; Heinonen et al., 1977).

A sex-specific effect of in utero progestogens was claimed in a case control study reporting a significant increase in congenital limb-reduction defects in males (Janerich, Piper, & Glebatis, 1974). One small supportive study from Sweden has been published (Hellstrom, Lindstein, & Nillson, 1976), but the Collaborative Perinatal Project data are inconclusive.

Although studies of general malformation rates after in utero exposure to progestogens tend to be inconclusive or negative (Oakley & Flynt, 1973; Harlap, Prywes, & Davies, 1975; Goujard & Rumeau-Rouquette, 1977), this does not negate the reports of associations with specific malformations. The absolute increase in the risk of having an infant with a congenital anomaly appears to be small with progestogen exposure, and the influence of confounding factors and of specific hormone preparations is still not clear. Although inadvertent exposure of the fetus to progestogens will continue to occur, iatrogenic exposure appears unwarranted as there is no evidence progestogens are effective therapy for threatened abortions (Glass & Golbus, 1978), and there are simpler, quicker, more accurate pregnancy tests.

Other than gonadotropins, the only pituitary hormone implicated as having a possible deleterious fetal effect is oxytocin. Mast et al. (1971) first suggested a relationship between the use of oxytocin and neonatal hyperbilirubinemia,

and verification by others followed (Davies et al., 1973; Chalmers, Campbell, & Turnbull, 1975). Hyperbilirubinemia secondary to oxytocin is independent of gestational age, birth weight, or Apgar score, but whether it is caused by a redistribution of blood between fetus and placenta or by a mechanism involving red blood cell breakdown or hepatic enzyme maturation is unknown.

Thyroid hormones have been used during pregnancy for many years, but they appear to cross the placenta very poorly if at all. In spite of widespread use there have been only a few isolated case reports of infants with congenital malformations after maternal thyroid ingestion. On the other hand, congenital goiter and/or hypothyroidism attributable to maternal intake of antithyroid drugs or iodine is well documented. The thiourea agents, which block the iodination of tyrosine and the coupling of diiodotyrosine, are widely used in the therapy of maternal hyperthyroidism. These drugs readily cross the placenta and may cause fetal hypothyroidism and a compensatory hypertrophic goiter. Neonatal goiter induced by thiourea derivatives tends to be minimal, and does not usually cause respiratory obstruction. Cretinism is avoided by administering a minimal maintenance dose to the mother and signs of neonatal hypothyroidism disappear over 2 to 6 weeks. Breast feeding is contraindicated if the mother continues on thiourea therapy. Burrow et al. (1968) compared children exposed to propylthiouracil in utero to unexposed siblings and found no significant differences in intelligence, height, or bone age. Only methimazole has been associated with a specific ulcer-like midline scalp defect; five infants showed this defect following in utero exposure (Milham & Elledge, 1972; Mujtaba & Burrow, 1975).

Inorganic iodides also have been used to treat hyperthyroidism and are often used as mucolytic agents in the treatment of asthma. Iodides readily cross the placenta, interfere with fetal thyroid production, and may cause massive thyroid enlargement. The huge goiter may prevent fetal swallowing, leading to polyhydramnios, and it has been responsible for a number of neonatal respiratory obstruction deaths. A high percentage of the survivors demonstrated varying degrees of cretinism (J. Wolff, 1969; Carswell, Kerr, & Hutchinson, 1970). The excessive use of iodides during pregnancy and breast feeding by women taking iodide-containing medications should be avoided. Finally, the fetal thyroid concentrates iodine after 10 weeks of pregnancy (Shepard, 1967); thus, radioactive iodine ingested by the mother after this time will destroy fetal thyroid tissue. The use of ^{131}I during pregnancy, even for diagnostic purposes, is therefore contraindicated.

Considering the known increased incidence in congenital anomalies in the offspring of diabetic women, it is difficult to evaluate the teratogenicity of the hypoglycemic agents. The relatively similar patterns of malformations (except for caudal regression syndrome) in babies of nondiabetic and diabetic mothers and the likely increased incidence of anomalies in offspring of nontreated class A diabetic women makes it improbable that insulin per se is teratogenic. The oral hypoglycemic agents, particularly sulfonylureas, have been criticized as potential teratogens. However, despite their marked teratogenicity in rodents, there is no evidence of an increased rate of anomalies in general nor of any specific anomaly in human neonates exposed in utero.

COMMONLY ABUSED CHEMICAL AGENTS

One of the most significant teratogenic risks to the fetus is maternal use of alcohol. Alcoholism is the most common drug abuse problem in contemporary society, and affects at least 1 to 2 percent of women of childbearing age. In 1973 Jones et al. reported eight unrelated children with similar malformations born to mothers with chronic alcoholism. The fetal alcohol syndrome includes IQ $\leq$ 79 (44 percent of exposed fetuses), intra-uterine growth retardation (32 percent), ocular anomalies (25 percent), joint anomalies (25 percent), and cardiac murmurs (25 percent) (Jones et al., 1974). Additionally, these neonates may go through an alcohol withdrawal reaction (Pierog, Chandavasu, & Wexler, 1977). More moderate alcohol consumption during pregnancy may also carry a significant risk (Streissguth, Hanson, & Smith, 1977). Of 16 women who drank 2 ounces or more of alcohol daily, 19 percent were delivered of infants with evidence of the fetal alcohol syndrome. Of 54 women who consumed between 1 and 2 ounces of alcohol daily, 11 percent of the children had at least some features of the fetal alcohol syndrome.

Maternal narcotic addiction is directly related to such obstetrical complications as intra-uterine growth retardation, premature labor, breech delivery, and toxemia. The neonate has a 65- to 75-percent chance of undergoing withdrawal, which has an associated 3- to 5-percent mortality rate. Several large series have demonstrated no increased risk of congenital anomalies per se (Reddy, Harper, & Stern, 1971; Zelson, Rubio, & Wasserman, 1971). Neonatal withdrawal also has been reported following excessive maternal propoxyphene use.

Maternal smoking is a well-established cause of intra-uterine growth retardation (W. Simpson, 1957). There is also evidence that smoking increases the risk of stillbirth, particularly for women who are already at risk (Rush & Kass, 1972), and that it increases the perinatal death rate (Meyer & Tonascia, 1977). The influence of maternal smoking on the incidence of congenital malformations is unclear; half the studies report an increased incidence, whereas the other half claim no such increase.

Lysergic acid diethylamide (LSD) use during pregnancy has been the subject of a number of reviews. Long (1972) studied 161 children born to parents who ingested LSD before conception and/or during the pregnancy. He found only 5 infants (3 percent) with limb reduction anomalies, which ostensibly could be explained in no way other than attributing them to the LSD usage. It must be recalled, however, that these patients often have a history of multiple drug ingestion and are generally at an increased risk of numerous reproductive complications. Jacobson and Berlin (1972) studied 148 pregnancies of LSD users and found that 3.4 percent of the infants had limb bud anomalies and a 9.6 percent had CNS defects. In view of this last finding it might be appropriate to offer LSD users both sonography and amniocentesis for an α-fetoprotein determination. Indeed, this is the policy of at least one large prenatal diagnosis program (University of California at San Francisco, unpublished data). This type of prospective data eventually will help establish the risk of a prenatally detectable neural tube defect. There has been much discussion of the effect of

LSD on chromosomal breakage, but Dumars (1971) found no increase in chromosomal breakage or rearrangements in 41 infants whose parents were LSD users.

The teratogenicity of marijuana is unknown. There is no evidence of human teratogenicity, but likewise no assurance that marijuana exposure is safe for the fetus. Women thus should be counseled not to use marijuana during pregnancy.

ANTIANXIETY AGENTS

This class of drugs is of particular interest because it contains the classic teratogen, thalidomide. Thalidomide is a hypnotic agent that was widely used outside of the United States as a tranquilizer and sedative. The spectrum of thalidomide embryopathy includes reduction deformities of the limbs, ear anomalies, nasal abnormalities, defects of the middle lobe of the right lung, cardiac malformation, pyloric or duodenal stenosis, and gastrointestinal atresias. Observations that an affected girl showed Müllerian aplasia suggest that gynecologists should become more involved with these patients (Hoffmann, Grospietsch, & Kuhn, 1976).

The antianxiety agents or minor tranquilizers are among the drugs most commonly used by American women. Several prospective studies have examined the safety of meprobamate and chlordiazepoxide. The best study is also the most disturbing. Milkovich and van den Berg (1974) observed severe anomalies in 12.1 percent of infants exposed to meprobamate, in 11.4 percent of infants exposed to chlordiazepoxide, and in 2.6 percent of infants born to mothers diagnosed as anxious but not treated. Drug exposure occurred in the first 43 days after the last menstrual period, and the children were followed for 5 years. Hartz et al. (1975) reported the Collaborative Perinatal Project data and found no increased risk of anomalies from exposure to these two drugs. This project reported all malformations found in the first year of life and had a base rate almost three times as high as the previous study. Thus, if the main drug effect was either severe defects or those more likely to be noticed after the first year of life, the different results of these studies would be understandable. One other smaller study recorded malformations noted in the first six weeks of life (Crombie et al., 1975). No adverse effects attributable to chlordiazepoxide were demonstrated, but meprobamate exposure in the first trimester was associated with a significant increase in malformations. The safety of meprobamate and chlordiazepoxide should be considered questionable but no definitive answer is currently available.

The studies on diazepam are all retrospective case control comparisons. Suspicions were first raised by a report from the Center for Disease Control, which demonstrated that in infants exposed to diazepam during the first trimester there was a fourfold relative risk for cleft lip with or without cleft palate (Safra & Oakley, 1975). However, this association was found during a multiple-association search; thus it needs to be interpreted with caution, as noted by the authors. This report prompted review of the Finnish Register of Congenital Malformations, which confirmed a significant increase in cleft palate in neo-

nates with first trimester exposure to diazepam (Saxén & Saxén, 1975). Further confirmation was furnished by a Norwegian study, which found a sixfold increase in oral clefts among newborns who had been exposed to diazepam during the first trimester (Aarskog, 1975). Pregnant women inadvertently exposed to diazepam should be forewarned, but it must be remembered that even a sixfold increase in oral cleft will produce less than a 1-percent incidence of affected infants.

ANTIPSYCHOTICS

Debate about the teratogenicity of the phenothiazine compounds has raged for almost two decades and still continues. Two large recent surveys serve to summarize the dilemma. A prospective French survey included 12,764 women of which 315 took phenothiazines during the first trimester (Rumeau-Rouquette, Goujard, & Huel, 1977). The malformation rate among the exposed infants was significantly higher than in the control group. Specifically, phenothiazines with a 2- or 3-carbon aliphatic side chain, rather than those with piperazine or piperidine side chains, appeared to be implicated as teratogens. However, analysis of the Collaborative Perinatal Project data found no increase in anomalies among phenothiazine-exposed neonates (Slone et al., 1977). Examining specific malformations for any association showed a significantly increased standardized relative risk only for cardiovascular defects, and even this association is doubtful in the context of multiple comparisons. However, so long as such uncertainty about the safety of these drugs remains, they should be employed in pregnancy only after extensive consultation between psychiatrist and obstetrician and only when absolutely necessary.

Haloperidol, a butyrophenone derivative, is used to treat schizophrenia and agitated psychoses. There have been two case reports of limb malformations in infants exposed to haloperidol early in the first trimester (Dieulangard, Coignet, & Vidal, 1966; Kopelman, McCullar, & Heggeness, 1975). However, there were no malformations among 189 neonates exposed in utero when their mothers took haloperidol as an antiemetic (Van Waes & Van de Velde, 1969). Until the safety of this drug in pregnancy is better established, it should be employed only where no safer alternative is available.

The possibility that tricyclic antidepressants might cause limb reduction defects was raised by McBride (1972), but no support for the teratogenicity of these compounds was found in later retrospective studies (Banister et al., 1972; Rachelefsky et al., 1972).

The amphetamine stimulants are also phenothiazine derivatives. A large retrospective study reported more congenital malformations among infants exposed in utero to amphetamines than to those not exposed (Nelson & Forfar, 1971), and a case-control survey of newborns with cardiovascular anomalies revealed an association with prenatal amphetamine exposure (Nora, McGill, & McNamara, 1970). The one large prospective study that speaks to the issue did not find an association with severe anomalies per se or specifically with cardiovascular defects; however a suggestive association with oral clefts was observed (Milkovich & van den Berg, 1977). Four cases of biliary atresia have

been reported in newborns exposed to amphetamines in the second and third gestational months, and this is noteworthy because of the rarity of the lesion (Levin, 1971). Thus, there may be some teratogenic risk to amphetamines, and continued surveillance is warranted.

ANTIMANIC AGENTS

Lithium carbonate has been widely employed for patients with manic-depressive psychosis. Because of concern about potential teratogenicity, a registry of lithium babies was established in Scandinavia and later in California. To these two registries 166 infants have been reported; 18 had malformations, 13 involving the heart and great vessels (Schou, 1976). The most common defect is the rare Ebstein's anomaly, which has occurred in at least four infants (Weller, 1974). A follow-up of the children born without malformations showed no increased frequency of physical or mental problems (Schou, 1976). At this time, lithium should be considered a teratogen and its use in pregnant women and in women likely to conceive avoided. Because lithium is present in breast milk, it also would be advisable for mothers receiving lithium not to nurse their infants.

ANTICONVULSANTS

Approximately 1 of every 200 pregnant women is epileptic, and anticonvulsant therapy is usually continued throughout the pregnancy. The teratogenicity of antiepileptic drugs was first questioned by Janz and Fuchs (1964), who retrospectively surveyed 246 epileptic women, finding five malformed infants in the treated group and none in the untreated group. Because only liveborns were studied and the numbers were small, they were unwilling to draw any conclusions. Over the next eight years there were many positive case reports and a few series that suggested that cleft lip with or without cleft palate occurred more frequently among infants exposed to antiepileptic medications in utero (Meadow, 1970; Elshove & van Eck, 1971). Starting in late 1972, however, more data implicating anticonvulsants as teratogens were reported, without mentioning specific agents. Speidel and Meadows (1972) found a threefold increase in the malformation rate among infants exposed in utero to antiepileptics compared to control infants, and similar ratios were reported by other series (Bjerkedal & Bahna, 1973; Lowe, 1973; Miller & Nevin, 1973). Niswander and Wertelecki (1973) claimed that one confounding factor was that untreated epileptic women were at increased risk of delivering a malformed infant. However, many other studies have found no difference in the malformation rate among control infants and infants of untreated epileptics (Elshove & van Eck, 1971; South, 1972; Koppe et al., 1973; Lowe, 1973; Monson et al., 1973; Annegers et al., 1974). Whether or not a woman actually experienced convulsions during the pregnancy does not influence the rate of either minor or major malformations among her offspring (Shapiro et al., 1976b). Our analysis of the literature indicates that exposure of the fetus to antiepileptics introduces a twofold to

fivefold increase in the risk of having an infant with anomalies, with specific increases in oral clefts and congenital heart defects (Annegers et al., 1974; Hill et al., 1974; Janz, 1975).

The concept of a specific syndrome caused by a specific anticonvulsant agent arose when digital hypoplasia and nail dysplasia were associated with diphenylhydantoin exposure (Barr, Poznanski, & Schmickel, 1974). A recognizable syndrome of intra-uterine growth retardation, microcephaly, mental retardation, a ridged metopic suture, inner epicanthal folds, eyelid ptosis, a broad depressed nasal bridge, nail and/or distal phalangeal hypoplasia, and hernias was identified as the fetal hydantoin syndrome (Hanson, 1976; Hanson & Smith, 1976; Hanson et al., 1976). A prospective study of 35 infants and a review of 104 infants from the Collaborative Perinatal Project, all of whom were exposed in utero to diphenylhydantoin, indicated that 11 percent of exposed newborns had sufficient features to be classified as having fetal hydantoin syndrome and an additional 31 percent displayed some features of this syndrome (Hanson et al., 1976).

Trimethadione, used to treat petit mal epilepsy, also has been implicated as causing a specific malformation syndrome (German, Kowal, & Ehlers, 1970; Zackai et al., 1975). The fetal trimethadione syndrome includes growth and development delay, V-shaped eyebrows, epicanthal folds, low-set ears, palatal anomalies, and irregular teeth. Serious cardiovascular and visceral anomalies have occurred in some affected infants. Eighty-seven percent of the 53 reported pregnancies with in utero exposure to trimethadione or paramethadione resulted in either fetal death or in a child with the fetal trimethadione syndrome (Feldman, Weaver, & Lovrien, 1977).

Phenobarbital has been in use for more than 60 years and from a teratologic point of view appears to be the safest antiepileptic. There has been only one report raising a question of a phenobarbital-induced dysmorphic syndrome (Béthenod & Frédérich, 1975). In view of this safety record we recommend a change to this medication when possible for epileptic patients considering pregnancy or who are already pregnant. However, barbituates carry an addiction liability, and could cause neonatal withdrawal symptoms. At the dosage levels usually required for epilepsy control, however, fetal addiction should be an extremely rare complication.

ANTICOAGULANTS

Warfarin derivatives are competitive inhibitors of vitamin K and cause decreased synthesis of clotting factors II, VII, IX, and X. It has been known for over 30 years that warfarin crosses the placenta and can produce fatal fetal hemorrhage (von Sydow, 1947). Chronic anticoagulation for deep thrombophlebitis or with prosthetic cardiac valves accounts for the greatest use in pregnancy. As patients with valve replacements began attempting pregnancy in the 1960s, the number of fetuses exposed to warfarin increased. Several reports of malformed neonates were published in that decade (Quenneville et al., 1959; DiSaia, 1966; Kerber, Warr, & Richardson, 1968) but a well-defined warfarin syndrome was not delineated until 1975, when data on approximately

20 abnormal infants exposed to warfarin in utero were collected (Holzgreve, Carey, & Hall, 1976; Pauli, Hall, & Shaul, 1977). First trimester exposure appears to result in nasal hypoplasia, chondrodysplasia punctata, and possibly retardation; whereas second and third trimester exposure results in retardation, optic atrophy, and microcephaly. Prospective estimates of the risk of embryopathy are not available, but estimates as high as 10 percent in the first trimester have been offered (Hall, Pauli, & Wilson, 1980).

Heparin does not cross the placenta and thus causes neither hemorrhage nor malformations (Hall, Pauli, & Wilson, 1980). A minor obstacle to using this agent for long-term anticoagulation during pregnancy is that the patients must be taught to self-administer the medication (Hill & Pearson, 1971; Stillman et al., 1977). Nonetheless, in pregnancy heparin is the anticoagulant of choice, and the use of warfarin derivatives is contraindicated.

MISCELLANEOUS DRUGS

General anesthesia is employed for surgery on 15,000 to 30,000 pregnant women annually in the United States. Early retrospective studies found no association between anesthetics in pregnancy and congenital malformations (Shnider & Webster, 1965; Smith BE, 1974). Recently, surveys have presented data suggesting a twofold to fourfold increase in the rate of spontaneous abortion in women chronically exposed to inhalation anesthetics (anesthesiologists and operating room nurses), and some studies even have implicated wives of male anesthesiologists to be at higher risk (Askrog & Harvold, 1970; Cohen, Belville, & Brown, 1971; Knill-Jones et al., 1972). Problems with these studies are that they are not well controlled, that no specific agent is implicated, and that there is no information about the aborted conceptuses. Some surveys observed an increased rate of congenital anomalies in neonates exposed in utero (Knill-Jones et al., 1972; Corbett et al., 1974), and some found no difference in malformation rates (Cohen, Belville, & Brown, 1971). The issue must be considered unresolved, awaiting a well-constructed prospective study that includes pathologic and chromosomal examination of abortuses. Until such data become available, each woman working in the operating room will have to make a personal decision whether or not to stop working before undertaking a pregnancy. Thus far, no adverse effects on human embryos from local anesthetics have been reported.

Acetylsalicylic acid and other salicylates are ingested by over 80 percent of pregnant women some time during their pregnancy. Some case-control surveys of aspirin teratogenicity have found significantly increased risks of malformations in exposed fetuses (Richards, 1969; Klemetti & Saxén, 1970; Nelson & Forfar, 1971; Saxen, 1975), whereas others have found no association between aspirin and anomalies (Crombie et al., 1970; Collins & Turner, 1975; Slone et al., 1976). These studies indicate that if aspirin has a teratogenic effect, the risk must be exceedingly low, and that there is no specific malformation syndrome implicated. Chronic consumption of large doses of aspirin prolonged the length of gestation and increased the perinatal mortality rate (Lewis & Schulman, 1973; Collins & Turner, 1975). Aspirin interferes with platelet aggregation and

factor XII synthesis, and the resultant bleeding predisposition in both mother (Lewis & Schulman, 1973; Collins & Turner, 1975) and newborn (Bleyer & Breckenridge 1970) is probably the cause of the increased perinatal mortality. Occasional consumption of lesser amounts of aspirin, as generally employed by American women, was not associated with any increased perinatal mortality (Shapiro et al., 1976a). Nevertheless, it appears prudent not to encourage aspirin usage in the third trimester for trivial reasons.

Antinausea medications are widely used during the first trimester, meclizine and Bendectin (Merrell-National) being the two most commonly prescribed drugs. After the thalidomide publicity, several small retrospective surveys implicated meclizine as a potential teratogen (Watson, 1962; Peterson, 1964); however, more extensive prospective studies found no association between meclizine and malformations (Smithells & Chinn, 1964; Yerushalmy & Milkovich, 1965; Milkovich & van den Berg, 1976). Similar large prospective studies have demonstrated the safety of the commonly used combination of dicyclomine, doxylamine, and pyridoxine during pregnancy (Shapiro et al., 1977; Smithells & Sheppard, 1978). Another drug in this class, diphenhydramine, is used for its antihistaminic and mild sedative actions. Although not extensively studied, one retrospective case control investigation of children with oral clefts showed a significant association between cleft palate and first trimester exposure to diphenhydramine (Saxén, 1974). Clearly, a prospective study of this widely used compound is needed.

Many diuretics are teratogenic to rodents, but no teratogenicity to human embryos has been demonstrated. Thiazides given to the mother have been associated with bone marrow depression, causing extremely low platelet counts in newborns (Rodriguez, Leiken, & Hiller, 1964). However, this reaction occurred in only a small group of infants, and the magnitude of the risk is not well defined. None of the cardioactive drugs have been associated with congenital anomalies, but both hypoglycemia and bradycardia may occur in the newborn after propanolol administration to the mother (Gladstone, Hordof, & Gersony, 1975; Habib & McCarthy, 1977). Likewise, the antihypertensive agents do not appear to have human teratogenic actions, although they may still compromise the neonate in other ways. Reserpine taken by the mother during the 24 hours before delivery causes nasal discharge and congestion in 10 to 16 percent of the exposed newborns (Budnick, Leiken, & Hoeck, 1955; Desmond et al., 1957). Hexamethonium compounds cross the placenta and cause a fetal ganglionic blockade leading to paralytic ileus (Morris, 1953).

Excess or substandard intake of vitamins has been a classic tool of experimental teratologists but appears to have limited significance in human gestation. Vitamin A is the most suspect, because two cases of urinary tract malformation were reported in infants exposed to excessive retinol in the first trimester (Pilotti & Scorta, 1965; Bernhardt & Dorsey, 1974). Gal and colleagues (1972) found higher maternal serum vitamin A levels in mothers of infants with CNS malformations than in control mothers. Two children malformed in association with hypovitaminosis A have been described; one had microcephaly and anophthalmia (Sarma, 1959), whereas the other had microphthalmia and coloboma (Lamba & Sood, 1968). Vitamin D also has drawn attention. Friedman (1968) correlated high doses of vitamin D and infantile hypercalcemia

with supravalvular aortic stenosis as part of a characteristic syndrome (Williams syndrome). However, excessive maternal intake of vitamin D has not been consistently observed in either mothers or infants with this lesion (Antia et al., 1967; Rowe & Cook, 1969).

Penicillamine is employed as a chelating agent in the therapy for Wilson disease and cystinuria. One neonate was born with generalized connective tissue defects, including lax skin, hyperflexible joints, vessel fragility, and impaired wound healing, after the mother was treated throughout pregnancy with 2 g of penicillamine daily for cystinuria (Mjolnerod et al., 1971). A similar case occurred when a woman was treated with 900 mg of penicillamine daily for rheumatoid arthritis (Solomon et al., 1977). However, a review of 27 women treated with unknown quantities of penicillamine for rheumatoid arthritis or cystinuria reported only one infant with a small ventricular septal defect (Lyle, 1978). Nineteen of these women were exposed only in the first trimester, whereas 8 took the drug throughout pregnancy. A review of 18 women with Wilson disease treated with penicillamine before and during 29 pregnancies resulted in no congenital anomalies in the offspring (Scheinberg & Sternlieb, 1975). The therapeutic maintenance dose of penicillamine for Wilson's disease is usually 1 g daily, and this may be an argument for using the lowest possible maintenance dose during pregnancy. Alternatively, the excess maternal copper in this condition may absorb the penicillamine and thus protect the fetus.

ENVIRONMENTAL AGENTS

Naphthalene is an example of an environmental chemical that can harm a specific risk group of fetuses because of their genetic predisposition. The agent is representative of a large number of oxidizing agents that produce hemolytic anemias in individuals lacking normal erythrocyte glucose-6-phosphate dehydrogenase (G6PD) activity. Naphthalene and many of the other oxidizing agents cross the placenta and can cause hemolysis in a susceptible fetus (Zinkham & Childs, 1958; Anziulewicz, Dick, & Chiarulli, 1959). In both documented cases the mother had ingested mothballs during the third trimester.

That environmental agents need not be ingested by the mother to affect the fetus is demonstrated by the chlorobiphenyls. An epidemic of skin eruptions in 1968 in Japan was caused by cooking oil contaminated with chlorobiphenyls. Nine pregnant women were affected, and each delivered an infant with skin stained dark-brown. Two infants were stillborn, and five showed intra-uterine growth retardation (Miller, 1971). Various environmental agents related to specific employment situations, such as laboratory solvents (Strandberg et al., 1978) and the chemicals involved in printing (Erickson, Cochran, & Anderson, 1978), have been implicated as teratogens.

Fetal toxicity from organic mercury has been known for years (Alfonso & de Alvarez, 1960), but has come to public attention only recently because of sporadic epidemics caused by consuming mercury-containing fish. Infants exposed in utero demonstrate a syndrome of CNS damage including cerebral palsy, chorea, ataxia, seizures, and mental retardation (Nelson, 1971). Since ingestion of methylmercury slows the metabolism and since maternal exposure

occurs primarily through fish consumption, it has been recommended that women of childbearing age should eat no more than 350 g/wk of fish (Koos & Longo, 1976).

DISCUSSION

Any statement about human teratogenesis must, by definition, be incomplete. The obstetrician is therefore inevitably left in a quandary. One problem is that many drugs and environmental agents have not been studied adequately for their teratogenic potential. Only a few have been studied well enough to define their hazards or safety. Also, new drugs are being marketed. Another problem is that even if an agent is found to be teratogenic there is a long lag time before reports appear in the literature and the evidence accumulates. This evidence may well be published in sources unfamiliar to the practicing obstetrician. The articles used to reference this chapter make the point. Only 19 percent of the articles were published in obstetric journals, whereas 39 percent were in general medical journals, 12 percent in teratologic or genetic publications, 12 percent in specialty publications other than obstetrics or pediatrics, 9 percent in pediatric journals, and 9 percent in foreign publications.

We suggest that obstetricians follow a course of therapeutic nihilism—expose the pregnant patient only to the most necessary therapeutic agents, and only after carefully considering the risks to the fetus. Perhaps such a policy, widely applied, would help decrease the 65 to 70 percent of developmental defects said to be of "unknown origin."

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Index*

*This index is not designed to serve in differential diagnosis. Thus, not every mention of a given anomaly (e.g., cardiac) will be cited. The reader should look up the disease in question (e.g., trisomy 13).