INTRODUCTION

Medical care of the female patient requires a thorough understanding of the normal development and physiology of the reproductive system. The purpose of the human female reproductive tract is to maintain our species by producing oocytes, thereby allowing for fertilization and development of the embryo and fetus. The reproductive system is governed by a complex series of regulatory mechanisms involving autocrine, paracrine, hormonal, and neural systems. Perturbations in any of these systems can result in abnormalities that can mimic or complicate pathophysiologic processes in other organs. This chapter will explore the normal development, growth, and function of the female reproductive system and how it applies to medical care of the female patient.

EMBRYONIC DEVELOPMENT

The Ovary

Genetic sex is determined at the time of conception when a spermatozoon fuses with the oocyte in the ampullary segment of the fallopian tube. The spermatozoon has a haploid number of chromosomes with either an X or Y sex chromosome. If the spermatozoon that fertilizes the oocyte has an X chromosome, then the fetus will usually develop along female lines; whereas, if the spermatozoon has a Y chromosome, then the fetus will develop into a male. The human fetus at 4 to 5 weeks gestation is sexually undifferentiated and has the potential to develop into a normal male or female.

The fetal gonad begins as a thickening along the urogenital ridge overlying the mesonephros, consisting of coelomic epithelial cells and underlying mesenchyme. The primordial germ cells begin development in the yolk sac at 4 weeks gestation and migrate by ameboid type movement and differential growth to the urogenital ridge (Fig. 12.1). In the developing ovary, these primordial germ cells undergo a remarkable, exponential increase in numbers through mitosis, from a few thousand in early development to over 6 million at 20 weeks gestational age (Fig. 12.2).

The primordial germ cells continue active development during gestation. Not only are the numbers of cells increasing exponentially by mitosis, but also they enter meiosis where they arrest in prophase of the first meiotic division. At this stage, the primordial germ cells become surrounded by a single layer of mesenchymal cells from the urogenital ridge (the future granulosa cells) and form a primordial follicle.

At 16 weeks gestational age, the process of atresia begins to affect germ cells, significantly reducing their total number. By birth, the total number of oocytes has been reduced to about 1 to 2 million. The fetal ovary is active throughout gestation, and follicles will be stimulated to various degrees of development during latter stages of gestation, including preantral and antral follicles. However, ovulation does not occur until maturation of the hypothalamic pituitary axis at puberty.

The Uterus

The uterus begins development as two longitudinal infoldings of the coelomic epithelium just lateral to the mesonephros (Fig. 12.3). These infoldings form two tubular structures known as the müllerian ducts, which later fuse to form the uterine body. The distal ends of the müllerian ducts remain unfused to form the fallopian tubes (Fig. 12.4).

The undifferentiated human embryo at 5 to 6 weeks gestational age has both mesonephric ducts (potential to develop into male internal genitalia) and müllerian ducts. Alfred Jost, in a classic series of experiments, demonstrated the dependence of ductal differentiation on gonadal secretions. In the developing male gonad, Sertoli cells produce anti-müllerian hormone, and the Leydig cells secrete androgens. Anti-müllerian hormone is a paracrine cells that causes ipsilateral regression of the müllerian ducts. Testosterone acts on the
developing mesonephric ducts in a paracrine fashion to stimulate their differentiation into epididymis, vas deferens, and seminal vesicles. Anti-müllerian hormone in the male fetus may be involved in descent of the testis into the scrotum. In contrast, the developing female gonad does not produce significant quantities of anti-müllerian hormone, and the müllerian ducts continue development into fallopian tubes, uterine body, and the upper part of the vagina. Low levels of androgens produced by the developing ovary are not sufficient to maintain the mesonephric ducts, and they undergo regression. Male development requires the active secretion of steroidal and nonsteroidal molecules from the gonad, whereas female development occurs as the default pathway of sexual differentiation.

Müllerian system development is intimately dependent on normal development of the renal system. Kidney development occurs in stages, and each stage is dependent on normal differentiation of the previous stage. The stages of renal development include the pronephros, mesonephros, and metanephros. The close association between renal and genital differentiation explains the association of kidney abnormalities with genital tract abnormalities.

**External Genitalia**

The external genitalia of male and female embryos is undifferentiated at 5 to 6 weeks gestational age with the potential to develop into external genitalia of either sex, depending on the hormonal milieu. In the male fetus, the testes begin to produce testosterone by 8 to 9 weeks gestational age. Mas-
culinization of the external genitalia can be detected as early as 1 week later, and is completed by 14 weeks gestational age. Masculinization of the external genitalia relies on the conversion of testosterone to dihydrotestosterone by 5-alpha reductase. Dihydrotestosterone results in (i) enlargement of the genital tubercle, which ultimately forms the penis; (ii) fusion of the folds of the urogenital sinus, which form the penile urethra; and (iii) fusion of the labioscrotal folds to form the scrotum.

The female fetus lacks adequate androgen levels, resulting in (i) the genital tubercle forming the clitoris, (ii) the folds of the urogenital sinus forming the labia minora, (iii) the urogenital sinus contributing to formation of the lower vagina, and (iv) the labioscrotal folds forming the labia majora (Fig. 12.5). If the female fetus is exposed to androgens during development, variable degrees of masculinization can result, depending on dose, duration, and timing of exposure.

Normal development of external genitalia in the male and female fetus depends on appropriate timing and level of exposure to the respective sex steroids. In a male fetus, reduced levels of androgens, either testosterone or dihydrotestosterone, will result in inadequate virilization, resulting in a male pseudohemaphrodite. The degree of genital ambiguity will correlate with the level of androgen to which the fetus is exposed during development. In the female fetus, elevated levels of androgens can result in masculinization of the external genitalia. Similarly, the degree of virilization of the female genitalia correlates with the intensity and duration of androgen exposure.

THE NEONATAL OVARY

The total endowment of germ cells has been reduced from 20 million to 1 to 2 million by birth. The neonate is separated from the maternal environment at birth, resulting in a fall in circulating steroid levels. This decrease in maternal steroid levels results in decreased negative feedback on the fetal pituitary gonadotrope with resultant release of gonadotropins.
follicle-stimulating hormone (FSH), and luteinizing hormone (LH).

In the female fetus, there are increased levels of pituitary and circulating levels of FSH with increased pituitary levels of LH. The male fetus has lower levels of gonadotropins, probably related to androgen and inhibin production by the developing testes. Inhibin is a dimeric polypeptide hormone secreted by Sertoli cells that produces a negative feedback effect on FSH secretion by the pituitary gland. After birth, the female neonate has a larger rise in levels of FSH than in LH, and this rise will remain for 12 to 24 months. This rise in gonadotropin levels can stimulate follicular development in the neonatal ovary, resulting in formation of cysts. One of the common causes of abdominal masses in the female neonate is ovarian cysts resulting from gonadotropin stimulation.

Gonadotropin levels remain elevated for 12 to 24 months in the female before reaching a nadir in early childhood that lasts until puberty. These low levels of gonadotropins result from a highly sensitive negative feedback mechanism coexisting with a nonsteroidal central inhibitor of gonadotropin secretion. Despite a quiescent hypothalamic pituitary gonadal axis, the ovary continues to show evidence of follicular development and atresia.

**PUBERTY**

Puberty is a time of transition from immaturity to a sexually mature adult. This transition results in numerous physical, hormonal, and psychologic changes in an individual. Puberty is usually heralded by the onset of a growth spurt, followed by breast budding (median age, 9.8 years). The development of the breast usually follows a well-defined sequence of events, which characterize the stages of pubertal development (Tanner stages) (Fig. 12.6). The development of axillary and pubic hair, followed by menarche at an average age of 12.8 years are the usual sequence. The development of the pubertal mechanism with positive feedback is a late event in normal puberty. The usual length of time to complete the pubertal process is 4.5 years for a healthy European girl. Any abnormality in the timing (age at onset or duration) of puberty may be a sign of a serious underlying disease and needs to be evaluated with a thorough, systematic approach.

The timing of puberty is primarily genetic, with significant environmental influences such as nutrition and psychologic stresses. Recent studies show a reduction in the mean age of onset of menarche in developing countries, reflecting an improvement in nutrition and general health. A critical weight for the onset of puberty has been hypothesized and probably reflects a shift in body composition to a higher percentage of fat in the premenarchal female. The percentage of body fat has an important role in initiation and maintenance of normal menstrual function during the female's reproductive life. Perturbations (high or low) of the percentage of fat may disturb ovarian function, resulting in oligoovulation or anovulation with an accompanying disturbance in menstruation.

Adrenarche results from increased secretion of adrenal androgens as manifested by the appearance of pubic and axillary hair. These androgens include dehydro-3-epiandrosterone (DHEA), its sulfate, and androstenedione. This increase in androgens first appears at about 6 to 7 years of age.
and continues to increase into mid-adolescence (about age 13 to 15), correlating with an increase in size of the zona reticularis of the adrenal cortex. Adrenarche usually occurs 2 years before the onset of pubertal changes, but is not believed to be a "trigger" for other pubertal changes. The exact initiating event for onset of adrenarche is unknown. This "trigger" mechanism does not appear to involve adrenocorticotropic hormone (ACTH) or cortisol because these hormones remain stable through the pubertal period. Recent evidence suggests another pituitary molecule as a possible initiating "trigger," a posttranslational cleavage product of proopiomelanocorticotropic hormone (POMC).

Puberty is heralded by activation and maturation of the hypothalamic-pituitary-gonadal axis, which culminates in sexual maturity. During early childhood, pituitary gonadotropins are suppressed to very low levels, thought to be the result of a highly sensitive negative feedback mechanism and an intrinsic central inhibitory influence. Puberty is marked by an orderly decline in the negative feedback mechanism and release from the central inhibitory influence.

The release of the hypothalamic pituitary axis from these suppressive influences releases gonadotropin-releasing hor-

---

### Figure 12.6

<table>
<thead>
<tr>
<th>STAGE 1</th>
<th>STAGE 2</th>
<th>STAGE 3</th>
<th>STAGE 4</th>
<th>STAGE 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image_url" alt="Image" /></td>
<td><img src="image_url" alt="Image" /></td>
<td><img src="image_url" alt="Image" /></td>
<td><img src="image_url" alt="Image" /></td>
<td><img src="image_url" alt="Image" /></td>
</tr>
</tbody>
</table>


---

mone (GnRH) from the hypothalamus, which stimulates the release of gonadotropins from the pituitary that interact with the gonad, resulting in production and release of sex steroids (Fig. 12.7). GnRH acts on gonadotrope cells in the anterior pituitary where it induces production of GnRH receptors. By up-regulating the GnRH receptors it acts as a "self-primer" and stimulates the synthesis and release of gonadotropins by the gonadotropes. As the gonadotropin levels rise, they increase the production and release of sex steroids by the ovary. This release of sex steroids by the gonad results in the development of secondary sexual characteristics in a well-described sequence.

In both sexes, the onset of GnRH pulses occurs initially during sleep. Sleep-associated GnRH pulses (measured as LH pulses) are an initial sign of puberty that concurs with the onset of LH release in response to exogenous GnRH. The GnRH responsiveness of LH correlates with "awakening" of the hypothalamic gonadotrope axis in the early stages of puberty. As puberty progresses and levels of sex steroids rise, the GnRH pulses extend through the day.

An initial event in puberty is the reduction of negative feedback of estrogen on the hypothalamic pituitary axis, resulting in increasing gonadotropin and estrogen levels. This change in the hormonal milieu is marked by the onset of secondary sexual characteristics. The development of estrogen-induced positive feedback on the hypothalamic pituitary axis is a late manifestation of puberty and correlates with ovulation. Positive feedback is necessary for normal

---

**FIGURE 12.7.** The release of the hypothalamic from inhibition both by a decrease in sensitivity of negative feedback by steroid hormones and a reduction in the central nonsteroidal inhibitory factor results in gonadotropin-releasing hormone (GnRH) production and secretion. The consequence of this process is maturation of the productive axis.
Precocious Puberty

Precocious puberty is defined as pubertal changes before the age of 8 years, and its occurrence requires a thorough, systemic evaluation. Recent studies suggest that pubertal maturation may be occurring at an earlier age, especially in African American girls; however, a thorough evaluation is still important in the girl who presents with precocious puberty before age 8. The causes of precocious puberty can be divided into GnRH-dependent and independent groups. GnRH-dependent precocious puberty is associated with premature maturation of the hypothalamic-pituitary axis with the onset of GnRH pulses resulting in release of gonadotropin and steroids. GnRH-dependent precocious puberty can result in ovulation with the potential for pregnancy. GnRH-independent precocious puberty does not result in maturation of the GnRH pulse generator, but is dependent on a peripheral source of gonadal steroids that results in the development of secondary sexual characteristics (Table 12.1).

Precocious puberty is seen five times more frequently in girls than in boys. In 75% of girls, no etiology can be found and it is known as idiopathic. The diagnosis of idiopathic precocious puberty is one of exclusion and requires a thorough evaluation. In girls, the younger the patient is on presentation the more likely she has a pathologic condition. The most common diagnosis in girls with GnRH-dependent precocious puberty is idiopathic. Approximately 7% will have central nervous system lesions that require further evaluation and treatment. Because of activation of the GnRH pulse generator, these patients can ovulate, and reports of early pregnancy document their sexual maturity. The onset of sexual development, however, does not require activation of the GnRH pulse generator with ovulation. Some examples of GnRH-independent causes of sexual precocity include ovarian tumors, adrenal tumors, McCune-Albright syndrome, and ectopic gonadotropin-producing tumors.

McCune-Albright syndrome is characterized by café au lait spots, polyostotic fibrous dysplasia of bone, and hyperfunction of a number of endocrine systems. One of the endocrine systems most commonly affected in females is ovarian, resulting in precocious development. Other systems that are frequently affected include the thyroid, adrenal, pituitary, and parathyroid. Molecular diagnosis reveals that McCune-Albright syndrome is caused by an activating mutation of a G protein.

The workup of precocious puberty should focus on defining the etiology and eliminating the possibility of serious illness. The initial evaluation includes a thorough history and physical examination with detailed historic evaluation of growth and the development of secondary sexual characteristics. Evidence of heterologous sexual development caused by excess androgen secretion in the female should be sought; it may be the first sign of an adrenal tumor or congenital adrenal hyperplasia (CAH). Thorough neurologic, abdominal, and pelvic examinations are important to discover signs of tumors involving these organ systems. The patient should also be examined for any signs of systemic illnesses that could result in precocious puberty (McCune-Albright syndrome).

Laboratory evaluation of patients should include serum gonadotropin and steroid levels [estradiol, progesterone, 17α-hydroxy progesterone, dehydro-3-epiandrosterone sulfate (DHEAS), and testosterone]. A GnRH test can be extremely helpful in determining pituitary gonadotropin reserve. Patients with early maturation of the hypothalamic-pituitary gonadal axis demonstrate release of gonadotropins on stimulation with GnRH. Important radiologic tests include bone age, imaging of the head with computed tomography (CT) or magnetic resonance imaging (MRI), CT imaging of the adrenals, and pelvic ultrasonography. This series of tests can help distinguish GnRH-dependent from GnRH-independent causes of precocious puberty. Once categorized to the GnRH dependency group, the previously

---

**TABLE 12.1. ETIOLOGIES OF PRECOCIOUS PUBERTY**

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH-dependent precocious puberty</td>
<td>Ovarian, Granulosa cell tumors, Granulosa-luteal cell cysts, McCune-Albright syndrome, Pfeutz-Jeghers syndrome</td>
</tr>
<tr>
<td>GnRH-independent precocious puberty</td>
<td>Ovarian tumors and disorders, Adrenal tumors and disorders, Congenital adrenal hyperplasia, Adrenal tumors, Adrenal adenomas, Adrenal carcinomas, Exogenous sex steroids, Primary hypothyroidism</td>
</tr>
</tbody>
</table>

CNS, Central nervous system; GnRH, Gonadotropin-releasing hormone.
described tests can further characterize the nature and localize the site of excess hormone secretion.

**Pubertal Delay**

Lack of sexual development may also be a sign of serious illness. The following presentations are signs of potentially serious pathology and demand a rapid, thorough evaluation: (i) lack of onset of menses by age 14 with no signs of secondary sexual characteristics, (ii) lack of menses by age 16 regardless of secondary sexual characteristics, or (iii) prolonged duration of puberty (greater than 4.5 years). Lack of pubertal development is rare in females and requires an evaluation directed toward abnormalities including genetic, hypothalamic pituitary, and anatomic (Table 12.2).

The initial evaluation should include (i) signs of past poor health, (ii) evidence of excess exercise or abnormal eating habits, and (iii) chronologic height and weight records. The physical examination should include accurate height and weight measurements as well as Tanner staging. Short stature may be the first clue that the subject suffers from (i) an isolated growth hormone deficiency, (ii) global pituitary hormone deficiency, or (iii) gonadal dysgenesis. Intracranial disease should be considered, and a detailed neurologic examination is essential.

The laboratory evaluation of patients with delayed puberty includes thyroid function tests; prolactin, gonadotropin, and steroid levels (both gonadal and adrenal). A bone age and skull imaging are important if the patient has low gonadotropin levels. Patients with high gonadotropin levels require cytogenetic testing to evaluate for sex chromosome abnormalities.

Determination of gonadotropin levels will help direct the evaluation. Elevated gonadotropin levels are consistent with gonadal deficiency. The most common cause of gonadal deficiency is gonadal dysgenesis caused by a privation of the X chromosome, which can occur as a complete absence, a mosaic condition, or a structural abnormality of the X chromosome. Many patients with gonadal dysgenesis will have a normal 46,XX karyotype. A number of etiologies exist for gonadal failure in these patients including torsion, inflammation, sickle cell disease, and enzymatic deficiencies. An example of an enzymatic deficiency is 17-hydroxylase deficiency, which results in a sexually infantile patient with hypertension and hypokalemia.

Low gonadotropin levels can be caused by constitutional delay of puberty or by pathologic conditions. Pathologic conditions include hypothalamic amenorrhea, Kallmann syndrome, and hyperprolactinemia. In this group of patients, panhypopituitarism and tumors of the pituitary or hypothalamic region should be ruled out. The most common neoplasm is craniopharyngioma, which is a tumor of Rathke pouch. The treatment for a craniopharyngioma is surgery with possible radiation therapy.

Eugonadal subjects most commonly have müllerian segmental abnormalities such as a transverse vaginal septum, complete müllerian agenesis, or androgen insensitivity. Patients with müllerian abnormalities will have 46,XX karyotypes with functional ovaries and a normal distribution of female pubic and axillary hair. Serum testing will show evidence of ovarian function with normal gonadotropin, estrogen, progestrone, and female testosterone levels. Individuals with müllerian abnormalities have an increased frequency of renal and skeletal abnormalities. Individuals with complete androgen insensitivity will present with 46,XY karyotypes, functional intraabdominal testes, and sparse or absent pubic and axillary hair. Serum testing will reveal male levels of testosterone.

Treatment of patients with delayed puberty is determined by the etiology. Constitutional delay should be treated by reassurance and counseling. If an XY cell line is discovered, gonadectomy is necessary to prevent ovarian neoplasms (most commonly dysgerminomas and gonadoblastomas). Delayed puberty should be treated with hormone replacement therapy to stimulate development of secondary sexual characteristics.

**TABLE 12.2. ETIOLOGIES OF DELAYED PUBERTY DEPENDENT ON GONADOTROPIN LEVELS**

<table>
<thead>
<tr>
<th>Elevated serum gonadotropins:</th>
<th>Chromosomally incompetent ovarian failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turner syndrome</td>
<td>45, X</td>
</tr>
<tr>
<td>45, X mosaics (46,XX or 46,XY)</td>
<td>X structural abnormalities</td>
</tr>
<tr>
<td>Chromosomally competent ovarian failure</td>
<td>Congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>17α-hydroxylase deficiency</td>
<td>Autoimmune oophoritis</td>
</tr>
<tr>
<td>Luteinizing hormone</td>
<td>Radiation therapy</td>
</tr>
<tr>
<td>Oophorectomy</td>
<td>Chemotherapy, especially alkylating agents</td>
</tr>
<tr>
<td>Galactosmia</td>
<td>Idiopathic (vanished testis syndrome)</td>
</tr>
<tr>
<td>Resistant ovary syndrome</td>
<td></td>
</tr>
<tr>
<td>Normal or decreased gonadotropin levels:</td>
<td>Constitutional delay</td>
</tr>
<tr>
<td></td>
<td>Hypopituitarism</td>
</tr>
<tr>
<td></td>
<td>Panhypopituitarism</td>
</tr>
<tr>
<td></td>
<td>Isolated gonadotropin deficiency (Kallmann)</td>
</tr>
<tr>
<td></td>
<td>Hypothryroidism</td>
</tr>
<tr>
<td></td>
<td>Chronic systemic illness</td>
</tr>
<tr>
<td></td>
<td>Anorexia nervosa</td>
</tr>
<tr>
<td></td>
<td>Sickle cell disease</td>
</tr>
<tr>
<td></td>
<td>Thalassemia</td>
</tr>
<tr>
<td></td>
<td>Chronic exercise</td>
</tr>
<tr>
<td></td>
<td>Hyperprolactinemia</td>
</tr>
<tr>
<td></td>
<td>Müllerian anomalies</td>
</tr>
<tr>
<td></td>
<td>Müllerian agenesis</td>
</tr>
<tr>
<td></td>
<td>Müllerian segmental anomalies</td>
</tr>
<tr>
<td></td>
<td>Complete androgen insensitivity</td>
</tr>
</tbody>
</table>
THE OVARIAN CYCLE

The ovary serves important roles in the production of gametes and ovarian steroids. The ovary is governed by complex regulatory mechanisms, with both positive- and negative-feedback control, resulting in the production of usually one egg per cycle. The human menstrual cycle is divided into three phases: follicular, luteal, and menstrual. The menstrual cycle is a continuum of follicular development, ovulation, and luteinization (Fig. 12.8).

The follicular phase is characterized by a series of events involving feedback mechanisms that allow for the sequential development of a follicle, usually resulting in the release of a single ovum at ovulation.

Primordial follicles are formed initially during embryonic and fetal development from germ cells, which arise in the endoderm of the yolk sac. A primordial follicle consists of an oocyte, arrested in the prophase of the first meiotic division, surrounded by a single layer of cells. This single layer of cells will subsequently become functional granulosa cells. The primordial follicle complement is formed by mitotic expansion, reaching maximal numbers in the second trimester of pregnancy. Continual reduction of the number of follicles occurs during the life of the ovary. This process of initiating growth and atresia of follicles continues without interruption until the ovary is completely devoid of follicles (the menopause).

The number of primordial follicles that begins growth in each cycle is unknown, but is related to the total number of follicles remaining in the gonads. The majority of follicles that begin development eventually undergo atresia. The dominant follicle, that follicle which is destined to ovulate, is selected in the first few days of the follicular phase and is dependent on elevations in FSH levels in the early follicular phase.

Follicular phase elevation of FSH follows the late luteal phase reduction in steroids and inhibin. This rise in FSH level in the early follicular phase is accompanied by morphologic changes in the follicle, including an increase in the size of the oocyte and a change in granulosa cells from flat to cuboidal. In addition, gap junctions form between the granulosa cells and the oocyte and serve as a mechanism of communication between these cells (allowing for effective paracrine actions). Under the continued stimulatory effect of FSH, the granulosa cells begin to proliferate and transform the primordial follicle into a primary follicle. Stromal development is also occurring under gonadotropin stimulation, resulting in the differentiation of two layers, theca interna and theca externa, in the primary follicle (Fig. 12.9).

The binding of FSH to gonadotropin receptors on the developing primary follicle results in proliferation of granulosa cells and steroidogenesis. As the primary follicle continues to enlarge, a membrane develops around the oocyte, the zona pellucida, changing the follicle into a preantral follicle. The preantral follicle depends on continued FSH production that stimulates cell growth and steroidogenesis.

Granulosa cells contain an enzyme with aromatase activity. This activity is stimulated by binding of FSH to receptors on the granulosa cell membrane. Aromatase converts androgens into estrogens, thus maintaining the estrogenic environment of the follicle. Estrogen and FSH combine to increase the number of FSH receptors on granulosa cell membranes, thereby increasing the cellular sensitivity to FSH. This increase in FSH sensitivity allows the cells to continue to respond as FSH levels fall in the latter follicular stage.

Aromatase is also sensitive to androgen production by theca cells. A low level of androgens stimulates aromatization, whereas higher levels suppress this process. This com-
Combination of follicular phase events results in the majority of follicles becoming atretic, with one or a few growing to maturity. Continued growth of the follicle is accompanied by the appearance of fluid around the granulosa cells, eventually forming a cavity within the follicle, transforming the preantral follicle into an antral follicle. The follicular fluid contains FSH, estrogen, and other granulosa cell metabolites in relatively high concentrations that bathe the developing oocyte.

Follicular development is a highly integrated process that involves effective communication between various compartments of the follicle. A two-cell model has been proposed to explain granulosa cell dependence on thecal cells. In this model, granulosa cells are predominantly FSH dependent, as reflected by FSH receptors on their surface, whereas thecal cells are LH dependent, as reflected by LH receptors on their surface. Thecal cells under the influence of LH convert cholesterol into androgens. The androgens then diffuse to the granulosa layer where they are aromatized into estrogens, thereby maintaining the estrogenic milieu of the follicle.

Conversion from an androgen-dependent to estrogen-dependent environment marks the selection of the follicle that is destined to ovulate. This process depends upon the interaction of FSH and estrogen within the follicle and the pituitary gland. Within the developing follicle, FSH and estrogen function to increase FSH receptor number, increasing the gonadotropin sensitivity of the follicle. The increased circulating concentration of estrogen exerts an inhibitory effect upon the pituitary, thereby decreasing circulating concentrations of FSH (classic negative feedback). This combination of events, an increased sensitivity of the dominant follicle and decreased concentration of FSH, selects the follicle destined to ovulate. The ovary that is supporting the dominant follicle can be distinguished from its counterpart by the fifth day of the follicular phase. In nondominant follicles, the fall in circulating FSH causes atresia (Fig. 12.10).

The increase in number of granulosa cells in the maturing follicle is accompanied by an increase in vascularity of the theca. The increase in vascularity results in preferential delivery of FSH to the follicle with the greatest blood flow, which can be detected by day 9 of the follicular phase.

Granulosa cells develop LH receptors, which allow them to respond to the luteinizing hormone surge at midcycle with completion of development and ovulation. LH recep-
tors first appear in large antral follicles at the time of falling FSH levels and increasing intrafollicular estrogen levels. LH receptors form in response to the estrogenic environment and local paracrine events.

Production of GnRH by the hypothalamus serves an obligatory role in stimulating the release of gonadotropins. Feedback of follicular-derived growth factors and hormones "fine tune" the secretion of gonadotropins required to stimulate ovulation. Estrogen, the primary steroidal hormone released from the developing follicle, has both positive and negative feedback effects on the hypothalamic-pituitary axis. Estrogen decreases gonadotropin secretion by reducing the secretion and response to GnRH in pituitary cells. The exact mechanism of this negative feedback is unknown. When estrogen reaches adequate concentrations for an extended period, a positive-feedback mechanism results in robust release of gonadotropins from the pituitary gland. This positive effect involves both the hypothalamus and the pituitary gland. In the hypothalamus, estrogen increases the amount of GnRH that is released with each GnRH pulse. In the pituitary gland, estrogen increases the number of GnRH receptors, which results in more gonadotropins being released with each GnRH pulse.

FSH is sensitive to negative feedback, even at low levels of estrogen. LH has a variable response to estrogen: sup-pression at low levels and stimulation at higher levels. The change from suppression to stimulation occurs when estrogen levels reach an adequate value for a sustained period.

The preovulatory follicle responds to different hormones and locally acting growth factors. In the preovulatory follicle, FSH promotes luteinization of the granulosa manifested by enlargement and appearance of lipid inclusions in the cells. Theca lutein cells simultaneously develop inclusions and become richly vascular. These morphologic changes in the follicle are accompanied by increased production of estrogen and progesterone. Midcycle progesterone production facilitates the LH surge and plays a dominant role in the production of the FSH surge. The LH surge results after adequate estrogen priming and provides the ovulatory stimulus for the dominant follicle.

Ovulation with the release of a mature oocyte occurs after the LH surge. Ovulation occurs 10 to 12 hours after the LH peak and 34 to 36 hours after the start of the LH surge. Resumption of meiosis and stimulation of a number of proteolytic enzymes, which digest the walls of the follicle, allow the oocyte to release; this occurs after the gonadotropin surge. Ovulation is not an "explosive" event, and studies have shown no increase in intrafollicular pressure before ovulation.

After ovulation, granulosa cells continue to enlarge and develop a vacuolated appearance while becoming the corpus luteum. Thecal cells also contribute to the formation of the corpus luteum. The postovulatory follicle accumulates a yellow pigment known as lutein, hence the name corpus luteum. A rapid period of vascularization with ingrowth of capillaries into the granulosa cells occurs immediately after ovulation. The vascularity of the corpus luteum ensures the continued supply of substrates to the metabolically active cells.

The corpus luteum produces ovarian steroids, primarily progesterone and estrogen. The secretion of sex steroids is episodic during the luteal phase and correlates with the pulsatile release of LH. Adequate production of sex steroids depends on adequate follicular growth and gonadotropin receptor formation during the follicular phase. The combination of estrogen and progesterone functions to transform the endometrium into an environment that will accept and nurture the developing embryo. A normal menstrual cycle luteal phase is about 14 days (range, 11 to 17 days). The luteal phase is the most constant part of the menstrual cycle in terms of length. The corpus luteum is programmed to undergo involution in 9 to 11 days after the LH surge, unless rescued by human chorionic gonadotropin (HCG), which is secreted actively by the developing fetoplacental unit. If implantation occurs, HCG maintains the corpus luteum until the ninth to tenth week of gestation.

Leptin

Leptin is a polypeptide hormone produced by the adipocyte, which plays an important role in energy homeostasis, feed-
Pregnancy is a unique time in ontogeny of the human. A semi-allograft thrives in the center of a potentially hostile maternal immune system. The exact mechanism for this apparent paradox of classical transplant immunology is unknown. Proposed mechanisms include such theories as (i) the uterus as an immunologically privileged site, (ii) production of maternal blocking antibodies, (iii) idiotype anti-idiotype antibody networks, and (iv) multiple other mechanisms.

Pregnancy is also a unique situation in terms of steroidogenesis when three different interacting systems exist: mother, placenta, and fetus. Each of these units contributes essential nutrients and metabolites to the other while allowing the conglomerate to function as an integrated whole (Fig. 12.11).

Progesterone is synthesized from cholesterol from any source: (i) conversion of acetate to cholesterol, (ii) hydrolysis of stored cholesterol esters, or (iii) from low-density lipoprotein (LDL) cholesterol. Maternal-blood LDL cholesterol is the usual source for progesterone synthesis during pregnancy.

**Diagram:**

**Fetal Compartment**
- Pregnenolone Sulfate
- 3β-al-dehydrogenase block
- Dehydroepiandrosterone Sulfate (DHEAS)
- 16 OH DHEAS

**Placenta**
- Cholesterol
- Pregnenolone
- Progesterone
- Estrone, estradiol
- 16α OH DHEA

**Maternal Compartment**
- Cholesterol
- Acetate
- Pregnenolone
- Progesterone
- Estrone, estradiol
- Dehydroepiandrosterone sulfate

**Figure 12.11.** Steroidogenic pathways during pregnancy. Interrelationship of maternal-placental-fetal compartments. (From Reece EA, Hobbins JC, Mahoney MJ, et al., eds. Medicine of the fetus and mother. Philadelphia: JB Lippincott Co, 1990, with permission.)
During pregnancy, progesterone is produced initially by the corpus luteum that has been rescued by HCG from the syncytiotrophoblast. The developing pregnancy depends entirely on the corpus luteum for progesterone until the seventh week of gestation. From 7 to 10 weeks gestation, both the placenta and corpus luteum are producing progesterone. After 10 weeks, the placenta is the primary source of production. Early miscarriage occurs if the corpus luteum is removed before 7 weeks gestational age, unless the pregnancy is "rescued" with exogenous progesterone.

Progesterone plays a number of important roles in pregnancy, including preparation and maintenance of the uterine endometrium. The endometrium is thought to suppress maternal immune response to the fetal allograft. Progesterone also supplies the fetus with precursors for the production of glucocorticoids and mineralocorticoids.

In addition, estrogen concentrations are elevated during pregnancy, but unlike progesterone, estrogen synthesis depends on the production of adequate precursor steroids by the developing fetus. There are three main categories of estrogen produced during pregnancy: estrone, estradiol, and estriol. Estrone and estradiol concentrations in the blood are about 100-fold greater in pregnancy than in the nonpregnant state, whereas estriol is over 1,000-fold greater.

Estrogens depend primarily on the 19-carbon steroid precursors, androgens, for their production. The placenta has a deficiency of cytochrome P-450 17-hydroxylase enzyme activity, which contains both 17-hydroxylase and 17-20 desmolase activity. Therefore, placental synthesis of estrogen depends on 19-carbon steroid precursors from both maternal and fetal sources. In early pregnancy, androgen precursors come primarily from the mother, whereas later in pregnancy, the majority of 19-carbon precursors come from the fetal adrenal.

The thin, outer definitive zone of the fetal adrenal can be differentiated from the thick inner fetal zone by 7 weeks gestation. The inner fetal zone is proportionately larger than the adult adrenal gland, rapidly undergoing involution after delivery (Fig. 12.12). Initially, the fetal adrenal develops under the control of HCG, independent of ACTH. After midgestation, the secretion of fetal ACTH by the developing hypothalamic pituitary axis assumes greater importance. ACTH is believed to play an obligatory role in steroidogenesis and development of the fetal adrenal, but may not be the only control mechanism. Previous studies have suggested a role for prolactin.

DHEAS is the primary 19-carbon precursor secreted by the fetus. The large quantities of estrogen produced during pregnancy inhibit 3β-hydroxysteroid dehydrogenase isomerase activity in the fetal adrenal. This inhibition of 3β-hydroxysteroid dehydrogenase isomerase results in the fetal adrenal, producing large quantities of delta-5 steroids, DHEA, and DHEAS.

DHEAS secreted by the fetal adrenal may be 16-hydroxylated by the fetal liver to form 16-hydroxy-DHEAS. DHEAS and 16-hydroxy-DHEAS are transported to the placenta, which has an active sulfatase enzyme encoded on the short arm of the X chromosome that will cleave the sulfate moiety from the 19-carbon precursor. DHEAS will then be converted into estrone and estradiol by the placenta, whereas 16-hydroxy-DHEAS will be converted to estriol. Hence, the production of estrogens by the placenta depends intimately on 19-carbon precursors from the fetus transferred to the placenta.

A number of clinical conditions exist in which alterations in fetal adrenal androgen production result in reduced estrogen production. In an anencephalic fetus, the adrenal is small and ill-developed. This condition does not produce adequate quantities of estrogen precursors, resulting in low estrogen levels. In situations where the fetus is under chronic stress, there will be a lowering of fetal adrenal androgen production resulting in low estrogen production. In the past, estriol was used as a method of determining fetal well-being; however, because of a lack of sensitivity and specificity, this test has been abandoned. Fetal biophysical testing, nonstress test, and ultrasound are the best predictors of fetal well-being.

The major protein hormones of pregnancy are produced by the placenta. The cytotrophoblast cell layer of the placenta is composed primarily of single, mononuclear cells that are precursor cells to the syncytiotrophoblast. The syncytiotrophoblast is a syncytium with multinuclei and is most active in hormone production (Fig. 12.13). The placenta releases a number of hormones similar to those produced by the hypothalamus and pituitary, leading to speculation that the placenta has a system of feedback control mechanisms. Hypothalamic hormones expressed in the placenta include corticotropin-releasing hormone, GnRH, thyrotropin-releasing hormone, and others.
HCG is a glycoprotein hormone produced by the syncytiotrophoblast of the placenta. The protein HCG is a dimer composed of an alpha chain and a beta chain. The alpha chain of HCG is the same as the alpha chain of a number of pituitary glycoproteins including LH, FSH, and TSH. The beta chains of these molecules are different in structure and presumably are the functional protein moieties. The beta chain of HCG is similar to the beta chain of LH, which accounts for the clinical use of HCG as a substitute for the LH surge in stimulated cycles.

Structural differences in beta subunits of the various glycoprotein hormones have allowed development of highly specific and sensitive immunoassays. The development of a radioimmunoassay for the beta subunit of HCG allows earlier detection of metabolically active placental tissue and pregnancy. Recent developments have included a sensitive radioimmunometric assay directed against the entire HCG molecule, with specific antibodies raised against the alpha and beta subunits. One antibody can be fixed on a solid surface like a microsphere and the other tagged with a detection device, such as a radioactive label for detection.

The concentration of HCG is 10 mIU/ml at the time of the expected missed menses. This level increases exponentially during early pregnancy until about 10 weeks gestation, when it reaches levels of 100,000 mIU/ml. The exponential increase in HCG levels has led to the clinical axiom that the HCG level should double every 2 to 3 days during the first weeks of a normal gestation. After the HCG level reaches its peak, it decreases and then plateaus for the remainder of pregnancy.

Early in pregnancy, HCG plays a vital role by rescuing the corpus luteum from demise. The corpus luteum will continue to produce progesterone to maintain the uterine endometrium and maintain pregnancy. The developing fetus depends on HCG for stimulation of the fetal testes to produce androgen and for stimulation of the fetal adrenal gland.

A number of pathologic conditions exist where HCG concentration can help establish the diagnosis. In early pregnancy, determination of serial HCG levels can predict pregnancy viability. If HCG levels do not increase in a normal progression, then the possibility of an abnormal pregnancy, such as an ectopic gestation or spontaneous abortion, should be considered.

Determinations of HCG are helpful in arriving at a correct diagnosis in a patient suspected of having an ectopic gestation. Ectopic gestation occurs when the pregnancy implants outside the normal intrauterine location, most commonly in the fallopian tube. A negative HCG determination essentially eliminates the possibility of a viable trophoblast and pregnancy. Approximately two thirds of ectopic pregnancies will have an abnormal rise in serial HCG titers obtained in early pregnancy. The other one third will have normal rising titers but may fall or plateau later in gestation.

Pelvic ultrasonography has added an important dimension to diagnosis of an abnormal gestation. A “discriminatory zone” is the HCG level where an intrauterine gestational sac should be detected in a normal gestation. With abdominal ultrasonography, the “discriminatory zone” is at 6,000 to 6,500 mIU/ml, whereas vaginal sonography has a discriminatory zone of 1,000–1,500 mIU/ml. The combination of serum HCG level and ultrasonography has proved helpful in the diagnosis of ectopic gestation.

Human placental lactogen (HPL) is also secreted by the syncytiotrophoblast and is composed of a single polypeptide chain with two disulfide bonds. This polypeptide hormone belongs to a group of hormones that also includes growth hormone and prolactin. HPL is produced throughout pregnancy, but tends to increase to high levels in the latter stages of gestation where it correlates with fetoplacental weight. HPL functions by inducing insulin resistance (carbohydrate intolerance) along with increasing insulin-like growth factor-1 (IGF-1) levels.

Glucose is the primary carbohydrate fuel for the developing fetus and is transported actively by the placenta. The placental hormones, including the sex steroids and HPL, induce a state of insulin resistance in the mother. When glucose levels are elevated (fed state), free fatty acids are stored as triglycerides. When glucose levels fall (fasting state), HPL levels increase, resulting in the mobilization of free fatty acids in the mother. By mobilizing free fatty acids, HPL maintains glucose levels for the developing fetus. Prolonged fasting results in mobilization of maternal adipose tissues and a rise in serum ketone levels. These ketones can be used by the developing fetus but, if persistent, may result in abnormalities in certain developing tissues.

α-Fetoprotein (AFP) is produced primarily by the fetal liver and has no known fetal function. AFP is a glycoprotein that resembles albumin and reaches high levels in the fetal circulation. This similarity to albumin has led to hypotheses that AFP may function as a carrier molecule in the fetal circulation much as albumin does in the adult.
Maternal serum levels of AFP may be abnormal in a number of abnormal pregnant conditions. Elevated levels of maternal serum AFP are seen with open neural tube defects, anterior abdominal wall defects, congenital nephrosis, fetal death, multiple pregnancy, fetal maternal hemorrhage, and other less-common causes. Data suggest that elevated levels of AFP of unknown etiology are associated with poor pregnancy outcome. Low maternal serum AFP levels have been useful in diagnosis of fetal aneuploidy, especially Down syndrome.

Prolactin is a polypeptide hormone belonging to the growth hormone family that is secreted actively in pregnancy. Prolactin is synthesized and secreted into three compartments during pregnancy: (i) the fetal bloodstream, (ii) amniotic fluid, and (iii) maternal bloodstream. Circulating prolactin, whether maternal or fetal, is produced by and secreted from the respective pituitary glands and maintains its regulatory pathways, primarily under negative control by dopamine.

Prolactin is also synthesized by decidua and is detected initially soon after decidualization of the endometrium. Prolactin in amniotic fluid is secreted primarily by the decidua that are not under the same control mechanisms as the pituitary. This conclusion results from no change in amniotic fluid prolactin in a patient on dopamine agonist therapy. Amniotic fluid prolactin may play an important role in fluid and electrolyte hemostasis.

A number of polypeptide hormones are produced by the placenta, including HCG, human chorionic thyrotropin, and human chorionic adrenocorticotropic. These hormones, along with a number of growth factors, play important roles in maternal fetal physiology during pregnancy.

Pregnancy results in a number of hormonal changes in fetal, amniotic fluid, and maternal compartments that help to initiate and maintain pregnancy. These hormonal alterations play an important role in each stage of pregnancy and its successful outcome.

LABOR AND DELIVERY

The embryo and fetus are nurtured within the uterine cavity until the developing human reaches maturity and can exist in the extraterine environment. During the latter stages of gestation, the human uterus begins to contract episodically. These contractions can occur for a period but are characterized by a lack of uterine effacement or dilation. Labor occurs only when the contractions become sufficiently coordinated to cause cervical effacement and dilation. This change in the cervix allows for the presenting part of the fetus to pass through the pelvis.

The exact mechanism that initiates labor is unknown; however, there are a number of theories that potentially explain it. One intriguing possibility is progesterone withdrawal. In sheep, the withdrawal of progesterone is felt to play a major role in the onset of labor. In humans, systemic progesterone levels do not change before the initiation of labor. The possibility remains of a local decrease in progesterone at the uteroplacental interface resulting in the start of uterine contractions. Another potential etiology for labor is an increase in oxytocin. Oxytocin (or Pitocin) has been used for a number of years to induce and augment labor, so it is a natural extension to consider it as a cause of labor. In normal labor and delivery, oxytocin levels are found to increase significantly only in the second stage of labor, and hence probably do not play an active initiation role. Investigators have found an increased concentration of oxytocin receptors in the mature uterus. These data support oxytocin as having a role in labor but not initiating the process.

A unifying concept for the initiation of labor involves communication between the fetal membranes and uterine decidua. The components of this communication network could function together to signal maturity of the fetus and trigger labor. One potential signaling mechanism is prostaglandins, which are formed actively by the decidua and are increased in concentration during normal labor.

Labor is characterized by an increase in the number of oxytocin receptors in the myometrium, increase in myometrial gap junctions, and cervical effacement and dilation. These changes allow for rhythmic, propulsive uterine contractions that propel the fetus through the bony pelvis.

Labor can be divided into three stages: stage 1, which involves effacement and dilation of the cervix; stage 2, which involves propulsion of the fetus through the bony pelvis; and stage 3, which starts after delivery of the fetus and ends with delivery of the placenta. Some authors describe a fourth stage that occurs for the first hour after delivery of the placenta.

The first stage can be divided into the latent and active phases. When one analyzes cervical dilation, it is found to follow a sigmoid curve. The latent phase is that period when the cervix is dilating slowly and the period ends when it enters the phase of rapid dilation, which is usually at 4 or 5 cm. The active phase is the linear portion of cervical dilation and extends from 4 or 5 cm until the cervix is dilated completely at 10 cm. During effacement and dilation of the cervix, the fetal presenting part will descend slowly into the bony pelvis. Only when the cervix is fully dilated will the second stage of labor begin. This stage of labor is marked by active pushing; the presenting part negotiates the bony pelvis and is delivered. The second stage concludes with the clamping and cutting of the umbilical cord.

The third stage of labor involves the time from delivery of the baby until delivery of the placenta and fetal membranes. This stage usually lasts between 15 and 30 minutes. Some authors describe a fourth stage, which is the hour after delivery of the placenta and is marked by a number of physiologic changes including contraction of the uterus (which reduces blood loss) and a rapid redistribution of maternal circulation.
The average amount of blood lost after a vaginal delivery is about 500 ml and after a cesarean delivery is about 1,000 ml. There are a number of etiologies for excess blood loss after delivery; the most common include uterine atony, retained placental fragments, and reproductive tract injuries. Less common causes of postpartum hemorrhage include uterine inversion, abnormalities of placentation (placenta accreta), and bleeding diathesis.

**MENOPAUSE**

Menopause is a retrospective diagnosis marked by the cessation of menses for 12 months resulting from follicular exhaustion. Menopause occurs at an average age of 52 years in the United States, and this age has remained constant over time. The climacteric is that period marked by waning ovarian function and culminating in hypogonadism. Menopause correlates with the morphologic finding of few gonadotropin-resistant follicles in the ovaries. This lack of follicles and low levels of gonadal steroids results in elevated gonadotropin levels, especially FSH.

Because of increased life expectancy, a woman from a developed country can expect to live one third of her life in the hypogonadal state of menopause. The loss of ovarian follicles results in a number of important physiologic changes. First, lack of responsive follicles results in sterility. Second is loss of gonadal steroids, both estrogen and progesterone. This hypogonadism increases the risk of vasomotor symptoms, urogenital atrophy, osteoporosis, heart disease, and possibly Alzheimer’s disease. These signs and symptoms of menopause can be reduced with the use of hormone replacement therapy.

**Vasomotor Symptoms**

The hot flush/flash is the most common reason that perimenopausal and menopausal women consult a physician during this time of their life. The prevalence of hot flushes varies across the world, with women of European origin experiencing these symptoms 60% to 80% of the time. Asian women complain of vasomotor symptoms only 10% to 20% of the time, and Mexican Mayan women rarely complain of hot flushes. There are a number of theories to explain these differences of hot flash prevalence, including social and cultural reasons and ingestion of variable amounts and types of phytoestrogens. Hot flushes typically occur in the years 1 to 5 following the menopause before disappearing.

The hot flush is described as a hot, flushing feeling occurring in the face, head, neck, and upper chest with a typical duration of 1 to 5 minutes. Before the flush, there is a small but significant increase in core temperature followed by peripheral vasodilation. This vasodilation results in increased blood flow to the skin, a rise in temperature, and sweating.

The loss of estrogen production plays an important role in the genesis of hot flushes. The faster and larger decline in estrogen equates with more severe and prevalent hot flashes. In premenopausal women after oophorectomy, more than 90% will experience vasomotor symptoms. However, estrogen loss does not totally explain the presence of these symptoms. Central nervous system neurotransmitters such as catecholamines—especially norepinephrine, dopamine, and serotonin—may play important roles.

Estrogen replacement therapy is the most efficacious therapy for vasomotor symptoms. If estrogen is not an option, other agents used include progesterins, clonidine, verapride, bromocriptine, and the selective serotonin reuptake inhibitors.

**Urogenital Atrophy**

Loss of estrogen also can result in urogenital atrophy and consequent recurrent vaginitis, painful intercourse, pruritus, and even vaginal stenosis. Other events can include dysuria, recurrent urinary tract infections, and possible urinary incontinence.

The treatment for urogenital atrophy is the local delivery of estrogen or the use of lubricants. The classic therapy has been the application of estrogen cream to the affected area. An area of concern with locally applied estrogen cream is consistency of serum levels and the potential of variable estrogen absorption. A new, novel therapy is the use of Estrin, which is an estrogen-impregnated ring that delivers a low dose of estrogen to the vagina for 3 months.

**Bone Loss**

Bone is a metabolically active tissue that is constantly undergoing remodeling, which is the removal of old and the laying down of new bone. This process is normally under tight control to maintain the integrity of bone. Remodeling is important for repairing injury, renewing aging, and maximizing flexibility and strength of bone. Any interference with the tightly coupled process of the osteoclast-osteoblast unit results in weakened bone and increased fracture risk.

Humans achieve peak bone mass between the ages of 25 and 35. This peak bone mass is multifactorial, with both strong genetic and environmental components. After achieving peak bone density both men and women experience a gradual decline with advancing age. Estrogen loss at menopause results in an accelerated osteoclast-mediated breakdown of bone without an increase in calcium deposition. This breakdown with decreased deposition may result in up to 3% loss of bone for the first 5 to 8 years following onset of menopause. If allowed to continue unabated, this loss of bone structural integrity results in clinically significant osteoporosis and an increased risk of fractures.

Osteoporosis is defined by the World Health Organization as "a disease characterized by low bone mass and mi-
croarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk.” Osteoporosis has been defined in terms of bone mineral density as a bone mineral density that is more than 2.5 standard deviations below the peak bone mineral density. Not all postmenopausal women will develop osteoporosis. A number of risk factors exist that increase a woman’s chance of developing osteoporosis including being thin, Caucasian, a smoker, and having a family history of osteoporosis. However, no risk factor or test exists that can be used to absolutely predict which women will develop osteoporosis.

Three major types of tests are used to determine bone health including biochemical tests, radiologic detection, and bone biopsies. Biochemical tests include bone formation markers such as serum alkaline phosphatase, serum osteocalcin, and type I collagen peptides. Tests of bone resorption include hydroxyproline, the pyridinoline cross-links, and tartrate-resistant acid phosphatase. These tests may be beneficial in determining which patient will respond to therapy and in monitoring adherence to therapy. At this time, these tests are limited in their usefulness because they cannot differentiate between focal and systemic disease. In addition, these tests do not quantitate or significantly differentiate between resorption and formation, and are affected by a number of other factors such as liver uptake.

Radiologic evaluation of bone density remains the gold standard for determining bone health or disease for osteoporosis. The test most commonly used for evaluating bone density is dual-energy x-ray absorptiometry (DEXA) scanning. DEXA uses two energy peaks, with the lower energy wave primarily absorbed only by soft tissue, whereas the high-energy wave is absorbed by soft tissue and bone. The differential absorption by bone and soft tissue allows calculation of bone mineral density for an individual. The bone density report will give both a z score and a t score. The z score compares a patient’s bone density to that of individuals of the same age, sex, and race. The t score compares a patient’s bone density to the expected peak bone mineral density. Other tests of bone density include the quantitative computerized tomography (QCT) scan and quantitative ultrasound. Quantitative ultrasound is increasing in use because of its speed, ease of administration, less radiation, and good detection of decreased bone mineral density.

The prevention and treatment of osteoporosis is multifactorial and includes dietary intervention by increasing calcium and ensuring adequate vitamin D intake, weight bearing exercise, reducing risk factors such as smoking and excess caffeine, and pharmacologic therapy consideration. The use of exogenous estrogen can markedly retard osteoporosis and improve bone mineral density in postmenopausal women. Epidemiologic studies in estrogen replacement demonstrate a 5% to 8% increase in vertebral and a 2% to 3% increase in femoral-neck bone mineral density. The minimum dose of estrogen needed to improve bone mineral density is equivalent to 0.625 mg of conjugated equine estrogens or 0.3 mg of conjugated equine estrogens with calcium and vitamin D supplementation.

Raloxifene hydrochloride (Evista) is the first selective estrogen receptor modulator (SERM) approved by the U.S. Food and Drug Administration (FDA) for the prevention and treatment of osteoporosis in menopausal women. Raloxifene is an estrogen with variable activities in different tissues. Studies show that raloxifene use improves bone mineral density and has a beneficial effect on serum lipids. Raloxifene, however, does not stimulate the uterine endometrium or the breast. The Multiple Outcomes of Raloxifene Evaluation (MORE) trial was a prospective, randomized, double-blind, multinational trial that evaluated the effect of raloxifene versus placebo on bone mineral density. The study results demonstrated an increase in bone mineral density in the lumbar spine and femoral neck, with a 55% reduction in fractures in women who did not have previous fractures and a 30% reduction in fractures in those women with previous fractures. Raloxifene improved total and LDL cholesterol and did not increase the incidence of vaginal bleeding, endometrial hyperplasia, or endometrial cancer. Raloxifene demonstrated a 65% reduction in the incidence of breast cancer. The decrease in breast cancer was in estrogen receptor positive cancers. This finding has led to a National Cancer Institute trial comparing raloxifene to tamoxifen for prevention of breast cancer in high-risk women.

Other pharmacologic agents with benefit in osteoporosis include bisphosphonates and calcitonin. All of these current agents (SERMs, bisphosphonates, and calcitonin) are antiresorptive and, despite differences in bone mineral density gain with their use, all reduce the incidence of fractures by about 50%. The similar reduction in fracture risk among the currently available antiresorptive agents is most likely the result of bone-architecture improvement by agents with similar effects on bone. Exciting new research is underway evaluating anabolic agents such as parathyroid hormone, androgens, growth hormone, and statins that may improve bone strength through different mechanisms.

Cardiovascular System

Heart disease is a significant cause of morbidity and the most common cause of mortality in postmenopausal women. There are a number of cardiovascular risk factors that increase with advancing age and the hypogonadal state of the menopausal woman. As a woman ages there is a general increase in body weight, central obesity, insulin resistance, and elevated blood pressure, all of which predispose to the development of heart disease. The loss of estrogen at menopause is believed to play a role in the development of cardiovascular disease. Epidemiologic studies demonstrate an increase in cardiovascular disease in women after the menopause and the male-to-female ratio of disease rates decrease after this time.

Estrogen has both direct and indirect protective effects on the cardiovascular system. In terms of direct effects, es-
Epidemiologic studies have shown estrogen replacement therapy to be beneficial for primary prevention of cardiovascular disease. The effects of hormone replacement therapy on cardiovascular disease were challenged with the publication of the Heart Estrogen/Progestin Replacement Study (HERS). The HERS trial was a secondary prevention trial in which women with a history of heart disease were randomized to placebo or estrogen and progesterone. At the end of the trial, the relative risk of having a cardiac event did not differ significantly between the two groups. Temporal analysis demonstrated that the women on estrogen/progesterone therapy had a higher incidence of events in the first year of the study, which slowly decreased over time. This suggests an immediate prothrombotic or proischemic effect of estrogen/progesterone therapy, with long-term benefit from improvements in lipid levels. Current studies include the Women's Health Initiative—a prospective, randomized study being performed by the National Institutes of Health and designed to evaluate the effects of hormone replacement therapy and dietary modification on cardiovascular disease.

Hormone Replacement Therapy

Estrogen replacement during the menopause is the treatment of choice for vasomotor symptoms, urogenital atrophy, and for the prevention of osteoporosis, cardiovascular disease, and other disease states. Conventional therapy includes estrogen alone for patients with no uterus but estrogen combined with a progestin in patients with a uterus. Unopposed estrogen has been shown to stimulate the endometrium and may result in endometrial carcinoma if used for a prolonged period. In postmenopausal women, the addition of progestin to estrogen therapy reduces the risk of developing endometrial cancer to baseline.

Breast cancer is a significant cause of morbidity and mortality in menopausal women. The risk factors for breast carcinoma suggest that the longer a woman is exposed to estrogen the higher her risk of developing cancer. The effect of hormone replacement therapy on breast cancer risk remains controversial. Studies suggest that women on hormone replacement therapy may have an increased risk of developing breast cancer, especially after more than 5 to 10 years. One study suggests that there is a higher risk of breast cancer associated with the combination of estrogen and progesterone than with estrogen alone.

Other adverse events associated with hormone replacement therapy include irregular vaginal bleeding, breast tenderness and pain, venous thromboembolism, and choleliithiasis. The risk of venous thromboembolism is three to four times greater in women on hormone replacement therapy than in menopausal controls, but this is still a low incidence of events.

CONCLUSION

The primary purpose of the reproductive tract is to allow for reproduction of the species. Many pathophysiologic processes can affect the reproductive system, and the reproductive system may modify the presentation of these same disease processes. In addition, alterations of the reproductive system may mimic or adversely affect other organ systems, especially intrauterine organs. An understanding of the reproductive system is vital for all health care professionals who provide care for women.

This chapter reviews the development, function, and final cessation of the activity of the female reproductive system. This entire process culminates in female reproductive maturity with the ability to carry a pregnancy to successful completion. After approximately 40 years of ovulatory competence, the ovaries cease functioning. This cessation of function results not only in lack of gamete production but also in substantially reduced hormone production. This loss of hormones, specifically estrogen and progesterone, results in menopause and the attendant risks of osteoporosis, heart disease, genitourinary atrophy, and the menopausal syndrome.

GLOSSARY

Cytotrophoblast Single mononuclear cells of the developing placenta that fuse and form syncytiotrophoblast.

Diploid karyotype: 2(n) Condition where a cell contains two complete sets of chromosomes. In the human, 46 chromosomes.

Ectopic pregnancy Implantation of a pregnancy outside of the normal intrauterine environment, most commonly in the fallopian tube.

Follicle-stimulating hormone (FSH) A dimeric glycoprotein composed of an alpha chain and a beta chain produced by gonadotrophs in the adenohypophysis. Important during folliculogenesis and ovulation.

Human chorionic gonadotropin (HCG) A dimeric glycoprotein produced by the syncytiotrophoblast of the placenta, which "rescues" the corpus luteum in early pregnancy.

Haploid karyotype (n) Condition where a cell contains one complete set of chromosomes. In the human, 23 chromosomes.
Hirsutism  Androgen-induced excess body and facial hair growth.

Luteinizing hormone (LH)  Dimeric glycoprotein produced by gonadotrophs in adenohypophysis.

Meiosis  Cell division occurring in germ cells that reduces chromosome number to the haploid state (2n to n).

Menopause  Cessation of menstrual cycles.

Menstrual cycle  Cyclic hormonal and anatomic changes that occur in preparation for ovulation and pregnancy. Can be divided into follicular, luteal, and menstrual phases.

Mitosis  Cell division in somatic cells that maintains a diploid set of chromosomes (2n to 2n).

Syncytiotrophoblast  Multinucleated, syncytium of cells of the placenta that functions in the production and secretion of a number of placental hormones.

Virilization  Signs of pronounced hyperandrogenemia including temporal balding, deepening of voice, increased muscle mass, and clitoris megaly.

SUGGESTED READINGS


The Writing Group for the PEPI (Postmenopausal Estrogen/Progestin Interventions) trial. Effects of hormone therapy on bone mineral density: Results from the postmenopausal estrogen/progestin interventions (PEPI) trial. JAMA 1996;276:1389.


THE BREAST

JAMES M. MCGREEVY

KIRBY I. BLAND

This chapter reviews the basic science facts that are essential for the clinician to understand breast disorders. To make this presentation readable and usable, it will be neither exhaustive nor comprehensive. The goal of the chapter is to present the basic science of the breast in a fashion that allows the clinician to recall the information easily for use in the practice of surgery.

THE NORMAL BREAST

Embryology

The breasts of mammals develop on the ventral aspect of the embryo in longitudinal bands called milk lines (Fig. 13.1). These milk lines extend from the axilla in a curvilinear fashion toward the midline, ending in the inguinal region (Fig. 13.2). Animals bearing multiple pairs of breasts develop these glands in the thoracic and abdominal portion of the milk lines. Humans have only one pair of breasts and develop the organ in the thoracic portion of the milk line. Between 2% and 6% of human females have an accessory nipple or extramammary collection of breast tissue (1). These extra glands (polymastia) or nipples (polymelhia) occur in the milk line. Extra nipples more commonly appear on the chest wall below the breast, whereas extra breast tissue more commonly occurs in the axilla. The milk lines are thickened epithelial bands derived from ectoderm. They begin to appear at the 6th week of gestational age. By the 9th week, the milk line has atrophied entirely, except in the pectoral region where the breasts develop. As the milk line regresses elsewhere in the embryo, the nipple bud appears. Between 10 and 16 weeks of gestation, this nipple bud develops by invagination of the overlying squamous epithelium (Fig. 13.3). As the invading squamous epithelium grows, it sprouts between 10 and 15 longitudinal fingers composed of cells. These epithelial extensions eventually develop into the mammary lobules. As the invading epithelium grows into the mesenchyme, differentiation begins. The mesenchyme forms the smooth muscle of the areola and supporting tissues of the breast as the epithelial cords elongate. Between 20 and 24 weeks gestation, these epithelial cords develop lumens. At 6 months, the lumens of the main epithelial cords extend into the secondary mammary branches. As the fetus approaches term, the mass of the epithelial cords increases four-fold, and lobular-alveolar formation begins at the end of the epithelial cords.

At birth, the breast of the neonate is a branching system of ducts arranged about the nipple with radial symmetry. The 15 to 25 individual mammary units drain into major ducts that converge into a retroareolar ampulla. Each ampulla then opens onto the nipple. At birth, a watery secretion may drain via the nipple, which is called witch’s milk, or colostrum. The colostrum is primarily water, fat, and cellular debris, which results from secretory activity in the cells of the alveoli (2). This secretion is stimulated by the withdrawal of placental steroids at the time of birth. From infancy until puberty, there is an increase in the supporting stromal structures of the breast and an elongation of the ducts in proportion to the increase in body size; no lobular development occurs before puberty (2).

Gross Anatomy

Topography and Surface Anatomy

The mature female breast varies tremendously in size and shape among individuals, and in the same individual, dramatic differences in configuration occur with pregnancy and advancing age. With pregnancy and lactation, the breast increases in turgor and weight; shape becomes more spherical. After pregnancy, the breast reduces in size and assumes a less round, more flattened, pendulous configuration. With advancing age, the breast substance is replaced with fat such that the elderly female breast is reduced in volume, is less firm, and remains flattened against the chest wall. The principal feature of the surface anatomy of the breast is the nip-
ple and areola. Together they comprise a circular, pigmented complex that varies between 2 and 3 cm in diameter. This area becomes more deeply pigmented and enlarged during pregnancy. The nipple is located in the center of the areola and is raised above the areola for a distance of approximately 1 cm. Less than 10% of the female population have inverted nipples (indentation of the nipple complex) (3). The milk ducts open in the base of this concavity. This normal variant must be acknowledged, because retraction of the nipple can be an early sign of malignancy in those patients who do not have an inverted nipple.

No subcutaneous fat exists directly under the areola. Instead there is a layer of smooth muscle arranged in both a circular and radial fashion. This arrangement produces erection of the nipple and a decrease in the diameter of the areola. Smooth muscle contraction results from both tactile sensory and autonomic sympathetic stimulation. Scattered throughout the areola are elevations of the skin that represent openings of the accessory areolar Montgomery glands. These glands are an intermediate stage between sweat glands and the mammary glands (2). They do not produce milk but may produce a scant secretion, which often contributes to the moisture of the areola. An increase in size is noted with pregnancy and lactation; these glands diminish after menopause.

**Glandular Anatomy**

Between 15 and 20 lactiferous ducts are found within the nipple. These ducts coalesce within the nipple such that the actual number of openings may not correlate with the number of breast lobules. The ducts within the nipple dilate to

**FIGURE 13.2.** Mammary milk line. After development of the milk buds in the pectoral area of ectodermal thickening, the milk streak extends from the axilla to the inguinal areas. At week 9 of intrauterine development, atrophy of the bud has occurred except for the presence of the supernumerary nipples or breast. (From Bland K, Romrell L. Congenital and acquired disturbances of breast development and growth. In: Bland KI, Copeland EM, eds. The breast: comprehensive management of benign and malignant diseases. Philadelphia: WB Saunders, 1991:70, with permission.)
form milk sinuses, which extend below the nipple to become the lactiferous sinuses. Each lactiferous sinus drains between 15 and 20 breast lobules, and each lobule drains between 10 and 100 alveoli (Fig. 13.4). The breast lobules are supported by stromal elements that interdigitate between the lobules to give the breast substance. There is no distinct fascia separating the lobules or encasing the breast parenchyma. Between the breast and the skin is a subcutaneous layer of fatty tissue. The plane between the subcutaneous tissue and the breast substance is well defined in younger women and may be used as a plane of dissection during mastectomy.

The upper and central portions of the breast are predominantly glandular tissue. The upper-outer quadrant has the largest volume of glandular tissue, which may explain the higher incidence of breast cancer in this area as well as the frequency of pain in this part of the breast caused by proliferative changes (2). The glandular tissue of the upper-outer quadrant extends up toward the axilla in the shape of a tongue. This elongation of breast tissue is called the tail of Spence; it proceeds through the axillary fascia and occasionally into the axilla where it is contiguous with the axillary lymph nodes. If a woman has glandular tissue extending into the axilla, she may experience axillary enlargement during pregnancy or menstruation. Scattered throughout the substance of the breast parenchyma are fibrous thickenings of the stromal connective tissue, which proceed from between the breast lobules and insert in a perpendicular fashion into both the skin and the underlying pectoral fascia. These ligaments, described by Cooper, serve a suspensory
function in that they attach the breast to the chest wall and to the skin. When cancer involves these ligaments, they shorten and can result in skin indentation which is recognized clinically as "peau d'orange" (2).

**Fascia**

Because the breast develops within an envelope of skin and subcutaneous tissue, actual fascia does not invest the breast substance. The organ rests on fascia overlying the pectoralis major muscle. Between the deep layer of the superficial pectoral fascia and the deep pectoral fascia is a potential space referred to as the retromammary bursa (2). This space is evident during mastectomy, as this portion of the dissection proceeds quickly and with minimal blood loss.

Another fascial layer of surgical significance is the clavpectoral fascia. This condensation of fascia covers the deep surface of the pectoralis major muscle and envelopes the pectoralis minor muscle. It then extends out into the axilla to form a sheath around the axillary vessels. The anterior thoracic vessels and nerves and the cephalic vein pass through the clavpectoral fascia (3). This fascia must be opened sharply to enter the axilla during the course of an axillary lymph node dissection.

**Microscopic Anatomy**

Before puberty, the epithelium of the ducts within the breast is a two-cell-layered basal cuboidal epithelium, whereas the alveolar lining is a low cylindrical-surface epithelium. With the onset of puberty, sex steroids (especially estrogen) initiate an increase in the number of layers that line both the ducts and the alveoli, with the formation of buds and papillae on some of these cells. In the mature nonpregnant breast, there are three cell types in the lining of the ducts: superficial (luminal) A cells, basal B cells, and myoepithelial cells (2).

The A cells of the duct mechanism are involved primarily in milk production. On routine histologic examination, they appear dark because of the rich cytoplasmic ribonucleic acid (RNA) and reticulum, and the many ribosomes within the cytoplasm. These cells can form bridges between themselves and sometimes proliferate into the lumen of the ducts.

The B cells, also called chief cells, are the most abundant cell type lining the duct. These cells have a clear cytoplasm and are not involved in the production of milk proteins. These cells seem to be energy providers for the luminal secretory cells. B cells contain distinctive intracytoplasmic filaments and fibrils, which are noncontractile. The filaments of these cells resemble the myofilaments of the myoepithelial cells.

Myoepithelial cells contain actual myofilaments, which insert on the base of the membrane of the cell and perform a contractile function. These cells are located in a basket-like network that surrounds the alveoli of the small milk ducts. They are stimulated to contract by sex steroids and prolactin. They do not have direct innervation, which allows contraction. With the suckling reflex, oxytocin release from the posterior pituitary results in contraction of the myoepithelial cells such that the milk-filled alveolus is emptied into the smaller milk ducts. Because the smaller milk ducts contain a network of myoepithelial cells, milk is evacuated into the larger ductules. No smooth muscle exists around the alveolus or the small milk ducts. These cells decrease in size and number after completion of lactation and are diminished in number after menopause. Because these cells are ectodermal in origin, they resemble their mesodermal counterparts, the smooth muscle cells (2).

**Hormone Regulation**

**Estrogen**

Estrogen is the principal hormone responsible for the development of the female breast and for maintenance of the glandular breast elements throughout reproductive life. Estrogen has potent mitogenic effects on mammary epithelium. This steroidal hormone is responsible for the initiation of ductal development, as it increases the number of estrogen and progesterone receptors on the mammary epithelial cells (4).

**Progesterone**

Progesterone, principally of ovarian origin, is responsible for the differentiation of epithelial cells and causes lobular development. This hormone may actually limit proliferation of the tubular system of the glandular breast elements and reduce estrogen binding to membrane receptors (4).

**Prolactin**

Prolactin is required, together with the presence of growth hormone and cortisol, for the development of mammary epithelium. This hormone also contributes to the development of the adipose tissue in the breast. Biochemically, it increases the number of estrogen receptors within epithelial cells. Prolactin can act synergistically with estrogen in ductal development and with progesterone in lobular-alveolar development. This compound is the primary hormone for lactogenesis in late pregnancy and in the postpartum period. It stimulates the differentiation of milk-producing cells and initiates the synthesis of the components of milk in those cells (4).

**Other Hormones**

The effects of growth hormone, cortisol, and thyroid hormone in the human female breast are not well established. However, each of them initiates a variety of effects on mam-
ary epithelial cells in experimental conditions. The secretion of the primary trophic hormones for the human breast (estrogen and progesterone) is under the control of the neurohormones of the hypothalamus (gonadotropin-releasing hormone, GnRH) and the trophic hormones of the pituitary gland (luteinizing hormone, LH, and follicle-stimulating hormone, FSH; both secreted by the basophilic cells of the anterior pituitary) (Fig. 13.5). Prolactin is secreted by the acidophilic cells of the anterior pituitary gland.

**Puberty**

With the onset of puberty comes the initiation of the secretion of GnRH, LH, and FSH from the anterior pituitary gland. This event results in estrogen and progesterone release from the ovary. Rising serum levels of estrogen result in breast bud development as the first noticeable event in breast maturation. Estrogen stimulates proliferation of the ductal epithelium, the myoepithelial cells, and the surrounding stroma (4). Progesterone, which is released from the ovary, initiates formation of the secretory components of the mammary epithelium, which is located at the terminal aspect of the ductules. The sex steroids also increase the amount of connective tissue and fat in the breast, so that within 2 years of the onset of menarche the breast has assumed a mature spherical configuration.

**Menstrual Cycle**

The changing serum and tissue concentrations of estrogen and progesterone associated with the menstrual cycle have several effects on the mature female breast, which are outlined in Table 13.1 (2). In the premenstrual phase of the cycle, rising levels of FSH and LH result in elevation of plasma estrogen values. Estrogen values peak in the late follicular phase of the cycle when ovulation occurs. After ovulation, the luteal portion of the cycle begins, estrogen continues to increase above that seen in the follicular phase of the cycle, and plasma progesterone rises. The elevated plasma estrogen and progesterone tissue concentrations result in an increase in volume of the breast. This increased volume results from water retention, an increase in basement membrane thickness, an increase in alveolar diameter, a stromal infiltration with fluid, lymphoid, and plasma cells, and stimulation of intraalveolar secretion. Consequently, breast volume is greatest in the second half of the menstrual cycle, just before the

---

**FIGURE 13.5. Overview of the neuroendocrine control of breast development and function with relationship to gonadotrophic hormones of the anterior pituitary and ovary. Basophil secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is responsible for ovarian synthesis and release of progesterone and estrogen, respectively. The mambrotropic effects of estrogen and progesterone initiate myoepithelial and alveolar development. Ductal and stromal enlargement with pregnancy occur as a result of progesterone and estrogen secretions in excess from the corpus luteum (first 12 weeks) and thereafter from the placenta. Acidophil cell secretion of prolactin (PRL) is initiated after evacuation of the gravid uterus and is mammatropic to the lobular alveoli. The suckling reflex initiates oxytocin release from the posterior pituitary and is stimulatory to alveolar myoepithelial cells to initiate milk release. Neuroendocrine organs other than the pituitary and ovary secrete hormones (glucocorticoid, GH, insulin, and thyroxine) that are trophic to ductal and glandular maintenance and growth. GH, growth hormone; CRF, corticotropin-releasing factor; LH-RH, luteinizing hormone-releasing factor; HCG, human chonic gonadotropin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.**
onset of menses, the size, density, nodularity, and sensitivity of the breast increases. With the onset of menses, tissue volume continuously increases, perhaps related to intraalveolar secretion brought about by sex steroid withdrawal, permitting partial stimulatory action by prolactin. In the postmenstrual period, breast volume decreases with a reduction in alveolar and glandular cell size, a regression of the edema, and a subsidence of the lymphoid inflammatory cell infiltrate. The mammary gland is smallest between the fourth and seventh day of the cycle. This mammary involution results from a fall in plasma levels of estrogen and progesterone after the onset of the menses. This involution is incomplete with each cycle such that mammary growth continues up to the age of 30 to 35 years.

**Pregnancy**

The changes in the breast induced by pregnancy are summarized in Table 13.2 (2). With the onset of pregnancy, high levels of plasma estrogen and progesterone are secreted by the corpus luteum and are maintained after the twelfth gestational week by the placenta. Plasma estrogen and progesterone values gradually increase throughout all trimesters of the pregnancy; in late gestation, the concentration of prolactin in the serum increases ten-fold. Under the effects of these increasing sex steroids, the areola and nipple become larger, more prominent, and more pigmented. At the microscopic level, sex steroids cause the ducts and lobules to proliferate; these hormones also induce alveolar development.

In the first trimester of pregnancy, lobular-alveolar formation is initiated. The proliferating glandular epithelium replaces resting adipose and connective tissue. The ducts proliferate and branch to begin development of multiple alveoli. In the second trimester of pregnancy, the proliferation of the ductal elements increases. A true lobular-alveolar system develops, and the secretory epithelium becomes active.

In the third trimester of pregnancy, the secretory activity of the epithelium increases. Fat droplets accumulate in the alveolar cells and colostrum fills the alveolar and ductal spaces. Blood flow increases and myoepithelial cells enlarge. In the last weeks of pregnancy, rising prolactin levels induce a limited synthesis of milk fats and proteins.

**Menopause**

Following withdrawal of the sex steroids with cessation of ovarian function, lobules and ducts in the breast reduce to an atrophic, hypoplastic epithelium. The periductal fibrous tissue becomes denser, and the lactiferous duct network di-

### Table 13.1. Breast Changes During Menstrual Cycle

<table>
<thead>
<tr>
<th>Phase of Cycle</th>
<th>Effect</th>
<th>Cause</th>
<th>Hormone Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenstrual</td>
<td>Increase in volume</td>
<td>Water retention, Basement-membrane thickening, Increase in alveolar diameter, Intraalveolar secretion</td>
<td>Estrogen increase</td>
</tr>
<tr>
<td>Menses withdrawal</td>
<td>Increase in volume</td>
<td>Intraalveolar secretion</td>
<td>Sex-steroid permitting limited prolactin action</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>Decrease in volume</td>
<td>Degeneration of glandular cells, Reduction in alveolar size, Smallest on days 4-7 of cycle</td>
<td>Estrogen withdrawal</td>
</tr>
</tbody>
</table>

### Table 13.2. Breast Changes During Pregnancy

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Histologic Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Terminal ductule sprouting, Lobular-alveolar formation begins, Glandular buds invade and replace connective tissue and fat, Alveolar epithelium changes from 2-cell to 1-cell layer</td>
</tr>
<tr>
<td>Second</td>
<td>Ductular proliferation, Increase in lobular-alveolar units, Lymphocytes in connective tissue, Activation of secretory epithelium resulting in colostrum</td>
</tr>
<tr>
<td>Third</td>
<td>Fat droplets in secretory alveolar cells, Filling of alveoli with colostrum, Replacement of connective tissue and fat by lobular-alveolar proliferation</td>
</tr>
</tbody>
</table>
lates. Cystic formation occurs as the epithelium in the lobular acini becomes atrophic. There is a loss in total mass of fat tissue within the breast. The end result of these degenerative changes is a decrease in breast volume and a change in shape of the breast from the original lobular structure to a flat, pendulous organ.

**Lactation**

During pregnancy, sex steroids may cross the placenta and stimulate the fonal breast; 80% to 90% of newborns have nipple discharge (colostrum milk) at the time of birth. This secretion comes from the one to two-cell-thick epithelium of the alveoli under the influence of prolactin. With delivery, the sudden withdrawal of the high levels of sex steroids of the placenta from the epithelium of the infantile breast allows prolactin to stimulate the mammary epithelium, producing this *witch’s milk*. This effect reaches a maximum in 4 to 7 days and subsides in 3 to 4 weeks (2) as the circulating hormones are metabolized.

**Blood Supply**

Approximately 60% of the total breast mass obtains blood supply from the internal mammary artery (3). Ventral branches of this artery (anterior rami mammarii) penetrate the intercostal muscles of the second to the fifth intercostal spaces approximately 1 to 2 cm lateral to the parasternal border. Once these arteries enter the breast, they pass through the breast substance transversely toward the nipple. Extensive collateralizing within the breast occurs via the two other major sources of blood supply: the lateral thoracic artery and the branches from the intercostal arteries. The lateral thoracic artery originates from the axillary artery proximal to the origin of the subscapularis artery. It passes along the lower border of the pectoralis minor muscle. At approximately the level of the median portion of the muscle belly of the pectoralis minor, the lateral thoracic artery gives off the external mammary artery, which courses ventrally around the muscle belly to supply primarily the upper-outer quadrant of the breast. The lower-outer quadrant of the breast is supplied by branches of the third through fifth posterior intercostal arteries. Other arteries that supply a minor amount of the nutrient blood supply to the breast are the thoracoacromial, subscapular, upper thoracic, and thoracodorsal arteries (Fig. 13.6).

The venous drainage of the breast follows the primary arterial supply. The principal veins are the intercostal veins, which drain to the internal mammary vein; the external mammary vein; and the lateral thoracic vein, which drains into the axillary vein.

There are two other arteries of surgical significance. The first is the thoracodorsal branch of the subscapularis artery. This vessel does not supply blood to the breast but is centrally located within the axillary contents and is contiguous with the central and scapular lymph node groups. Because of its deep location, this vessel can be difficult to control if injured operatively. It lies in close proximity to the long thoracic and thoracodorsal nerves and may be injured during isolation of those structures. A second artery of surgical significance is a large branch of the lateral thoracic artery (Fig. 13.6), which is the primary blood supply to the pectoralis major muscle. This branch comes directly from the groove formed by the pectoralis minor and major muscles, approximately 3 to 5 cm below the axillary vein. This large branch enters the pectoralis major in the laterally exposed portion

**FIGURE 13.6.** Arterial distribution of blood to the breast, axilla, and chest wall. The breast receives its blood supply via three major arterial routes: (i) medi ally from anterior perforating intercostal branches arising from the internal thoracic artery, (ii) later ally from either pectoral branches of the thoracoacromial trunk or branches of the lateral thoracic artery (the thoracoacromial trunk and the lateral thoracic arteries are branches of the axillary artery), and (iii) from lateral cutaneous branches of the intercostal arteries that are associated with the overlying breast. The arteries indicated with a dashed line lie deep to the muscles of the thoracic wall and axilla. Many of the arteries must pass through these muscles before reaching the breast. (From Romrell L, Bland K. Anatomy of the breast, axilla, chest wall, and related metastatic sites. In: Bland KI, Copeland EM, eds. The breast: comprehensive management of benign and malignant diseases. Philadelphia: WB Saunders, 1991:26. With permission.)
of the muscle belly. If this artery is interrupted, the pectoralis major muscle may undergo severe atrophy.

**Lymph Drainage**

The axillary lymph nodes that drain the breast are grouped into three levels, defined by their relationship to the pectoralis minor muscle (Fig. 13.7). Level I nodes are those that are lateral to or below the lower border of the pectoralis minor (the external mammary, axillary vein, and scapular lymph node groups). Level II lymph nodes are those that are deep to or behind the pectoralis minor muscle (the central lymph node group and some of the subclavicular nodes). Level III nodes are those that are above the upper border of the pectoralis minor muscle (the subclavicular or apical node group) (Fig. 13.8).

There are four major routes for lymphatic drainage from the breast: the lateral, medial, transsectoral, and retrosectoral routes (3). The lateral route is the most important from an operative standpoint. The skin and the majority of the breast drain lymph via the external mammary node group, and lymph flows into the lateral node group thereafter. Subsequent drainage into the proximal aspects of the axilla pass through the subscapular, central, apical, and supraclavicular node groups. Each of these groups are detailed below. The second major route of drainage is the medial route. Lymph from the skin and the central and medial parts of the breast follow the major vessels that perforate the intercostal muscles from the internal mammary artery and vein. These lymphatics drain into the internal mammary lymph node chain. The third major lymphatic drainage route is the transsectoral route. Lym-
phatics from the retromammary plexus penetrate the pectoralis major muscle to drain into the interpectoral nodes, which are also known as Rotter nodes. These nodes are located beneath the lateral aspect of the pectoralis major where it forms a groove in contact with the pectoralis minor muscle. Rotter nodes drain into lymphatics that course along the thoracoacromial artery to the subclavicular group of nodes. A fourth major route of lymphatic drainage from the breast is the retropectoral route. Lymphatics from the superior and internal portions of the breast drain into lymphatics on the lateral and inferior portion of the pectoralis major and minor muscles. These lymphatics eventually terminate in the apex of the axilla and the subclavicular node group. The nodes that drain the lateral aspect of the breast are divided into the following groups.

Axillary Vein Group (Lateral Group)
The lateral group is located within level I of the axilla. These veins are the most lateral and numerous group of nodes in the axilla and are located ventral and caudal to the axillary vein. They receive the majority of lymph flow from the arm.

External Mammary Group (Anterior or Pectoral Group)
The anterior group is also located in level 1 of the axilla. These veins are found along the lower border of the pectoralis minor muscle in association with the lateral thoracic vessels. These nodes receive the major portion of the lymph flow from the breast and drain into the central group of axillary lymph nodes.

Subscapular (Scapular or Posterior) Group
The subscapular group is also located in level I of the axilla, along the lateral border of the scapula. This group lies on the posterior wall of the axilla in association with the thoracodorsal branches of the subscapular vein. These nodes drain into the central and subclavicular groups.

Central Group
The central group is located in level II of the axilla behind the pectoralis minor muscle wall and is surrounded by fat. These nodes are called the central group because of their location midway between the anterior and posterior axillary line. These nodes are often close to the skin and are, therefore, frequently palpable when they are clinically involved with metastases. They drain into the subclavicular group of nodes.

Subclavicular (Apical) Group
The subclavicular group is located in level III of the axilla at the point where the axillary vein passes under the subclavius muscle to become the subclavian vein. These nodes are the highest and most medial nodes in the axilla and receive lymph from all the other groups of axillary nodes. They drain directly into either the internal jugular vein, the right lymphatic duct, or the thoracic duct.

Interpectoral Group (Rotter Nodes)
Rotter nodes (named after a German pathologist) are located between the pectoralis major and minor muscles in association with the pectoral branches of the thoracoacromial vessels. Rotter nodes drain directly into the central and subclavicular groups of nodes.

Innervation
The breast is supplied by both somatic sensory and autonomic innervation. The nipple and areola have abundant somatic sensory and sympathetic autonomic innervation that, when activated, result in contraction of the areola and erection of the nipple (2). Parasympathetic autonomic innervation of the breast exists. Furthermore, the parenchyma of the mammary gland has no innervation (3); lactation is stimulated exclusively by hormonal influences (prolactin). Somatic sensory innervation to the skin overlying the breast has origin from three sources. There are lateral branches of the thoracic intercostal nerves 3 to 7, which supply the skin of the lateral aspect of the breast. Skin of the medial aspect of the breast is innervated from branches of the thoracic intercostal nerves 2 to 6. The superior aspect of the breast is also supplied by branches from the supraclavicular nerve, which arises from the brachial plexus.

Several nerves are of surgical significance during operative procedures on the breast and the axillary contents. The first is the long thoracic nerve (respiratory nerve of Bell). This nerve arises from the brachial plexus and passes inferiorly in the medial border of the axilla within a plane dorsal to the axillary vein. The long thoracic nerve is found within the serratus fascia millimeters from the chest wall as it courses to innervate the serratus anterior muscle. Transection of this nerve results in postoperative disability known as a winging scapula. The second nerve of surgical significance is the thoracodorsal nerve. This nerve arises from the posterior cord of the brachial plexus and passes inferiorly into the axilla dorsal to the axillary vein. It is usually found in the same plane as that of the long thoracic nerve and approximately 1 to 3 cm lateral to that nerve. The thoracodorsal passes caudally along the lateral border of the axilla for 4 to 7 cm, then turns medially to enter the latissimus dorsi muscle. This nerve is usually exposed in its entire length during an axillary dissection. It can be injured in the lower axilla when the operator feels that the axillary dissection has been completed safely. Additional nerves of surgical significance are the intercostal brachial nerves, which arise from intercostal nerves 2 and 3. These nerves are large and they pass directly across (transverse) the axilla through the axillary contents, which are removed in the axillary dissection. They are transected frequently with dissection, resulting in numbness of the upper inner arm. Another nerve of significance during a surgical procedure is the lateral pectoral nerve. This nerve accompanies the lateral thoracic artery into the belly of the pectoralis major muscle. Transection of this nerve will denervate the
majority of the pectoralis major muscle and result in significant cosmetic disability with atrophy of this important functional muscle of the chest wall.

**BENIGN CLINICAL CONDITIONS**

**Fibrocystic Disease**

Fibrocystic disease is a term that has been used widely in the past but is being replaced appropriately in medical lexicon. The reason for its disuse can be appreciated readily by a consideration of the term's inaccuracy and imprecision. Fibrocystic disease has two definitions, a clinical one and a histologic one. Clinically, the term refers to the presence of palpable lumps in the breast, which fluctuate in size and discomfort with the menstrual cycle. Symptoms become worse with advancing age until menopause, when symptoms cease. When one applies this clinical definition, at least 50% of females have fibrocystic disease as a normal (physiologic) condition (5). Because the breasts are composed of elements that are remarkably sensitive to sex steroids in terms of their growth and development, changes in size and sensitivity during the cycle are evident. In addition, it is not unusual to have variations in responsiveness to hormonal stimulation that result in variations of tissue density (lumps) between breasts in the same woman and even among quadrants within the same breast. These physiologically induced changes in breast texture and tenderness are so frequent that a precise distinction between clinical disease and physiologic variation is impossible.

Attempts to define fibrocystic disease histologically have been equally problematic. Foote and Stewart (6) attempted to define the pathologic basis of fibrocystic disease by listing five microscopic findings associated with the condition that they referred to as chronic cystic mastitis. Those changes include macrocysts and microcysts, sclerosing adenosis, fibrosis, papillomatosis (epithelial hyperplasia), and apocrine change. Unfortunately, these histologic variants are seen frequently in women with or without clinical fibrocystic disease. Love et al. (7) summarized the literature on the subject and noted that 58% of women in eight different autopsy studies had these changes in the normal breast. In addition, women over 70 for whom there was no antemortem diagnosis of fibrocystic disease had the histologic changes noted by Foote and Stewart 89% of the time. Furthermore, 69% of the women had histologically confirmed epithelial hyperplasia. Therefore, both definitions are imprecise because the clinical definition fits 50% of the population and the histologic definition applies to 70%. Thus the term fibrocystic disease should not be used (7), because of its vague and imprecise connotation. Fibrocystic changes are normal changes expected within the breast that become more evident with aging. The most important task for the surgeon is an understanding of the relationship of fibrocystic change to the risk for developing cancer. The classification shown in Table 13.3 of benign histologic breast conditions has been devised to identify those changes that are associated with the subsequent development of breast cancer (8).

**TABLE 13.3. CLASSIFICATION OF BENIGN HISTOLOGIC BREAST CONDITIONS**

<table>
<thead>
<tr>
<th>Lesion Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonproliferative lesions</td>
</tr>
<tr>
<td>Cysts and apocrine metaplasia</td>
</tr>
<tr>
<td>Duct ectasia</td>
</tr>
<tr>
<td>Mild epithelial hyperplasia</td>
</tr>
<tr>
<td>Califications</td>
</tr>
<tr>
<td>Fibroadenoma</td>
</tr>
<tr>
<td>Proliferative lesions</td>
</tr>
<tr>
<td>Sclerosing adenosis</td>
</tr>
<tr>
<td>Radical scar and complex sclerosing lesions</td>
</tr>
<tr>
<td>Moderate and florid epithelial hyperplasia</td>
</tr>
<tr>
<td>Intraductal papillomas</td>
</tr>
<tr>
<td>Atypical proliferative lesions</td>
</tr>
<tr>
<td>Atypical lobular hyperplasia</td>
</tr>
<tr>
<td>Atypical ductal hyperplasia</td>
</tr>
</tbody>
</table>

**Nonproliferative Lesions**

**Cysts and Apocrine Metaplasia**

Both macrocysts (larger than 1 cm) and microcysts (smaller than 1 cm) are common in the premenopausal female breast. In an autopsy study of 725 women, 58% had microcysts and 21% had macrocysts (9). The smaller cysts most likely result from lobular involution caused by hormone imbalance. These cysts are lined with cells of apocrine derivation with secretory snouts. The fluid that fills these smaller cysts contains proteins and enzymes that are found only in apocrine epithelium. Also, the lobular component of these microcysts has been demonstrated to contain elastic fiber when stained by using immunohistochemical techniques (10). The larger cysts are probably the result of ductal obstruction secondary to sloughed epithelium or from angulation of the duct. Because the processes that produce the cysts are different, one would expect two distinct types of fluid to occupy the cysts. In the macrocyst, the type of fluid is the same as in other cysts that are lined with flattened cells. These cells are nonsecretory with passive barriers through which the cyst fluid equilibrates with the plasma. Macrocystic fluid has a high sodium content and low potassium concentration, similar to plasma. Macrocysts also contain albumin, nonsecretory 75 immunoglobulin A (IgA), and low levels of apocrine cyst proteins. The second type of cyst fluid is that associated with tall columnar cells that have secretory function. This fluid, therefore, has a high potassium concentration, a low sodium concentration, low levels of albumin, and high levels of apocrine cyst proteins. Secretory IgA, epidermal growth factor, and dehydro-3-epiandrosterone sulfate are also found in this fluid.
TABLE 13.4. RELATIVE RISK FOR INVASIVE BREAST CARCINOMA BASED ON HISTOLOGIC EXAMINATION OF BREAST TISSUE WITHOUT CARCINOMA

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>No increased risk (no proliferative disease)</td>
<td></td>
</tr>
<tr>
<td>Apocrine metaplasia</td>
<td></td>
</tr>
<tr>
<td>Duct ectasia</td>
<td></td>
</tr>
<tr>
<td>Mild epithelial hyperplasia of unusual type</td>
<td></td>
</tr>
<tr>
<td>Slightly increased risk (1.5–2 times)</td>
<td></td>
</tr>
<tr>
<td>Hyperplasia of usual type, moderate or florid</td>
<td></td>
</tr>
<tr>
<td>Sclerosing adenosis, papilloma</td>
<td></td>
</tr>
<tr>
<td>Moderately increased risk (4–5 times) (atypical hyperplasia or borderline lesions)</td>
<td></td>
</tr>
<tr>
<td>Atypical ductal hyperplasia and atypical lobular hyperplasia</td>
<td></td>
</tr>
<tr>
<td>High risk (8–10 times) (carcinoma in situ)</td>
<td></td>
</tr>
<tr>
<td>Lobular carcinoma in situ and ductal carcinoma in situ (non-comedo)</td>
<td></td>
</tr>
</tbody>
</table>

Cysts tend to be multiple and recurrent. In a study of 352 women followed for 5 years after their initial presentation with a breast cyst, 54% subsequently developed additional cysts and 12% presented with multiple cysts (11). A total of 10% of cysts that were aspirated in this study refilled to the extent that they became palpable again. The apocrine-lined cysts may be more prone to recur. The presence of breast cysts is not considered a risk factor for the development of breast cancer (Table 13.4). However, three epidemiologic reviews suggest an increase of 1.7 to 4.0 times in the risk of breast cancer in women with breast cysts (12–14). These reviews did not control for the association of proliferative disorders with breast cysts. Any increase in the risk of breast cancer seen in women with breast cysts is most likely the result of epithelial proliferation, not cystic disease alone.

Apocrine metaplasia refers to the transition of the ductal epithelium to the tall columnar cell type with a rounded apical aspect similar to that of secretory cells in the breast. This metaplastic change is found most often in cysts and has not been confirmed to manifest an increased incidence of breast cancer.

Duct Ectasia

Duct ectasia is a benign condition resulting in dilatation, periductal inflammation, and fibrosis of the retroareolar ducts just beneath the nipple. A common disorder, it is the presenting complaint of between 1% and 4% of women who present with breast symptoms (15). The incidence of duct ectasia in asymptomatic women has been reported to be as high as 20% to 24% (15). The plasma cell is a predominant feature of the inflammatory process around the ducts, thus the term plasma cell mastitis has been applied. Other names for this clinical entity include mammary duct ectasia, periductal mastitis, comedo mastitis, and secretory disease of the breast. This process presents different clinical symptoms manifested at different stages of presentation.

The early symptoms are nipple discharge and pain. Late in the course of the disease, the pain becomes more pronounced as the periductal inflammation becomes more profound. Subareolar abscess and mammary duct fistula are other features of this disease in its intermediate stages of development. The late symptoms are those of fibrosis from the periductal inflammation: diminished pain, nipple retraction, mass behind the nipple, skin fixation, and nipple discharge. For obvious reasons, advanced stages of duct ectasia are indistinguishable from those of ductal adenocarcinoma.

Two theories regarding the development of ductal ectasia are considered. An older theory holds that the genesis of the process is an abnormal dilation of retroareolar ducts of the nipple. According to this hypothesis, periductal inflammation is caused by extrusion of ductal debris into the periductal stroma across the damaged ductal epithelium. The second theory holds that periductal inflammation is the primary and essential feature of this disorder; ductal ectasia is a result of the destruction of the periductal stroma by the inflammation (15). Duct ectasia is not associated with an increased risk of breast cancer.

Mild Epithelial Hyperplasia

Epithelial hyperplasia refers to an increase in the number of cells in relation to the basement membrane. There are normally two cell layers that line the ducts, thus a thickness exceeding three cells represents hyperplasia. This process presents in a spectrum of mild epithelial hyperplasia to moderate hyperplasia, for which there are five or more cell layers. Finally, florid hyperplasia refers to the process in which ducts are packed with solid sheets of cells. Inflammatory cells may be interspersed with the epithelial cells. In the moderate forms of the disease, the cells can form bridges as they cross the duct. As the hyperplastic cells fill the duct space, they may initiate ductal dilatation. Mild-to-moderate epithelial hyperplasia without cellular atypia is common. These changes may be found in up to 20% of breast biopsies (16). Patients observed to have epithelial hyperplasia without cellular atypia have a slightly increased risk of breast cancer, which is 1.5 to 2.0 times greater than that of the general population.

Calcifications

The widespread application of mammography as a screening modality for breast cancer has resulted in better documentation of the frequency with which microcalcifications occur in the female breast. Approximately 90% to 95% of breast calcifications are associated with benign conditions. Calcium deposits can occur within the ducts or lobules, in the stroma, or within the epithelium. Widely scattered calcium deposits are a common feature with sclerosing adenosis. A total of 5% to 10% of patients in whom microcalcifications are identified mammographically will have breast cancer. Although radiographic features may be suggestive of
a benign or malignant process, a biopsy is often necessary to establish histology. The characteristics of benign calcifications include long parallel lines that resemble the cast of a blood vessel; the burst or “popcorn” pattern of coarse calcifications within a fibroadenoma; blunt calcifications with radiolucent centers; uniformity of size; and widely dispersed and well-defined deposits without spicules, branches, or comma shapes. Features that are more frequently associated with a malignant process include irregular, poorly defined deposits that have branches, spicules, or comma shapes that are clustered within 1 cm². These microcalcifications are often identified as five or more densities within 1 cm² (17). The absolute number of clustered calcifications is not necessarily of value.

**Fibroadenoma**

Fibroadenoma is a common clinical entity observed in young women, age 15 to 25. The clinical features of the mass are distinct enough to allow diagnosis by physical examination alone. They are discrete, well defined, firm, freely movable, and nontender. The mass is composed of elements thought to be derived from the terminal duct lobular unit (18). Fibroadenomas are probably not a true neoplasm but rather an exuberant overgrowth of normal tissue constituents (18). Immunohistochemical and electron microscopic evidence (19) suggest the cell of origin for fibroadenomas may be the fibroblast rather than the myoepithelial cell. In addition, estrogen receptor (ER) and progesterone receptor (PR) are commonly expressed biochemically in fibroadenomas (20). The tumor appears to be sex-steroid dependent, a trait it shares with fibrocystic disease. Fibroadenomas can regress, remain the same size, or enlarge. Fibroadenomas will regress with aging, as noted by the low incidence of these tumors in mastectomies and at autopsy (21).

The cellularity of fibroadenomas has been shown to diminish with aging (22). In a prospective follow-up of 63 patients with 20 fibroadenomas, 31% resolved, 12% became smaller, 25% had no change, and 32% enlarged (18). The risk for breast cancer in patients who have had a fibroadenoma excised is 2 to 4 times higher than for the general population (23). In addition, cancer can develop from the epithelial elements of the fibroadenoma stroma; this is an unusual occurrence. Two literature reviews have collected only 96 such cases (24,25). When carcinoma appears in a fibroadenoma, it is usually in older patients (40 to 45 years old) and contained within the fibroadenoma (50%). Half of the cancers are lobular carcinoma in situ.

**Proliferative Lesions**

**Sclerosing Adenosis**

Sclerosing adenosis, a histologic condition, can present as a painful mass (26) or as an incidental finding in a biopsy specimen obtained for other reasons. It can be confused microscopically, grossly, and mammographically with cancer. Histologically, this process resembles cancer because there is proliferation of both the stromal and epithelial elements of the terminal duct lobular units in such a manner that cords of epithelial cells are isolated within the stroma. These cords can also be found near nerves and blood vessels. This process is called pseudoinfiltration and can resemble invasive adenocarcinoma. In sclerosing adenosis, the normal two-cell layer is maintained in relation to the basement membrane. Therefore, sclerosing adenosis can be distinguished from cancer by electron microscopy, because the infiltrating cells of invasive cancer have breached the intact basement membrane of the ductal epithelium (26). The fibrosis (desmoplasia) that occurs with this process distorts the normal lobular architecture in a stellate or whorled pattern that may resemble cancer when examined grossly. This fibrosis may present as a dense opacity on mammography with architectural distortion that is often associated with scattered or clustered microcalcifications. Patients who have sclerosing adenosis on biopsy have a slightly increased risk for development of breast cancer (1.5 to 4.0 times) compared with the normal (index) population (16).

**Radial Scar and Complex Sclerosing Lesion**

Radial scar and complex sclerosing lesion refer to the same rare pathologic entity. Radial scar is applied to lesions up to 1 cm in size, and complex sclerosing lesion is reserved for lesions larger than 1 cm. The identifying characteristic of these entities is a central scar with proliferating epithelial elements emanating from the center in a stellate fashion. The central sclerosis can be surrounded by various degrees of cystic dilation, epithelial hyperplasia, apocrine metaplasia, and papillomatosis. This sclerosing process can resemble cancer grossly and mammographically for the same reasons that sclerosing adenosis resembles cancer. There is a slight increase in risk for breast cancer (1.5 to 4.0 times) in patients found to have these lesions on breast biopsy (16).

**Moderate and Florid Epithelial Hyperplasias**

Moderate and florid epithelial hyperplasias are the most common of the proliferative breast lesions, being identified in 20% of breast biopsies (27). Moderate hyperplasia is defined as cellular proliferation more than three cell layers above the basement membrane. Florid hyperplasia involves many cell layers such that greater than 70% of the duct lumen is filled with cells. The duct is often packed and distended with cells, which can form clefts, papillomas, arches, and bridges within the lumen. The cells have no atypical characteristics. Patients with mild to florid hyperplasia have a slight (1.5 to 4.0 times) increase in risk for breast cancer (16).
Intraductal Papilloma

Intraductal papilloma usually occurs in the lactiferous ducts and sinuses. It commonly presents as bloody nipple discharge and less commonly as a palpable mass. The process results from a neoplastic alteration of the epithelial lining of the duct. The tumor (0.5 to 5.0 cm) is attached to the duct wall by a fibrovascular stalk of connective tissue. This stalk branches many times to support a villous pattern of epithelial cells. The entire polyp or papilloma is a discrete tumor that is identified easily by galactogram and occasionally by mammogram. There is an increased risk (1.5 to 4.0 times) of breast cancer in patients with papillomatosis. Multiple papillomas result in higher cancer risk than do single papillomas (28).

Atypical Proliferative Lesions

Atypical Lobular Hyperplasia

As with the other proliferative lesions of the breast, the principal reason for identifying and defining atypical hyperplasia is to define the risk of cancer development in a breast with these histologic features. Atypical hyperplasia is identified by features similar to those that characterize carcinoma in situ; however, the histologic changes associated with atypical hyperplasia are less abnormal than those of carcinoma in situ. For this reason, an understanding of the pathologic spectrum of atypical lobular hyperplasia (ALH) begins with the definition of lobular carcinoma in situ. Page and Simpson define lobular carcinoma in situ as “filling, distortion and distortion of more than half of the acini of a lobular unit by a uniform population of characteristic cells” (16). The diagnosis of ALH thus requires that less than half of the acini are completely destroyed and/or less than half of the acini are filled by the uniform population of characteristic cells (16). This abnormality is associated with a four-to-five-times increased incidence of the development of breast cancer compared with the normal population. A great enhancement in cancer risk is noted for patients who have atypical lobular or ductal hyperplasia and a positive family history. Approximately 20% of these patients develop invasive breast cancer within 15 years of the diagnosis of atypical hyperplasia (28).

Atypical Ductal Hyperplasia

As with the lobular variety, atypical ductal hyperplasia (ADuH) is defined as an incomplete expression of the features of ductal carcinoma in situ. Page and Dupont (28) define ductal carcinoma-in-situ as two or more ductal spaces that are filled completely with a uniform population of neoplastic appearing cells; there also are intercellular bridges and arches composed of evenly placed, uniform cells (16). ADuH has more variants in the histologic patterns of presentation and, therefore, is harder to recognize than ALH.

Biopsies that demonstrate ADH often have areas of ductal carcinoma in situ, but there are also cells in the involved area that are histologically normal. The hyperplastic cells are oriented properly in regard to the basement membrane and they do not have nuclear abnormalities that are characteristic of the atypical cells (28). Florid epithelial hyperplasia can be confused with ADuH. The cancer risk for florid epithelial hyperplasia is much less (1.5 to 2.0 times index) than the risk of ADuH (4 to 5 times index).

Inflammatory Lesions

Abscess

Two major types of abscesses are recognized in the breast: periareolar (nonlactational) and puerperal (related to childbirth and breast-feeding). The relative incidence of these two abscesses has changed such that the periareolar variant is now more common than the puerperal (29). Benson (29) reports that the number of puerperal breast abscesses seen at the Leeds General Infirmary decreased from approximately 20 per year in 1975 to 5 or less per year in 1987. The most plausible explanation for this change is the prompt recognition of mastitis in lactating women and early treatment with antibiotics. Puerperal mastitis develops in a breast segment when a major duct is obstructed with inspissated milk. The segment becomes engorged with milk, causing mastalgia, swelling, and redness. Bacteria thrive in the stagnant milk, producing a characteristic wedge-shaped abscess, with the base at the edge of the breast and the apex near the nipple. The evolution of the abscess can be aborted if the blocked duct is opened by sucking or if the patient receives antibiotics in early phases of the disease. For this reason, breast-feeding may be allowed to continue in patients with early mastitis as it prevents engorgement and promotes drainage. However, lactation frequently stops spontaneously in patients who develop a puerperal abscess (30). The bacteria commonly involved in puerperal abscess are skin organisms, so the drug of choice is a penicillinase-resistant penicillin. The differential diagnosis for breast abscess in a nonlactating woman includes chronic, recurring periareolar abscess, carcinoma, tuberculous, inflammatory cysts, and duct ectasia (31). The most common of these disease states is the periareolar abscess. As discussed earlier, periareolar abscess is commonly associated with primary ductal disease in the lactiferous sinuses. Recurrent periareolar abscess is commonly associated with mammary duct fistula. The recurrence rate for periareolar abscess is 38% (30). The rate is higher once a periareolar abscess has recurred (32). Recurrence is commonly related to the persistence of a fistulous tract from the lactiferous sinuses to the periareolar skin. The tract remains open as a result of granulation tissue and provides a locus for contaminating bacteria to develop an abscess similar to a fistula in ano. The management of this problem requires excision of the fistula tract (Fig. 13.9). Table 13.5 contrasts the
two major abscesses that commonly occur in the female breast.

**Mondor Disease**

Mondor disease is a nonsuppurative thrombophlebitis of the thoracopigastric vein. This vein courses along the lateral segment of the breast where it may be exposed to trauma. This process is easy to recognize clinically as a long, linear, tender, cord-like mass located in the lateral anterior aspect of the breast. It is associated with erythema and inflammation. Heparin is not needed; the process is self-limited and resolves within 3 weeks. Heat compresses to the affected site and aspirin therapy are useful to alleviate symptoms.

**Hormone Relationship to Benign Breast Disease**

Fibrocystic breast disease is usually recognizable clinically at menarche; it ceases with menopause, resumes with the institution of postmenopausal hormone replacement (estrogen), and can worsen with use of oral contraceptives. For these reasons, the lumpiness and pain associated with fibrocystic breast changes have been attributed to a hormonal abnormality. The proposed etiology of the entity includes an es-

---

**TABLE 13.5. BREAST ABSCESSSES**

<table>
<thead>
<tr>
<th>Puerperal Abscess</th>
<th>Duct Ectasia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>&gt;40</td>
</tr>
<tr>
<td><strong>Cause</strong></td>
<td></td>
</tr>
<tr>
<td>Blocked duct in lactating breast</td>
<td>Ectatic central ducts with inflammation</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td></td>
</tr>
<tr>
<td>Peripheral breast</td>
<td>Central breast</td>
</tr>
<tr>
<td><strong>Organism</strong></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>Streptococcus sp</td>
</tr>
<tr>
<td></td>
<td>Bacteroides sp</td>
</tr>
<tr>
<td></td>
<td>Enterococcus sp</td>
</tr>
<tr>
<td><strong>Prognosis</strong></td>
<td></td>
</tr>
<tr>
<td>Resolves after drainage</td>
<td>Chronic until ducts excised</td>
</tr>
</tbody>
</table>
triglyceride excess; progesterone insufficiency; and prolactin, thyroid hormone, or androgen alteration (33). The hyperestrogen theory has never been substantiated with documented increases in plasma or urinary estrogen levels (34). Likewise, a progesterone deficiency has never been established in patients with fibrocystic breast disease (35). Prolactin secretion has been evaluated in patients with benign breast disorders. Although absolute plasma levels are the same as those in normal controls, abnormalities in the chronobiology have been identified (33). Nocturnal peak values and stimulated levels are higher in patients with fibrocystic changes than in controls (33). It may be that isolated measurements of plasma hormones do not adequately document the influence of sex hormones on the production and maintenance of fibrocystic changes. Tissue homogenates contain higher concentrations of sex steroids than does plasma (36). Alterations in the metabolism of sex steroids in the breast microenvironment are possibly responsible for abnormalities seen in patients with proliferative and nonproliferative breast disease.

The Relationship of Silicone to Connective Tissue Disease

The Nurses' Health Study cohort was used to evaluate the relationship between silicone breast implants and connective tissue diseases. Among 87,501 women who were eligible for follow up, 516 were confirmed as having definite connective tissue disease and 1,083 as having breast implants. Comparison of these two populations did not find an association between silicone breast implants and connective tissue diseases, as defined by a variety of standardized criteria, signs, or symptoms of these diseases (37).

Gynecomastia

Enlargement of the male breast can occur in the normal course of development and with aging, without an apparent endogenous or exogenous cause. Gynecomastia before puberty is rare, so rare that its occurrence should prompt a search for an endocrinologically active tumor (adrenal or testicular) or enzyme deficiency (11-hydroxylase deficiency). The male breast is commonly enlarged in the neonatal period from the influence of placental estrogen. This enlargement regresses within 3 to 6 weeks of age. Puberty is a common time for the male breast to become enlarged and tender. In 60% to 70% of normal males, physiologic gynecomastia occurs within 1 year of the onset of testicular development. Gynecomastia begins most commonly between 12 and 25 years of age, resolves within 12 to 24 months, and is unusual after the age of 20 years (38,39). Breast enlargement and tenderness at the time of adolescence occurs secondary to an increase in serum estrogen relative to serum testosterone (40). Sarcopenic gynecomastia may be a normal developmental occurrence in the elderly male. Niewohner and Nutter (41) found an incidence of 65%, based on an autopsy study of 214 men. They concluded that palpable bilateral gynecomastia is present in most older men. Its presence correlates with body fat and does not require investigation, unless the condition is symptomatic, unilateral, or of recent onset. Breast enlargement with advancing age may occur for several reasons: (i) a relative decrease in serum total and free testosterone (42), (ii) an increase in serum luteinizing hormone, (iii) maintenance of a normal serum estrogen, and (iv) an increase in the conversion of androgen to estrogen by aromatization in peripheral fat stores (43).

Histologically, gynecomastia is composed of elongated major ducts that exhibit branching and proliferation of periductal fibroelastic stroma. In the normal male breast, no branching of the major ducts is evident when epithelial and stromal elements both enlarge proportionately. The ducts in gynecomastia appear to be lined by normal epithelium. Any benign histologic alteration that occurs in the female breast can also be found in the male breast, with the exception of fibrocystic changes, which are extremely rare in the male (44). The increase in epithelial and stromal elements most likely occurs secondary to an increase in the estrogen-progesterone ratio. Prolactin does not seem to have a direct effect on the development of gynecomastia in males. Hyperprolactinemia states are not associated with gynecomastia. Serum prolactin levels in patients with gynecomastia are normal (45). Prolactin may have an indirect effect on gynecomastia in that it influences the estrogen-androgen ratio (46). Patients with gynecomastia do not have an increased risk for the development of breast cancer. Only Klinefelter syndrome is associated clearly with an increased risk of cancer in the male breast (47).

The three principal nonphysiologic causes for gynecomastia are estrogen excess, androgen deficiency, and drug effect (40). Estrogen excess in males that results in gynecomastia can occur secondary to several different disorders. Excess estrogen from a gonadal source may occur in true hermaphroditism, germ cell tumors of the testes, and gonadal stromal tumors. The excess estrogen may be produced by nongonadal tumors. Of these, the most common are bronchogenic carcinoma, hepatocellular carcinoma, and adrenal neoplasms. Furthermore, gynecomastia can be associated with hyperthyroidism or hypothyroidism (48) and is most likely the result of an alteration in the metabolism of estrogen (49). Another common clinical cause for gynecomastia is liver disease, mediated by estrogen excess. Cirrhosis, from whatever cause, as well as hemochromatosis and fatty metamorphosis may be responsible for this entity. Finally, starvation has been associated with gynecomastia (50).

The second major nonphysiologic cause for gynecomastia is androgen deficiency. The clinical conditions associated with this mechanism are aging, primary testicular failure (Klinefelter syndrome), secondary testicular failure (trauma, viral infection, irradiation, and chemotherapy), and renal failure.
TABLE 13.6. DRUGS ASSOCIATED WITH GYNECOMASTIA

<table>
<thead>
<tr>
<th>Drugs with estrogenic or estrogen-related activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabolic steroids (nandrolone, testosterone cypionate)</td>
</tr>
<tr>
<td>Clomiphene citrate</td>
</tr>
<tr>
<td>Diethylpropion hydrochloride</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
</tr>
<tr>
<td>Digitalis</td>
</tr>
<tr>
<td>Estrogens</td>
</tr>
<tr>
<td>Heroin</td>
</tr>
<tr>
<td>Oral contraceptives</td>
</tr>
<tr>
<td>Tetrahydrocannabinol (cannabis, marijuana)</td>
</tr>
<tr>
<td>Drugs that inhibit the action and/or synthesis of testosterone</td>
</tr>
<tr>
<td>Antineoplastic agents (vincristine, nitrosourea, methotrexate)</td>
</tr>
<tr>
<td>Cimetidine</td>
</tr>
<tr>
<td>Cyproterone acetate</td>
</tr>
<tr>
<td>d-Penicillamine</td>
</tr>
<tr>
<td>Diazepam</td>
</tr>
<tr>
<td>Flutamide</td>
</tr>
<tr>
<td>Ketaconazole</td>
</tr>
<tr>
<td>Medroxyprogesterone acetate</td>
</tr>
<tr>
<td>Phenytoin</td>
</tr>
<tr>
<td>Spironolactone</td>
</tr>
<tr>
<td>Drugs that enhance estrogen synthesis by the testes</td>
</tr>
<tr>
<td>Human chorionic gonadotropin</td>
</tr>
<tr>
<td>Drugs with idiopathic mechanism for induction of gynecomastia</td>
</tr>
<tr>
<td>Amiodarone</td>
</tr>
<tr>
<td>Bumetaneide</td>
</tr>
<tr>
<td>Busulfan</td>
</tr>
<tr>
<td>Droperidone</td>
</tr>
<tr>
<td>Ethionamide</td>
</tr>
<tr>
<td>Furosemide</td>
</tr>
<tr>
<td>Ibomiazide</td>
</tr>
</tbody>
</table>

The third major cause for nonphysiologic gynecomastia is drug related. A list of medications and hormones that are associated with breast enlargement is given in Table 13.6; these represent an ever-increasing etiologic basis of gynecomastia.

MALIGNANT CLINICAL DISORDERS

Abnormal Cell Growth

Our understanding of basic breast cancer tumor biology is rapidly evolving. There are few areas of human biology research that produce as many major findings each year. This section will focus upon the process that allows a normal mammary epithelial cell to bypass the usual control mechanisms that check abnormal growth. Even though it is difficult to keep up with the changes in this field, the effort is essential. In 2001, the first rationally selected therapy directed against a molecular determinant of malignant transformation produced a marked increase in survival in patients with metastatic breast cancer (51). A humanized monoclonal antibody, trastuzumab, was given to patients along with conventional chemotherapy. Trastuzumab has an affinity for the extracellular domain of HER2. It binds with HER2 and prevents signal transduction. Combination therapy with trastuzumab and chemotherapy produces longer survival than chemotherapy alone. This is one example of a gene that influences breast cancer growth. Other genes can have an effect by regulating progression through the cell cycle, by influencing resistance to programmed cell death (apoptosis), by detecting and repairing damaged deoxyribonucleic acid (DNA) or by mediating the response to signals that direct cell differentiation (52). In addition to genes, this section will examine the influence of hormones, growth factors and environmental factors on abnormal cell growth.

Estrogen

The profound influence of estrogen in breast development, growth, and maintenance was outlined previously. Estrogen has growth-promoting effects on malignant as well as benign mammary epithelium. Estrogen receptors are necessary for normal duct development. Knock-out mice lacking ER proteins develop only vestigial ducts at the nipple (53). Similarly, estrogen promotes growth in ER-positive tumors (54). This direct tumor effect may occur through the induction of enzymes and proteins involved in nucleic acid synthesis and through activation of oncogenes (55). Clinical evidence for estrogen's mitogenic role is varied. Breast cancer occurs less commonly in men (1% of all breast cancers), oophorectomy before age 30 results in a 70% risk reduction, and dogs who have oophorectomy before the development of the estrus cycle develop breast cancer with less than 1% of the incidence for normal dogs (56). Further indirect evidence for a mitogenic effect for estrogen is that 33% of patients will experience a regression of their breast cancer after oophorectomy or after the initiation of antiestrogen therapy (tamoxifen). Approximately 66% of ER-positive tumors will respond to these manipulations (56). Table 13.7 lists the mechanism through which estrogen might exert control on mammary cell growth. Estrogens may initiate the production of locally acting hormones that further influence (regulate) epithelial growth and development. These polypeptide growth factors are capable of initiating the cell cycle in epithelium that would otherwise be at rest (G0). They may induce the production of other local hormones or growth factors that can induce oncogenes, specifically c-fos,

TABLE 13.7. ESTROGEN AND REGULATION OF MAMMARY CELL GROWTH

| Induction of growth factor production                             |
| Modulation of gene expression                                    |
| Induction of enzymes responsible for DNA synthesis              |
| Induction of progesterone receptor                               |
| Induction of plasminogen activators and collagenolytic enzymes  |
| Induction of receptor binding protein for laminin              |

DNA, Deoxyribonucleic acid.
c-myc, and c-nis (57). The specific growth factors that have an effect on mammary epithelium include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor α (TGFα), insulin-like growth factor 1 (IGF-1), and somatomedin C. These growth factors will be discussed later.

Since 1980, 24 studies and 3 metaanalyses have failed to consistently demonstrate an increased risk of breast cancer among women who have ever used estrogen therapy (58). One of the largest studies to demonstrate an association between estrogen use and the development of breast cancer is the Nurses’ Health Study. These investigators also looked at the effect of adding progestins to estrogen therapy in postmenopausal women. They found that risk of breast cancer was increased significantly among women who were currently using estrogen only or estrogen plus progesterone as compared to postmenopausal women who had never used hormones. This increase was most pronounced in women over the age of 55 and was limited largely to women who had used estrogen therapy for 5 or more years (59). The increased risk of breast cancer related to estrogen replacement therapy is limited to the actual duration of therapy and a short time after cessation (60). The increased risk of breast cancer associated with oral contraceptives is only slightly higher than the general population after long use (61), with no increased risk 10 years after cessation (62,63). In postmenopausal women, serum estrogen levels correlate with breast cancer risk; women with higher levels are at higher risk of developing cancer (60). Since mammographic density correlates with serum estrogen, postmenopausal women with radiographically dense breasts are a population that requires more careful screening (64–66).

**Growth Factors**

Good experimental evidence from the past 10 years has established the role of growth factors in the regulation of mammary cell growth (57). As mentioned in the beginning of this section, a new therapy directed at a transmembrane tyrosine kinase receptor, HER2, has increased survival of breast cancer patients with HER2-positive metastatic disease (51). The following growth factors have an established role in mammary cell growth regulation and, therefore, may be the molecular basis of some novel treatment in the future.

**Epidermal Growth Factor Family**

This family includes HER1 or epidermal growth factor receptor (EGFR), HER2, HER3, HER4, epidermal factor, TGFβ, amphiregulin, and crypto-1. In experimental tumor models, EGF and TGFβ promote tumorigenesis. Several members of this family are overexpressed in human breast cancer (54). One third of breast cancers overexpress the receptors HER2 or EGFR. These tumors are associated with ER negativity, a high S phase, and a poor prognosis.

**Insulin-Like Growth Factor Family**

This group contains two growth factor ligands, IGF-1 and IGF-2, two transmembrane tyrosine kinase receptors, IGF-1R and IGF-2R, and seven IGF-binding proteins. IGF-1 and -2, also called somatomedins, are produced by the majority of normal tissues. They are also found in blood and urine (57). In contrast to insulin, which is made only in the pancreas, IGF-1 and IGF-2 polypeptides are synthesized in nearly all organs, including the liver (57). The somatomedins differ from insulin in structure. Insulin has two, disulfide-linked chains, whereas the somatomedins have a single chain (57). IGF-1 is mitogenic for some cultured breast cancer cells. All human breast cancers make an IGF-1-related growth factor (67). And finally, human breast cancer cells in culture increase production of IGF-1 when exposed to estrogen and decrease production when treated with antiestrogens (57).

**Fibroblast Growth Factor Family**

Fibroblast growth factor (FGF) is required for growth of normal mouse mammary cells in culture (68). FGF stimulates neovascularization (69). Human breast cancer cells in culture produce an FGF-related polypeptide (70). FGF-1, FGF-3 and FGF-4 promote growth; FGF-2 inhibits growth. Expression of this family in human breast cancer varies so that the role of FGFs in tumorigenesis is unclear (34).

**Transforming Growth Factor**

This group inhibits growth of most epithelial cells and promotes the growth of stromal cells (34). TGFβ induces apoptosis as well as cell cycle arrest in mammary epithelial cells (71). Normal mammary epithelium stops growing in culture when exposed to TGFβ, and normal mammary duct development is inhibited by local application of TGFβ. This factor is found in human milk (57). Because its actions are antagonistic to those of the other growth factors, TGFβ may be part of the normal hormonal balance that controls mammary growth. This inhibitory effect may be operative in breast cancer cells that secrete a TGFβ-like factor (58). The role of TGFβ in breast cancer is not yet known. TGFβ may participate in the growth-inhibitory effects of antiestrogen therapy.

**Platelet-Derived Growth Factor**

High concentration of PDGF is found in platelets and in a diverse number of tumors (72). Several human breast cancer cell lines produce a factor with PDGF-related activity. PDGF mediates proliferation of stromal cells (73) and causes the release of prostaglandins I2 and E2 (74) to induce fibroblast proliferation, collagen secretion, and production of IGF-1 (75).
Other Growth Modulation

Many other hormones have growth regulatory activity in normal and malignant mammary cells. A pituitary-derived factor augments the mitogenic effects of estrogen on the MCF-7 human breast cancer cell line (76). Growth hormone-releasing factor from the pituitary may directly inhibit breast cancer cell growth in culture (77), and prolactin stimulates growth in some cell lines (78). Among the many molecules to affect growth of MCF-7 cells in culture, glucocorticoids, iodothyronines, androgens, and retinoids are stimulatory (57); progesterone, somatostatin, interleukins (1 and 6), tumor necrosis factor, and interferon are inhibitory (57).

Environmental Factors

The environmental forces that are responsible for the large geographic variation in the incidence of breast cancer have not been elucidated. A five-fold difference exists in incidence among Japan (12.1 cases/100,000 women/year), England (54.5 cases/100,000/year), and the United States (57.2 cases/100,000/year) (79). This difference is substantially reduced in first-generation Japanese women raised in the United States (79). The incidence of breast cancer and mortality from the disease are higher in the upper socioeconomic groups (80). This effect of social status seems to be independent of the effects of reproductive factors. The early onset of menses and the early establishment of regular menses are associated with an increase of two-to-three times in breast cancer risk (81). Other reproductive influences that are associated with a decreased risk include early age at the time of first birth, menopause before age 45, and high parity (81). Even though longer exposure to endogenous estrogen seems to be associated with an increased breast cancer risk, epidemiologic studies of patients exposed to exogenous estrogens (hormone replacement or birth control) have not found a significantly increased risk of breast cancer (56). Ionizing radiation increases the risk in direct proportion to the dose and in inverse proportion to the age at the time of exposure (82). Lactation and breast-feeding have a controversial effect on cancer risk. If there is a benefit to breast-feeding, it is minimal (83). Many environmental factors that have been considered as risk factors may actually be operative through an influence on estrogen levels. Studies of obesity, exercise, and dietary intake (alcohol, fat, and anti-oxidant vitamins and fiber) have produced conflicting results (60). This may be in part because of differences in end-organ sensitivity to estrogen. Human breast cells can have variable amounts of estrogen receptors and, therefore, vary in sensitivity. In addition, there are two kinds of estrogen receptors, alpha and beta. The alpha receptor has a higher affinity than the beta receptor for estrogen. The distribution of estrogen receptor types in any given tissue may account for differences in end-organ sensitivity (60).

Genes Associated with Breast Cancer

Abnormal mammary cell growth may be caused by changes in certain genes. Such changes can result in uncontrolled replication or failure of programmed cell death (84,85). Genetic changes can participate in carcinogenesis in one of three broad categories: protooncogenes, tumor suppressor genes, and genes that recognize and repair damaged DNA (85). Genes with an established relation to breast cancer are: BRCA1, BRCA2, p53 (Li-Fraumeni syndrome), PTEN (Cowden disease), and AT (ataxia telangiectasia).

Genetic Predisposition to Breast Cancer

BRCA1 and BRCA2 are tumor suppressor genes. They function as genetic caretakers, acting as guardians of DNA integrity (85). Mutations in these genes result in an increase in genomic instability that promotes neoplasia (86,87). BRCA1 and BRCA2 are associated with medullary breast cancer more often than is usually seen in the general population. BRCA-related tumors are more often high-grade, aneuploid tumors with a high S-phase fraction of cells. They tend to be ER and PR negative and exhibit a high mitotic rate (88–91). Families with the BRCA gene have a higher risk of prostate and colon cancer. Patients with the BRCA1 gene have an extremely high risk of developing breast cancer. That risk exceeds 50% before the age of 50 and 80% by the age of 65 (92). BRCA1 is on 17q; BRCA2 has been mapped to chromosome arm 13q (93).

The American Society of Clinical Oncology has provided some guidelines to help identify families that may have the BRCA1 or BRCA2 gene. The criteria are (i) families with two or more breast cancer cases and one or more ovarian cancer cases diagnosed at any age, (ii) families with more than three breast cancer cases diagnosed before age 50, and (iii) sister pairs with two of the following cancers diagnosed before age 50 years—two breast cancers, two ovarian cancers, or a breast and ovarian cancer (94).

Li-Fraumeni cancer syndrome is one of many human cancer syndromes associated with p53. The p53 protein responds to DNA damage by arresting the cell cycle, inducing DNA repair or cell death. In the absence of functional p53, a cell can escape apoptosis and replicate an unstable genome (95). The Li-Fraumeni cancer syndrome consists of childhood sarcomas, breast cancer, brain and adrenal tumors, and leukemia (96). Breast cancer associated with this syndrome occurs in younger women, ages 20 to 30 years.

The PTEN gene is an autosomal dominant on 10q. It is associated with Cowden syndrome. These patients have an increased risk of bilateral breast cancer. They also have multiple facial trichilemmomas, acral keratoses, oral papillomatosis, gastrointestinal polyposis, female genital tract tumors, and malignant thyroid tumors (97). The risk of breast cancer in PTEN patients is 30% to 50% by age 50 (98).
The AT gene is an autosomal recessive that confers a seven-fold increase in the risk of breast cancer. Patients with the AT gene also have progressive neurologic degeneration, telangiectasias of the skin and eyes, immunodeficiency, developmental abnormalities, and hypersensitivity to ionizing radiation (99). This increased radiosensitivity needs to be considered when recommending mammography for patients with the AT gene and the p53 gene.

Oncogenes

The relationship of oncogenes to breast cancer is undefined. Oncogenes are mutated forms of genes (protooncogenes) that have been transformed from the DNA of host cells. Protooncogenes are most likely involved in the control of normal cell growth and differentiation. When altered in structure and/or function, the protooncogenes become oncogenes, which are thought to contribute to malignant cell transformation (100). Oncogene products that could contribute to carcinogenesis include nuclear proteins that act as transcriptional activators, transmembrane tyrosine kinases that function as growth factor receptors, intracellular serine kinase, tyrosine kinase, and membrane-bound G protein analogues. Overproduction of these proteins may allow the cells to grow more rapidly and develop invasive properties (100). Overproduction occurs through a process called amplification. Amplification is an activation of oncogenes resulting in an excess of DNA template, which leads to an overproduction of oncogene-specific RNA and protein. Amplification and oncogene overexpression have been correlated in certain tumors with aggressive behavior. The first oncogene alteration with clinical significance is the association of the oncogene N-myc with more aggressive neuroblastomas (101). Although research may confirm that oncogene amplifications may be a marker for poor prognosis in breast cancer, the evidence at this time does not support this conclusion. Jandrig (102) examined more than 1,000 breast cancers for amplification of the oncogene c-erb-B. He concluded that the association of this oncogene with a poor prognosis is not convincing. However, Borst and Miller (103) reviewed evidence that oncogene amplification correlates with poor prognosis in breast cancer patients with positive axillary lymph nodes. Some studies have found a significant association between amplification of either c-myc, c-erb-B, or int-2 with a high risk for relapse or poor survival. Despite these reports, the status of oncogenes as outcome predictors continues to be debated (104).

The precise role of the oncogene products and the advantage bestowed on a cell as a result of oncogene amplification has not been established in molecular biology. All five oncogenes that have been implicated in breast cancer (int-2, c-erb-B, c-myc, c-ras, and Rb-1) have different cellular functions involving growth and differentiation. In cancers, these oncogenes change their function such that they may contribute to the malignant characteristics of the tumor. The c-myc oncogene is commonly rearranged, amplified, or both, in human breast cancer (105). Animal data suggest that c-myc expression is related causally to the development of breast cancer. Oncogene c-erb-B, which is analogous to the EGF receptor, is commonly overexpressed in ER-negative tumors that portend a poor prognosis and high degree of invasiveness (106). Amplification and protein expression of c-erb-B has been correlated with the number of positive lymph nodes (67). The ras family of oncogenes may contribute to malignant progression (107). Insertion of the c-ras oncogene into MCF-7 cells increased the ability of the cells to invade the basement membrane (108) and increased the production of polypeptide growth factors (109). In spite of this experimental evidence, the precise role of oncogenes in any cancer, including the breast, remains conjectural.

Flow Cytometry

Flow cytometry has been used to identify cancers that have a high likelihood of recurrence. This may be particularly useful in determining which patients with stage I (node negative) breast cancer are likely to develop a recurrence and, therefore, are most likely to benefit from adjuvant chemotherapy. Flow cytometry evaluates the DNA content of a population of cells (approximately 50,000 cells) from a tumor. The ploidy of the tumor is determined by the DNA content of tumor cells in G0 phase relative to nonmalignant G0 cells. Diploid tumors are those in which the stem-line DNA content is not measurably different from nonmalignant reference cells. Aneuploid tumors are those whose stem-line DNA content is altered. The DNA index is the numerical ratio of the mean DNA content of phase G0 tumor cells to that of normal cells (110). Tumor proliferative activity or index is the percentage of cells in the S and G2 phases combined (110). Analysis of old breast biopsy specimens by flow cytometry has been correlated with survival and tumor recurrence. Aneuploidy occurs more commonly in ER-negative tumors, poorly differentiated tumors, and tumors with a high proliferative index that are more likely to be ER negative and poorly differentiated (110). Ewers et al. (111) evaluated 97 stage I patients and 140 node-negative stage II patients. Only 3 of 98 (3%) patients with diploid tumors suffered a relapse at 16 months, whereas 20 of 139 (14%) patients with an aneuploid tumor had recurred. Kallioniemi et al. (112) reported a 5-year survival of 95.5% for diploid stage I tumors and 80.2% for aneuploid tumors. In node-positive disease, both ploidy and proliferative activity have been linked strongly to clinical outcome (110).

Estrogen Receptor

The ER protein must be present in a tissue, whether it is breast or some other organ, for estrogen to influence development and function. The presence of the ER in tumor cells has clinical implications that will be addressed later, but in
general, ER-positive tumor cells have retained some of the cell’s regulatory mechanism present before the transformation to cancer. Normal breast epithelial cells have specific binding proteins for estrogen, progestins, glucocorticoid, and androgen. The estrogen receptor is most probably located in the nucleus (113) (Fig. 13.10). Three serum proteins bring the estrogen molecule to the target cell: albumin, testosterone-estradiol-binding globulin (TeBG), and corticosteroid-binding globulin (CBG). Estrogen is fat-soluble and easily passes into the cell by simple diffusion. Once in the cell, estrogen binds with the receptor protein. The estrogen-ER complex is then incorporated into the nuclear matrix and chromatin (a process known as retention). After nuclear integration occurs, the specific gene affected by the estrogen is activated to produce the specific breast cell protein that characterizes the effect of estrogen on the cell. The exact mechanism by which the estrogen-ER complex interacts with the cell’s nucleus is not known.

The clinical usefulness of the ER-protein determination in breast tumors is well established. The presence of the estrogen and progesterone receptors in a breast tumor predicts the effectiveness of hormonal therapy for that tumor. Approximately 53% of ER-positive tumors respond to hormone therapy (Table 13.8), compared with only 6% of ER-negative tumors (113). The presence of the progesterone receptor enhances the likelihood of hormonal response. The

![Diagram of breast cancer progression](image)

**FIGURE 13.10.** Schematic representation of hypothetical intracellular cascade of events following steroid receptor interaction with its receptor in a target cell. Steroid hormones (SH) normally circulate in the blood bound to albumin and certain specific serum proteins (SP) such as testosterone-estradiol-binding globulin (TeBG) and corticosteroid-binding globulin (CBG). As lipid molecules, steroids move across the cell membrane into the cytoplasm in a passive fashion and interact with their intracellular receptor proteins (R) in a reaction exhibiting high affinity and specificity. The exact location of the true receptor protein is unknown, but possibilities include association with the nuclear membrane (Rn), nuclear matrix (Rm), and chromatin (Rc). After association with the steroid, an apparent activation takes place that may involve phosphorylation. The activated steroid hormone-receptor (SH-R) complex associates with acceptor sites in chromatin and stimulates synthesis of nucleic acids and, subsequently, proteins characteristic of the biologic response (differentiation and growth) to the specific steroid hormone. In addition, steroid hormones may be associated with low-affinity sites (LAS) whose subsequent pathway is uncertain. The details of these intranuclear events are presently unclear. However, the presence of the receptor protein in a cell appears to be a prerequisite for response to a steroid hormone stimulus. (From Wittliff JL. Steroid receptor analyses, quality control, and clinical significance. In Donegan WL, Spratt JS, eds. Cancer of the breast, 3rd ed. Philadelphia: WB Saunders, 1998:303-335, with permission.)
TABLE 13.8. RELATIONSHIP BETWEEN ESTROGEN RECEPTOR STATUS OF BREAST TUMOR AND PATIENT’S OBJECTIVE RESPONSE TO ENDOCRINE THERAPY

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Responses/ER+ Tumors</th>
<th>Responses/ER- Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blamey et al.</td>
<td>13/30</td>
<td>5/27</td>
</tr>
<tr>
<td>Dao and Namoto</td>
<td>64/119</td>
<td>4/56</td>
</tr>
<tr>
<td>DeSombre and Jensen</td>
<td>39/62</td>
<td>4/103</td>
</tr>
<tr>
<td>Maas et al.</td>
<td>64/93</td>
<td>3/76</td>
</tr>
<tr>
<td>Manni et al.</td>
<td>68/105</td>
<td>0/12</td>
</tr>
<tr>
<td>McCarty et al.</td>
<td>32/58</td>
<td>3/20</td>
</tr>
<tr>
<td>Nomura et al.</td>
<td>29/45</td>
<td>0/36</td>
</tr>
<tr>
<td>Osborne et al.</td>
<td>70/145</td>
<td>5/53</td>
</tr>
<tr>
<td>Paridaens et al.</td>
<td>14/38</td>
<td>0/11</td>
</tr>
<tr>
<td>Rubens and Hawyard</td>
<td>46/146</td>
<td>5/55</td>
</tr>
<tr>
<td>Singhakowinta et al.</td>
<td>20/30</td>
<td>2/25</td>
</tr>
<tr>
<td>Skinner et al.</td>
<td>17/30</td>
<td>5/44</td>
</tr>
<tr>
<td>Wittliff</td>
<td>46/76</td>
<td>0/44</td>
</tr>
<tr>
<td>Total</td>
<td>522/977 (53%)</td>
<td>36/567 (6%)</td>
</tr>
</tbody>
</table>

ER, Estrogen receptor.

response rate for ER-positive and PR-positive tumors is 78%; for ER-positive and PR-negative tumors, 34%; for ER-negative and PR-negative tumors, 6%; and for PR-negative and PR-positive tumors, 45% (113). The ER status of male breast cancer also predicts the response to hormonal manipulation, in that 65% of ER-positive male breast cancers will respond. A total of 90% of male breast tumors are ER positive; 50% are PR positive (114). Tissues from benign breast processes usually contain less than 10 fmol of ER (ER negative) for each milligram of protein. Wittliff et al. (113) report that 60% to 65% of female breast cancers are ER positive and that 45% to 55% of recurrent and metastatic tumors are ER positive. Approximately 45% to 60% of female breast cancers are PR positive. A study of the tumor registry from Portland Kaiser Permanente noted that between 1975 and 1985, ER-negative cancer rose 22%, whereas ER-positive tumors increased 131% in the same period (115).

The levels of ER protein in breast cancer vary between 0 and 6,000 fmol/mg protein. No histologic characteristic exists that correlates with the ER level. The ER status of a tumor does not correlate with size, location, nodal status, or clinical stage. Various studies have demonstrated a slight correlation of ER status with several tumor characteristics, including histologic classification, nuclear grade, DNA ploidy, proliferative activity, and lymphocytic infiltration. However, not all authors are in agreement, and additional study is needed (113).

Estrogen receptor status correlates with age. The ER protein quantity is lower and less frequently positive in premenopausal than in postmenopausal women (116). The PR is also found less frequently in younger than in older women (117). Tumors tend to lose the estrogen receptor with duration of the tumor and with recurrence (118). Synchronous tumors have a similar ER status 80% of the time (119). Because the ER status varies with the concentration of serum estrogen and progesterone, it follows that hormonal and cytotoxic therapy can alter the ER-receptor assay by killing cells containing the receptor or by changing the number of binding sites. Tamoxifen or other hormone treatment should be stopped 3 weeks before a biopsy for the purpose of determining ER status. The ER protein is labile and undergoes degeneration in a warm environment (120,121). Studies on the half-life of ER degeneration have resulted in estimations that vary from 30 minutes to 6 hours. The specimen is best handled by freezing to −70°C within 30 minutes of removal. Although at least 200 mg of tumor should be frozen, 400 to 500 mg is optimal.

Estrogen can also induce the production of the PR. The presence of the PR is an indication that the cell’s growth is influenced by estrogen. The presence of the PR in breast tumor cells is an indicator of the probability that the tumor will respond to antiestrogen therapy (57). Estrogen also increases the synthesis of plasminogen activators and collagenolytic enzymes. These proteins may function to facilitate cellular invasiveness and, therefore, support cell metastasis. The exact role of these proteins in the regulation of mammary cell growth remains unclear (56). Another estrogen-induced receptor protein in the MCF-7 cell culture line is the receptor-binding protein for laminin. The laminin receptor mediates the attachment of the cells to basement membrane and facilitates the metastatic potential of the cell (57).

Sentinel Node Concept

A sentinel node is the first lymph node in the lymphatic drainage pathway from an organ (e.g., leg, breast) to the central circulation. The concept of a sentinel node has clinical utility for melanoma because the sentinel node accurately predicts the disease status of the nodal group. If the sentinel node does not contain melanoma, no lymphadenectomy is needed. This concept is being examined for breast cancer to prevent the morbidity of an axillary dissection in those who do not need it (stage I). As of 2001, the sentinel lymph node concept for breast cancer is experimental and should be done only in a trial (122).

The technique uses two tracers to find the axillary sentinel node, a blue dye and a gamma emitter. The tracers are injected into the interstitial space around the tumor or around the areola. The sentinel node can be localized after a few minutes when the tracers have found their way into the lymphatic channels to the axilla. The dye stains the main lymph trunk and the sentinel node blue. The radioactive tracer is located with a handheld radiation detector. A multicenter trial found considerable variability among surgeons
in their success at locating the sentinel node, suggesting that this method is difficult to master (123). In this study, a hot spot was identified 93% of the time. When identified, the sentinel node accurately predicted the status of the axillary nodes 97% of the time. The false negative rate, that is, the frequency of a negative sentinel node and a subsequent positive axillary dissection, was 11%. This compares unfavorably with the false negative rate of 2% to 3% for a standard axillary dissection. In patients who have negative level I and II nodes, there is a 2% to 3% chance that the level III nodes will be positive. Another conceptual problem with the sentinel node concept for breast cancer is the unpredictable lymphatic drainage of the breast. In a study of 250 normal women, the primary lymphatic drainage went to the internal mammary lymphatics 20% to 86% of the time depending upon the breast quadrant injected (124). Metastasis to internal mammary nodes occurs in 5% to 10% of breast cancer patients who have negative axillary nodes (125). Further evidence of the importance of the internal mammary nodes is the fact that 90% of patients who die of breast cancer have internal mammary node metastasis at autopsy (126). The usefulness of the sentinel node concept is being validated in a large clinical trial and should become standardized soon. Whatever technique is used, there is substantial evidence that axillary dissection provides excellent local disease control in those breast cancer patients with axillary nodal metastasis (127–131).

Biology of Metastasis

The spread of a cancer from its primary site to distant organs is a complex cascade of incompletely understood biologic events. For a cancer cell to leave the tumor successfully and establish itself as a secondary growth, many stages in the process must be accomplished successfully in sequence. If the cell fails to accomplish any one of a number of the steps in the process of metastases, the cell will not survive. The events of metastases, as described by Price (132), are given in Table 13.9.

Breast tumors appear to be heterogenous populations of cells. Clones of cells from the same tumor have different phenotypes, including karyotype, proteolytic enzymes secreted, hormone receptors, and metastatic potential. Therefore, all cells of a tumor cell line will not have the necessary properties for successful metastasis. This is also important from the standpoint of tumor sampling. A small incisional biopsy may not accurately reflect biochemically or molecularly the entire tumor in terms of receptors, DNA ploidy, etc. And finally, Price points out that heterogeneity is important in terms of tumor kill. Even if a 99.9% kill rate is achieved in a 1-cm3 tumor, there will be 106 residual viable cells to contribute to the viability of the cancer. The understanding of the events that contribute to successful metastasis are just beginning to be defined.

The initial event in metastases is the growth of the primary tumor. The breast cancer cells are able to secrete polypeptides with paracrine and autocrine effects that support growth. One of the most important of these events is angiogenesis. If the tumor is not able to stimulate the growth of new nutrient blood vessels, it will quickly outstrip its blood supply and die. FGF stimulates angiogenesis (133). As the tumor grows, the next step is invasion of the surrounding stroma and entry into the circulation via blood vessels and lymphatics. This process is facilitated by the secretion of lytic enzymes from the tumor cells. Enzymes produced by human breast cancer cells that could augment local invasion as the tumor grows include such factors as cathepsin D, cathepsin B, plasminogen activator, collagenase, and heparinase (132). The fact that any given tumor secretes these enzymes is not necessarily a marker for metastatic potential. Some tumors, such as basal cell carcinoma, secrete proteolytic enzymes but have a low incidence of metastasis. This emphasizes the need for a metastatic cancer cell to meet multiple requirements to be successful. In a similar way, mere entry into the bloodstream does not guarantee an established metastasis. In fact, few cells survive hematogenous invasion. Less than 1% of radiolabeled cells placed into the circulation survive for 24 hours, and less than 0.1% become established metastasis (134).

Once the cells are in the blood, several other steps must be accomplished. Cells are destroyed in the circulation by trauma and by activated macrophages. Survival of tumor cells in the blood of the host is enhanced by aggregation of tumor cells into clumps and the specific ability of individual tumor cells to adhere to and penetrate the endothelium (132). The number of tumor emboli entering the blood and

<table>
<thead>
<tr>
<th>TABLE 13.9. STEPS IN THE DEVELOPMENT OF METASTATIC DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steps in Metastases</strong></td>
</tr>
<tr>
<td>1. Primary tumor growth</td>
</tr>
<tr>
<td>2. Invasion</td>
</tr>
<tr>
<td>3. Survival in the circulation embolization</td>
</tr>
<tr>
<td>4. Arrest in organ sites</td>
</tr>
<tr>
<td>5. Extravasation</td>
</tr>
<tr>
<td>6. Growth in the organ environment</td>
</tr>
</tbody>
</table>
the lymphatic systems correlates with the size of the tumor, its duration, and the degree of necrosis and hemorrhage (133).

Once in the lymphatics, the cells pass to regional lymph nodes. In 1894, Halsted (135) described his rationale for the radical mastectomy with his initial report of a remarkably low 6% local recurrence rate at a 2-year follow-up. He thought that hematogenous spread of breast cancer did not occur, but rather the cancer spread by direct extension through the lymphatics (135). He believed that the lymph nodes were actual filters that prevented more distant spread of the disease until they were packed with cancer themselves.

The concept of lymph nodes as barriers to the spread of cancer has been challenged by several pieces of evidence. First, tumor cells can pass directly through lymphatics without nodal arrest when placed into distal lymphatic channels of experimental animals (136). Second, in humans, more proximal lymph node groups may harbor metastatic deposits without evident disease in the more distal groups, indicating that tumor cells can traverse lymph nodes without arrest (137). And finally, National Surgical Adjuvant Breast Project (NSABP) trial B-04 included a subset of patients who did not have an axillary dissection. The other groups in the trial that had an axillary dissection had positive nodes in approximately 30% of the patients. Because the trial was randomized prospectively, it is valid to assume that 30% of the patients in the group that did not have an axillary dissection also had positive nodes. The survival for all patients in this trial is identical in more than 10 years of follow-up (138). Therefore, it does not appear to be a variable that positive lymph nodes serve as the source of systemic failure in patients with breast cancer. Based on the NSABP results, the role of the lymph node in breast cancer has been redefined. Instead of barriers to systemic spread of the disease, the nodes most likely serve a role in immunomodulation of the cancer.

Once the neoplastic cells enter the circulation, the next step toward establishment of the successful metastasis is arrest in organ sites. Breast cancer has preference for lung, liver, bone, soft tissue, and brain. These sites are more receptive to breast cancer cell growth because of the presence of a favorable milieu. This idea was called the soil and seed theory by Paget (139) in 1889. The theory that cancer cells grow only in organs that are favorable is supported by the recent use of the peritoneal metastatic shunt in patients with malignant ascites. These patients receive a direct infusion of tumor cell-rich ascitic fluid into the superior vena cava, yet they do not support the growth of these metastatic lesions in every organ (140).

The influences that could contribute to a favorable environment for tumor growth include the ability of the tumor cell to adhere to the capillary endothelium of the organ, the secretion of proteolytic enzymes by either the organ or the tumor cell to enhance the movement of tumor cells into the stroma of the organ, and organ-derived growth-modulating factors. Such local factors most likely control growth and development of the organ as well as tissue repair and, therefore, may enhance growth of the tumor cell (132). It is these differences in the local environment that explain the predilection of ER-positive, slow-growing breast metastasis for bone and the tendency for ER-negative, fast-growing metastasis to adhere and proliferate in viscera. The polypeptides that have been isolated from some human breast cancers and that could participate in the modulation of metastatic cell growth include TGF, IGF-1 TGFα, and FGF (132).

Chemotherapy

Cytotoxic therapy for node-negative disease has evolved from the anatomic, pharmacologic, and molecular biologic considerations for the treatment of breast cancer. The Halstedian view of cancer dissemination by direct extension has been replaced by Fisher’s alternative hypothesis that the disease is a systemic one from the beginning (141). This hypothesis attempts to account for systemic failure, which occurs in 25% of node-negative patients within 10 years. Clinical evidence suggests that these systemic failures result from established metastases that were present as occult foci at the time of the operation. Systemic disease does not commonly result from showering of tumor cells to the periphery with tumor manipulation (142). Therefore, perioperative chemotherapy does not address systemic metastasis in the same manner that prophylactic antibodies address postoperative infection. Even though operative manipulation may initiate systemic spread of cancer cells, early systemic treatment (i.e., before nodal spread) is justified for other reasons. A greater probability of tumor kill exists in smaller tumors, which have, on a percentage basis, a relatively larger volume of mitotically active cells than do large tumors (143).

Another justification for early therapy is the relative infrequency of chemoresistant strains in smaller tumors; consequently, a larger tumor volume would escape kill. Spontaneous mutations occur with neoplastic growth, and larger tumors are more heterogeneous and are more likely to contain one or more drug-resistant subpopulations. Several clinical trials, including the initial NSABP trial, B-01, have demonstrated the clinical usefulness of single-agent chemotherapy (144). NSABP B-01 randomized 826 women after Halsted radical mastectomy to either adjuvant chemotherapy with thiotepa for 6 months or placebo. No difference in overall survival was observed, but premenopausal women with more than four positive nodes benefited from the chemotherapy (145). Subsequent multidrug trials have confirmed this beneficial effect for premenopausal women with positive lymph nodes at the time of presentation. Adjuvant chemotherapy is less effective for postmenopausal women. This may be related to the greater tumor cell heterogeneity seen in older women, the medical oophorectomy that results from chemotherapy in the premenopausal patient, or the inability of older women to tolerate intense cytotoxic treatment regimens (142).
After demonstration of the efficacy of chemotherapy for premenopausal, node-positive patients in several different trials, the possibility of enhancing disease-free survival and overall survival in node-negative patients has been evaluated (146). This question was prompted by the high disease recurrence rate in stage I breast cancer and by the demonstrated success of chemotherapy in stage II patients. For instance, in NSABP trial B-06, the tumor recurrence rate was higher in the node-negative patients who did not receive chemotherapy than in the node-positive patients who were treated with melphalan and 5-fluorouracil (147). Three studies of adjuvant chemotherapy have been reported early in their course because of possible positive results. An Intergroup Study and NSABP B-13 and B-14 all have suggested an increase in disease-free survival but no increase in overall survival with various regimens in node-negative patients (147-149). The treatment-related morbidity and mortality varied with the chemotherapeutic regimen. As these studies approach maturity, the greatest interest will be the subset of node-negative patients who are more likely to benefit from adjuvant chemotherapy. Fisher and others (150) have evaluated 950 node-negative patients from NSABP B-06 in an attempt to define pretreatment parameters that might predict recurrence. They noted that studies using DNA ploidy, proliferative activity reflected by S phase or thymidine tumor labeling, EGFR, and erb2 oncogene expression have given inconsistent results. Of the 26 parameters examined, Fisher et al. report that only three correlated with tumor recurrence. These were histologic type, nuclear grade, and race of the patient. Patients with mucinous, tubular, or papillary cancer survived longer than those with typical ductal adenocarcinoma or atypical medullary tumors. Blacks had significantly higher recurrence than whites, but the number of blacks in the study was small. There were 276 paraffin blocks available for flow cytometry. DNA ploidy correlated with nuclear grade but not with survival. In addition, 193 tumors were evaluated for immunohistochemical demonstration of erb2 expression. The authors did note that erb2 expression accurately predicted survival among patients with good nuclear grade.

The duration of adjuvant chemotherapy has been addressed by several studies that have given the same regimen of drugs over different periods. Five separate randomized trials seem to support the conclusion that there is little benefit to a course of treatment longer than 6 months (142).

At this time, standard chemotherapy regimens have been defined for certain subsets of breast cancer patients. Carbone (151) summarized the results of 61 trials involving approximately 29,000 women. Results indicate that chemotherapy is effective for premenopausal, node-positive (stage II) women with both ER-positive and ER-negative tumors. Chemotherapy has not been universally effective in postmenopausal patients, with the possible exception of those with ER-negative tumors. For postmenopausal patients, the only standard regimen, as outlined by the 1985 Consensus Development Conference on Adjuvant Chemotherapy of Breast Cancer (152), is the use of tamoxifen in stage II, ER-positive patients. Node-negative, ER-negative postmenopausal patients have had an increase in disease-free survival but not overall survival with adjuvant chemotherapy. Tamoxifen may also be added to the regimen of these patients in lieu of, but not in addition to, chemotherapy. The addition of tamoxifen to chemotherapy does not enhance the effectiveness of the chemotherapy (151). Node-positive, ER-negative postmenopausal patients have no defined, standard therapy. For stage III disease, combination chemotherapy containing doxorubicin followed by operation and radiation therapy is commonly used (153).

Histologic Types of Breast Cancer

Approximately 85% of breast cancers comprise a group that is identified histologically as invasive ductal adenocarcinoma. More recently, this common histologic type has been called not otherwise specified (NOS) to distinguish it from the less-frequent histologic variants with more-distinctive histologic characteristics. Those types and their frequency of occurrence are medullary (6%), colloid (2%), Paget disease (2%), tubular (less than 2%), papillary (less than 2%), adenoid cystic (less than 0.1%), apocrine (less than 0.1%), secretory (less than 0.1%), and infiltrating lobular carcinoma (5% to 10%). Up to 33% of the invasive ductal adenocarcinomas can be mixed with one or more of the distinct histologic types. A small focus of a histologic subtype within a dominant pattern of NOS does not alter the prognosis. Mixed tumors with a dominant NOS pattern clinically behave as invasive ductal adenocarcinoma. Most of these tumors are moderately to poorly differentiated (Table 13.10).

Paget disease presents as a crusting erosion of the nipple. It can also be less subtle, presenting with thickening, redness or roughness of the nipple often associated with itching and burning. The histologic diagnosis depends on the presence of the pagetoid cell in the epithelium. This cell is clear with a large vesicular nucleus and prominent nucleoli (154). The cell can be identified immunohistochemically with carcinoembryonic antigen (CEA) stain. The origins of these cells are controversial; two leading theories are migration of the cells into the epithelium from the tumor and cellular transformation as a result of metaplastic change within the epithelium. This entity is nearly always associated with subareolar invasive or intraductal cancer. In approximately 60% of patients, the associated cancer can be felt on examination as a distinct breast mass. Survival with Paget disease depends on the stage of the accompanying breast cancer.

Medullary cancer of the breast is bilateral in approximately 20% of patients. This histologic type has one of the fastest growth rates of any breast cancer. Less than 10% of these tumors are ER and PR positive. Approximately 50% are associated with a prominent intraductal component. Despite these characteristics, medullary cancer has a better prognosis than NOS. The gross appearance is softer and
bulker than the usual breast cancer, and it usually presents with a circumscribed, distinct capsule. Cyst formation and hemorrhagic necrosis are common. Microscopically, the tumor contains a dense infiltrate of lymphocytes and plasma cells throughout a uniform, sheet-like growth of large cells with pleomorphic nuclei and frequent mitoses (154).

Tubular cancer is common in younger patients: this tumor is often small (less than 1 cm) and has an excellent prognosis because most patients are node negative at presentation (more than 90% are stage I) (155). Microscopically, this tumor is characterized by a haphazard array of randomly arranged tubular structures. The important distinguishing histologic feature of this cancer is a single cell layer within the tubular architecture with absence of the basement membrane or myoepithelial cells. This pattern must be present in more than 75% of the tumor to be classified as a tubular cancer.

Colloid cancer is also called mucinous cancer because the cells produce large quantities of mucin pools around the cells. This produces a gelatinous surface on gross inspection. Two thirds of these tumors are ER positive. This histologic type has a highly favorable prognosis.

Papillary cancer is identified by distinctive, well-defined fibrovascular stalks covered with multilayered epithelium (156). This tumor is associated with a more favorable prognosis than NOS, even when positive nodes are evident (154).

Adenoid cystic cancer is rare in its pure form. The microscopic features of this tumor are identical to those of the salivary gland cancer that bears the same name. Axillary metastases are rare but pulmonary metastasis has been described (157,158).

Apocrine cancer of the breast is exceedingly rare. The histologic characteristics resemble those of the apocrine glands common to the axillary, anogenital, and groin regions. This can be an aggressive tumor.

Secretory cancer is the most common histologic variant of breast cancer that occurs in children. Metastatic disease is unusual, and local excision is recommended in most cases.

Infiltrating lobular carcinoma is bilateral in at least one third of patients. It also has a high incidence of multicentricity in the ipsilateral breast. This tumor may produce intracytoplasmic mucin such that some cells have a signet ring-like appearance. Signet ring metastatic deposits can be distinguished from gut tumors with an ER stain. This neoplasm commonly metastasizes to the meninges and serosal surfaces (peritoneum and pleura). The prognosis is similar to that of the NOS variant.

Grading systems have been described in an effort to classify tumors with reference to the degree of variation from normal histologic patterns. Tumors are graded for nuclear appearance (159) and architectural organization (160). Nuclear grading segregates tumors according to differentiation (154). A well-differentiated tumor has small, uniform, round nuclei with few nucleoli and mitoses. Moderately differentiated tumors have an increase in nuclear size, pleomorphism, and chromatin variability. Mitoses are frequent; nucleoli are large and irregular. Poorly differentiated tumors have large nuclei with variable chromatin patterns, prominent nucleoli and frequent, often bizarre, mitotic figures. The architectural grading system is a description of the degree of organization of the tubular-acinar unit (154). Well-differentiated tumors have a prominent tubular pattern. Moderately differentiated tumors have identifiable tubular structures arranged in small groups, nests, or cords. Poorly differentiated tumors have absence of tubular structures, as the cells tend to grow in sheets.

### Factors that Predict the Likelihood of Tumor Recurrence

The College of American Pathologists released a consensus statement in 1999 (161) listing features of breast cancer in three categories of prognostic significance. Category I factors are those proven to have prognostic significance and are useful in patient management. Category II factors are tumor, node, metastasis (TNM) staging information, histologic grade, histologic type, mitotic figure counts; and hormone receptor status. Category III factors are those whose importance remains to be validated in statistically robust studies. Category II factors are c-erbB (HER2-neu), proliferation markers, lymphatic and vascular channel invasion, and p53.

### Table 13.10. Comparison of Histologic Types and Outcome

<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>Infiltrating Duct Carcinoma</th>
<th>Infiltrating Lobular Carcinoma</th>
<th>Medullary Carcinoma</th>
<th>Mucinous Carcinoma</th>
<th>Papillary Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Node involvement</td>
<td>60%</td>
<td>60%</td>
<td>44%</td>
<td>32%</td>
<td>17%</td>
</tr>
<tr>
<td>Crude survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td>54%</td>
<td>50%</td>
<td>63%</td>
<td>73%</td>
<td>83%</td>
</tr>
<tr>
<td>10 years</td>
<td>38%</td>
<td>32%</td>
<td>50%</td>
<td>59%</td>
<td>56%</td>
</tr>
<tr>
<td>Actuarial survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td>59%</td>
<td>57%</td>
<td>69%</td>
<td>75%</td>
<td>89%</td>
</tr>
<tr>
<td>10 years</td>
<td>47%</td>
<td>42%</td>
<td>68%</td>
<td>72%</td>
<td>65%</td>
</tr>
<tr>
<td>20 years</td>
<td>38%</td>
<td>34%</td>
<td>62%</td>
<td>62%</td>
<td>65%</td>
</tr>
</tbody>
</table>
Male Breast Cancer

Breast cancer is an uncommon disease in males. Only 1% of all breast cancers occur in this gender. No clearly defined relationship of gynecomastia to breast cancer exists. Microscopic gynecomastia is so common in older men that it is frequently found in association with cancer. However, no evidence exists to support the concept of a malignant progression from gynecomastia to breast cancer. Hormonal factors may be important, however, in that Klinefelter syndrome is 20 times more common in male breast cancer patients than the normal population (162). In addition, mumps orchitis in men older than 20 years increases the risk of breast cancer.

Male breast cancer is highly responsive to hormonal therapy. More than 80% of male breast carcinomas are ER positive (163). Approximately 50% of patients respond to hormone manipulation. Orchiectomy, tamoxifen, and aromatase inhibitors have been used effectively in therapy, but further study is needed to define optimum hormonal management (164,165). Chemotherapy has been less effective, producing objective responses in approximately 35% of patients with either the Cooper five-drug or doxorubicin-containing combination (166). Despite these hormonally responsive, the relationship of male breast cancer to estrogen production and sensitivity has not been defined. Studies linking the cancer to hyperestrogenism have not been substantiated (167). Approximately 85% of male breast cancer is ductal adenocarcinoma; 5% is papillary. Lobular, tubular, and colloid patterns are rare. Paget disease may occur in males with a presentation and prognosis that are similar to females.

REFERENCES

6. Fuote FW, Stewart FW. Comparative studies of cancerous versus noncancerous breasts. I. Basic morphologic characteristics.


