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Human Breast Development

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This review presents an atlas of the histology of the normal physiological states of the human breast including prenatal, prepubertal, and pubertal development, adult resting gland, pregnancy, lactation, and postinvolution. The aim is to produce a pictorial overview of the main stages in development and the common findings in the adult that are considered to be within the range of normality. Unlike inbred strains of animals, in humans it is clear that the chronology of ductal and lobular development is not predictable, either in the fetus, the infant, the peripubertal breast, or the adult. This is probably due to the individual variation in hormone levels both *in utero* and after birth. For many of the developmental time points there are very little data available. In this review we indicate the current state of knowledge of human breast development and some of the main similarities and differences with the rodent, the main animal model. The major phases of growth and development are described and accompanied by photographs that are representative of each stage. Stress is placed on terminology as there is confusion in the literature. This article is written as an accessory to the companion review on breast cancer.

KEY WORDS: Breast development; histology; atlas.

INTRODUCTION

Current Knowledge of Breast Development

Very little is known about the molecular mechanisms that initiate breast formation. Breasts are formed after the three major axes have formed and segmentation has occurred. It seems likely that positional information within the vertebrate body plan is conveyed to the cells that will become the breast anlage by as yet unknown mechanisms.

Breasts are assumed to arise as the result of reciprocal epithelial and mesenchymal interactions, as has been demonstrated experimentally in mice (see Refs. 1 and 2 for review). How the future site of the breast anlage is determined is unknown. This initial step of organ formation is significant since it also determines which cells will become the stem cells (3). Once this area is determined, growth, morphogenesis, and cytodifferentiation occur so that eventually a rudimentary system of ducts is formed by the time of birth (4). The molecular mechanisms that regulate these developmental processes are probably common to different tissues and species. Studies of model systems such as the chick limb (5–8) and the mouse tooth bud (9,10) that are amenable to genetic and experimental manipulations have shown that morphogenesis is controlled through compartmental and coordinated expression of an ever-increasing number of proteins. Expression studies in the mouse mammary gland and observations in knockout mice have suggested that these same proteins are temporally expressed at critical times in mammary gland development and probably serve similar functions (11–13). These studies are now being supported by functional analyses using tissue recombination experiments, where tissues (separated epithelium and mesenchyme) can be derived from knockout mice. The importance of individual components of the hormone (12) and growth factor pathways and the

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compartments in which they are expressed are being dissected even in mutations which are embryonic lethals (11,13).

Due to constraints on the availability of tissues, comprehensive studies of human breast development do not exist. The best that can be achieved in the human breast is to attempt to define whether the stages seen in the mouse and human have parallels and if there are any notable differences that could undermine the application of the mouse model for human breast diseases. Such comparisons of tumors that arise in humans with those that arise in mice will help to identify issues that need to be addressed in developing more appropriate model systems. Mouse models exhibiting postnatal mammary gland developmental defects have been recently reviewed and will not be addressed here (14). A comparison of human and mouse pathology has also been reviewed recently (15). This review concentrates on the development of the human breast.

Owing to the limited availability of material, there have been very few recent studies on the development of the human breast. There are a number of older reviews (16) and publications (17,18), but these are mainly based on line drawings from histology sections and schematic impressions, making interpretation difficult. These reviews also provide summaries of some of the original references in the field that date from the 1820s. Recently we have collected fetal, infant, and peripubertal breast to reevaluate the development of normal breast and the cell types present. Much of this review is based on our personal experience. For a detailed review of the literature on the development of the normal human breast see Ref. 19. This review also covers what is known about the hormonal control of human breast development. Much can be learned from studying breast growth in an abnormal hormone environment such as those present in human syndromes that result from endocrine deficiencies and excess. The histology of the developing breast has also been reviewed (20). The clinical variations in the breast have been much more extensively described (21–23). The size, shape, and rate of development of the human breast vary enormously between individuals, so it is not surprising that the same variations are seen at a microscopic level.

Developmental Abnormalities During Early Breast Development

Several human syndromes exist that affect the early stages of breast development during organogenesis. Amastia, polymastia, and polythelia occur as single phenotypes as well as in pleiotropic syndromes (21,24–26). Some of these syndromes have been reported to have genetic bases. Mutations in the human *TBX-3* gene (a member of the T-box gene family) cause ulnar-mammary syndrome, a pleiotropic disorder (27). Limb, apocrine gland, tooth, breast, and genital development are affected in this syndrome, suggesting that epithelial–mesenchymal inductions during formation of these organs are altered (27). Women and men with ulnar-mammary syndrome may have hypoplastic, absent, or extra breast structures (27). Another recently described human disorder with pleiotropic genetic features is limb mammary syndrome (LMS),[3] which maps to human chromosome 3q27 (28). Clinical features of LMS patients include severe hand and/or foot anomalies and hypoplasia or aplasia of the breast and nipple. The phenotypes of affected individuals suggest that single genes are involved in both limb and breast development as well as other organs that develop as a result of epithelial and mesenchymal interactions, such as apocrine glands and teeth. The variable phenotypes suggest that genes affecting breast development may be especially prone to modification by other genes and environmental factors.

Developmental Abnormalities During Postnatal Breast Development

Other breast developmental abnormalities that occur postnatally (23,29) are sporadic conditions of unknown etiology or are due to hormonal imbalances. Adolescent, juvenile, or virginal hypertrophy is a condition that results in the formation of extremely large breasts after the onset of puberty. No underlying hormonal imbalance has been identified in this condition. Fibroadenomas are often referred to as benign lumps, but these well-circumscribed nodules are best considered as abnormalities of breast development. They are a common finding in young women and account for 60% of breast lumps in women under 20 years of age and 13% of all breast lumps. They are under normal hormonal control and are sometimes multiple. They have been associated with a 4:12 translocation t(14;12) (q27;q15) indicating

[3] Abbreviations: LMS, limb mammary syndrome; TDLU, terminal duct lobular unit; EGFR, epidermal growth factor receptor; DPP IV, dipeptidyl peptidase IV; TGFα, tumor-derived growth factor alpha.

that they are clonal lesions (30). This breakpoint region on chromosome 12 is a frequent finding in lipomas and salivary gland tumors, where the third intron in the gene for HMGI-C is involved. Fibroadenomas can be variable in size and so-called giant fibroadenomas are over 5 cm in diameter. Juvenile fibroadenomas are found in adolescent girls and grow rapidly. The most common male breast condition is gynecomastia, which is an enlargement of the ductal and stromal tissues that presents as female-appearing breast development. There are many causes of this condition, but the majority are due to hormonal imbalance either at puberty (25%) or due to secondary factors such as drugs, cirrhosis, or hypogonadism. The majority of the female benign breast ''abnormalities'' that present to the clinician and pathologist are simply examples of what falls within the spectrum of normality.

The human breast is a dynamic organ which is continuously remodeled under the influence of hormones and growth factors. It is thought that this intrinsic dynamic ability of breast cells to be continuously influenced and remodeled makes them especially susceptible to carcinogenesis. In this atlas, we will demonstrate the major changes that are seen in the breast during development and involution. An understanding of the plasticity of the normal breast provides insights into the large number of lesions that are seen in the adult breast and the types of tumors that arise in the epithelium and stroma (31).

MATERIALS AND METHODS

Tissues

All tissue samples were obtained with the consent of the family or patient. Fetal samples were obtained following medically induced terminations with parental consent. Fetal breasts were fixed in Methacarn, Robert's, or Bouin's fixatives and processed for whole-mount analysis, paraffin embedding, and light microscopy as previously described (32,33). Pubertal breast tissue was obtained and fixed as described (34). Details of collection, fixation and staining protocols can be found in Refs. 32, 33, and 35.

We have chosen specimens that we believe are representative of key developmental stages. Plate 1 figures were selected from an analysis of 46 fetal samples. Of these, 11 were female, 29 were male, 4 were of unknown sex, and 2 were of intermediate sex. Plate 2 involved the analysis of 72 infant breast specimens of which 37 were male and 35 were female. Twenty seven female peripubertal specimens were analyzed. Thousands of adult stages have been analyzed.

Photography and Imaging

Fetal whole mounts were photographed using an Olympus SC35 (Type 12) camera mounted on an Olympus SZ-ST dissecting microscope using a tungsten lamp. The 35-mm slides were scanned using a Polaroid SprintScan 35 scanner and Adobe Photoshop 5.0.

STAGES OF HUMAN BREAST DEVELOPMENT

Prenatal Development

The formation of the breast is a critical stage of morphogenesis. It has been suggested that some of the initial events of breast carcinogenesis may occur *in utero* and at early stages of postnatal development (35–37). Prenatal breast development has been extensively described, but these classical descriptions in the older European literature (38–42) accompanied by drawings of the various stages have many inconsistencies. The major problem that we have encountered is that the 'stages' in development do not follow the age of the fetus or of the infant. It is of interest to note that the best study of fetal breast development was based on the length of the embryo (17). In these studies, Hughes was able to follow a clear progression in 70 human embryos. It is difficult to correlate historical data based on embryo measurements with more recent data based on weeks of gestation. The association between size and maturity may be important as recent data would indicate that women from economically deprived areas have lower birth weights and a decreased incidence of breast cancer. Low-protein diet in rodents is known to affect breast development in the newborn (43).

A mammary band was described in a 15-mm embryo by Schultz in 1892 and its presence was confirmed by subsequent observers. The relevance of this band to breast development is doubted as an identical structure is also seen in the embryos of reptiles and birds. An epithelial crest or milk line has been observed in 15-mm embryos extending along the lateral wall of the trunk. In animals such as pigs

and cats that have rows of nipples, this crest segments and is reduced to a series of nipples. No segmentation has been described in humans and there is no real evidence that the nipple is derived from the crest. Evidence for any of the statements concerning a mammary band, mammary crest, or milk line is difficult to substantiate in relation to the formation of the breast and nipple anlage. The fact that supernumerary nipples can be found from the groin to the axilla, however, supports the concept of a milk line or crest (22). A few of our samples did appear to have a thickening that extended across the embryo, in which a condensation of epithelial cells appeared in the thoracic/pectoral region where the breast bud forms (Fig. 1A). According to Hughes, the nipple primordium is first seen in 7.0- to 8.0-mm embryos as a narrow collection of ectodermal cells, which by 10 mm has a single layer of closely applied mesenchyme. From 11.0 to 14.0 mm, the mesenchyme is reported to differentiate beneath the nipple primordium into four layers (17). At 14.0 mm, the nipple has moved from a relatively dorsal to a ventral position and the epithelium has proliferated to form a nodule that is pushing into the mesenchyme. The growth of the nodule then continues to form the breast bud. The bud is fully enclosed in the mesenchyme and there is an indentation over the surface (Fig. 1B). The basal layer of cells is tall and vacuolated with the nucleus displaced apically (not discernible at this magnification). The mesenchyme is closely applied to the basement membrane of the bud, and the deeper mesenchymal cells form a further condensation around the bud (44,45). A stereotypical pattern of blood vessels can be seen around the breast anlagen (Fig. 1C). In all fetal samples examined, we observed an inhibition of hair follicle/peg formation in the immediate vicinity of the breast anlagen. These hair peg anlage appear to display tissue polarity and are distributed at regular (periodic) intervals except in the region where the breast anlagen forms (Fig. 1C). Incipient organs in other systems are known to produce inhibitors (46) and the pattern of hair pegs around the breast anlagen is suggestive of lateral inhibition.

In contrast to rodent development, in which testosterone induces destruction of the breast bud via condensation of the mesenchyme around the neck of the gland on E14 (47–49), both the female and male glands develop similarly *in utero* (data not shown). There is no difference between male and female breast buds with respect to breast epithelial cells or mesenchyme. Turner reported that bovine male mammary buds are more spherically shaped and of greater volume than female buds, which are more elongated (50,51). We have not observed any differences in shape between the sexes in human mammary buds.

There have been suggestions that the breast is a modified apocrine, sebaceous, or eccrine gland, but we can see no evidence to support this (31). The bud changes from the solid structure seen in Fig. 1B. The first sign that we identified in the whole mounts was a small cleft in its center (Fig. 1C). Different planes of sectioning through the same bud will produce variable views as is predicted from the whole-mount appearances seen from above through the periderm (Fig. 1F, H). As the clefting deepens over the surface of the bud (Fig. 1E, G), the bud starts to branch (Fig. 1F–H). Secondary buds appear as the bud lumen enlarges and a change in shape of the bud to a clover-shaped structure occurs. Secondary buds elongate, become canalized, and branch. Ductal morphogenesis occurs as the sprouts elongate and invade the mesenchymal tissue. Studies of expression of growth factors and extracellular matrix proteins in the early

Fig. 1. (A) Carmine-stained whole-mount preparation of an 18-week male fetal breast bud. Arrows indicate breast bud, which is evident as an epidermal thickening along the ventral surface. ×15.75. (B) Histology section of a 16-week male fetal breast stained with hematoxylin and eosin. The developing breast bud is composed of large, clear cells, with nuclei of the basal layer displaced toward the center of the bud. Note the condensed stromal cells (arrows) surrounding the breast bud. ×170. (C) Carmine-stained whole-mount preparation of 16-week male fetal breast bud (arrow). Note the absence of hair pegs (unfilled arrowheads) around the breast anlage and the surrounding pattern of blood vessels (solid arrowheads), which is similar in all developing breasts examined. ×5.0. (D) Histology section of an 18-week male fetal breast bud immunostained for keratin 14, demonstrating the immunonegative precursors of the breast (arrows). The arrowheads indicate the positive-staining cells of the basal cells of the periderm. ×165. (E) Histology section of an 18-week female breast bud immunostained for Bcl-2. The basal cells (arrows) are strongly positive. Note the neck (N) of the breast bud, which has elongated (compare to panels B and D). ×165. (F) Whole-mount preparation of a 16-week male fetal breast bud stained with carmine. Ductal elongation is evident in this view taken from above through the periderm. Ducts are indicated by arrows. ×15.75. (G) Histology section of an 18-week male fetal gland breast stained with hematoxylin and eosin. The breast bud shows branching (arrows indicate the two branches). Note the elongation of the neck (N) of the breast bud. ×170. (H) Whole-mount preparation of a 14-week male fetal gland stained with carmine. Ductal elongation has progressed so that a rudimentary ductal system (arrows) is present. This specimen is viewed from above through the periderm. ×15.75.

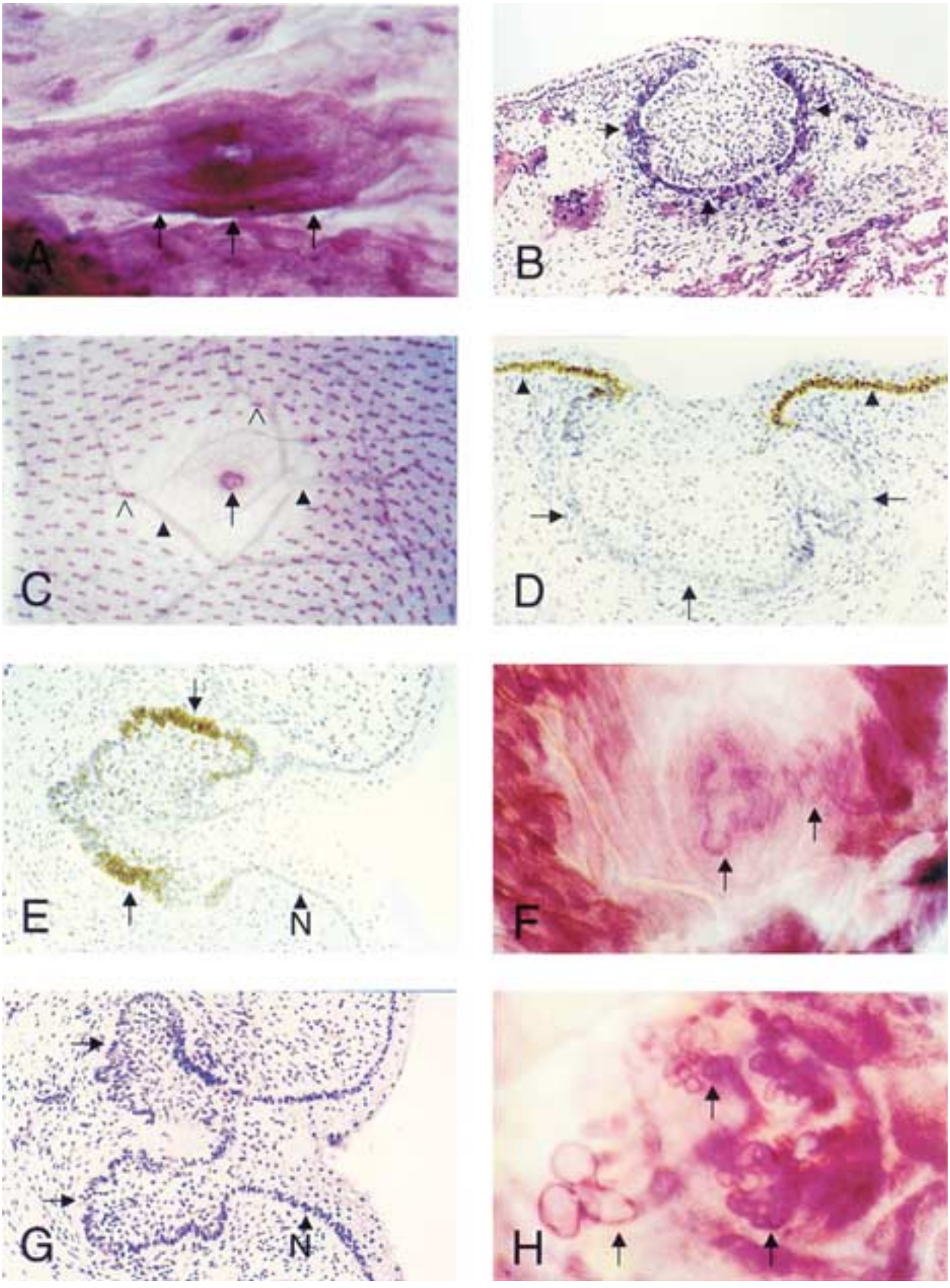

Plate 1. Prenatal breast development.

developing human breast suggest that these proteins have roles in differentiation, growth, and morphogenesis. At the bud stage (18 weeks), the primordial breast cells are clearly delineated as a discrete population by their negative staining for keratin 14 (Fig. 1D) and smooth muscle actin (not shown) compared with the basal layer of the adjacent periderm (33). By 28 weeks, the basal cells in the developing breast are positive for both typical basal cell markers, keratin 14 and smooth muscle (33). At the same time (28 weeks), there is a high expression of epidermal growth factor receptor and TGFα in these cells, suggesting an autocrine stimulation to proliferation. High levels of expression of BCL-2 are observed as early as 18 weeks (Fig. 1E); this molecule would prevent apoptosis and permit expansion of this cell population (52). There is overexpression of BCL-2 in the stromal cells adjacent to the bud at this stage (not visible in this section; see Ref. 52), suggesting that these are also protected from apoptosis and permitting their expansion as the future specialized breast fibroblasts (52). There is very little information in the literature concerning the distribution of extracellular matrix molecules during breast development. TGFβ is expressed in the extracellular matrix at all stages examined; tenascin and type IV collagen are concentrated in the extracellular matrix at the neck of the developing buds (31). These distributions are all consistent with the proposed roles of these molecules in morphogenesis in other tissues (31).

Table I. Summary of Morphological Types and Functional Stages of Infant Breast[a]

Morphological type	
I (MT I)	Rudimentary ductal system composed of elongated ducts with no branchings or less than two dichotomous branchings
II (MT II)	Branching ductal system with more than two dichotomous branchings, but without the development of terminal lobular units
III (MT III)	Branching ductal system with number of branchings and well-developed terminal lobules
Functional stage	
I (FS I)	All ducts and ductules are lined by secretory type of epithelium
II (FS II)	Mixture of ducts lined by secretory epithelium and ducts lined by apocrine type of epithelium
III (FS III)	Almost all ducts lined by apocrine type of epithelium
IV (FS IV)	Mixture of ducts lined by apocrine type of epithelium and involving ducts lined by multilayered epithelium
V (FS V)	Almost all ducts showing involution and often lined by multilayered epithelium

[a] Studies of infant breast from birth to 2 years of age in both sexes show variable features that have been described in terms of the degree of glandular development and branching complexity (morphological type) and the amount of functional differentiation, relating to secretory activity and glandular involution (functional stage). These are summarized here and are useful descriptive terms as the degree of development of the gland is not predictive of functional activity and various combinations can be seen.

Infant Development

As in the fetus, from birth to puberty there are no identifiable morphological differences in the development of the breasts in boys and girls. Similarly, there are wide variations in the degree of glandular development (branching and acinar formation) and in the functional differentiation of the cells lining the ducts and acini. In a study of 72 developing infant breasts, it was decided that the best method of describing these diverse changes was to produce a simple classification representing the morphological type and the functional stage (see Table I). Many combinations of types and stages were observed, and the complexity of the gland in terms of branching and acinar formation could not be directly correlated with the ability of the lining epithelium to be secretory. At birth, the ductal system opens onto the surface through the breast pit, a small depression on the skin's surface. The underlying mesenchyme proliferates to form an everted nipple. The skin surrounding the nipple also proliferates to form the areola. Hair is only found at the periphery of the areola.

Whole-mount analyses at birth demonstrate the diversity in the degree of development present, which can range from simple blunt-ended tubular structures to well-developed branching with acinar development (compare Fig. 2B, C). The infant breast is able to respond to the secretory stimuli that arise from maternal hormones (20,53) and the production of milk by 80–90% of both sexes is due to the effects of prolactin on withdrawal of the sex steroids (16). This is true whether acini are present or not. Thus, some breasts have dilated ducts distended by secretions (Fig. 2A), whereas in others, the acini are secretory with casein

production and a specialized stroma is seen surrounding the acini (Fig. 2D). In those cases where the breast has a well-developed branching pattern and acini, it is possible to see the development of the specialized interlobular and intralobular stroma (see Fig. 4A–C for the adult pattern for comparison). These two fibroblast populations differ in their synthesis of type IV collagen (54) and the intralobular fibroblasts give rise to breast-specific stromal tumors (55). This is a major difference with the rodent breast, where the epithelium is not encased within a specialized fibroblastoid stroma, but is mainly surrounded by fat. The cell-surface peptidase dipeptidyl peptidase IV (DPP IV) is expressed specifically on interlobular fibroblasts in the adult breast (55), whereas aminopeptidase N is expressed on both populations of fibroblasts (56). In Fig. 2E the developing inter- and intralobular stroma are clearly delineated by the expression of DPP IV. Extramedullary hematopoiesis is present in the stroma (Fig. 2F). This specialized stroma differs in a number of respects. The interlobular fibroblasts are distinguished from the intralobular fibroblasts by their expression of TGFα (Fig. 2G). In the newborn, the tips of the tubular ducts and the peripheral acinar structures have a solid appearance reminiscent of terminal end buds in rodent glands. These bud-like structures have a high proliferation rate (Fig. 2H) and a low level of expression of integrins α6 and β1 (57). Cap cells similar to those in rodent terminal end buds have not been identified.

In a recent review, Bartow described estrogen receptor expression in the infant, pubertal, and cycling adult breast taken from autopsies, indicating that this source of material may provide valuable information in the future (59). Keeling *et al.* (58) recently described the distribution of both estrogen receptor and progesterone receptor in fetal, infant, and prepubertal breast tissue and concluded that estrogen receptor is present in the epithelium from the third trimester of pregnancy and progesterone receptor from term to 2–3 months after birth. It is also of interest that estrogen receptor was also present in the stromal cells in the areas of extramedullary hematopoiesis.

During the first 2 years of life, the lining epithelium differentiates and involutes sequentially (48). The infant breast appears to undergo involution once it is removed from the influence of maternal hormones. The changes that take place include apocrine and cystic changes very similar to those seen in the postmenopausal woman. By 2 years of age, all that remain are small ductal structures in a fibroblastic stroma. In the few samples examined, the breast remains in this state in both sexes until puberty.

Pubertal Development

Whereas the gross anatomical changes that occur during puberty have been well documented (reviewed in Ref. 19), the sequence of events at the cellular level is less well understood (20,34). Changes occur in both the epithelium and the stroma. In the stroma, there is an increase in the amount of fibrous and fatty tissue, with the adult nonlactating breast consisting of 80% or more of stroma. Indeed, the extension of ducts is preceded by proliferation of connective tissue, and the fatty tissue is thought to inhibit the growth of the epithelium (60). The ducts elongate and branch dichotomously and sympodially (20).

Sexual dimorphism of the breast occurs during puberty under the influence of hormones. In females, ductal elongation and formation of lobular structures occurs. During puberty, end-bud-like structures are the major sites of proliferation (34) and the terminal duct lobular units are formed. In males, no further development normally occurs. The occurrence of gynecomastia in males demonstrates that ductal and stromal (but not lobular) developmental potential is present in the male breast. Whole-mount analysis of the female pubertal breast displays changes in the ductal system, especially in the types of termini present. The previously blunt-ended ductal termini undergo dichotomous branching, with lateral bud formation with bulbous and solid epithelial tips (Fig. 3A, B). The extending ducts and tips in this 13-year-old female breast are surrounded by a tight glove of fibroblasts, which can be seen in the whole-mount preparations (Fig. 3A). The type of branching that is seen during puberty is very variable, but eventually a typical structure develops. The primary ducts that reach the nipple give rise to a complex branching pattern of subsidiary ducts that lead to the so-called segmental ducts and to smaller subsegmental ducts (Fig. 3C, D, F and Fig. 4A). The subsegmental ducts lead to terminal ducts that give rise to blind-ended ductules called acini (Fig. 3F, Fig. 4A, B). A collection of acini arising from one terminal duct and embedded in intralobular stroma is referred to as a terminal duct lobular unit (TDLU), which is considered as the functional unit of the breast (Fig. 3F). More detailed descriptions of these early stages of peripubertal

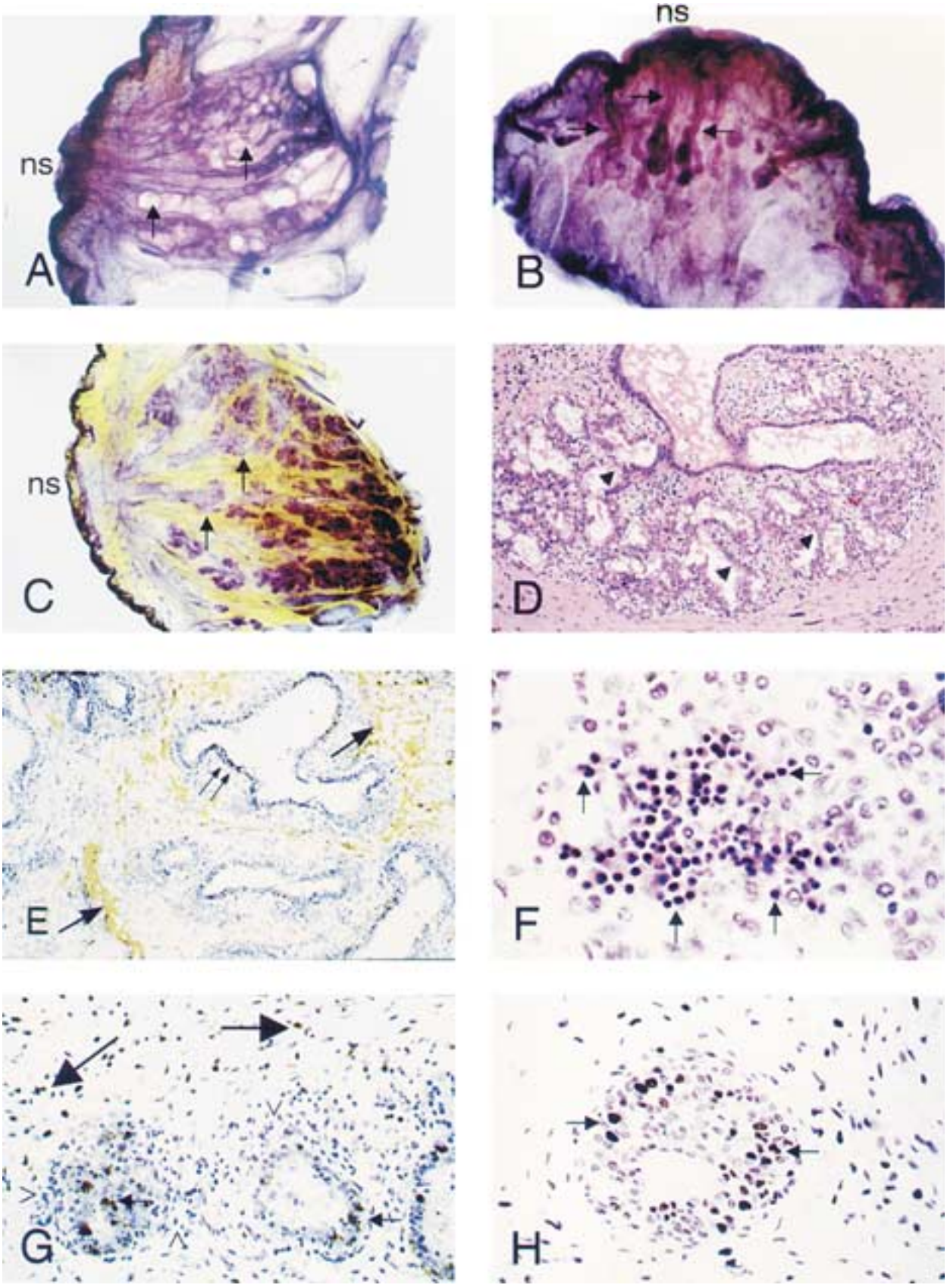

Plate 2. Infant breast development.

breast development can be found in the literature (18,61–63).

In the 15-year-old breast (Fig. 3C, D), the architecture pattern is complete in the center of the breast, but the gland is still expanding at the periphery where the epithelium is pushing forward into the fat pad (Fig. 3C). At this growing front, terminal end bud-like structures and lateral buds are still seen (Fig. 3E).

The 18-year-old breast in Fig. 3F shows the completed typical nulliparous adult gland. Russo and Russo (20) classified the degree of lobular complexity into types 1–3. Type 1 lobules consist of a short terminal duct, ending in a cluster of alveoli. The more differentiated types 2 and 3 have terminal ducts which branch into numerous ductules, giving rise to increased numbers of alveoli. We have not assessed whether a progression of lobular types occurs, but it is possible to see type 3 lobules in very young nulliparous breasts. Our studies of many hundreds of reduction mammoplasties indicate that there is enormous variation in both the epithelium and the stromal content in the normal nulliparous breasts both within the same breast and between breasts. Page and Anderson also noted the wide individual variations seen in the normal breast (64).

Cell Populations Within the Breast

From 28 weeks of intrauterine life, we have been able to distinguish two cell populations in the developing breast epithelium. The inner luminal cell population is separated from the basement membrane by an outer basal or myoepithelial layer. In the fully developed gland, some cells with a luminal surface do meet the basement membrane, and in the acini, the luminal cells often touch the basement membrane between the processes of the stretched myoepithelial cells (65). In the human breast, the term basal was introduced to distinguish the cells in the ducts in a basal position from the myoepithelial cells in the acini. This classification was partly based on cell shape as the cells on the basement membrane in the ducts are cuboidal and those in the acini are flattened. There is, however, no clear functional distinction and the term myoepithelial reflects the fact that these cells have high concentrations of actin microfilaments and stain for alpha smooth muscle actin.

Ultrastructurally, distinctions of the two cell populations are clearly seen (Fig. 3G). The luminal cells have a less electron-dense cytoplasm and an open nuclear chromatin pattern. The myoepithelial cells have an electron-dense cytoplasm and coarse, peripherally arranged heterochromatin. The myoepithelial cells also have basal projections into the stroma, producing an uneven basal lamina. Lymphocytes are a normal transient population in the breast (Fig. 3G). It is possible to identify so-called 'basal clear cells,' usually in the region of the terminal ducts, but occasionally anywhere in the breast epithelium. These have been the subject of much controversy and some authors have claimed that they are not epithelial in origin. Figure 3H clearly demonstrates that these basally situated cells have microfilaments that under high power they have focal densities and form desmosomes with the luminal cells (66). These cells may be analogous to the proposed stem cells in the mouse mammary gland (3). Thymidine-labeling experiments have demonstrated that they divide and they have been proposed as a potential stem cell population. They are found in the same sites as a rare keratin 14/keratin 18-positive cell population described by O'Hare (67), but it has not been possible to confirm that they are the same cell type. Recent *in vitro* cloning experiments on sorted myoepithelial and luminal cells demonstrated the conversion of a

Fig. 2. (A) Whole-mount preparation of a 2-month-old female infant breast stained with carmine. There is extensive dilation of the ducts (arrows), which are filled with secretions. ns, Nipple sheath. Functional stage III. ×12. (B) Whole-mount preparation of a newborn female infant breast stained with carmine. Arrows indicate ducts. ns, Nipple sheath. Morphological type I (rudimentary ductal system composed of elongated ducts with no branching or less than two dichotomous branchings). ×56. (C) Carmine-stained whole-mount preparation of 2.5-month-old male infant breast showing a branching ductal system with terminal lobular units (arrows). ns, Nipple sheath. Morphological type III. ×12. (D) Histology section of 5-day male breast stained with hematoxylin and eosin. A well-developed lobule is lined by secretory epithelium (arrowheads). Functional stage I. ×80. (E) Histology section of a 6-week female infant breast immunostained for DPP IV. Fibroblasts in the developing intralobular stroma are negative (small arrows), whereas fibroblasts outside the forming lobular units (interlobular, large arrows) are immunopositive. ×150. (F) Histology section of 2.5-month-old female breast stained with hematoxylin and eosin showing a focus of extramedullary hematopoiesis (arrows). ×300. (G) Histology section immunostained for TGFα showing positive staining of a subpopulation of luminal epithelial cells (small arrows) and intralobular fibroblasts (large arrows). The periepithelial stromal cells (open arrowheads) are unstained and are associated with extramedullary hematopoiesis. ×200. (H) Histology section of a gland from a newborn infant immunostained with anti-PCNA antibody. This terminal end bud-like structure has a large number of positive cells (arrows). ×260.

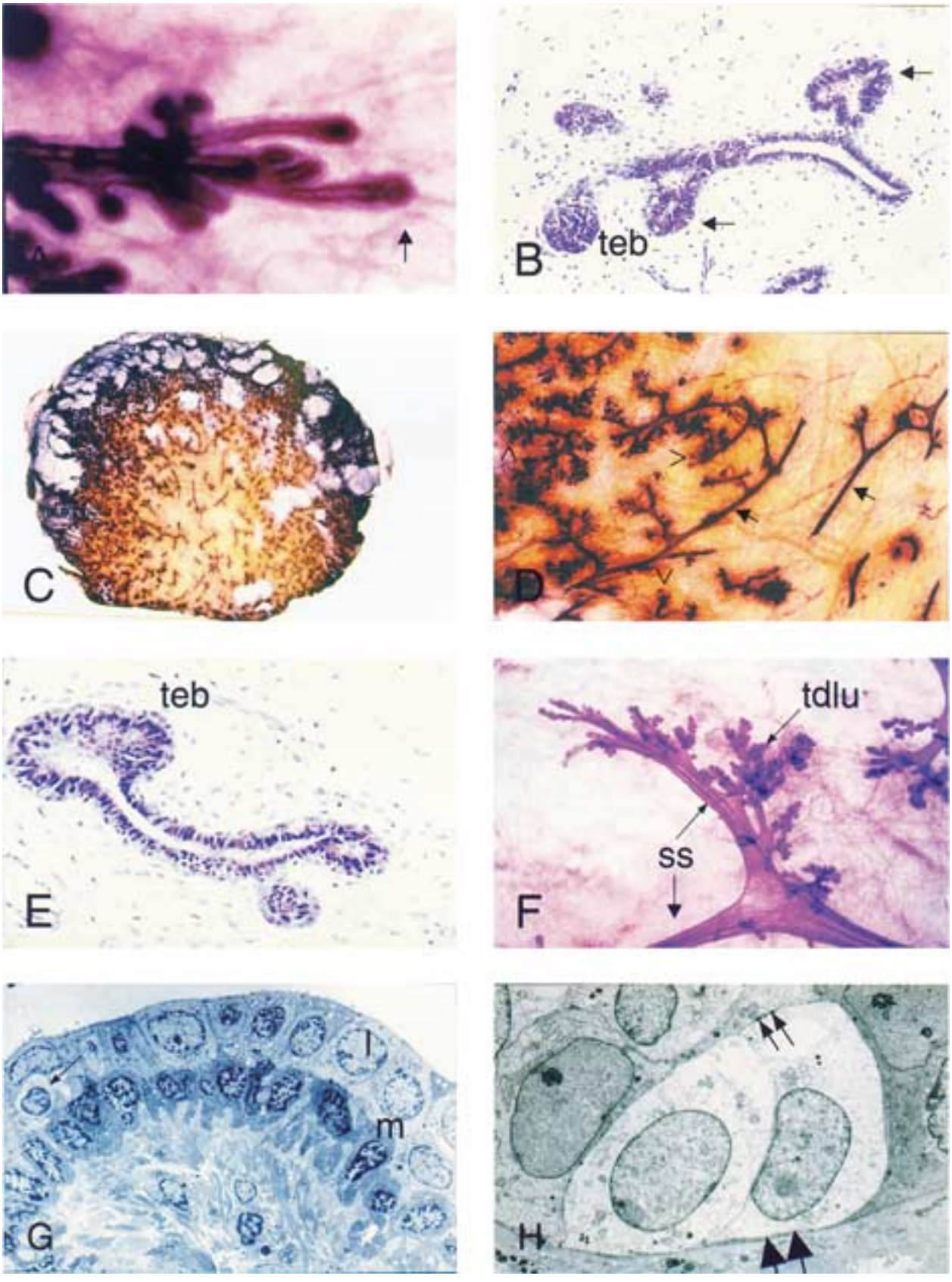

Plate 3. Peripubertal and adult breast development.

subpopulation of luminal cells to a myoepithelial cytoskeletal phenotype (68). This finding supports the view that myoepithelial cells may be derived from the luminal 'lineage.'

Adult Development

Whole-mount analysis of the adult, nulliparous breast shows the mature functional unit of the gland, the terminal duct lobular unit (Fig. 3F). Fig. 4A shows the typical architecture of a premenopausal adult breast at higher power (also see Fig. 4B), illustrating a terminal duct entering a terminal duct lobular unit. The loose cellular connective tissue of the intralobular stroma and the myosin-positive myoepithelial cells are also seen. The TDLU is well circumscribed and a delimiting layer of fibroblasts has been described ultrastructurally around the acini (69). The fibroblasts can be seen to make physical contacts with each other. We have seen, ultrastructurally, a basket-like pattern of fibroblasts arranged concentrically around the TDLUs that have processes that contact and interdigitate (Gusterson and Monaghan, unpublished observation). This appearance suggests that these stromal cells are flattened rather than spindle-shaped *in vivo*.

The immunocytochemical distribution of the cell-surface enzyme DPP IV has been studied in the human breast at the light microscopic and ultrastructural level (55). The presence of the enzyme was demonstrated on the cell membranes of interlobular fibroblasts, whereas intralobular fibroblasts were DPP IV-negative. This clear delineation of two functionally distinct subpopulations of breast fibroblasts was maintained in fibroadenomas and in the stromal tumor, cystosarcoma phyllodes. Both lesions have the phenotype of intralobular stroma. During a process to identify fibroblast subpopulations, we used membranes from purified intralobular fibroblasts (70) to make antibodies. One such antibody clearly reacts preferentially with the intralobular stroma (Fig. 4C), further indicating the specialized nature of this structure.

Unfortunately, it is very difficult to study the human breast during pregnancy and lactation. Most specimens are from operations on cancers arising during this period. Page and Anderson provide the best available description of changes in the human breast during pregnancy and lactation (64). The few specimens examined, however, show similar features to those in rodents, with an increase in the number of lobules and a loss of the fat (Fig. 4D). An interesting question is why we do not see large numbers of nuclei from the compressed fat-depleted adipose cells lined up beside the acini. The lactating gland is composed of dilated acini containing milk (Fig. 4E). In the figure, the expanded TDLUs are still separated by bands of interlobular connective tissue. The budding of the milk fat globule can be seen at high power (not shown). At weaning, with the end of the suckling stimulus, the breast involutes, and, as in the rodent, secretory epithelial cells are removed by apoptosis and phagocytosis. A characteristic feature of the human breast, however, is the heterogeneity seen in different parts of the breast and from one TDLU to another. This is a feature that has been noted by many researchers when looking at proliferation in the normal breast. In the pregnant breast, it is not unusual to see acini that are completely resting while others have expanded with a dramatic proliferative response. As can be seen in Fig. 4F, a similar situation is seen in postlactational involution.

Adolescent or virginal hypertrophy was first reported in 1669 and may be unilateral, suggesting that the overgrowth of the breast postpuberty is due to local rather than systemic factors. No evidence of abnormal systemic hormones has been identified. The breast can weigh several kilograms and the overgrowth is a mixture of stroma and epithelium. On

Fig. 3. (A) Carmine-stained whole-mount preparation of the advancing edge (arrow) of the parenchyma from a 13-year-old female. ×50. (B) Histology section (stained with hematoxylin and eosin) of the developing breast from a 13-year-old showing solid end bud-like structures (denoted teb) and lateral buds (arrows). ×100. (C) Carmine-stained whole-mount preparation of the breast from a 15-year-old female. This is a coronal section. ×0.5. (D) Higher power view of area in panel C. Arrows indicate ducts and unfilled arowheads indicate ductal termini. ×2.5. (E) Histology section (stained with hematoxylin and eosin) of the peripheral region of the parenchyma in the breast seen in panel C. Here teb denotes terminal end bud. ×180. (F) Carmine-stained whole-mount preparation of breast tissue from an 18-year-old nulliparous female. A segmental duct divides into two subsegmental ducts (ss), which then lead to the terminal duct lobular units (tdlu). ×5. (G) Electron micrograph of a normal adult subsegmental duct. Note the paler luminal cells (l), the darker basal (myoepithelial) cells (m), and an intraepithelial lymphocyte (arrow). ×1650. (H) Electron micrograph of a terminal duct lobular unit showing two 'basal clear cells.' These have microfilaments in the basal part of the cell (large arrows) and desmosome attachments with the luminal cells (small arrows). ×6500.

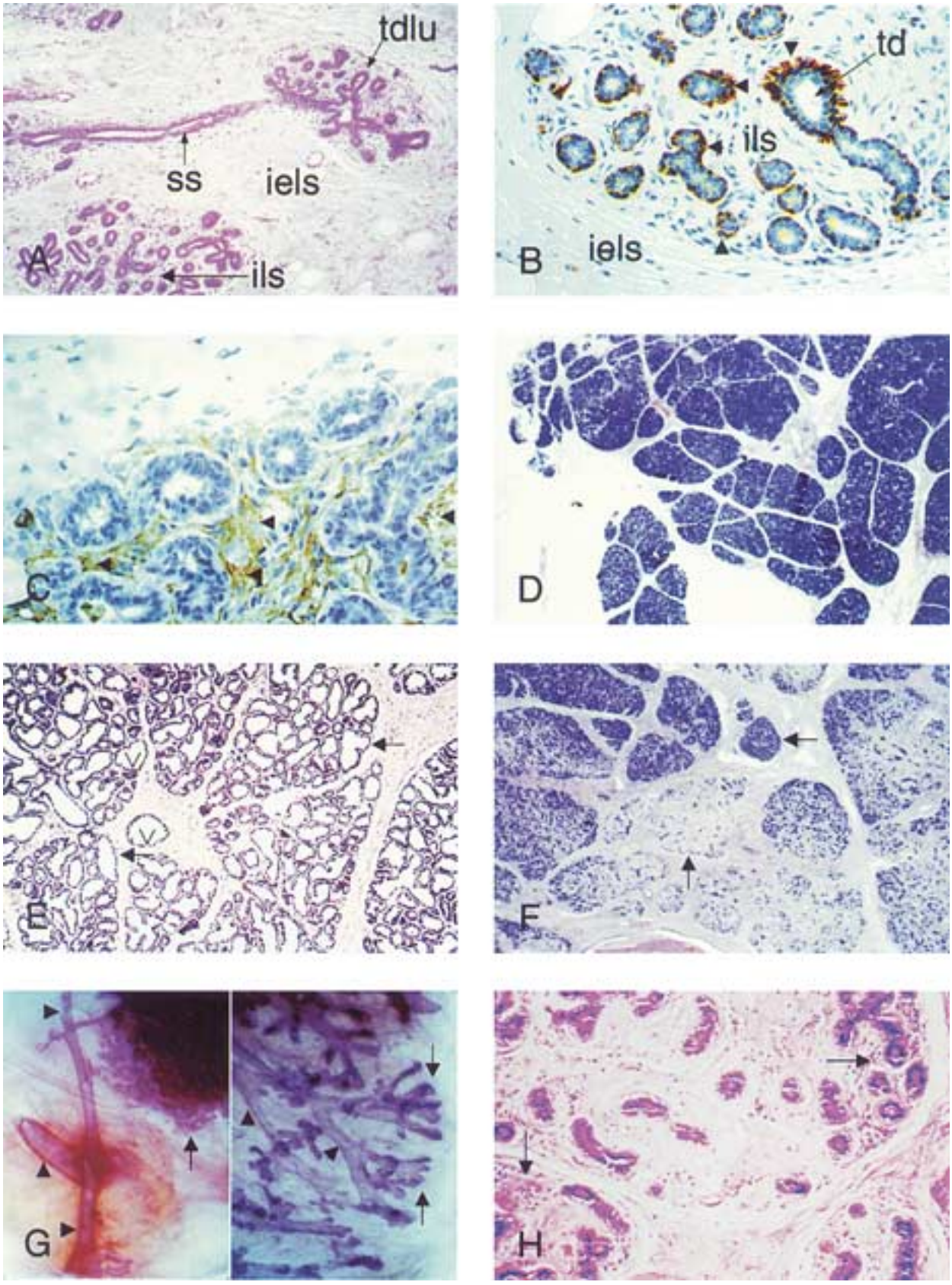

Plate 4. Adult resting, pregnant, and lactating breast.

slicing of the breast, large, well-circumscribed nodules are found which, on whole-mount examination (Fig. 4G), show large ducts with expanded TDLU structures (see low power), and on high power, branching ductules and acini in intralobular stroma can be observed.

Postmenopausal Involution

Following weaning, involution occurs. The two-layered epithelium of the resting breast is re-formed, but the cells that repopulate the epithelium of the gland have not been defined. During successive rounds of pregnancy and involution, the TDLUs expand and decrease in size with an increase and subsequent decrease in the number of acini. The ducts are not obviously involved in this process. This is in contrast to postmenopausal involution, where both lobules and ducts are reduced in number. The intralobular stroma is replaced by collagen (Fig. 4H), whereas the glandular epithelium and the interlobular connective tissue regress and are replaced by fat. Eventually all that remain are a few acini and ducts embedded in thin strands of collagen that are widely dispersed throughout the fat (Fig. 5A). The replacement of the dense fibrous tissue of the premenopausal breast by fat explains why mammographic screening in women over 49 years of age is much more effective in picking up small tumors that are dense against the contrasting fat.

Common Benign Proliferative Breast Conditions

A large number of relatively common lesions are found in the premenopausal and postmenopausal breast. A key issue which has yet to be adequately addressed in the human breast is which cell type or types are the origins of human epithelial-derived breast disease, whether benign or malignant. From immunocytochemical studies, using markers of luminal and basal (myoepithelial) cells, it can be concluded that the majority of human breast cancers arise in a cell population that is committed to a luminal phenotype. This is in contrast to the majority of the benign proliferative conditions, where it is clear that the lesions are derived from a pluripotent cell or there is a coordinated proliferation of both luminal cells and myoepithelial cell populations. In addition to the proliferative conditions, a large number of entities come to the notice of the pathologist and the clinician because they present signs and symptoms that can be confused with cancer. Breast lumps and nipple discharge result from benign conditions in the majority of cases. For a full description of benign disorders and diseases of the breast the reader is referred to excellent reviews (23,71). For an introduction to the clinical manifestations of breast diseases and their management, the *ABC of Breast Diseases* is a good starting point (22).

There is no clear definition of what constitutes a benign breast disease; most disorders belong to a spectrum from normal to presenting sufficient symptoms to bring them to the attention of the clinician. Disease is therefore a clinicopathological description and many of the so-called diseases are asymptomatic and common autopsy findings in the general population (72). The critical question here is whether any are premalignant. Many of the disorders can be classified under the convenient heading of aberration of normal development and involution (ANDI). Included in this group are (i) developmental disorders, such as adolescent hypertrophy, which was considered earlier, and fibroadenoma and (ii) involutional changes including cysts and sclerosing adenosis. Duct ectasia or periductal mastitis will not be considered in detail here, but this common condition is of unknown origin

Fig. 4. (A) Histology section of adult breast stained with hematoxylin and eosin. The subsegmental duct (ss) leads to the terminal duct lobular unit (tdlu). Note the cellularity of the intralobular stroma (ils) and the relatively acellular interlobular stroma (iels). ×90. (B) Histology section immunostained for smooth muscle myosin to delineate the myoepithelial cells (arrowheads). Note the cellularity of the intralobular stroma (ils) and the relatively acellular interlobular stroma (iels). Here td denotes terminal duct. ×220. (C) Histology section of adult breast immunostained with an antibody to an intralobular stroma-associated antigen. Arrowheads indicate positively stained intralobular stroma cells. ×250. (D) Histology section (stained with hematoxylin and eosin) of breast from a pregnant woman showing uniform lobular development. ×30. (E) Histology section (stained with hematoxylin and eosin) of lactating breast showing dilated acini (arrows) lined by a low cuboidal epithelium (open arrowheads). ×65. (F) Histology section from a postlactational breast stained with hematoxylin and eosin. Involution is patchy. Compare the variable lobular development in adjacent regions marked by two arrows with the uniform development seen in panel D. ×30. (G) Carmine-stained whole mounts from the breast of an 11-year-old female with adolescent virginal hypertrophy. Arrows indicate ductal termini which vary considerably. Arrowheads indicate ducts. Left, ×2.5; right, ×8.25. (H) Histology section of involuting, postmenopausal breast (stained with hematoxylin and eosin) showing replacement of the cellular intralobular stroma by dense acellular collagen (arrows). ×250.

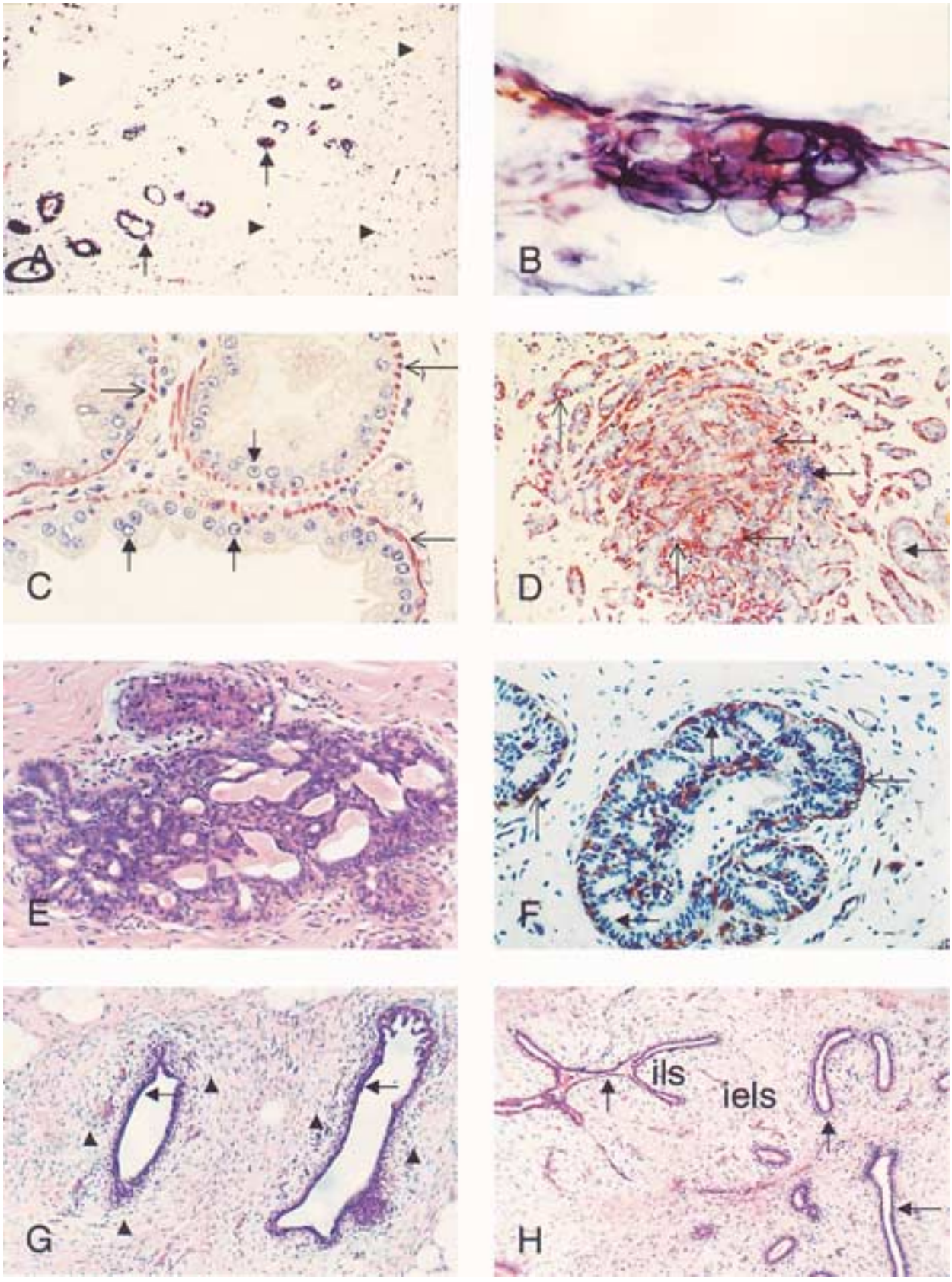

Plate 5. Involuting breast and benign breast conditions.

and is characterized by dilated ducts, periductal fibrosis, and periductal chronic inflammation. The cause is unknown, with suggestions varying from a primary autoimmune reaction in the breast to a component of the involutional process with stagnation of secretions leading to secondary inflammation. Duct ectasia like breast cancer, can present as a nipple discharge (73). Epithelial hyperplasias are considered in detail in the review on breast cancer, but it should be noted that some degree of hyperplasia has been reported in 59% of breasts of women over the age of 70 years. Other benign conditions such as breast abscess and fat necrosis are outside the scope of this review.

Here we will provide some examples of some of the more commonly encountered aberrations of normal development and involution. Now that reduction mammoplasties are a common cosmetic operation, the pathologist is able to appreciate the variations of breast composition that exist in the young adult breast. There is a wide difference in the proportions of connective tissue and fat present, but there have been no comprehensive studies to assess whether there is less epithelial tissue in a small breast than in a large breast. Small breasts are equally successful in breast feeding. For many scientists, the only source of normal breast tissue is reduction mammoplasties. The question therefore arises as to whether this tissue is 'normal' since it is obtained from larger breasts. We have seen over 300 reduction mammoplasties in the last 10 years and the variation in the pathology presents with the same spectrum as that seen in any comparable group of premenopausal breast samples.

The breast is continuously undergoing changes throughout life, both as part of the normal menstrual cycle and in pregnancy. During each estrus cycle, proliferation occurs, followed by apoptosis of the epithelial cells. In addition to the epithelial changes, the stroma is also under hormonal control. With these constant fluxes of proliferation and regression, it is not surprising that abnormalities occur. When a pathologist looks at a breast section from a postmenopausal woman she or he can see many changes in one microscopic field. It is not unusual to see a normal lobule, an area of fibrosis with involutional microcysts, apocrine metaplasia, and intraductal hyperplasia all in the same field. This type of observation demonstrates the range of normality for a postmenopausal breast. Many of these lesions can produce benign lumpy breasts, which is why they are biopsied. These are not diseases, but manifestations of a tissue that has been constantly exposed to remodeling.

One of the most common benign conditions is breast cysts. Cysts are thought to arise through obstruction of the ducts. Secretions build up and cysts like those in Fig. 5B are formed. It was mentioned under development in the infant that apocrine metaplasia was seen as part of the postneonatal involution. Apocrine metaplasia is a change whereby the lining epithelium takes on the cytological features of the apocrine glands of the axilla and perineum. As can be seen in Fig. 5C, these apocrine cells have a very characteristic appearance, with a granular cytoplasm due to the large numbers of mitochondria and large nuclei with prominent nucleoli. This metaplastic change can be seen as a feature of many benign and malignant conditions and can produce difficulties for the pathologist in the diagnosis of some benign conditions, where the nuclear characteristics can be confused with the nuclear atypia seen in association with cancer.

Sclerosing adenosis is a lobular proliferation in which the acini at the margins are infiltrative. It is possible to see perineural and vascular invasion in this condition, although there is no association with risk of cancer. There is an early proliferative phase followed by involution with fibrosis; the latter

Fig. 5. (A) Histology section from a normal postmenopausal involuting breast (stained with hematoxylin and eosin). The regression of both epithelial (arrows) and stromal (arrowheads) elements of the lobules is seen. ×90. (B) A carmine-stained whole-mount preparation of a postmenopausal breast showing cystic change. ×12. (C) Type 1 cysts are usually lined by apocrine type of epithelium. This section is immunostained for smooth muscle myosin to mark the myoepithelial cells (unfilled arrows). It is the luminal cells (solid arrows) that undergo this metaplasia and they have characteristic nuclei, with prominent nucleoli. ×300. (D) Histology section of sclerosing adenosis immunostained for myosin. Note that both luminal cells (solid arrows) and myoepithelial cells (open arrows) are involved in this condition. It can have an infiltrative growth pattern. ×200. (E) Histology section (stained with hematoxylin and eosin) showing a typical example of ductal hyperplasia of usual type. ×200. (F) A similar lesion to that of panel E, immunostained for myosin, clearly demonstrating the two cell types involved in this proliferation (luminal cells, solid arrows; myoepithelial cells, open arrows). ×220. (G) Histology section (stained with hematoxylin and eosin) from a case of male gynecomastia showing hyperplasia of the epithelium of the ducts (arrows) in loose edematous stroma (arrowheads). ×90. (H) Histology section (stained with hematoxylin and eosin) of a typical fibroadenoma. These localized nodules have characteristic features, with ducts and lobules (arrows) surrounded by intralobular stroma (ils) and intersected by interlobular stroma (iels). ×75.

is reflected in the term sclerosis. As can be seen in Fig. 5D, the proliferation involves both luminal cells and myoepithelial cells.

Epithelial hyperplasia is a condition in which there is expansion of the lobules through an intraluminal proliferation of a mixed population of luminal cells and myoepithelial cells. Figure 5E shows a typical example. Within the proliferative area, irregular-shaped spaces usually lined by nonpolarized luminal cells are formed (Fig. 5F). The so-called bridges of cells are formed of both luminal and myoepithelial cells (Fig. 5F).

Two additional conditions are considered here, one as an example of a developmental abnormality (fibroadenoma) and the other as an abnormal overgrowth of breast tissue in males (gynecomastia). The normal male breast consists of widely spaced ducts in a fibrous stroma with no TDLU. Gynecomastia may present like a well-formed breast, clinically resembling that of an adolescent female. There are two peaks of incidence, adolescence and middle age, with 75% of cases being unilateral. The histological appearance (Fig. 5G) shows an increase in the number of ducts, which often have proliferation of the lining epithelium. The stroma around the ducts is usually very cellular, vascular, and often myxoid. Although many cases are associated with systemic hormonal abnormalities, the high incidence of unilateral gynecomastia suggests that local factors are also important. Fibroadenomas are round, firm nodules that have been described in up to 25% of women at postmortem. It has been suggested that they are present in all breasts (23). They are of lobular origin and are composed of ductules and acini in intralobular stroma. In some cases, the epithelial component is elongated and the lumina are compressed (Fig. 5H). Apocrine metaplasia, epithelial hyperplasia, and sclerosing adenosis can be found in fibradenomas and they respond normally during pregnancy and lactation. They are best considered as hamartomas of the breast.

DISCUSSION

Most of the literature concerning embryonic and fetal breast development is descriptive, accompanied by drawings and sketches. Inconsistencies in the literature exist concerning whether a milk line is formed in mice, although it has been documented in other mammals. We observed no human specimens which displayed the ''milk streak,'' a two- to four-cell-layered, thickened epithelial band that runs from the axilla to the groin. The youngest fetal specimen that we examined was 10 weeks old, which is past the time (week 4) that this structure has been reported to occur. We did observe a banding pattern in a few of the fetal samples (Fig. 1A), but it is possible that this was an artifact of the fixation process. Only with a comprehensive analysis and documentation of multiple samples can these issues be resolved. It would be useful if researchers with other examples of these specimens made them available for analysis within the community so that the issue of how representative the examples presented here are could be addressed. Ethical and practical considerations make it difficult to obtain these types of samples, but the collection of breast tissue from all stages of development could prove to be an invaluable resource for understanding variations in development and the extent of disease in given populations. Due to the small number of specimens available for analysis, developmental anomalies may be present so we should avoid drawing conclusions from single specimens. From our fetal gland analysis, we analyzed 46 specimens and chose what we thought were the most illustrative examples. This stratagem, however, imposes our own preconceived notions about how an organ ought to develop. We did not have staging (i.e., crown–rump length) for these specimens and the age of the specimen does not correlate with the extent of development. One of the youngest samples (14 weeks) we examined displayed the most advanced stage of development (Fig. 1H).

Unlike mice and rats, which can be inbred and maintained under strictly maintained diets and environmental conditions, human subjects are genetically heterogeneous and have been exposed to variable environments. Genes, diet, and environmental influences probably play important roles in human breast development, but are not documented.

CONCLUSION

Breast development is currently receiving more attention mainly due to its important link to understanding breast cancer. It is now clear that many genes involved in cell cycle regulation, for example, those for cyclin D1 and p53, may have a role in breast development. Genes such as those for BRCA1 and BRCA2, when mutated in the germ line, predispose to breast cancer and are expressed in the gland during development. It is becoming clear that the cellular

context of gene expression is very important. Thus studies of development can provide insights into cancer (31).

The use of the breast as a paradigm for early developmental studies has lagged behind studies of other organ systems. Mammary gland patterning was analyzed by Bateson in his classic treatise, *Material for the Study of Variation*, over a century ago (74). The origin of mammary glands raises many basic biological questions concerning development, evolution, and specialization. Novel structures are thought to arise through changes in developmental programs. The developmental and genetic mechanisms that produce structures and patterns must first be elucidated to address these issues. Fundamental questions concerning how pattern formation of the breast is determined remained unanswered. Why do different species have different numbers and locations of mammary glands? How is the number and positioning of these determined?

We have presented examples of various developmental stages of normal breast development. This atlas should be useful as a tool to researchers in breast biology. In addition to its biological interest, a more comprehensive knowledge of how the gland forms and then develops may help us to understand why the breast is especially susceptible to carcinogenesis.

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REFERENCES

1. T. Sakakura (1987). Mammary embryogenesis. In M. C. Neville and C. W. Daniel (eds.), *The Mammary Gland*: *Development Regulation and Function*, Plenum Press, New York, pp. 37–66.
2. G. W. Robinson, A. B. Karpf, and K. Kratochwil (1999). Regulation of mammary gland development by tissue interaction. *J. Mammary Gland Biol. Neoplasia* **4:**9–19.
3. G. Chepko and G. H. Smith (1999). Mammary epithelial stem cells: Our current understanding. *J. Mammary Gland Biol. Neoplasia* **4:**35–52.
4. I. Thesleff, A. Vaahtokari, and A. M. Partanen (1995). Regulation of organogenesis. *Int. J. Dev. Biol.* **39:**35–50.
5. C. Tickle (1995). Vertebrate limb development. *Curr. Opin. Genet. Dev.* **5:**478–484.
6. R. L. Johnson and C. J. Tabin (1997). Molecular models for vertebrate limb development. *Cell* **90:**979–990.
7. J. W. R. Schwabe, C. Rodriguez-Esteban, and J. C. I. Belmonte (1998). Limbs are moving: Where are they going? *Trends Genet.* **14:**229–235.
8. L. Niswander (1997). Limb mutants: What can they tell us about normal limb development. *Curr. Opin. Genet. Dev.* **7:**530–536.
9. H. Peters and R. Balling (1999). Teeth. Where and how to make them. *Trends Genet.* **15:**59–65.
10. I. Thesleff and P. Nieminen (1996). Tooth morphogenesis and cell differentiation. *Curr. Opin. Cell Biol.* **8:**844–850.
11. J. F. Wiesen, P. Young, Z. Werb, and G. R. Cunha (1999). Signaling through the stromal epidermal growth factor receptor is necessary for mammary ductal development. *Development* **126:**335–344.
12. M. E. Dunbar, P. Young, J. P. Zhang, J. McCaughern-Carucci, B. Lanske, J. J. Orloff, A. Karaplis, G. Cunha, and J. J. Wysolmerski (1998). Stromal cells are critical targets in the regulation of mammary ductal morphogenesis by parathyroid hormone-related protein. *Dev. Biol.* **203:**75–89.
13. J. Sebastian, R. G. Richards, M. P. Walker, J. F. Wiesen, Z. Werb, R. Derynck, Y. K. Hom, G. R. Cunha, and R. P. DiAugustine (1998). Activation and function of the epidermal growth factor receptor and erbB-2 during mammary gland morphogenesis. *Cell Growth Differ.* **9:**777–785.
14. L. Hennighausen and G. W. Robinson (1998). Think globally, act locally: The making of a mouse mammary gland. *Genes Dev.* **12:**449–455.
15. R. D. Cardiff and S. R. Wellings (1999). The comparative pathology of human and mouse mammary glands. *J. Mammary Gland Biol. Neoplasia* **4:**105–122.
16. H. Vorherr (1974). *The Breast*: *Morphology*, *Physiology*, *and Lactation.* Academic Press, New York.
17. E. S. R. Hughes (1949). The development of the mammary gland. *Ann. R. Coll. Surg. Engl.* **6:**99–119.
18. E. K. Dawson (1934). A histological study of the normal mamma in relation to tumour growth. 1—Early development to maturity. *Edinburgh Med. J.* **41:**653–682.
19. D. J. Laurence, P. Monaghan, and B. A. Gusterson (1991). The development of the normal human breast. *Oxf. Rev. Reprod. Biol.* **13:**149–174.
20. J. Russo and I. H. Russo (1987). Development of the human mammary gland. In M. C. Neville and C. W. Daniel (eds.), *The Mammary Gland*: *Development Regulation and Function*, Plenum Press, New York, pp. 67–93.
21. R. R. Gates (1946). Inheritance of various sexual and intersexual conditions, *Human Genetics*, Macmillan, New York.
22. J. Dixon (1995). *ABC of Breast Diseases*, BMJ, London.
23. L. E. Hughes, R. E. Mansel, and D. J. T. Webster (1989). *Benign Disorders and Diseases of the Breast*: *Concepts and Clinical Management*, Baillière Tindall, London.
24. F. C. Fraser (1956). *Dominant Inheritance of Absent Nipples and Breasts*, Instituto Gregorio Mendel, Rome.

25. H. Goldenring and E. S. Crelin (1961). Mother and daughter with bilateral congenital amastia. *Yale J. Biol. Med.* **33:**466–467.
26. G. H. Klinkerfuss (1924). Four generations of polymastia. *JAMA* **82:**1247–1248.
27. M. Bamshad, R. C. Lin, D. J. Jaw, W. S. Watkins, P. A. Krakowiak, M. E. Moore, P. Franceschini, R. Lala, L. B. Holmes, T. C. Gebuhr, B. G. Bruneau, A. Schinzel, C. E. Seidman, and L. B. Jorde (1997). Mutations in human TBX3 alter limb, apocrine and genital development in ulnar-mammary syndrome. *Nat. Genet.* **16:**311–315.
28. H. van Bokhoven, M. Jung, A. P. Smits, S. van Beersum, R. S. F. M. van Steensel, M. Veenstra, J. H. Tuerlings, E. C. Mariman, H. G. Brunner, T. F. Wienker, A. Reis, H. H. Ropers, and B. C. Hamel (1999). Limb mammary syndrome: A new genetic disorder with mammary hypoplasia, ectrodactyly, and other hand/foot anomalies maps to human chromosome 3q27. *Am. J. Hum. Genet.* **64:**538–546.
29. E. F. Scanlon (1986). Benign diseases of the breast. In R. J. McKenna and G. P. Murphy (eds.), *Fundamentals of Surgical Oncology*, Macmillan, New York, pp. 548–556.
30. B. Staats, U. Bonk, S. Wanschura, P. Hanisch, E. F. Schoenmakers, W. J. Van de Ven, S. Bartnitzke, and J. Bullerdiek (1996). A fibroadenoma with a t(4;12) (q27;q15) affecting the HMGI-C gene, a member of the high mobility group protein gene family. *Breast Cancer Res. Treat.* **38:**299–303.
31. P. P. Osin, R. Anbazhagan, J. Bartkova, B. Nathan, and B. A. Gusterson (1998). Breast development gives insights into breast disease. *Histopathology* **33:**275–283.
32. R. Anbazhagan (1993). Foetal and infant breast development. In *Pathology*, University of London, London, p. 292.
33. R. Anbazhagan, P. P. Osin, J. Bartkova, B. Nathan, E. B. Lane, and B. A. Gusterson (1998). The development of epithelial phenotypes in the human fetal and infant breast. *J. Pathol.* **184:**197–206.
34. P. Monaghan, N. P. Perusinghe, P. Cowen, and B. A. Gusterson (1990). Peripubertal human breast development. *Anat. Rec.* **226:**501–508.
35. R. Anbazhagan, B. Nathan, and B. A. Gusterson (1992). Prenatal influences and breast cancer [letter; comment]. *Lancet* **340:**1477–1478.
36. R. Anbazhagan and B. A. Gusterson (1992). Reversed cerebral asymmetry and breast cancer [letter; comment]. *Lancet* **339:**1056.
37. R. Anbazhagan and B. A. Gusterson (1994). Prenatal factors may influence predisposition to breast cancer. *Eur. J. Cancer* **1:**1–3.
38. J. F. Meckel (1820). *Handbuch der menschlichen Anatomie*, Halle, Germany.
39. C. Langer (1850). Milchdruse. *Idenkschr. Akad. Wiss. Wien.*
40. M. Huss (1873). Entwicklungsgeschichte der Milchdrusen. *Jena Z. Naturw.* **7:**176.
41. A. Kolliker (1879). Brustdruse. *Verh. Med.-Physik. Ges. Wurzb.* **14:**198–202.
42. G. Rein (1896). Hyperthelie menschlichen Embryonen. *Anat. Anz.* **11:**702.
43. M. C. Alvarez Sanz, J. M. Liu, H. H. Huang, and E. J. Hawrylewicz (1986). Effect of dietary protein on morphologic development of rat mammary gland. *J. Natl. Cancer Inst.* **77:**477–487.
44. H. Salazar, H. Tobon, and J. B. Josimovich (1975). Developmental, gestational and postgestational modifications of the human breast. *Clin. Obstet. Gynecol.* **18:**113–137.
45. P. Kellokumpu-Lehtinen, R. M. Johansson, and L. J. Pelliniemi (1987). Ultrastructure of human fetal mammary gland. *Anat. Rec.* **218:**66–72.
46. B. L. Hogan (1999). Morphogenesis. *Cell* **96:**225–233.
47. K. Kratochwil (1971). *In vitro* analysis of the hormonal basis for sexual dimorphism in the embryonic development of the mouse mammary gland. *J. Embryol. Exp. Morphol.* **25:**141–153.
48. R. Anbazhagan, J. Bartek, P. Monaghan, and B. A. Gusterson (1991). Growth and development of the human infant breast. *Am. J. Anat.* **192:**407–417.
49. K. Kratochwil and P. Schwartz (1976). Tissue interaction in androgen response of embryonic mammary rudiment of the mouse: Identification of target tissue for testosterone. *Proc. Natl. Acad. Sci. USA* **73:**4041–4044.
50. C. W. Turner (1931). The anatomy of the mammary gland in cattle. II. Fetal development. *Missouri Agric. Exp. Sta. Res. Bull.* **160:**5–39.
51. C. W. Turner (1930). The anatomy of the mammary gland of cattle. I. Embryonic development. *Missouri Agric. Exp. Sta. Res. Bull.* **140:**3–34.
52. B. Nathan, R. Anbazhagan, P. Clarkson, J. Bartkova, and B. Gusterson (1994). Expression of BCL-2 in the developing human fetal and infant breast. *Histopathology* **24:**73–76.
53. J. Hiba, E. D. Pozo, A. Genazzani, E. Pusterla, I. Lancranjan, D. Sidiropoulos, and J. Gunti (1977). Hormonal mechanism of milk secretion in the newborn. *J. Clin. Endocrinol. Metab.* **44:**973–976.
54. A. J. Atherton, M. J. Warburton, M. J. O'Hare, P. Monaghan, D. Schuppan, and B. A. Gusterson (1998). Differential expression of type IV collagen/undulin by human mammary gland intralobular and interlobular fibroblasts. *Cell Tiss. Res.* **291:**507–511.
55. A. J. Atherton, P. Monaghan, M. J. Warburton, D. Robertson, A. J. Kenny, and B. A. Gusterson (1992). Dipeptidyl peptidase IV expression identifies a functional subpopulation of breast fibroblasts. *Int. J. Cancer* **50:**15–19.
56. A. J. Atherton, P. Monaghan, M. J. Warburton, and B. A. Gusterson (1992). Immunocytochemical localization of the ectoenzyme aminopeptidase N in the human breast. *J. Histochem. Cytochem.* **40:**705–710.
57. R. Anbazhagan, J. Bartkova, G. Stamp, M. Pignatelli, B. Gusterson, and J. Bartek (1995). Expression of integrin subunits in the human infant breast correlates with morphogenesis and differentiation. *J. Pathol.* **176:**227–232; Erratum (1996), *J. Pathol.* **178**(2):235.
58. J. W. Keeling, G. King, and F. Walker (1998). Oestrogen and progesterone receptors in fetal, infant and childhood mammary tissue. *J. Pathol.* **186:**21A.
59. S. A. Bartow (1998). Use of the autopsy to study ontogeny and expression of the estrogen receptor gene in human breast. *J. Mammary Gland Biol. Neoplasia* **3:**37–48.
60. A. Dabelow (1957). Die Milchdruse. In A. Benninghoff *et al.* (eds.), *Handbuch der mikroskopischen Anatomie des Menschen*, Springer-Verlag, Berlin, pp. 277–512.
61. K. C. Richardson. (1947). The structural features of the mammary tissues. *Br. Med. Bull.* **5:**123–129.
62. J. O. Drife (1986). Breast development in puberty. *Ann. N.Y. Acad. Sci.* **464:**58–65.

63. J. Fraser (1929). The breast in health and disease. *Edinburgh Med. J.* **36:**217–241.
64. D. L. Page and T. J. Anderson (1987). *Diagnostic Histopathology of the Breast*, Churchill Livingstone, Edinburgh.
65. H. Hamperl (1970). The myothelia (myoepithelial cells). Normal state; regressive changes; hyperplasia; tumors. *Curr. Top. Pathol.* **53:**161–220.
66. C. A. Smith, P. Monaghan, and A. M. Neville (1984). Basal clear cells of the normal human breast. *Virchows Arch. A Pathol. Anat. Histopathol.* **402:**319–329.
67. T. Kamalati, B. Niranjan, A. Atherton, R. Anbazhaghan, and B. Gusterson (1996). Differentiation antigens in stromal and epithelial cells of the breast. *Cancer Treat. Res.* **83:**227–242.
68. C. Pechoux, T. Gudjonsson, L. Ronnov-Jessen, M. J. Bissell, and O. W. Petersen (1999). Human mammary luminal epithelial cells contain progenitors to myoepithelial cells. *Dev. Biol.* **206:**88–99.
69. B. P. Eyden, R. J. Watson, M. Harris, and A. Howell (1986). Intralobular stromal fibroblasts in the resting human mammary gland: Ultrastructural properties and intercellular relationships. *J. Submicrosc. Cytol.* **18:**397–408.
70. A. J. Atherton, R. Anbazhagan, P. Monaghan, J. Bartek, and B. A. Gusterson (1994). Immunolocalisation of cell surface peptidases in the developing human breast. *Differentiation* **56:**101–106.
71. J. R. Harris (1996). *Diseases of the Breast.* Lippincott-Raven, Philadelphia.
72. W. M. Kramer and B. F. Rush, Jr. (1973). Mammary duct proliferation in the elderly. A histopathologic study. *Cancer* **31:**130–137.
73. E. Mallon, P. Osin, N. Nasiri, I. Blain, B. Howard, and B. Gusterson (2000). The basic pathology of human breast cancer. *J. Mammary Gland Biol. Neoplasia*, this issue.
74. W. Bateson (1894). *Materials for the Study of Variation*, Macmillan, London.