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Proliferative Lesions of the Mammary Gland in Rats

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INTRODUCTION

The rat mammary gland has been utilized extensively as a model for mammary carcinogenesis. The strains of albino rat commonly used in chronic toxicology studies have varying susceptibility to chemical- or radiation-induced neoplasia; the Fischer (F344) strain being less susceptible than the Sprague-Dawley or Wistar derived strains (7, 12). In the Fischer rat, the mammary gland is one of the more common sites of carcinogenic effects after exposure to chemicals. The National Toxicology Program has exposed Fischer rats to over 250 different chemicals, and mammary gland tumors were induced in females by 13 of these chemicals (2). In lifetime studies using Sprague-Dawley rats, the incidence of spontaneous tumors often approaches 50% in control animals; these rats are also extremely sensitive to radiation-induced mammary tumors (6, 12).

Several factors greatly influence the susceptibility, magnitude and type of neoplastic response, and growth rate of mammary neoplasms in the rat. These include genetic factors, degree of differentiation of the mammary gland at the time of chemical exposure, physiological and hormonal status, and diet (2). The rat mammary gland becomes less susceptible to chemical carcinogenesis during pregnancy and lactation and with increasing age (9). With decreased sensitivity there is not only a reduction in the total number of induced neoplasms, but also an increase in the number of benign tumors (fibroadenomas and adenomas) compared to malignant lesions (adenocarcinomas). Studies with chemical carcinogens, such as 7,12-dimethylbenz[*a*]anthracene (DMBA) (9, 11), or irradiation (12) suggest that a common pathogenic pathway for mammary carcinogenesis exists for different etiologic agents.

The developing mammary gland in the young virgin rat consists of a branching parenchyma in which the terminal ductal structures end in a terminal end bud (TEB). Over time, these TEBs progressively differentiate into alveolar buds and alveolar lobules. The number of proliferating cells is greatest and the cell cycle shortest in the least differentiated structure, the TEB, while the alveolar buds have the fewest proliferating cells and the longest cell cycle. The number of TEBs in the mammary gland is not equal throughout the animal; thoracic glands contain more TEBs than do abdominal glands. The greatest number of tumors are observed when DMBA is administered between 40-46 days of age, the period during which TEBs are most actively differentiating into alveolar buds. After 55 days, the number of TEBs drops significantly, as does the incidence of induced tumors. Thus, it is the presence of the TEB that makes the mammary gland a target for DMBA chemical carcinogenesis (8), while benign lesions such as adenomas, cysts, and fibroadenomas appear to arise from the more differentiated alveolar buds (9).

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Histologically, the mammary glands are classified as compound tubuloalveolar glands (10). They consist of an arborescent system of ducts, with the secretory portions located at the ends of the branches. The terminal secretory portions of the gland form irregular branching tubules with many evaginations from their walls and from their blind ends. This terminal secretory portion is referred to as a ductule or alveolus, the latter term being used when the terminal epithelial unit is dilated and filled with secretion. The ductules (or alveoli) form a compact cluster around the small intralobular duct; this entire structure is a lobule. Intralobular ducts run within individual lobules. The interlobular ducts from many lobules form larger ducts which unite to form the main lactiferous duct. In the rat, a single main lactiferous duct, or galactophore, passes through the nipple by way of the nipple sinus to the nipple canal and to the exterior surface of the nipple.

MORPHOLOGY

NON-NEOPLASTIC LESIONS

Lobular Hyperplasia (Figures 1 & 2)

Lobular hyperplasia consists of enlarged lobules of relatively normal appearing alveoli. The alveoli are increased in both number and size, and may be filled with a proteinaceous secretion that may contain lipid droplets. Alveolar cysts (*described below*) are often present within the hyperplastic lobules. The alveolar epithelial cells are one layer thick, well-differentiated, and cuboidal. Although larger ducts may be surrounded by a thicker collagenous band, the individual alveoli within a hyperplastic lobule are usually separated by a thin connective tissue stroma. This lack of a prominent collagenous stroma aids in differentiating lobular hyperplasia from fibroadenomas, which also form discrete nodules. Because of the wide variation in size of normal lobules in young rats, it may be difficult to diagnose minimal or mild degrees of lobular hyperplasia.

Atypical Hyperplasia (Figure 3)

Atypical hyperplasia consists of focal irregular proliferation of epithelium within ducts or alveoli and is distinguished primarily by cellular atypia. Epithelial hyperplasia may be present as papillary infoldings, arches, solid nests, or plaques which extend into the lumen from the epithelial layer. Small alveoli may become solidly filled with cells, while larger dilated alveoli may exhibit stratification of the epithelium with one or more layers. Features of cellular atypia may include enlarged cells with vesicular or hyperchromatic nuclei and cytoplasm which may be intensely eosinophilic or basophilic. Eosinophilic cells resemble secretory cells and contain clear lipid droplets, while basophilic cells resemble duct epithelium and usually are devoid of vacuoles. Atypical hyperplasia may be associated with lobular hyperplasia or fibroadenomas.

Cysts (Figures 4 & 5)

Cystic changes are the most common non-neoplastic changes in the mammary gland of the rat. These thin-walled, epithelial-lined spaces are often round and may arise from either ductal or lobular elements. *Ductal cysts* may have a diameter 10 to 100 times larger than normal, and are lined by a single layer of flat, cuboidal epithelial cells. Myoepithelial cells are present and are compressed against the basement membrane. The distended lumen is filled with a granular, eosinophilic, proteinaceous material. Cholesterol crystals and laminated, partially-mineralized concretions may be present. *Alveolar cysts* are lined by a low cuboidal epithelium with round or oval nuclei which are compressed against the basement membrane. Some of the epithelial cells may contain large vacuoles or exhibit decapitation of the apical cytoplasm. Some alveolar cysts have an irregular contour due to multiple papillary ingrowths of hyperplastic epithelium.

BENIGN NEOPLASMS

Fibroadenoma (Figures 6-10)

Fibroadenomas are the most common benign neoplasms occurring in the rat mammary gland. They are composed of both connective tissue and mammary epithelial cells. The proportion of these two cell types varies considerably, from tumors which are mainly epithelial to those which are composed almost entirely of connective tissue. This variability has led to a number of subclassifications of fibroadenoma. Since many or all of the subclassifications may be encountered in a single tumor, there seems to be little value in subclassifying these lesions.

A common type of fibroadenoma has a lobular pattern and consists of ductules lined by a single layer of epithelium evenly distributed throughout the tumor and separated from one another by numerous concentric layers of mature collagenous connective tissue. The single layer of epithelium has small nuclei and a single nucleolus; lipid vacuoles may be present in the cytoplasm. The alveolar lumina may be dilated and contain secretory material.

At the other end of the spectrum is a fibroadenoma which consists of multiple concentric layers of dense connective tissue with a small number of widely dispersed ductules. The ductular epithelium is often attenuated or atrophic. Numerous mast cells may be present in

the dense connective tissue. Some fibroadenomas may contain large cysts. On rare occasions, mature adipose cells may be a prominent stromal component of the fibroadenoma.

Some fibroadenomas contain focal areas of epithelium exhibiting unusual growth patterns, cellular pleomorphism, and atypia similar to atypical hyperplasia. The epithelial changes may include small papillary projections, bridging septa, papillary infoldings, or solid nests of epithelial cells. The epithelial cells are basophilic and have a high nuclear/cytoplasmic ratio. Increased numbers of mitoses are often present as well.

Fibroma (Figure 11)

Fibromas are tumors composed entirely of collagenous connective tissue without any epithelial component and which arise in the subcutaneous regions normally occupied by the mammary glands. This diagnosis should be made with great care because of the extensive variation of the epithelial component in fibroadenomas as mentioned above. The criteria for diagnosing a fibroma in the mammary gland are the same as those used for diagnoses in other parts of the body.

Adenoma (Figures 12 & 13)

Adenomas are discrete, non-encapsulated, circumscribed masses composed almost entirely of glandular epithelial structures with a scant connective tissue stroma. The alveolar lumina may range from empty to widely distended by proteinaceous secretion. The epithelium is usually uniform, cuboidal to columnar, and is often vacuolated, especially in those tumors with distended alveoli. Nuclei are small and a single nucleolus may be present. The lumenal surface is generally round and may be either smooth or serrated. Focal areas exhibiting an altered growth pattern with pleomorphism and/or cellular atypia may be present, as well as a slight increase in mitotic figures. The more commonly seen forms include tubular, secretory, papillary, and cystic papillary patterns.

Adenomas with a *tubular pattern* consist of small, tightly packed, regularly arranged, round alveoli or ductules. The alveolar lumina are generally empty or contain minimal secretion. The characteristic change of *secretory pattern* adenomas is the wide distention of the ductules or alveoli by proteinaceous secretory material. Adenomas with a *papillary pattern* are characterized by papillary projections of epithelium which protrude into dilated ductal lumina. The papillae consist of a fibrous connective tissue core lined by a single layer of cuboidal or low columnar epithelium which may be vacuolated or appear pseudostratified. The *cystic papillary pattern* consists of numerous simple or multiloculated cystic spaces with many papillary projections protruding into the luminal space which is often filled with abundant secretory material.

MALIGNANT NEOPLASMS

Although distant metastases have long been considered the hallmark of malignancy, mammary tumors seldom metastasize in rats. Similarly, the incidence of local invasion by mammary tumors is quite low. Transplantability also has been suggested as evidence of malignancy; however, mammary fibroadenomas have been shown to be transplantable under appropriate experimental conditions (8).

In the case of mammary tumors of the rat, other histologic and cytologic features may also be used as criteria of malignancy. These include altered and variable growth patterns; cellular atypia characterized by increased nuclear/cytoplasmic ratio, altered chromatin content, and prominent nucleoli; increased numbers of mitoses and the presence of abnormal mitotic figures; and cellular and nuclear pleomorphism. Mammary carcinomas induced by chemical carcinogens often have a prominent stromal mononuclear cell infiltrate.

Adenocarcinoma (Figures 14-19)

Adenocarcinoma may be invasive, may metastasize, and may exhibit a broad range of histologic patterns. The more commonly seen forms include papillary, tubular, cribriform, and comedo patterns. The neoplastic epithelial cells are generally from one to several cell layers thick, are uniform with basophilic to eosinophilic cytoplasm, and contain round to oval nuclei which are located either centrally or near the base of the cell. Chromatin tends to be clumped and a single small nucleolus is often present. Mitotic figures sometimes exceed 10-15/high power field. The areas of atypia vary from focal to extensive.

The *papillary pattern* consists of multiple branching papillae covered by one or more layers of cuboidal to columnar epithelial cells oriented perpendicular to the fibrovascular core. *Tubular patterns* are characterized by a monotonous expanse of closely packed tubular structures which vary from round to elongated. One or more layers of epithelial cells line the tubules. In general, the tubular lumina are small and empty. The *cribriform (or sieve-like) pattern* is due to numerous secondary lumina which are small, round spaces, often filled with proteinaceous secretion, dispersed throughout the solid tumor mass. The *comedo pattern* is characterized by distended ductules filled with sheets of neoplastic cells which have become centrally necrotic. The central cavity contains cellular debris and sometimes calcifying concretions.

Squamous metaplasia is occasionally present in carcinomas with the cribriform or comedo pattern; however, mammary carcinomas with a predominantly squamous pattern are not reported in the rat. Sebaceous metaplasia may also occur in mammary carcinomas of the rat.

Adenocarcinoma Arising in a Fibroadenoma (Figures 20 & 21)

This neoplasm consists of two distinct tumor types: an adenocarcinoma arising in a primary fibroadenoma. In general, this lesion is considered a locally malignant transformation of the epithelial component of a fibroadenoma. The histologic patterns of the two tumor types are variable; see above discussions of adenocarcinoma and fibroadenoma for applicable diagnostic criteria.

DISCUSSION

In addition to the tumor types described above, a number of rare mammary tumors have been described in the rat. Although the rarity of each of these tumors precludes its inclusion as a separate entity, they are described here for the sake of completeness.

The ***adenolipoma (Figure 22)*** is a benign neoplasm which consists of glandular epithelial cells, mature adipose tissue, and fibrous connective tissue. The components are intermingled in most areas, but one or another may predominate in some sections of the neoplasm. In some systems of nomenclature, the adenolipoma is described as a type of benign mixed mammary tumor (1), while others believe these are variants of fibroadenomas. The term mixed mammary tumor to describe a neoplasm with an epithelial component and a mesenchymal, generally chondroid, component is well described in the dog (5), and has been reported in a CD rat (4). Both benign and malignant variants of this tumor type exist. Neoplasms consisting of both malignant epithelium and stroma, which have been previously called sarcocarcinoma (13) or carcinosarcoma (12), could also be described as malignant mixed mammary tumors (1, 3). The epithelial portion of such tumors consists of irregular tubular structures which are surrounded by poorly defined anaplastic stromal cells with numerous mitoses. In some areas there appears to be a transition between the epithelial and stromal cells, leading some authors (2) to suggest that the stromal cells are actually anaplastic epithelial cells rather than stromal cells and that the tumor would be better called an ***anaplastic carcinoma (Figure 23)***.

Occasionally, adenocarcinomas of the mammary gland are observed adjacent to and/or invaded by sarcomas. Although such lesions have been called collision tumors (4), it is appropriate to diagnose the tumors separately. Such lesions must be differentiated from carcinosarcoma or malignant mixed mammary tumors. In addition, fibrosarcomas arising in fibroadenomas may occur.

RECOMMENDED NOMENCLATURE AND DIAGNOSTIC CRITERIA

NON-NEOPLASTIC LESIONS

Lobular Hyperplasia

1. Lobule enlarged by increased number of apparently normal alveoli
2. Alveoli separated by a delicate connective tissue stroma
3. Single layer of well-differentiated alveolar epithelium without atypia
4. Epithelium may contain lipid vacuoles; alveoli may be filled with proteinaceous secretion
5. Alveolar cysts often present within hyperplastic lobule
6. Larger ducts may be surrounded by dense collagen collars

Atypical Hyperplasia

1. Focal irregular proliferation of epithelium within ducts or alveoli
2. Epithelium forms small papillae, arches, nests, or plaques extending into lumen
3. Cellular atypia/pleomorphism present

Cysts

1. Distention of normal lumenal diameter of ducts or alveoli
2. Lumen filled with eosinophilic granular material composed of lipids and proteinaceous material
3. May be lined by flat, cuboidal epithelium with myoepithelial cells compressed against the basement membrane (*ductal cyst*)
4. May be grape-like or become confluent, forming one large cyst, lined by low cuboidal or flattened epithelium (*alveolar cyst*)

BENIGN NEOPLASMS

Fibroadenoma

1. Composed of glandular epithelium and fibrous connective tissue
2. Epithelium is generally single-layered and uniform and may contain lipid vacuoles
3. Connective tissue distributed within and between lobules ranges from well-differentiated to dense hyalinized collagen with few interspersed fibrocytes
4. Epithelium often forms ductules, alveoli, or small cysts
5. Focal areas of atypia and/or cellular pleomorphism may be present
6. Mast cells may be frequent

Fibroma

1. Consists entirely of collagenous connective tissue
2. A subcutaneous tumor in an area normally occupied by mammary tissue

Adenoma

1. Discrete, non-encapsulated mass consists of proliferating alveolar structures in clusters separated by scanty connective tissue septa
2. Alveoli have a single layer of low cuboidal to columnar epithelium with small nuclei and a single nucleolus; epithelium often vacuolated
3. Alveolar lumina may be empty or filled with minimal secretion (*tubular pattern*) or may be widely distended by proteinaceous secretion (*secretory pattern*)
4. Luminal surface of epithelium is generally round, may be smooth or serrated
5. Focal areas of atypia and/or pleomorphism may be present
6. Papillary projections of epithelium may occur, either protruding into dilated ductal lumina (*papillary pattern*) or into cystic spaces which may be simple or multiloculated (*cystic papillary pattern*)

MALIGNANT NEOPLASMS

Adenocarcinoma

1. May be non-invasive or invasive
2. May have distant metastases
3. Neoplastic epithelial cells vary from one to several cell layers thick, are uniform, and have basophilic to eosinophilic cytoplasm
4. Centrally located round to oval nuclei; clumped chromatin; single small nucleolus; and numerous mitotic figures
5. Multiple branching papillae covered by cuboidal to columnar epithelium (*papillary pattern*)
6. Closely packed, generally empty, tubular structures which may be round or elongated (*tubular pattern*)
7. Neoplastic epithelial proliferation in a solid sheet with the formation of secondary lumina (*cribriform pattern*)
8. Distended ductules lined by a multilayered epithelium surrounding a central core of necrotic tumor cells (*comedo pattern*)

Adenocarcinoma Arising in a Fibroadenoma

1. Adenocarcinomatous change within a fibroadenoma
2. Histologic pattern of fibroadenoma component variable
3. Typical histologic appearance of adenocarcinoma present

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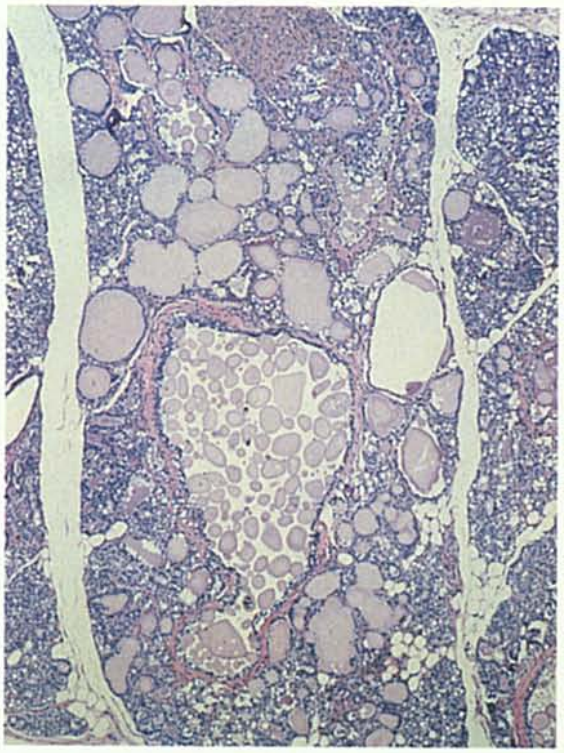

***Fig. 1** – Lobular hyperplasia; note lack of prominent collagenous stroma and alveoli filled with proteinaceous secretion (H&E).*

***Fig. 2** – Lobular hyperplasia; single layer of alveolar epithelial cells (H&E).*

***Fig. 3** – Atypical hyperplasia; note papillary infoldings of epithelium (H&E).*

***Fig. 4** – Multiple ductal cysts (H&E).*

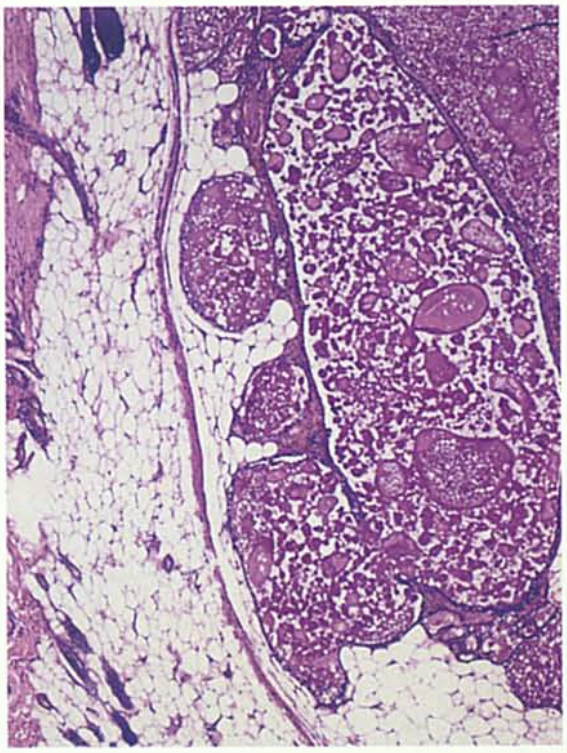

Fig. 5 – Alveolar cyst filled with granular proteinaceous material (H&E).

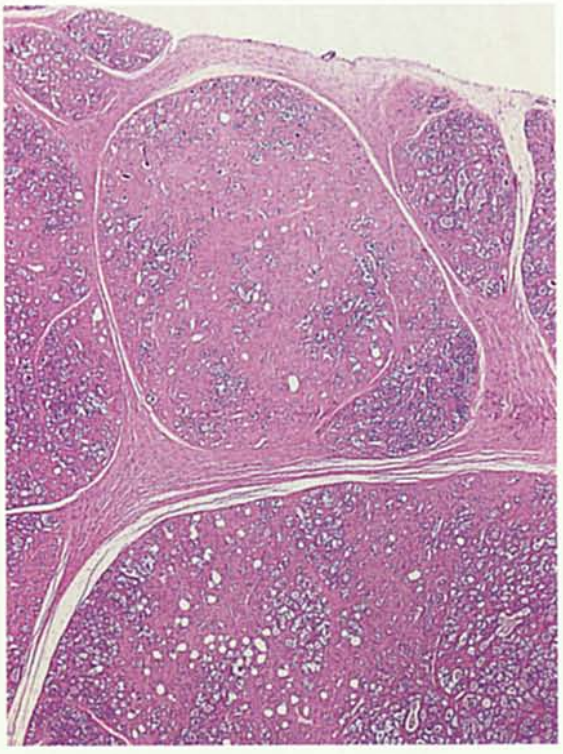

Fig. 6 – Fibroadenoma, lobular pattern (H&E).

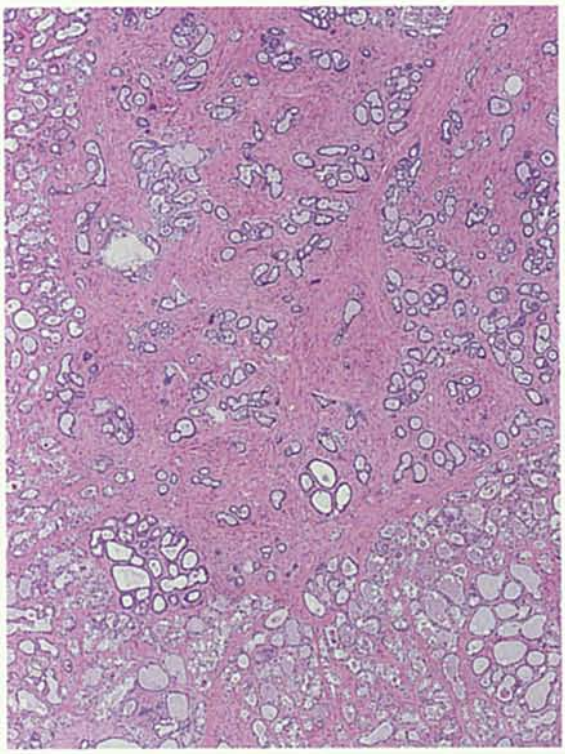

Fig. 7 – Fibroadenoma, mixed stromal and epithelial pattern (H&E).

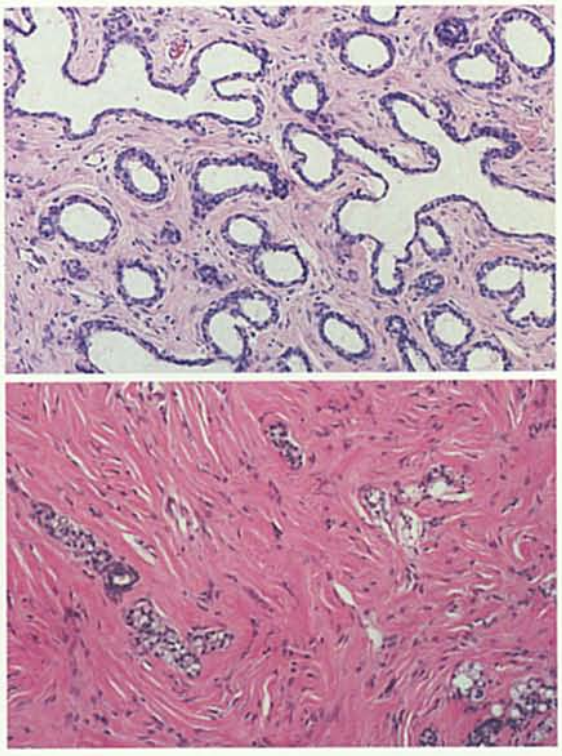

Fig. 8 – Fibroadenoma. Top*: multiple alveolar ductules (H&E);* Bottom*: connective tissue with few ductules (H&E).*

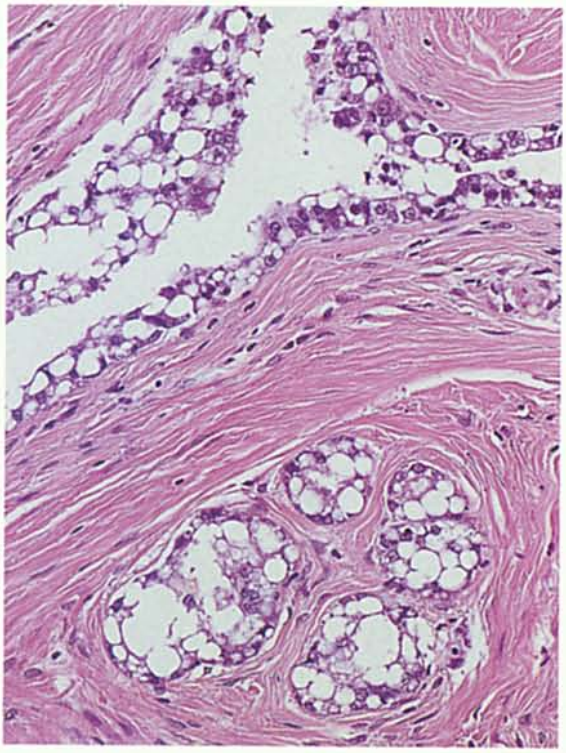

Fig. 9 – *Fibroadenoma; concentric layers of connective tissue (H&E).*

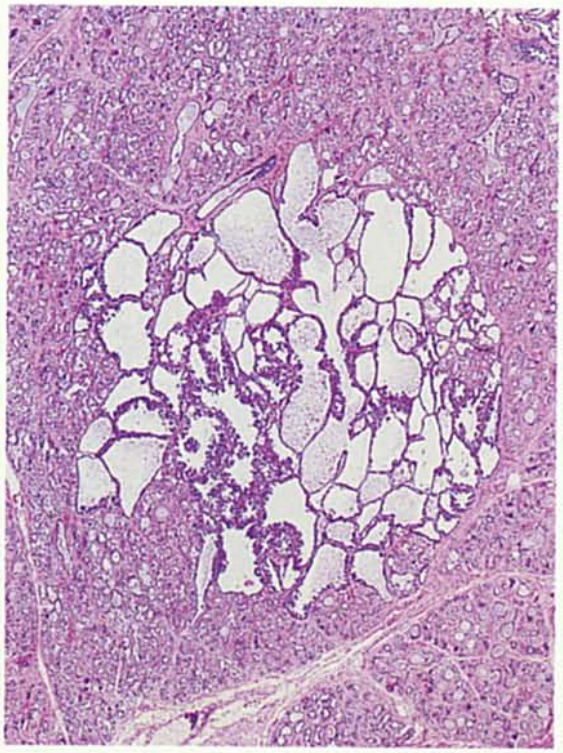

Fig. 10 – *Fibroadenoma; focal atypia (H&E).*

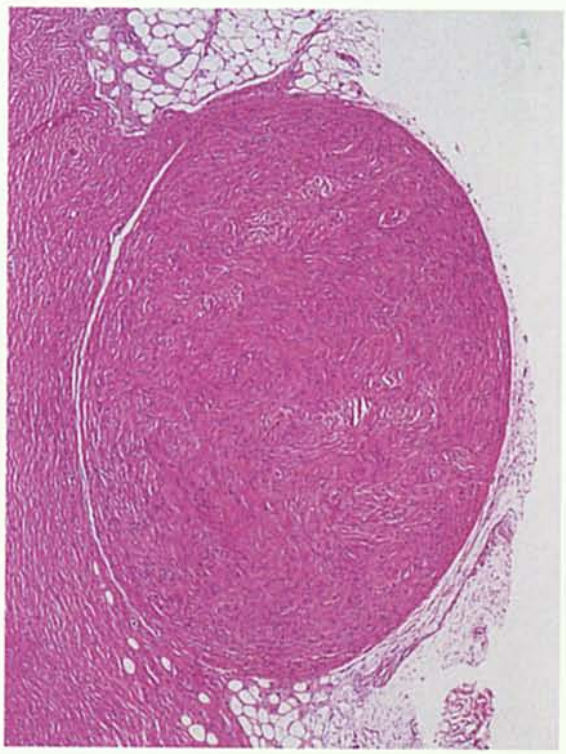

Fig. 11 – *Fibroma; there is no epithelial component (H&E).*

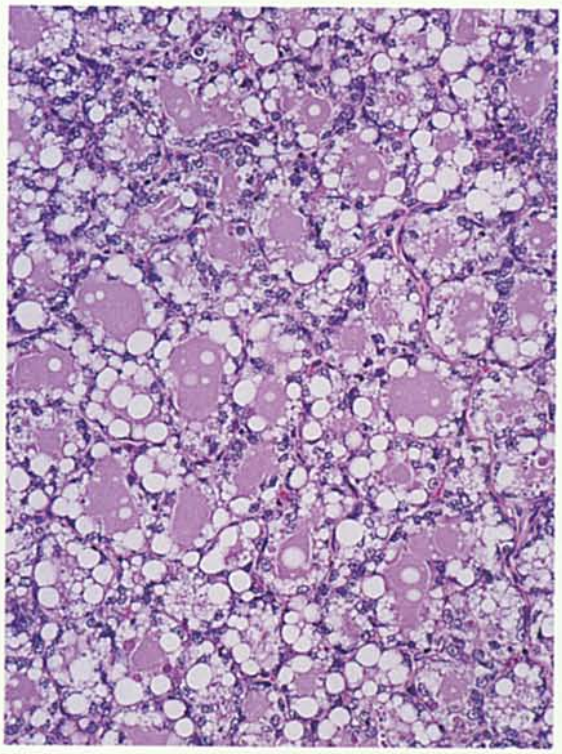

Fig. 12 – *Adenoma, secretory pattern. Ductules filled with secretory material (H&E).*

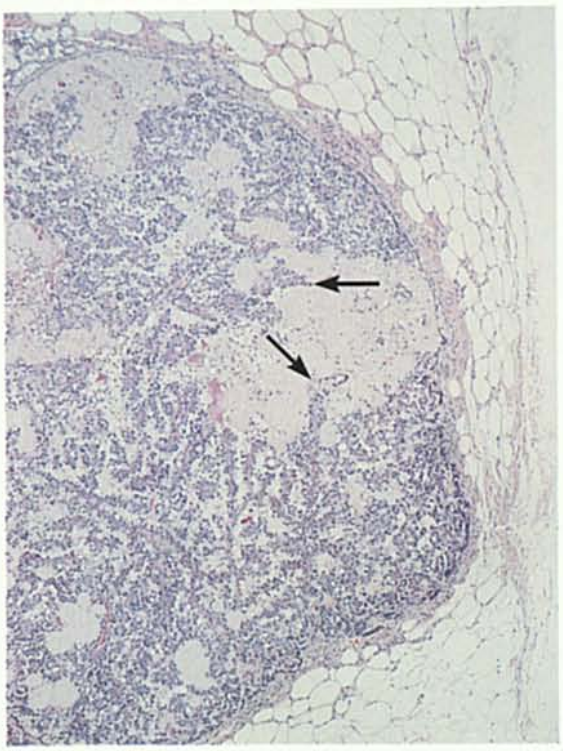

Fig. 13 – *Adenoma, cystic papillary pattern. Epithelial projections protrude into distended lumen (H&E).*

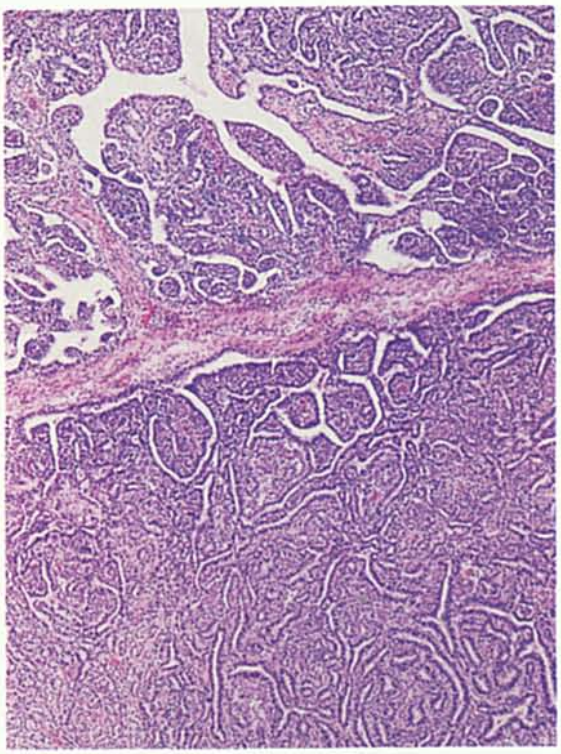

Fig. 14 – *Adenocarcinoma, papillary pattern (H&E).*

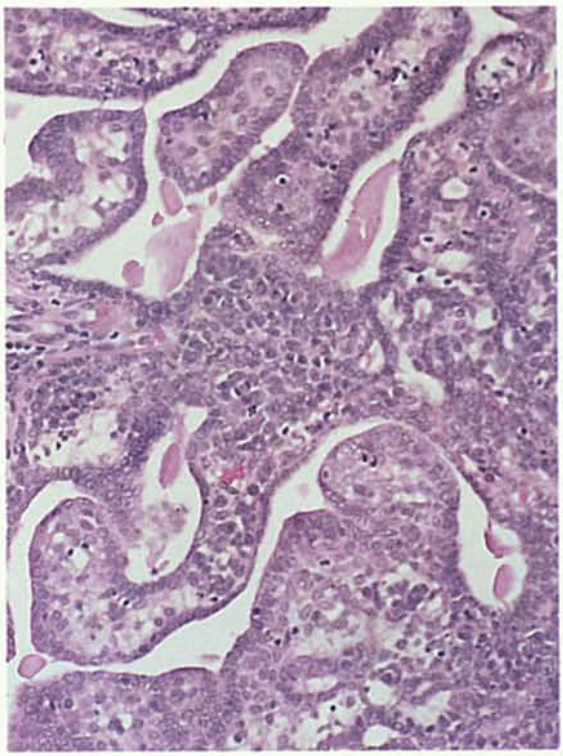

Fig. 15 – *Adenocarcinoma, papillary pattern. Multiple papillae are covered by neoplastic cells (H&E).*

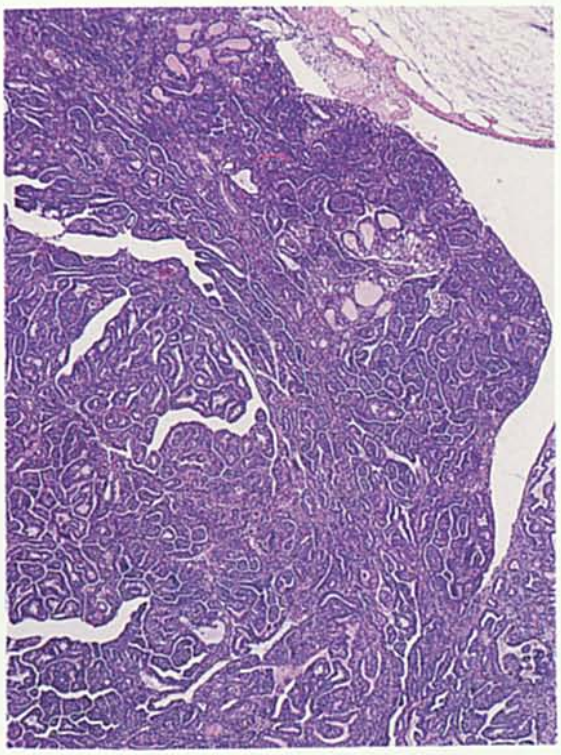

Fig. 16 - *Adenocarcinoma, tubular pattern (H&E).*

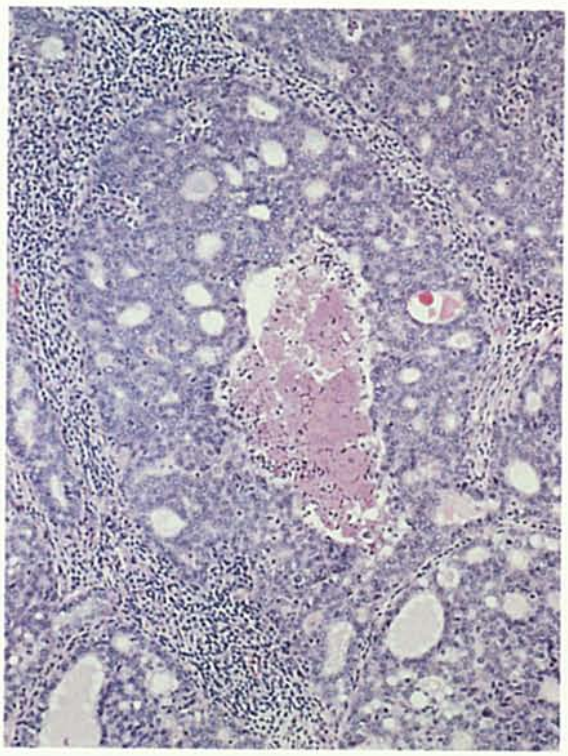

Fig. 17 – Adenocarcinoma, comedo-cribriform pattern (H&E).

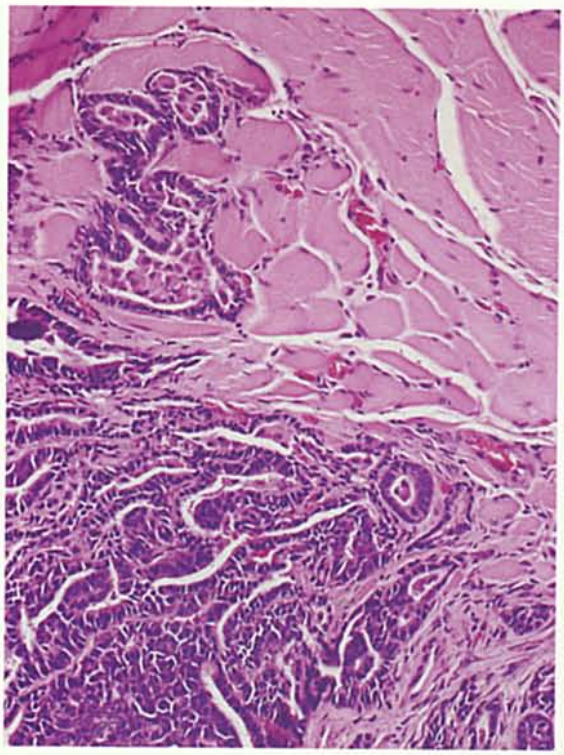

Fig. 18 – Adenocarcinoma; invasion of adjacent muscle (H&E).

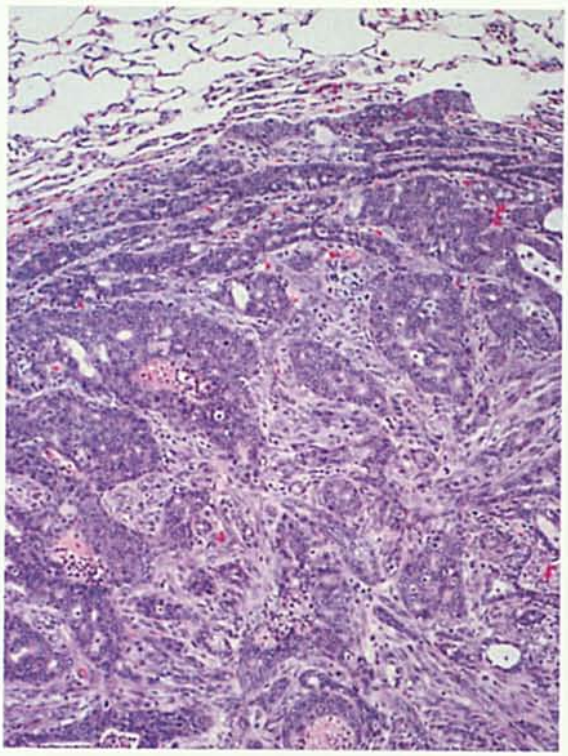

Fig. 19 – Adenocarcinoma; metastasis to the lung (H&E).

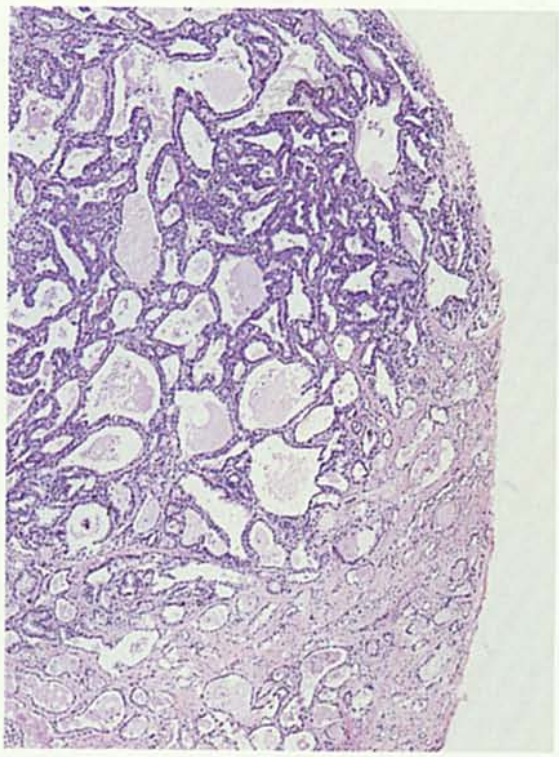

Fig. 20 – Adenocarcinoma arising in a fibroadenoma (H&E).

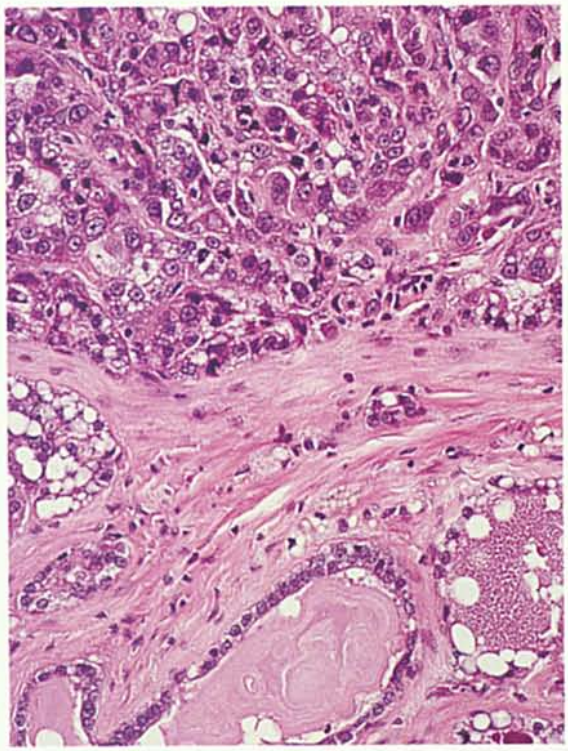

Fig. 21 – *Adenocarcinoma arising in a fibroadenoma, papillary malignant pattern. Cystic ducts in fibroadenoma (H&E).*

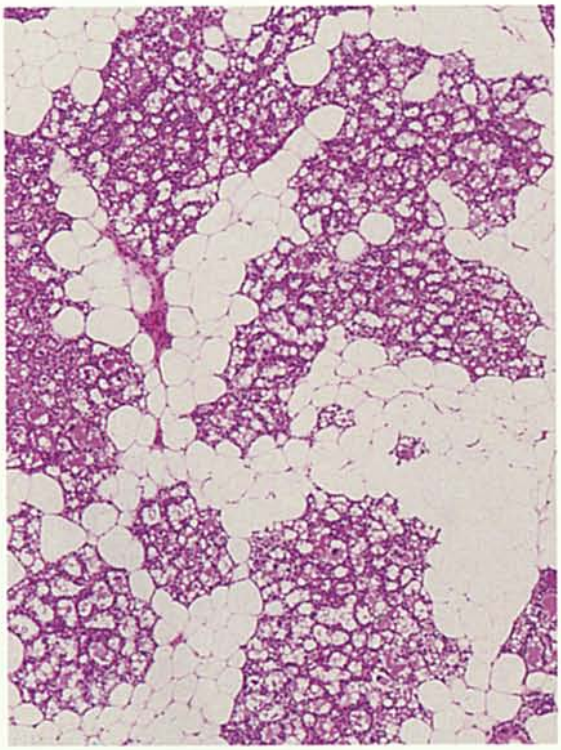

Fig. 22 – *Adenolipoma; note large amount of mature adipose tissue along with glandular epithelium (H&E).*

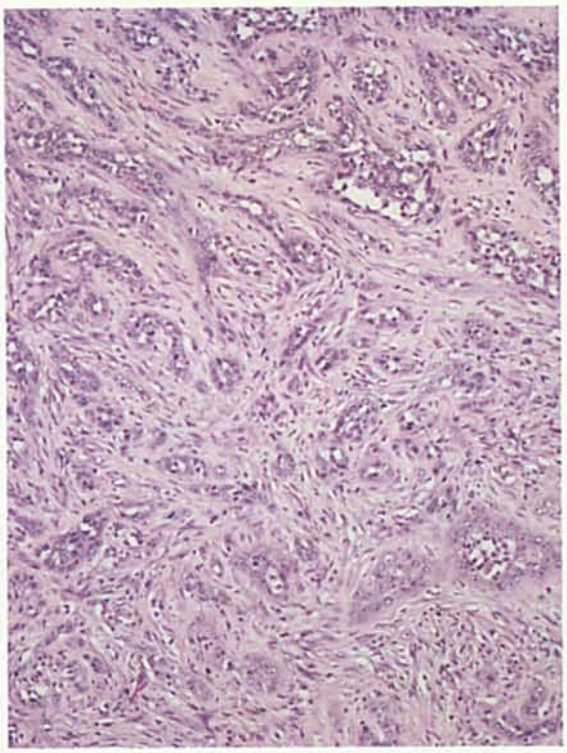

Fig. 23 – *Anaplastic carcinoma; note appearance of both stromal and epithelial malignant cells (H&E).*