

Morphology of Mammary Tumors in Mice

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VALUE OF MAMMARY TUMORS IN CANCER RESEARCH

A most significant event in experimental cancer research, which marked its real beginning, was the publication of Jensen's work in 1903.²⁹ He performed the first successful serial transplantation of a mammary tumor in the mouse, and understood the importance of this accomplishment in relation to the biology of cancer. (Previously a transplantation had been done by Morau,³⁰ but he supposed that he was transplanting an infectious agent.) Since this beginning the mammary tumor of the mouse has probably been the most completely studied of all tumors. Jensen's original (tumor was distributed to other laboratories in Europe and America and this stimulated cancer research at many different centers. These tumors were easily observed, they were frequent, and they were transplantable even in the period before inbred strains were available. These facts made them easily accessible to many scientists. Working with these tumors, they quickly recognized that tumor transplantation depended upon a transfer of living cells and not upon the propagation of some extracellular organism. This separated cancer from the group of bacterial diseases which were so vigorously and profitably studied at that time, and directed research into the complex and baffling problems of cell morphology and physiology.

It would be impossible in a brief survey to recount all the important advances in our knowledge of neoplastic growth which have been aided by the use of the mammary tumor of the mouse.³⁵ This concentration of effort on one small tumor may seem excessive, but like Tennyson's "flower in a crannied wall," it holds great secrets in a small space. If we could understand it, "root and all," we might understand the whole process of neoplasia. The study of its morphology has disclosed many general principles concerned with the histogenesis of cancer and the correlation of histologic structure with biologic behavior.

A special advantage of the mammary tumor in experimental work is its accessibility to palpation, so that size and growth rate can be estimated with reasonable accuracy. When tumors develop internally, or become generalized, as in leukemia, the scientist is usually unable to determine the time of the initiation or the extent of

the process before the death of the host. The mammary tumor has proved an invaluable tool in investigations in genetics, nutrition, endocrinology, chemotherapy, and virology.

Investigation of the genetics of cancer began when it was observed that many females in certain colonies of mice had mammary cancer. This clue was followed, and patient endeavor finally led to the development of inbred strains of mice having a high and fairly predictable incidence of mammary tumors. As a further development, strains were established that had a high or extremely low incidence of different forms of tumors. By use of these diverse strains many variations in environment and extrinsic stimuli have been tested, and reductions or increases in the expected incidence of tumors have been produced. The cancer biologist, therefore, has a quantitative measure of the effect of many procedures.

The nutritionist has found that the incidence and growth rate of spontaneous mammary tumors have responded sensitively to variations in diet, and the endocrinologist has noted a similar delicate response to the hormonal status, so that the effects of caloric and other dietary restriction or of hormonal variation have been accurately reflected by the number and size of developing tumors.

In attempts to find a chemotherapeutic substance the mammary tumor of the mouse has been a favorite test object because of its availability and high degree of standardization. Natural regression in spontaneous or induced mammary tumors has seldom been observed,⁴⁷ and for this reason experiments with these tumors have disclosed the ineffectiveness of most of the chemotherapeutic agents tested in the past. When transplanted tumors have been used, however, unless highly inbred strains of animals were employed and the experimental animals and procedures rigidly controlled, some misleading data have been reported.

Finally, the recognition of the milk agent has greatly stimulated research on mammary tumors in mice and the virologists have taken part in many recent investigations. This extrachromosomal factor is transmitted by the milk of high mammary strain females to the young and in some way so modifies the responsiveness of the mammary gland tissue, or the stimuli acting upon it, that with a favorable genetic constitution and the proper hormonal stimulation, mammary tumors develop at a comparatively early age and in high incidence.⁶² The interrelationship of these three factors, genetic constitution, hormonal stimulation, and the milk agent has been discussed by Heston.⁸⁰ The number of tumors and the age at which they appear shows great variation in different strains. When studying morphology it is important to keep these conditions in mind. For this reason, it is desirable to consider separately descriptions of morphology during an early period, before inbred strains were generally used and the milk agent recognized, and at a later period when inbred strains with or without the agent have usually been employed.

EARLY WORK

The early work on the morphology of mammary tumors was practically complete by 1913, at which time Woglom⁴⁸ published a review on experimental cancer research and summarized what had been done previously. Apolant³ in 1906 made the first detailed histologic study of mammary tumors. He had 76 tumors

available and from these he devised a classification which was standard for many years. Apolant emphasized the same features which we recognize as characteristic today: a basic acinar structure, or "mother terrain," on which were developed innumerable variations resulting from such secondary modifications as cyst formation, papillary ingrowth into the cysts, solid cellular growths without lumen formation, cordlike structures of one to several layers of cells, and various other architectural arrangements of a glandular tumor. He tried to separate these varying forms into strict categories, but at the conclusion of the work he reported that it might be of no practical value since it appeared, first, that mammary tumors in the mouse formed a structural entity, and second, that different types were not sharply defined, but blended gradually into each other so that systematic divisions were more or less illusory. Apolant attempted to distinguish between benign and malignant forms, and remarked that while a malignant change in mammary gland adenomas was rare in human pathology, it was an everyday occurrence in the mouse: when a tumor in the mouse had grown to the size of a cherry, a malignant change could nearly always be found. He attempted to identify the histologic features of this malignant change, using the criteria relied upon in human pathology. Later experience has proved that such criteria are unreliable, and that, in mice, a high degree of histologic differentiation may accompany a rapid growth rate and early metastasis. The proposal of Nicod²⁴ that no designation be used to indicate the benign or malignant nature of mammary tumors in mice should be adopted. Most of the so-called adenomas show continuous growth and there is no characteristic structure in those that metastasize. To decide by morphology alone that one lesion is benign and another malignant has led to many errors in both animal and human pathology. In tumors in experimental animals, where neither a prognosis nor a form of therapy is required, allocation to a benign or malignant category may dull the perception of the innumerable morphologic features of tumors and their biologic modifications such as progression and responsiveness.¹⁷

Following the report of Apolant, the most important papers on the morphology of mammary tumors came from pathologists working at the laboratories of the Imperial Cancer Research Fund in England. Murray³⁷ agreed with Apolant on one essential point, which is that in all these tumors the basic type was more or less the same and reproduced acini, although all gradations of structure were to be found, from this "adenomatous" form to a solid carcinoma. A suggestion was made by Haaland²⁹ that while in most tumors a relation to the mammary acini was evident, in others where cordlike and tubular formations were frequent, an origin from ducts had to be considered. He did not insist, however, that any sharp distinction could be drawn, since the two forms blended. Like many other pathologists who have attempted to classify mammary tumors, he provided categories for each tumor and then admitted that the tumors did not always fit them snugly.

A great service of the English group was a study made of mammary tumors during serial transplantations and a description of concomitant morphologic changes.

Bashford⁴ described variations in the degree of keratinization and noted that extreme keratinization was correlated with a slow growth rate. Haaland¹⁹ reported

the transformation of pure epithelial tumors to morphologic types indistinguishable from fibrosarcoma during transplantation, and observed that an increased growth energy accompanied this change. Two of our most frequently employed transplantable tumors, sarcoma 37 and sarcoma 180,¹⁰⁸ which grow in many different strains of mice, were derived from mammary tumors by this process. A number of other epithelial tumors, among them pulmonary tumors¹¹ and hepatomas⁷¹ have later been reported to undergo such a change, usually in early transplant generations. On the other hand, apparently identical epithelial tumors have been carried in transplants for many years without any change in morphology. A satisfactory explanation for this behavior has been sought by many pathologists. If cancer tissue can infect or by some means alter other tissue contiguous to it, new concepts as to its nature must be entertained. An interesting experiment pertinent to this problem was the growing of mammary tumor cells and normal fibroblasts together in tissue culture by Ludford and Barlow.:⁷ A change developed in the fibroblasts. Some objections to this study have been raised, for the identification of the cells in tissue culture was questioned and the technic may have allowed for mingling of the cells. Further efforts are required to solve this puzzle.

The early workers also described several special forms of mammary tumor which are usually found in any large collection. These will be discussed in a later section of this chapter. The early investigators also noted that mammary tumors were usually multicentric in origin, and this stimulated histologic investigations of the mammary glands of old mice, in an attempt to identify precancerous lesions. Studies of this same type have been repeated in recent years on inbred strains of mice with a predictable tumor incidence, and known presence or absence of the milk agent. Discussion of the early work on precancerous changes is, therefore, postponed for a later section in which the morphology of the precancerous lesion is considered.

When inbred mice became available, it was noted that the mammary tumors still followed the same well-recognized pattern, and no strain differences were detected.¹¹ When estrogen-induced tumors from male mice were examined, they also were histologically similar in all aspects to those arising spontaneously in the female of the same strain.²⁹

In a later report, mammary tumors were reported in untreated male mice of an exceptional strain. The tumors are probably attributable to hormonal abnormality, consequent on testicular lesions also present in these mice. The tumors were described as adenocarcinomas similar to those arising in females.⁷¹

Mammary tumors were grown in tissue culture by Santesson.¹⁰⁴ He agreed with others that the extremely variable histological appearance made it impossible to establish sharply defined classification groups. He divided the tumors into adenomas, adenocarcinomas, and carcinomas. An obvious similarity existed between the morphology of the epithelial formations in vitro and the structure of the tumor in vivo. The more differentiated tumors produced more liquefaction of the medium, and they did not infiltrate connective tissue, but always preserved a distinct border against such tissue. The cells were of uniform appearance, and mitoses were few. In comparison the less differentiated formed no border against connective tissue, but actively infiltrated it.

Metastasis of mammary tumors was of interest from the first days of experimental cancer research, for this was important in establishing the truly malignant nature of the tumors and their reliability as a tool for investigations of neoplasia. Jensen failed to observe metastasis of his tumors, but **Borrel**¹⁴ described metastases in the lungs which were of two general types: (1) an embolus within the larger blood vessels, sometimes covered by proliferating endothelium; and (2) a type in which the cancer cells reached the smaller pulmonary vessels and infiltrated the tissue. We see the same forms of pulmonary metastasis today. Metastases to lymph nodes are always extremely rare. **Ashburn** in 1937 investigated metastasis of mammary tumors. He found no correlation between morphology and metastatic growth, with the possible exception of an almost solidly cellular histologic form in which the number of metastases was rather high. On the other hand, such factors as the multiplicity, the duration, the size, and the rapidity of growth of the tumors were correlated with metastases in the lungs.

LATER WORK

With the development of inbred strains of mice, and our present ability to add or subtract the milk agent in many of these strains, there has been a renewed interest in the morphology of mammary tumors, and the changes in the mammary gland which may lead to the development of neoplasms. Close comparison of the morphology of tumors from different sources is now warranted, since such other factors as genetic constitution, hormonal effects, and the **presence** of the milk agent can be controlled. Knowledge from these new investigations is still accumulating, and it will be some time before all the reports can be properly evaluated. **Some** of them now appear to be contradictory, but this may be due to differences in the {trains of mice.

PRENEOPLASTIC AND EARLY NEOPLASTIC CHANGES

Many efforts have been **concentrated** on the recognition and identification of the earliest neoplastic change to be found in a tissue or cell. Animal experiments are indispensable for this, since tumors in man are usually of considerable **bulk** before they are removed and examined histologically. The mammary glands from strains of mice with a high incidence of tumors have been much utilized for these **studies**.

The **most important** precancerous lesion in many high tumor strains with the **agent** appears to be the "hyperplastic nodule" consisting of a localized area of **acinar** or alveolar proliferation. The total number of these lesions is significantly increased in mice that develop tumors, although only a small percentage of the individual lesions progress to true neoplasms.

Haaland²⁰ described "chronic inflammation" of the interstitial tissue, and a dilatation of the ducts with consequent cyst formation in the mammary glands of old female mice bearing tumors. Occasionally these cysts were lined by squamous **epithelial** cells, but Haaland considered this epidermoid lesion of less consequence than another which he called "nodular hypertrophy," that resulted from an initial increase in the number of apparently normal **acini** which proceeded to the

formation of large accumulations of acinar-like structures no longer connected with the mammary ducts. These nodules were regarded as probable precancerous foci which passed imperceptibly to cancer. Our present knowledge supports this suggestion, and recent investigations have the advantage of employing inbred high-mammary-tumor strains of mice with the agent, which provide a gland in which many precancerous foci are expected. Morphologists also employ a technic for making whole mounts of the mammary glands, which affords a means for an over-all survey of the mammary tissue. Suspicious areas can then be selected from the whole mounts and prepared for histologic study.

Fekete⁶⁸ compared the mammary tissue of a high cancer strain with a low cancer strain through successive pregnancies. She noted the development of "abnormal areas" in the high cancer strain, which corresponded to the areas described by Haaland. Gardner and co-workers⁷² studied the glands of multiparous females from five strains of mice varying in susceptibility to spontaneous tumors. They found that localized areas of alveolar development were most frequent in the strains having a high incidence of spontaneous tumors. Huseby and Bittner,²⁵ using the whole mount method, investigated the mammary glands of high and low tumor strain female mice in which the three "primary" factors--milk influence, proper hormonal stimulation, and genetic influence-varied. They described a hyperplastic lesion composed of budding alveolar tissue; this was generally considered to be the most significant precancerous lesion. This formation was found in highest number only in mice of the proper genetic background with adequate hormonal stimulation and with the milk agent. Areas composed of an overgrowth of fine ducts were also reported as frequent in the breast tissue of mice of a high cancer strain but such lesions were less common than the acinar proliferations. On the other hand so-called inflammatory foci were not shown to be associated with an increased incidence of mammary tumors. This paper reviews preceding work related to mammary tumor development in high-cancer-strain mice.

Mühlbock and co-workers⁸⁹ observed hyperplastic nodules in strain DBA mice with and without the agent, and concluded that the mammary tumor agent and pregnancy exerted considerable influence on their development. In mice with the agent the significant influence of pregnancy was obscured, since so many nodules developed in the virgins that the number was not much increased by pregnancy. The frequency of nodules was completely parallel to the later development of carcinomas. Groups of mice in which nodules were numerous likewise had a high incidence of tumors, although only a fraction of the nodules became manifest as true neoplasms. All this suggested that the agent should be regarded as an accelerator and intensifier of developments in the mammary gland that would take place at a later period in the absence of the agent. It was suggested that the effect of the mammary tumor agent consisted exclusively in an increase of hyperplastic nodules and their earlier occurrence.

It is now recognized that the incidence of nodules increases with age, irrespective of the agent. Jones⁷⁷ has shown that hyperplastic nodules may be numerous in very old female mice from the inbred strain C3H freed from the agent. In a study of RIIL mice, Pullinger^{93, 94} reported that adenomatous nodules provided valid

evidence of the action of the milk agent only when the females, whether virgin or parous, were less than 12 months old. In old breeders the nodules might be due to the agent or to other "cause or causes unknown."

Yet another mode of origin in untreated mice has been observed by Foulds.⁷⁴ The material studied came from hybrids of strains RIII and C57BL and their inbred offspring, some with and some without the milk agent. Early neoplasia was often represented by a "plaque" or disc, which grew only during pregnancy and regressed after parturition. The plaques were made up characteristically of radially disposed branching tubules in symmetrical arrangement. These plaques were "responsive" to pregnancy; they were conditional growths dependent on hormonal stimulation. Rarely the epithelium of the plaque was also responsive to lactogenic hormones, a milky fluid was secreted, and the plaque became a cystadenoma. Hyperplastic nodules and plaques were considered as alternative, not consecutive, manifestations of early mammary neoplasia. Progression in the plaques might be of a focal type, or of a **lobular** or **diffuse** type. In addition to the origin from plaques, some tumors in this material probably began in hyperplastic nodules, some within ducts, and some directly from apparently normal mammary gland.

Browning⁷⁵ by means of heterologous and homologous growth of transplants during the course of development of spontaneous mammary tumors in C3H mice tried to determine when the tumors became autonomous according to Greene's criterion. No correlation was found with histologic structure or growth rate in homologous transfers. Squamous cell metaplasia in the mammary tissue did not show autonomy or homologous growth.

In addition to the tumors developing in untreated mice, others have been induced and the process of neoplasia under artificial conditions observed. One method has been to treat male mice with estrogenic hormones, and observe the formation of tumors in the mammary glands. **Chemical** carcinogens have also been employed, and irradiation.

In a study by Bonser⁷⁶ castrated male mice from a cancer-susceptible and a cancer-resistant strain were treated with estrone. In the cancer-susceptible mice, active, localized, acinar proliferation commenced after about 40 weeks, and in 3 of 4 animals this was associated with the development of carcinoma. In the **cancer-resistant** mice, a generalized growth and proliferation of acini was apparent, but it was never associated with the development of carcinoma. It was, however, associated with a gradual cystic distension and accumulation of secretion within the ducts. In a later **paper**⁷⁷ when the results of this and other experiments were discussed it was concluded that the effect of the milk agent was mainly upon the **acini**, and the resulting tumor reproduced acini. The author pointed out that in human beings, on the contrary, cancer usually begins in the ducts, and the question was raised as to whether a milk factor exists in the human subject, and if so whether it must not be of a different kind acting primarily upon the ducts.

Carcinogenic hydrocarbons were employed by Bonser and Orr⁷⁸ in an attempt to induce mammary tumors. The substances were injected into the subcutaneous tissues of high and low cancer strain mice. The majority of the resulting tumors

were sarcomas. Adenocarcinomas appeared only in females in both the low and high tumor strains. Kirschbaum and co-workers²⁸ reported a high percentage of adenoacanthomas after methylcholanthrene injection in certain strains of mice in which the presence or absence of the agent was known. DBA mice with and without the agent were given percutaneous applications of methylcholanthrene by Andervont and Dunn' and a number of adenoacanthomas and carcinosarcomas were induced. In a later paper Bonser⁵⁴ described a method for the local application of minimal doses of methylcholanthrene to the breast tissue of IF mice, a strain highly susceptible to the induction of mammary carcinoma by this chemical. The hormonal state was diversified by spaying, administration of hormones, or lactation. Carcinoma arose following a graded series of epithelial changes starting with intra-acinous and intraduct hyperplasia, which proceeded to papilloma and lumenated carcinoma, and finally infiltration of the surrounding tissue. Squamous metaplasia was a rare occurrence, and it was concluded that squamous metaplasia was not an essential feature of the chemical induction of mammary carcinoma. This paper includes a valuable discussion of different etiologic factors in the development of mammary tumors.

Irradiation also appears to act as a carcinogenic agent to mammary tissue for it increased the numbers of both adenocarcinomas and sarcomas in Strain C3H mice without the milk agent.⁵⁵ These same mice, however, had a high incidence of granulosa cell tumors of the ovary or mixed tumors of the ovary containing granulosa cell elements and the mammary tumors were always associated with the ovarian tumors. Tests on the mammary tumors that developed following irradiation failed to show the presence of the milk agent.

The study of these precancerous lesions in the mouse has been of great importance in analyzing the histogenesis of the fully developed tumor. Despite the confusion on certain points, the following facts seem to be established: (1) The "hyperplastic nodule" consisting of a large cluster of budding acini is probably the most frequent precancerous lesion in mice of a susceptible strain that have an adequate hormonal stimulation and the milk agent: (2) Squamous-cell hyperplasia or metaplasia has been reported as a significant precancerous lesion in mice without the agent treated with methylcholanthrene. However, results of this treatment have varied depending upon the strain used and the dose and method of applying the carcinogen. (3) A subcutaneous epithelial proliferation arising in pregnancy, and receding after delivery, has proved to be significant in one group of hybrid mice. (4) Studies on the precancerous lesion are difficult to standardize and interpret and mice of known ancestry in which the presence or absence of the milk agent is known should be selected for such studies.

THE FULLY DEVELOPED MAMMARY TUMOR

Macroscopic. Mammary gland tissue in the female mouse extends from the cervical region, where it is in close contact with the parotid salivary gland, to the vulva on the ventral surface and almost to the mid-line in the back. There are five pairs of glands, arranged symmetrically along the ventral and lateral surfaces of the body.⁶⁹ Because of the extent of the mammary tissue, tumors may be found

at almost any subcutaneous site on the body. The development of tumors, however, is not strictly correlated with the amount and distribution of mammary tissue; In old breeders of the substrain **RIIIB** (deprived of the milk agent) the second pair of nipple regions was almost twice as often the site of hyperplastic nodule formation as the 4th pair and four times as often as any other pair.^{97, 98} A survey from the National Cancer Institute on the location of 3,612 tumors disclosed that the relative number of anterior tumors (those in the pectoral area) was increased when the tumor agent was absent, whereas a comparatively even distribution occurred when the agent was present. It was not possible to dissociate the effect of the agent itself and the effect that the earlier age of the mice might have in determining the **morphology** of the tumors. A close correlation was found between age and tumor location, for in older mice there were more anteriorly located tumors. It was suggested that age might be the determining factor. "Atypical" tumor types tended to be evenly distributed at all ages. Differences in genotype presumably accounted for slight differences in **distribution**.⁹⁹

Olivi and co-workers⁹⁴ found that the greatest number of tumors occurred in the right thoracic mammary gland in three high mammary tumor strains.

On gross inspection mammary tumors often show the same variability in different areas that is observed on histologic examination. The following features are sufficiently characteristic to suggest the diagnosis: round, oval, or coarsely nodular in shape; well circumscribed, and easily separated from surrounding structures; greatly dilated vessels around the tumor; on cut surface, tumor tissue usually grayish white and soft, often with many blood-filled cysts and central necrosis. Acanthomatous areas may contain flaky white material, and some tumors exude a milky fluid when sectioned. Gross examination should always be confirmed by a microscopic section.

All the early **work** on mammary tumors in the mouse indicated that morphologically they showed a limited range of modification from an easily recognized basic structure. Variation from one area of a tumor to another was usual and different tumors from the same **mouse** showed different histologic structures. The gradual merging of one form into another, depending upon the degree of cyst formation, keratinization, and the proportion of areas reproducing **acini** or tubules, was recognized by all who examined large tumor collections. No correlation between biologic behavior and tumor morphology had been discovered. For these reasons most **pathologists** were agreed that no rigid scheme of classification could be devised for the mammary tumor, and that there would be no practical value in such an attempt. However, with the present use of inbred strains, the control of the milk agent, and the ability of such external agents as carcinogens and irradiation to elicit breast tumors, we have found it desirable to devise a "sorting scheme" by which these tumors can be grouped. The one in current use at the National Cancer Institute will be presented,^{21 23} because it is the one that we have found most convenient and most readily understood by those who have used it here. It is not urged that it be rigidly adhered to, for when a number of tumors are to be reviewed, it should always be the practice first to sort out the tumors, placing all similar ones together without any reference to predetermined categories. Any biological form such as a tumor has many possibilities of variation, and each

tumor has individual characteristics. Any of the classifications so far proposed may need to be altered to accommodate new types that may be developed as a result of the many different conditions under which mammary tumors are now arising spontaneously or being induced. If the ancestry, presence or absence of the agent, exposure to carcinogens, breeding experience and hormonal state, are known for any large group of mice, then it may be worthwhile to analyze the morphology of the mammary tumors that arise. Significant variations have been found in the proportionate number of the different types of mammary tumors. If single tumors only are considered, no histologic features can be selected that reveal the strain of the mouse, the presence or absence of the milk agent, or the effect of carcinogenic agents such as methylcholanthrene or x-ray. Tumors of each of the types described below have been seen in nearly every large group from whatever source, but the proportion of certain types in special groups of mice has been found to vary greatly.

Other laboratories have adopted other schemes of classification that they have considered more satisfactory for their material. These schemes will be discussed later. It is urged that photographs and descriptions always be adequate for understanding the different categories used. If this is done there should be no difficulty in translating from one scheme to another or in recognizing that some tumors which may be reported are different from any commonly seen or previously described.

MAMMARY TUMORS CLASSIFIED BY HISTOLOGIC CHARACTERISTICS

ADENOCARCINOMA, TYPE A

Other terms used: Typical mammary tumor, mammary adenoma, mammary tumor of basic acinar structure, alveolar carcinoma, small tubular carcinoma, microtubular carcinoma.

A fine uniform acinar structure occupies over one half of the section (Fig. 8). All other tumor tissue in the section is of glandular epithelial origin, except for insignificant areas of squamous epithelium that may appear in many of these tumors. The tumor is composed predominantly of rather small cuboidal epithelial cells arranged in single rows surrounding small to medium-sized cavities that are generally round but may be elongated and tubular. A difference of opinion exists as to whether these cavities are circular like acini^{65, 92} or whether they are small, tightly coiled tubules^{10, 14, 20, 70}. This divergence of interpretation is of little consequence, so long as the identity of this tumor type is clear.

Histologically the tissue appears well differentiated and the orderly arrangement of cells, their small and uniform size, and the paucity of mitotic figures in some tumors led some of the early pathologists to the conclusion that this represented a benign adenoma. Tumors of this type, however, metastasize readily, often reproducing the primary structure, are easily transplanted, and may continue the adenomatous pattern indefinitely. Vacuoles may appear in the cell cytoplasm, and the luminal spaces may be distended with fluid (Fig. 9). This vacuolation, which is probably a manifestation of a secretory function, was first clearly described by Bonser¹⁰ in tumors from lactating females and estrogenized males. Foulds (IV)⁷⁰ studied secretion in certain mammary tumors and concluded that it was usually "the

manifestation of a particular type of responsiveness to the hormonal stimulus, provided by pregnancy, lactation, or artificially administered estrogens." Vacuolation has been seen in both our Type A and Type B tumors. This modification is usually restricted to small areas in a section, and does not involve the whole tumor.

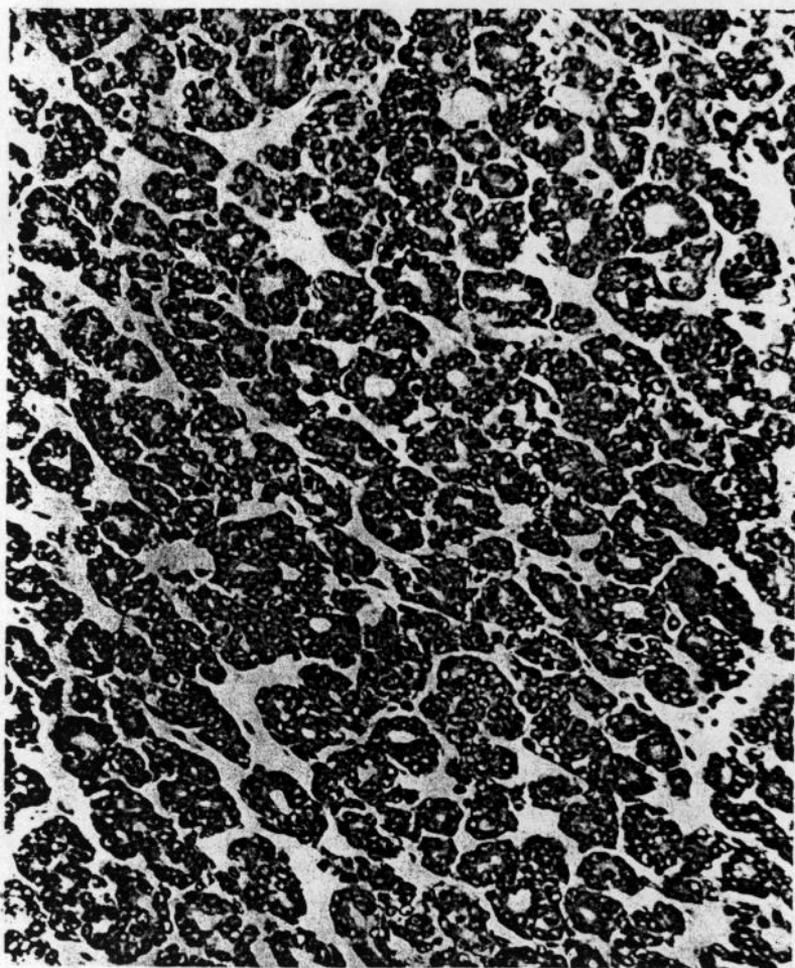


FIG. 8. Adenocarcinoma, Type A, with characteristic acinar structure. Note frequent mitotic figures. Hematoxylin and eosin. $\times 265$.

In our experience at the National Cancer Institute, Type A is remarkably frequent in strain C3H with the agent. Much of our experimental work has employed this strain, and the types of tumors that it develops may be receiving undue emphasis when compared with reports from other laboratories. Type A and Type B tumors represent the "typical" adenocarcinomas in mice with the agent, and the two types have not been separated in some reports.

ADENOCARCINOMA, TYPE B

Other terms used: Variable tumor, papillary cystadenocarcinoma, carcinoma simplex, intratubular carcinoma.

This represents a diversified, multiform group in which the tumors are clearly of



FIG. 9. Adenocarcinoma, Type A, with secretory activity, or lactation effect, in cells of one area. Hematoxylin and eosin. $\times 265$.

glandular epithelial origin, but in which there are no predominant features such as acinar formation that would warrant allocation to Type A, or cystic structure such as is found in Type C, or the squamous cell component of the adenoacanthomas. The value of segregating these variable tumors is debatable, but it has proved a convenient category for a large number of tumors not easily separated into smaller

groups, and for which individual descriptions would be tedious. Even in a single section, Type B will rarely show the same pattern throughout (Fig. 10). Acinar areas are frequently found but they do not comprise most or all of the section as in Type A. However, transitions from predominantly acinar tumors to variable Type

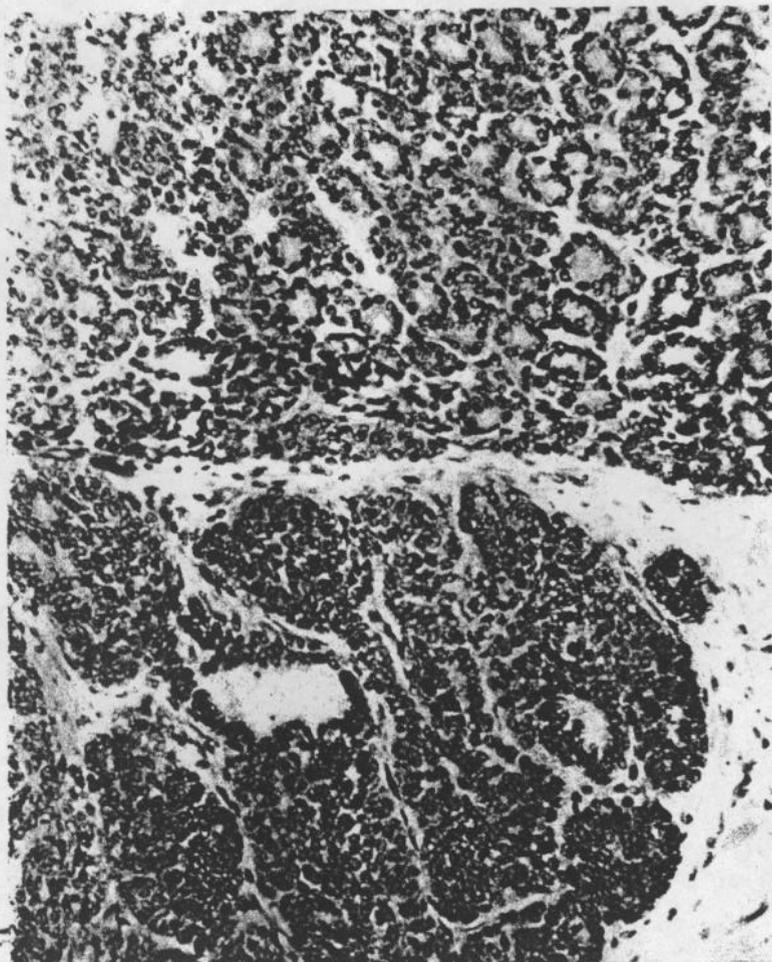


FIG. 10. Adenocarcinoma, Type B, showing the diversified, multi-form character of this type. Area above reproduces acinar structure of Type A, present in only a small portion of this tumor, while area below shows an irregular epithelial structure. Hematoxylin and eosin. $\times 265$.

B tumors may be gradual, and distinctions between Types A and B is often arbitrary. Cysts filled with blood or clear fluid, intracystic papillary projections (Fig. 11), cords, and tubes of cells (Fig. 12), such as were described by Apolant³ and separated by him into different categories have been included under Type B. The amount of stroma may vary (Fig. 12), and occasionally distinctive patterns may occupy a

part of the section (Fig. 13). Size of cells and intensity of staining show great variation. Occasionally the cells may be arranged in solid sheets or nests, with no appearance of gland formation, and this solid form has been considered especially malignant (Fig. 14). When tumors of this structure were fixed and stained to dem-



FIG. 11. Another pattern frequent in adenocarcinoma, Type B, showing cyst formation and papillary ingrowth. Small glandular structures are present in the more cellular areas. Hematoxylin and eosin. $\times 265$.

onstrate Golgi material, the arrangement of the Golgi apparatus revealed a polarization characteristic of the acinar structure, supporting the idea that there was a close relation to Type A tumors.¹⁵

Foulds (I)⁷⁰ has pointed out that tubular formations in mammary tumors in mice may be double layered, consisting of an outer layer composed of paler cells, and an inner layer of darker cells. This double-layered formation has been seen

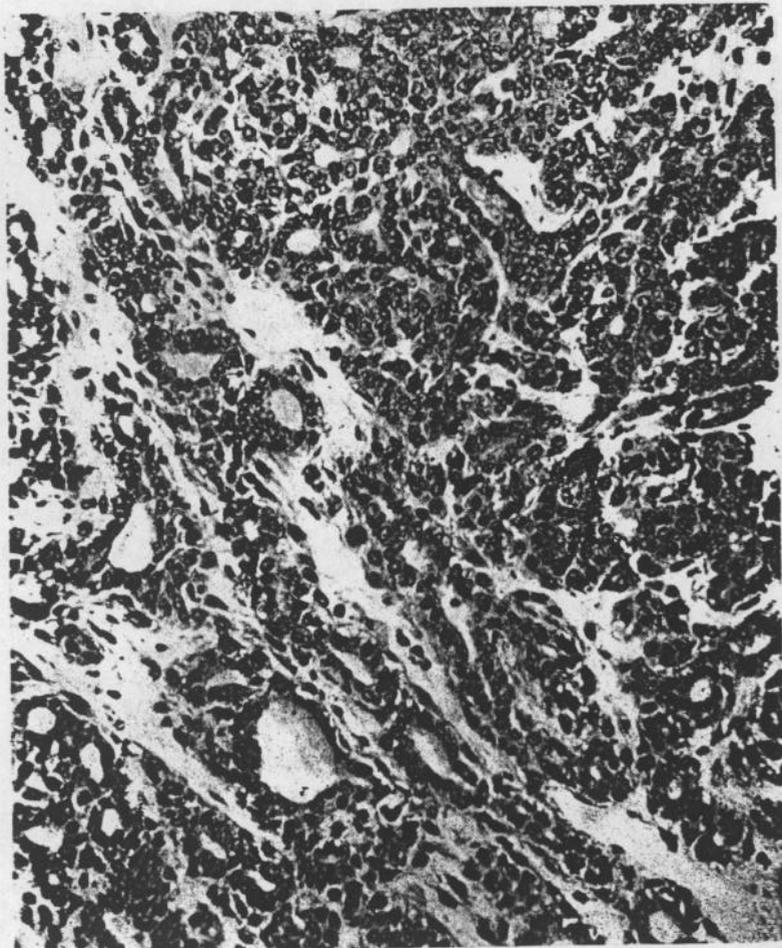


FIG. 12. Adenocarcinoma, Type B, showing irregular glandular spaces, and variable amounts of intervening stroma. Hematoxylin and eosin. $\times 270$.

especially frequently in our Type B tumors, and in some "miscellaneous" (Fig. 25). In women Dawson⁶⁰ described a somewhat similar outer layer of basal cells and an inner layer of lining cells in resting mammary epithelium.

ADENOCARCINOMA, TYPE C

Other terms used: Fibroadenoma, adenofibroma.

Microscopically this tumor is composed of multiple cysts, some extremely small in size (Figs. 15 and 16). The cysts are lined by a single layer of cuboidal epithelial cells which are closely invested by a layer of varying thickness composed of spindle cells. The spindle cells sometimes appear to fray out into the connective tissue lying between the cysts. It has been suggested that the spindle cells are myoepi-

thelial. They stain yellow with van Gieson and do not form collagen. The connective tissue stroma is often distinctive, for it generally appears edematous, with few cells and few collagen fibers, and it may be basophilic in its staining reaction when hematoxylin and eosin are used. This is a peculiar form of mammary tumor readily separated from other adenocarcinomas. In other types of mammary tumors small areas are occasionally seen in which the Type C formation appears, but the characteristic

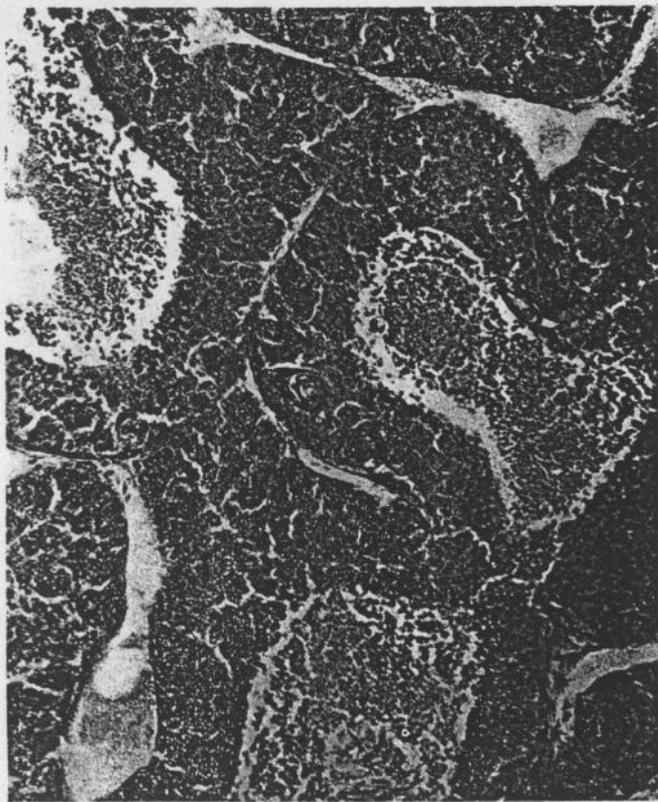


FIG. 13. A pattern sometimes found in small areas of Type B tumors. Necrotic material is found between broad bands of epithelial cells. Small masses of squamous cells are seen in center. Hematoxylin and eosin. $\times 200$.

Type C tumor is of uniform structure throughout the section. Its unique composition may reflect an unusual etiology since it has nearly always been found in very old mice that usually lacked the agent. Many of the mice were hybrids, often with one strain BALB/C parent, but it has also been seen in old C3Hf females. In a previous publication from this laboratory, the nature of this lesion was not recognized.⁶⁵ It was photographed and presented as a nonneoplastic lesion found in old mice, and it was thought that it represented the "precancerous cystic lesion" described by Haaland.²⁰ Further opportunities to observe this lesion, however, leave no doubt

that it is a distinctive tumor, for it often attains a diameter of a centimeter or more, and is unlike the rather diffuse areas of cystic dilatation found in mammary glands of old mice. Cloudman¹⁴ has photographed and described mammary tumors that appear to be identical with our adenocarcinoma Type C. He called them fibroade-

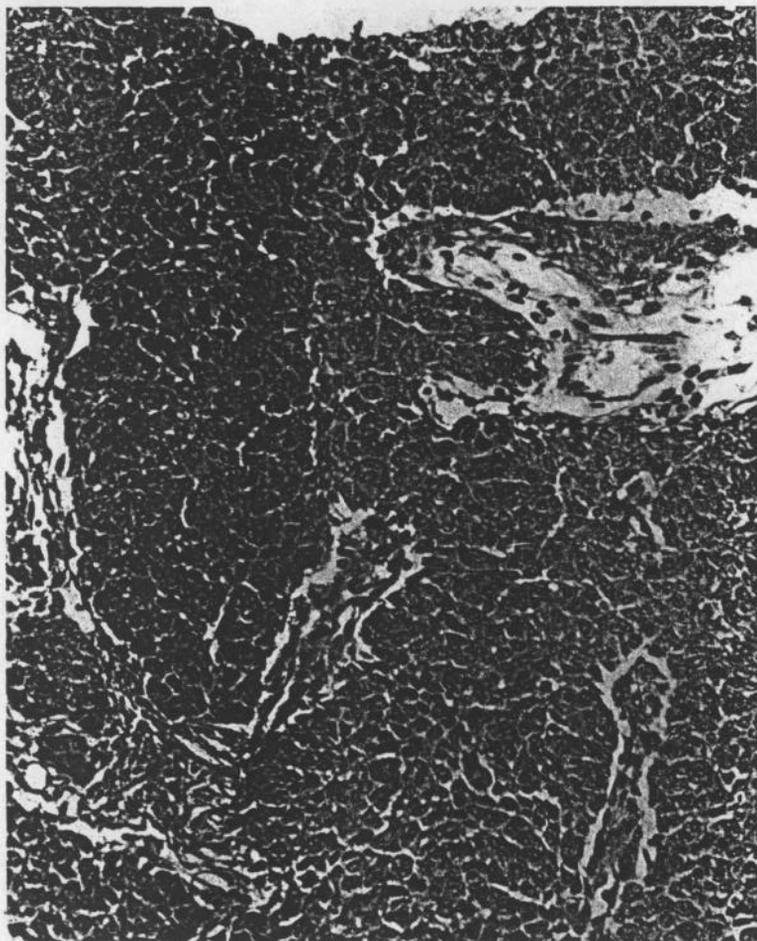


FIG. 14. Adenocarcinoma, Type B, showing solid cellular formation with no apparent glandular structure. Hematoxylin and eosin. $\times 265$.

nomas or adenofibromas and described the peculiar spindle cells that distinguish this tumor from others, but he regarded these cells as fibroblastic.

ADENOACANTHOMA

Other terms used: Keratinized mammary tumor, adenosquamous carcinoma, adenocarcinoid, mammary tumor with squamous metaplasia.

These are tumors in which a considerable area of the section, usually estimated

at one fourth or more, shows an epidermoid structure (Fig. 17). Although the squamous component is often referred to as "metaplasia" the epidermoid formation appears to be an integral part of the tumor development, and the capacity for epidermoid differentiation is inherent in the tumor cells. Indications of malignancy

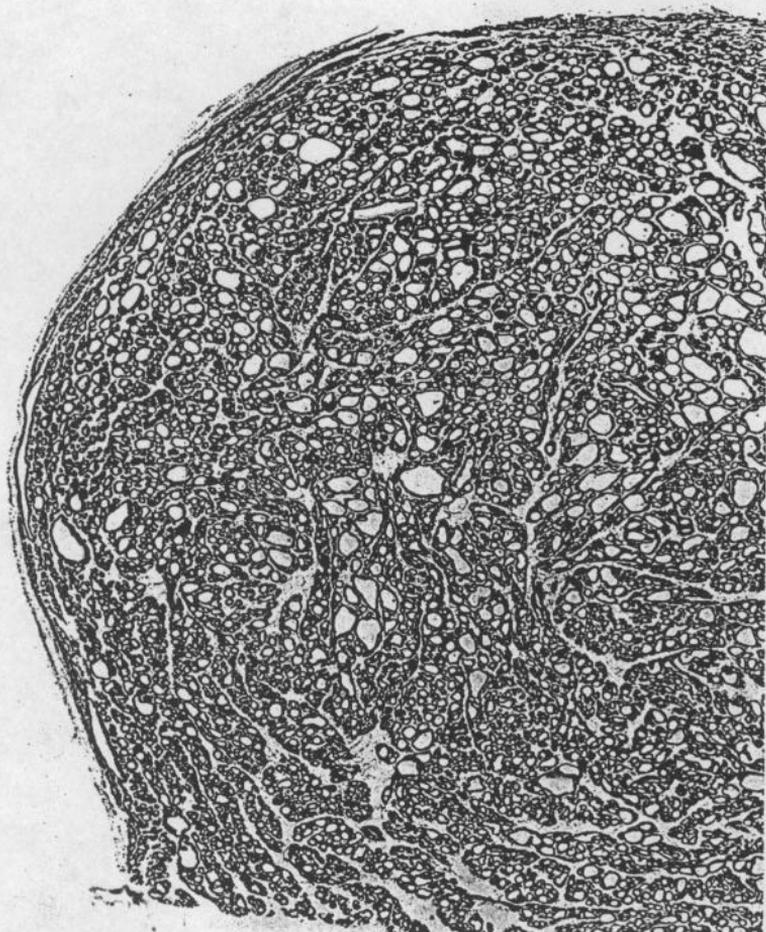


FIG. 15. Adenocarcinoma, Type C, showing uniform structure of entire tumor, which is composed of small cysts of varying sizes lined by cuboidal epithelium. Hematoxylin and eosin. $\times 20$.

such as an increased number of mitoses can be seen in the squamous epithelial component as well as in the glandular portion, metastases may contain epidermoid tissue, and transplantation usually reproduces both components, at least for a few generations.³⁷

Since the glandular element of this tumor is similar to the structure of adenocarcinoma Types A and B, which may have varying amounts of an epidermoid



FIG. 16. Adenocarcinoma, Type C. Higher magnification showing epithelial cells lining cysts invested by spindle cells. These spindle cells take a yellow color with the the van Gieson stain, and are characteristic of this tumor type. Hematoxylin and eosin. $\times 235$.

component, it is not always possible to separate these merging forms exactly. In examining large groups of tumors, however, those in which the squamous element is significantly increased can be separated from other tumors.

SPECIAL FORMS

Mollusoid. A variant of the adenoacanthoma is a form that Haaland⁷⁸ designated as "molluscoide" (Figs. 18 and 19) because of a supposed resemblance to molluscum contagiosum. He described the tumor as composed of lobes radiating like the spokes of a wheel from a central core of squamous epidermoid tissue. Varying amounts of epidermoid tissue are found in the center of this tumor and the radiating spokes suggest a reproduction of mammary ducts. The distal end of ductlike struc-

tures may be surrounded by and apparently connected with a neoplastic tissue that reproduces mammary glands. While perfect examples of these formations are rare, imperfect formations of this type, which may involve only a small area of a large tumor, often without the central epidermoid core, are not infrequent. This tumor is of peculiar interest, for it may furnish a clue to the understanding of many other

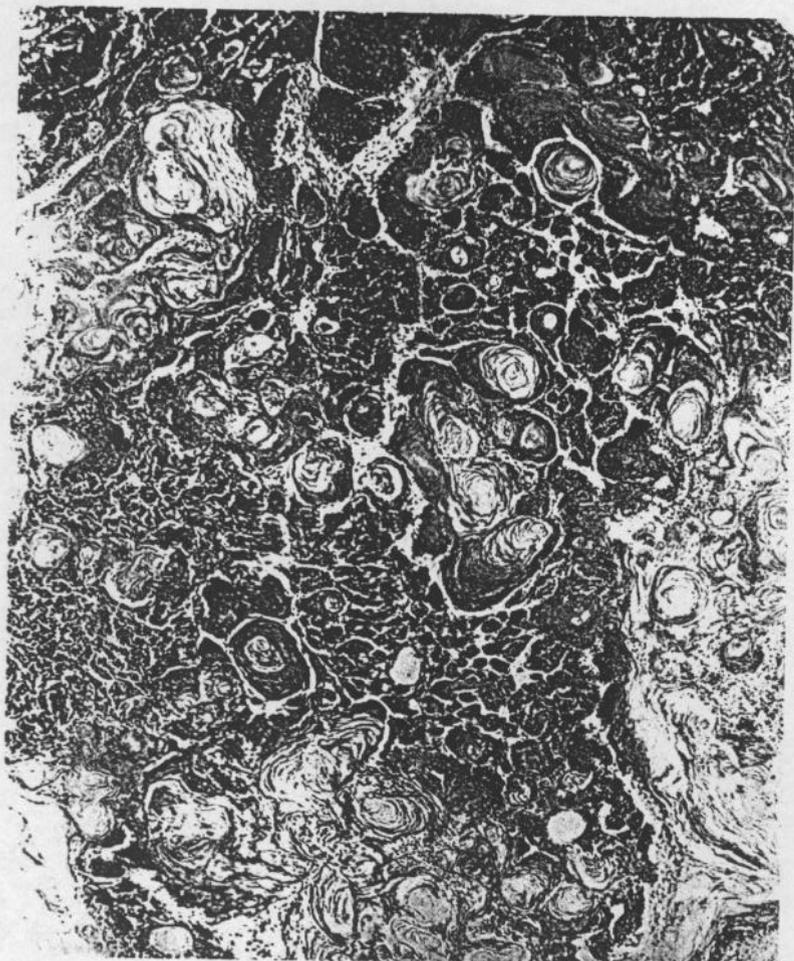


FIG. 17. Adenocanthoma, showing intimate mixture of glandular tumor tissue and epidermoid tissue. Hematoxylin and eosin. $\times 65$.

mammary tumors. It suggests that all epithelial elements that the mammary gland is capable of forming may participate in the neoplastic process, and that we have a malignant transformation in a complex organ, and not a change in a single cell or small group of identical cells. The diversity of structure in mammary tumors often suggests an imitation of acini, ducts, and epidermoid epithelium, so that imperfect and unbalanced organoid formation is reproduced. Attempts at classification, therefore, may do no more than name the predominant element in one section of a tumor.

Under the name "Hornstrahlen" or "horny-ray" tumor, Teutschlaender¹⁰⁹ described two molluscid tumors from his own mice, and discussed seven previously reported. He considered this tumor to be organoid in development, for its complicated growth was organized into a systematic glandular pattern. It did not invade along the paths of least resistance but maintained its organoid structure as the peripheral tumor tissue advanced. He discussed the possibility that the tumor originated from hair follicles rather than mammary tissue.



FIG. 18. Adenoacanthoma, molluscid form. Central portion of this tumor type shows epidermoid formation, with rows of epithelial cells radiating outward. The periphery shows a more glandular structure. Hematoxylin and eosin. $\times 18$.

Organoid. These tumors resemble the general architecture of the molluscid tumor, but they develop very little central keratinization (Fig. 20). Foulds (II)⁷⁰ considered the molluscid tumors as a special case of the more general phenomenon of organoid tumors. An organoid growth pattern was frequent in transitory early stages of neoplasia, referred to as "plaques," and already discussed under early cancerous changes. The tumors showed the same radiate structure described by Haaland⁷⁸ in the "tumeur molluscoide." Compound organoid tumors resulted when radiate tumors developed from multiple centers. One of these organoid tumors was

successfully transplanted, and all the resulting tumors contained some areas of radiate organoid growth. Invasive growth was observed, the invading tips retaining their sharp outlines, and maintaining their radial course. The tumors grew rapidly, tripling in size in about two weeks. True "molluscoid" tumors with keratinizing centers grew more slowly. A small area within an organoid tumor might not conform



FIG. 19. Higher magnification of margin of tumor shown in Figure 18. Central portion (at bottom) composed of dilated tubular structures lined by squamous cells. Periphery shows glandular formation. Hematoxylin and eosin. $\times 58$.

to the over-all structural plan. Such areas reproduced the patterns commonly found in mammary tumors, and suggested that some varied or commonplace mammary tumors might originate by progression in an originally organoid growth. Foulds, after studying these tumors, suggested that "histologically and biologically the smallest significant unit of an organoid tumor is not a malignant cell but a branching differentiating neoplastic tubule." Also, that "many of the structural variants familiar in the commonplace mammary tumors originate on occasion by progression or

modulation in organoid plaques and radiate tumors" and that "organoid growth is possibly the basis of all mammary neoplasia in mice."

A few perfect examples of organoid tumors (Fig. 20) and mullusoid tumors (Figs. 18 and 19) have been seen in collections from the National Cancer Institute,

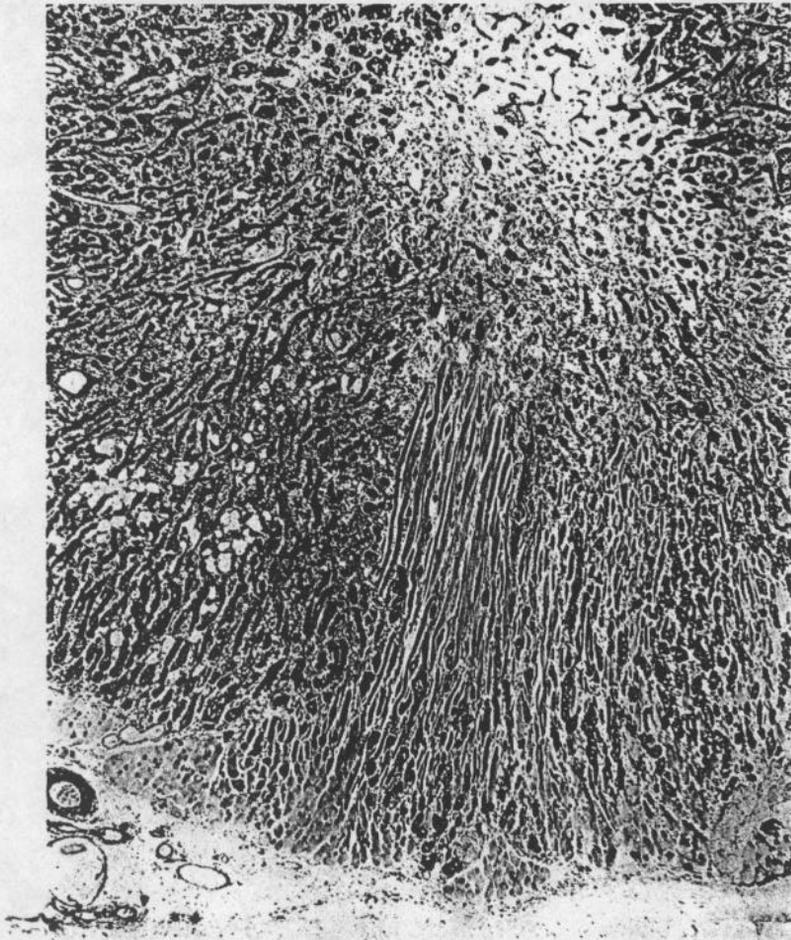


FIG. 20. An organoid tumor. The architectural pattern resembles that of the mollusoid form, but central keratinization is less evident. Note how this tumor has infiltrated muscle, yet maintained an organoid structure. Hematoxylin and eosin. $\times 40$.

but these are rare. On the other hand, tumors in which traces of an organoid structure are retained, usually at one margin of a section, are not uncommon.

CARCINOSARCOMA

Other terms used: Carcinoma with spindle cell formation, anaplastic carcinoma, mixed tumor.

Tumors of this type described by early pathologists⁷⁰ have developed during

transplantation of purely glandular tumors. These passed through a carcinosarcoma stage before becoming pure sarcomas. Primary carcinosarcomas are also found (Fig. 21). This tumor form exhibits a blending of cuboidal or round epithelial cells and elongate spindle cells resembling fibroblasts. Both components may show numerous mitotic figures, and both may appear malignant. In some areas the

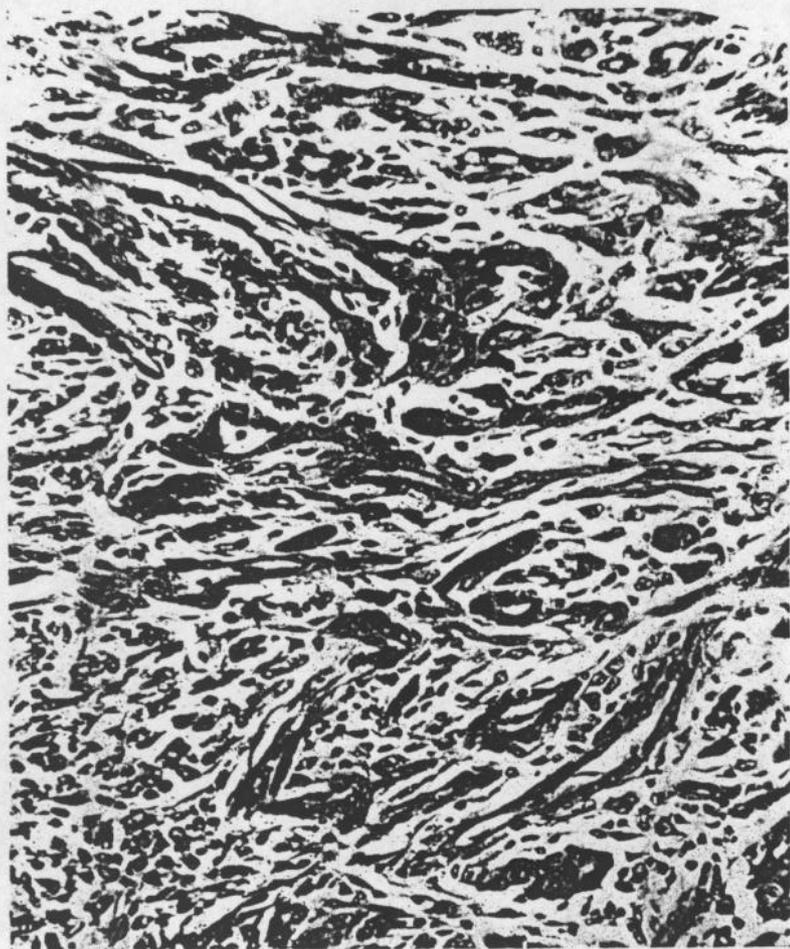


FIG. 21. Carcinosarcoma, showing a blending of neoplastic epithelial cells, and neoplastic spindle cells. Hematoxylin and eosin. $\times 265$.

epithelial cells appear to be assuming a spindle form. Whether this represents anaplasia in carcinoma cells, or whether a malignant change occurs in the stromal cells, either independently or under the influence of the growing carcinoma, has been the subject of much speculation and study, but no convincing experiment has yet been performed to prove how this transformation occurs.

Bonser and Orr⁵⁵ found this type frequently among tumors induced by carcinogenic hydrocarbons, and discussed its histogenesis.

SARCOMA OF THE MAMMARY GLAND AREA

Other term used: Mammary sarcoma.

These tumors are composed of interlacing bundles of spindle cells and are indistinguishable from fibrosarcomas originating at other sites, except that the spindle cells envelop the mammary ducts (Fig. 22). Tumor giant cells are not in-

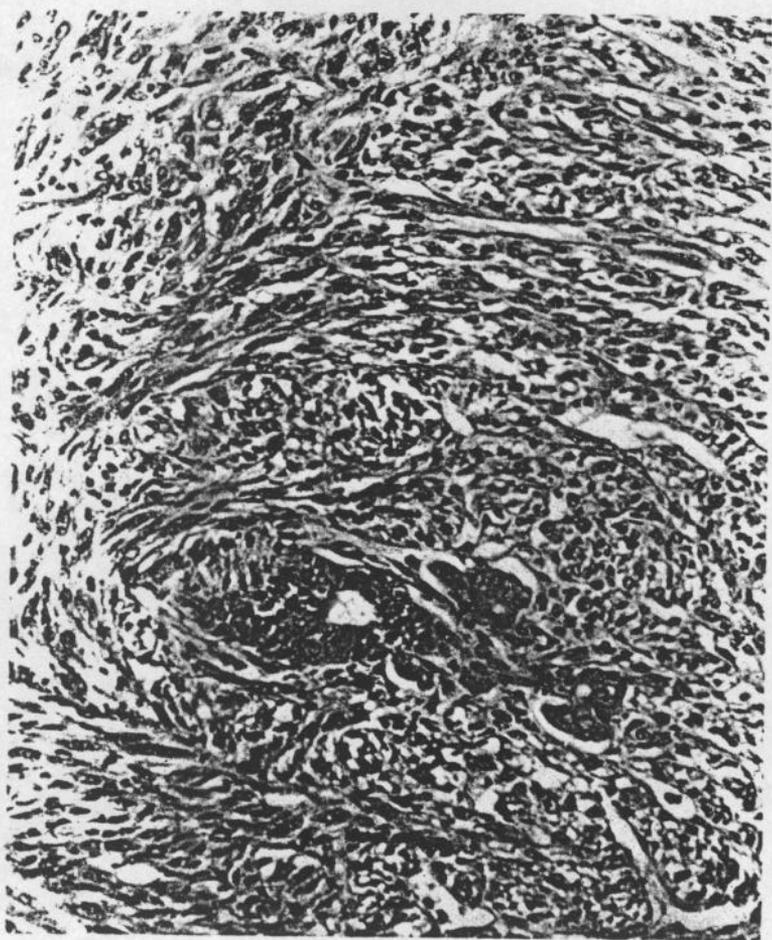


FIG. 22. Sarcoma of mammary gland area, showing typical fibrosarcoma surrounding mammary ducts. Hematoxylin and eosin. $\times 265$.

frequent. These tumors have been recorded with mammary tumors because they occur with remarkable frequency in some collections of subcutaneous tumors removed for histologic confirmation. They appeared to be increased in number after some experimental procedures that also increased the incidence of epithelial tumors, and they were frequent in one group of irradiated mice.³¹ Slye, Holmes, and Wells¹⁰⁶ described subcutaneous sarcomas in non-inbred mice, and concluded that about one

half originated from mammary gland tissue. In some of these the relation to the tubules was so close as to resemble a "pericanalicular fibrosarcoma."

MISCELLANEOUS

Unfortunately, very few classifications can avoid a "miscellaneous" category. It is reserved for tumors that do not fit any of the foregoing groups, yet do not form a

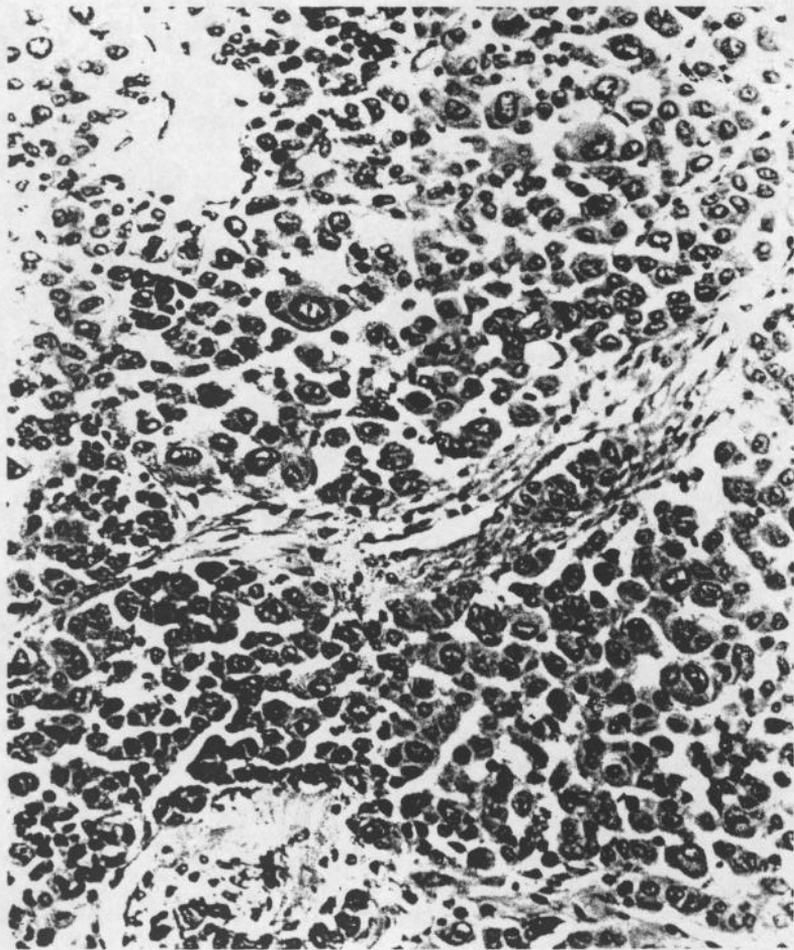


FIG. 23. "Miscellaneous" tumor of a bizarre type, showing detached cells of varying size. Hematoxylin and eosin. $\times 235$.

uniform type among themselves. In all large groups of mammary tumors, particularly in mice freed of the tumor agent, some very bizarre tumors may appear. Tumors composed of extraordinarily large cells, sometimes rounded off and dissociated from the stroma and from other tumor cells, have been seen (Fig. 23). Occasional tumors are composed of epithelial cells having no suggestion of acinar formation, or other structures resembling mammary tissue (Fig. 24). Other tumors

may form an abundant connective tissue stroma far beyond what is usual for "typical" mammary tumors and resembling "scirrhous" carcinoma in the human breast. Still others show a more lightly stained epithelioid element. In one rare tumor received from Dr. W. U. Gardner, almost the entire tumor was composed of these

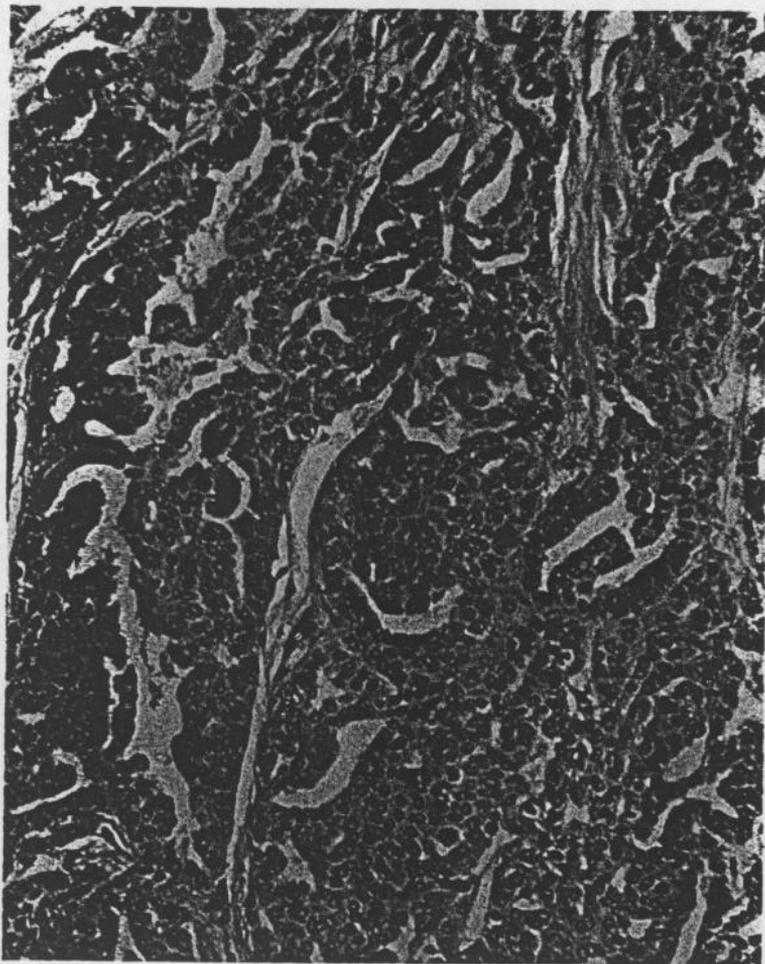


FIG. 24. "Miscellaneous" tumor of an unclassified type, showing epithelial cells with no evidence of an acinar arrangement. Hematoxylin and eosin. $\times 250$.

lightly stained cells (Fig. 25). A number of other variations may appear. All tumors placed in this miscellaneous group should be individually described and separately catalogued. If enough of a distinctive form are found they may warrant a separate category.

Pathologists should also be cognizant of other tumors in the mouse that arise in the area of the mammary gland and may be mistaken for mammary tumors. Fibrosarcomas are probably the most frequent.¹⁰⁶ Myoepitheliomas originating in

the salivary gland, or rarely in the region of the mammary gland but without any glandular element may be confused with mammary cancers.⁵² Rhabdomyosarcomas and mast cell tumors have been seen at the site of the mammary gland, though very rarely. Squamous cell carcinomas from the skin, or tumors derived from sebaceous



FIG. 25. "Miscellaneous" tumor, largely composed of pale-staining, round to oval epithelioid cells having no glandular pattern. Infrequent cleftlike glandular spaces were found lined by a double-layered epithelium in which the luminal layer was darker than the outer layer. Hematoxylin and eosin. $\times 210$. (Courtesy Dr. W. W. Gardner.)

glands have also been encountered.⁵⁰ Lymphosarcomas are readily distinguished by their location in lymph nodes and by their characteristic histology.

SPECIAL HISTOLOGIC FEATURES

In addition to this sorting process, when large groups of mammary tumors are examined it may be worthwhile to note certain histologic or histochemical features.

Secretory activity, iron content, mast cells, and alkaline phosphatase have been investigated.

Evidence of secretory activity (or lactation) may be revealed by vacuolation of the cell cytoplasm, and filling of the lumen with secretion. Correlation of this apparent functional activity with experimental procedures has not been determined in tumors examined in this laboratory but reports from other laboratories have indicated that it may be significant. Foulds has made a special study of this (IV).⁷⁹

A report on iron deposition in spontaneous mammary tumors in DBA mice has been given by Rawlinson.¹⁰² Although iron was present in large amounts in the nonneoplastic tissue, it was never found in the definitely neoplastic cells.

Olivi¹⁰¹ described mast cells and mucin containing epithelia in mammary glands of mice subjected to hormonal stimulation. The number of mast cells increased in females subjected to intense estrogenic stimulation.

Alkaline phosphatase activity during carcinogenesis of mammary tumors in mice implanted with stilbestrol pellets has been determined.¹⁰³ Activity was not dependent upon the "malignancy or anaplasia" of the tumor, but upon the activity exhibited by the luminal borders, myoepithelial cells, and endothelial-lined spaces. There was a notable lack of basic differences in the intensity of the reaction during various stages of tumor formation.

MORPHOLOGY OF TUMORS FROM THE NATIONAL CANCER INSTITUTE

Using the foregoing system of classification, tumors from various groups of mice autopsied at the National Cancer Institute have been sorted and tabulated (Table 1).

TABLE 1 MORPHOLOGY OF MAMMARY TUMORS IN MICE WITH AND WITHOUT THE MILK AGENT

Strain	Milk agent	Number of tumors	Morphologic type				
			Adeno-carci-noma A and B	Adeno-carci-noma C	Adeno-acanthoma	Car-cino-sarcoma	Mis-cellane-ous
C	+	94	92		1		1
C3H	+	123	122		1		
C3H	-	2	60		17	1	4
(C x C3H) _{F1}	?	190	172	17	8		1
Hybrids*		17	5	12			
DBA	+	40	40				
DBA	+(+MCA)†	29	25	2			2
DBA	- (+MCA)	86	30		30	20	4 2

* _{F1} hybrids derived from Strains C57 black, I,C,C3H, and DBA.

|| Treated with methylcholanthrene.

Notable features in the table are the high incidence of adenocarcinomas, Types A and B, in Strain C3H, Strain C, and Strain DBA mice with the mammary tumor agent; the relatively high incidence of adenoacanthomas in Strain C3H mice

without the agent; the number of adenocarcinomas Type C in old hybrid mice, and the number of carcinosarcomas in DBA mice without the milk agent but treated with methylcholanthrene. The same group of mice in which carcinosarcomas were most frequent had the largest number of sarcomas. A recent paper from Heston's laboratory⁷⁷ shows in general the same distribution of morphologic types as appears in Table 1. Six hundred and forty-two mammary tumors were found in 4,049 female mice from strains C3H (with and without the agent), C57BL₁ and F₁ and backcross hybrids of these strains. The mammary tumors that developed at an early age in females with the mammary tumor agent were for the most part adenocarcinomas, Types A and B. Some tumors of these types did, however, occur in older females, some with and some without the agent. Adenocarcinomas, Type C, and adenoacanthomas occurred mostly in females of an advanced age.

Heston and Deringer⁸¹ have also reported on the morphologic types of mammary tumors in virgin and breeding agent free strain C3H_f mice, and reciprocal hybrids with C57BL. Tumors in the F₁ hybrid females arose at a very advanced age and most of them were of histologic types rarely found in high-tumor strains with the mammary tumor agent.

CLASSIFICATION OF MAMMARY TUMORS IN OTHER LABORATORIES

Although the scheme in use at the National Cancer Institute has so far proved satisfactory for collections of mammary tumors examined here, no rigid classification for the tumors is advocated since we now know that the predominant morphologic type will probably vary with different inbred strains and be influenced by the presence of the agent. Some recent reports in which the morphology of mammary tumors has been considered significant have used the scheme described above. Dmochowski⁸¹ compared the development of mammary tumors in C57 X RIII hybrid mice, and in RIII high-cancer-strain mice. The majority of tumors in the agent harboring strain were Type A or Type B. In the hybrids, Type A and Type B tumors also predominated, but over 2 per cent of the tumors were adenoacanthomas. These occurred in mice over 12 months of age, and it was suggested that the frequency of various types of mammary cancer and the age at which they develop was influenced by genetic constitution. No connection was found between the appearance of tumors in hybrid mice and the presence or absence of the agent in the individual tumors.

Olivi and co-workers⁸⁴ used the National Cancer Institute scheme, and introduced a new Type M, characterized by the capacity of the neoplastic cells to produce mucus.

Bonser⁵⁴ describing tumors induced in IF mice by methylcholanthrene preferred to use a "less rigid classification," where the categories were determined by well-defined histologic features, in which tubular and intratubular growths were described, the relative amount and nature of the stroma, and areas of squamous metaplasia. This method proved satisfactory for a group of tumors in which there was a great variety of structure. Jull⁸⁴ used the same classification scheme as Bonser in describing the effects of estrogen and progesterone on the chemical induction of mammary cancer in mice of the IF strain. Carcinoma with varying

degrees of squamous metaplasia was the most frequent type, followed by intra-tubular carcinoma.

Guérin⁷⁹ divided mammary tumors into two groups: A, "Les formes glandulaires," with 2 subgroups, (a) acinar, composed of acini forming small cavities, or tubular, where the cavities were elongated and (b) a papillary cystic type, often hemorrhagic. B, "Les formes derides," consisting of 3 subgroups, (a) an undifferentiated type where gland formations were absent or rare, (b) a metaplastic type, with keratinization and (c) a sarcomatoid type, in which fusiform cells appeared.

Miihlbock *et al.*,⁸⁹ in describing the development of mammary tumors in dilute brown DBA mice with and without the agent, divided the tumors into two main groups: (1) Carcinomas with an acinar structure together with solid and cystic variations of this type, and (2) "deviating" types including tumors with marked keratinization, papillary epithelial proliferations, tubulo-adenocarcinomatous forms with abundant stroma, or fusiform carcinoma cells resembling the cells in sarcoma. The first group would comprise our Types A and B, and the second group our adenoacanthoma, carcinosarcoma, and certain "miscellaneous" forms. The simplicity of Miihlbock's method recommends it, because this division into only two general groups still indicates the morphologic differences that have proved to be significant in large collections of mammary tumors from different sources. DBA mice without the agent showed a relatively larger number of "deviating" types than did DBA mice with the agent and the percentage of tumors with an acinar solid structure and its variations was not predominant as in animals with the agent. However, the absolute number of carcinomas having the variable or deviating forms in DBA with the agent, where they formed "a few of a large number of tumors," differed little from the absolute number in DBA without the agent where they were "many of a small number of tumors." The agent appeared to act as an accelerator and intensifier of developments in the mammary gland that also take place in the absence of the agent.

Miihlbock and van Rijssel⁹⁰ also found the morphology of mammary tumors in the 020 Amsterdam strain of mice, which has a low incidence of mammary tumors, to be roughly the same as in other strains, although tumors with a purely aciniform structure were rare, and squamous metaplasia was not encountered. "Carcinosarcomatous" areas, in which spindle cells had formed in a highly cellular stroma were frequent, and sarcomatous transformation on transplantation occurred in 8 of 11 tumors. When the agent was introduced, the aciniform structure was slightly more marked, and no sarcomatous transformation was found in 2 transplanted tumors. The sarcomatous change was regarded as the result of "epithelial anaplasia" rather than a transformation to fibrosarcoma. Genetic constitution was considered an important factor in determining the form and frequency of mammary tumors.

RELATION BETWEEN MORPHOLOGY AND ETIOLOGIC FACTOR

The foregoing discussion on the morphology of mammary tumors and the effect of the tumor agent, hormonal stimulation, and genetic constitution may be summarized as follows:

7. The agent appears to promote the development of tumors with a predominantly acinar (Type A), or varied epithelial (Type B) structure. This has been notable in strains examined at the National Cancer Institute. The tumors develop at a relatively early age and cause death. More unusual forms emerge when the agent is absent and the mouse survives to an older age. However, typical Type A and B tumors may appear with considerable frequency in old mice without the agent; it is the *relative* number that is reduced when the agent is removed. It is impossible to tell on morphology alone in a single tumor whether or not the agent is present. The hyperplastic nodule, consisting of proliferating acinar or alveolar tissue, appears to be the most significant precancerous lesion in mice of a **high-tumor** strain with the agent.

It is impossible to determine the effect of age alone on the morphology of mammary tumors in mice since tumors ordinarily do not appear at an early age unless the milk agent is present. Old mice with mammary tumors are seldom found in strains with the agent.

2. *Hormonal stimulation* apparently has little influence in determining the type of mammary tumors. Thus castrated male mice receiving estrogenic hormones or untreated male mice with testicular lesions have developed mammary tumors similar to those appearing in females of the same strain.

3. Genetic *constitution* appears to be a very important factor in determining the morphology of mammary tumors, but more information is needed before the influence of this factor can be evaluated. With the removal of the agent, an increase in certain tumor types that appear to be characteristic of particular inbred strains may be recognized more readily.

With the removal of the agent it has **been** possible to study the effect of carcinogenic agents on mammary tissue. Sarcomas and carcinosarcomas have frequently been induced, but with a sufficiently small dose applied to the area of the mammary gland, epithelial tumors have **developed**. A squamous cell lesion has been described.

Removal of the agent also allows for a more **accurate** estimate of the effect of hormonal factors. Pregnancy greatly increases the number of mammary tumors in mice without the agent.

COMPARISON OF MAMMARY TUMORS IN MICE TO BREAST CANCER IN WOMEN

PRECANCEROUS STAGES

Attempts have been made to correlate the histologic appearances in the mammary tissue of old female mice of high cancer strains, with supposedly precancerous conditions in women.^{13, 18, 40} Such attempts have been much hampered by the fact that there has been no clear agreement as to what constitutes a precancerous condition in either species. It is an attempt to check one unknown against another unknown. An analogy has been made between cystic and inflammatory lesions described in old female mice and chronic cystic mastitis of the human female. "**Inflammatory** foci" in the mammary tissue of the mouse have not

been identified as precancerous. Proliferations of mammary acini, areas of squamous, cell metaplasia or hormonally responsive "plaques" have been identified as important preneoplastic lesions in mice. Doubt has been expressed that a condition simulating chronic cystic mastitis occurs in the mouse, although the dilatation and secretory activity following estrogenic administration is said to have some of the features of the human disease. Confusion is added by the varying conceptions which pathologists have of "chronic cystic mastitis" in the human breast. Foci of acinar proliferation, or "hyperplastic nodules," which are definitely precancerous in the mouse, are very infrequent in women, in whom the most significant precancerous lesions are now considered to be proliferations of duct epithelium. The differences in the histologic pattern of the developed tumors in the two species are consistent with the differences in the preneoplastic lesions.

DEVELOPED TUMORS

Breast cancer in the human female is nearly always derived from the ducts; alveolar cancer is rare. In the mouse, on the other hand, acinar or alveolar cancer is the most frequent type, especially in the presence of the milk agent. Statements by Willis⁴⁹ regarding interpretations of the structure of mammary carcinoma in human beings apply equally well to histologic examinations of mammary tumors of the mouse. He wrote that different parts of one tumor commonly show different structural variants, and terms such as "comedo," "scirrhous," "medullary," "adeno," and "simplex" when applied to cancer in women have only locally descriptive, not classificatory, value. Further, while individual tumors often show predominance of one or another type of structure, the structure of a breast tumor is rarely uniform throughout. He considered that histologic subdivisions of mammary carcinoma are arbitrary and when they are based on single or few sections of each tumor, they are often misleading. The same lack of uniformity exists in mammary tumors in the mouse. In the absence of the milk agent, when mice reach an advanced age many of the mammary tumors which develop show an adenoacanthomatous structure, which is, like the acinar formation, very rare in human tumors. As more tumors from mice without the milk agent are accumulated, more may be found resembling human breast cancer histologically. Occasional tumors in mice have an extreme degree of fibrosis resembling scirrhous carcinoma in women. The opinion is now widely held that breast cancer in women is often multicentric, which has long been recognized as the usual condition in mammary tumors in the mouse.

1

THE MILK AGENT

The possibility that a tumor agent may exist in human milk by which mammary cancer is transmitted from the mother to female offspring has been seriously considered.⁹ No convincing evidence for this has yet been presented; and it seems highly inadvisable to advocate any radical changes in infant feeding on this possibility. All the facts gathered from animal experiments make it appear more and more improbable that such an agent exists in any other species except the mouse. No agent of this type has been demonstrated in any other animal, although the incidence of mammary tumors is high in one strain of rats.¹¹⁰ A similar agent

transmissible through the milk has not been found for any other form of tumor. The histologic type of mammary tumor most closely associated with the milk agent, the acinar (or alveolar), is of rare occurrence in women. Moreover, removal of the tumor agent in mice has not prevented the development of all

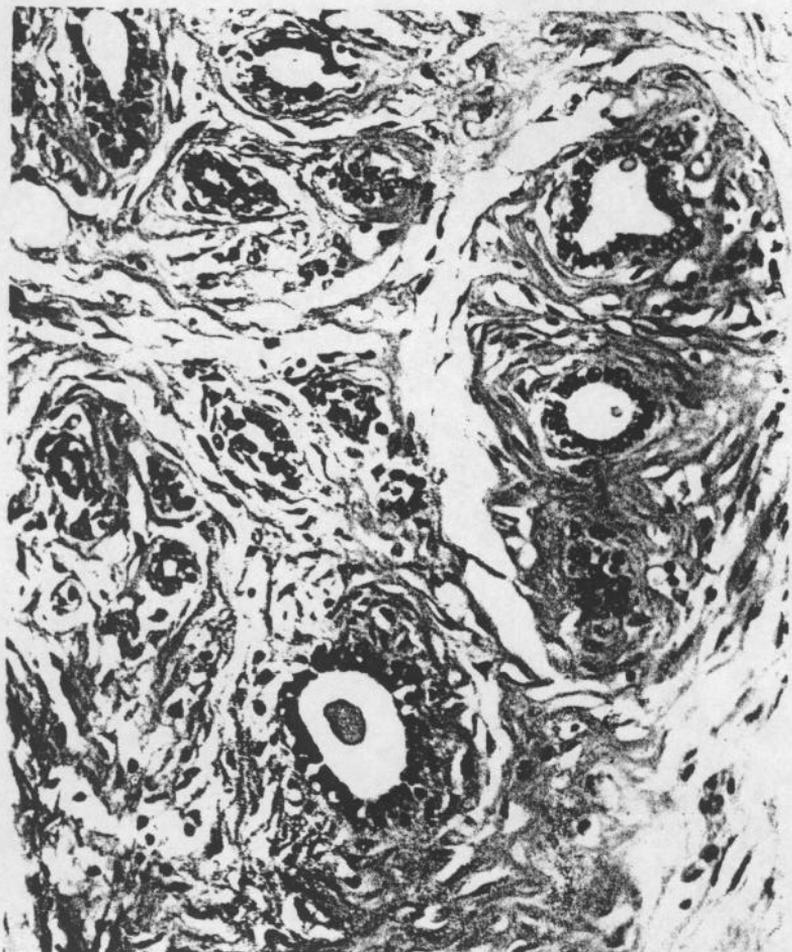


FIG. 26. This illustration and Figs. 27, 28, and 29 are examples of mammary tumors from four mammalian species. *Rat*. "Fibroadenoma" with an abundant fibrous tissue surrounding glandular spaces. $\times 290$.

mammary tumors. Records of a number of women with breast cancer who never received human milk²⁴ do not support the opinion that a milk agent exists in human beings. Many scientists who have been most concerned with investigations of this agent in the mouse decry the impetuosity with which these findings have been applied to the human being.

MAMMARY TUMORS IN OTHER SPECIES

Mammary neoplasms have not been as exhaustively studied in other mammals, although they are frequent in some strains of rats¹² and in dogs. A distinctive type of tumor has been found in all mammalian species in which the tumors have been

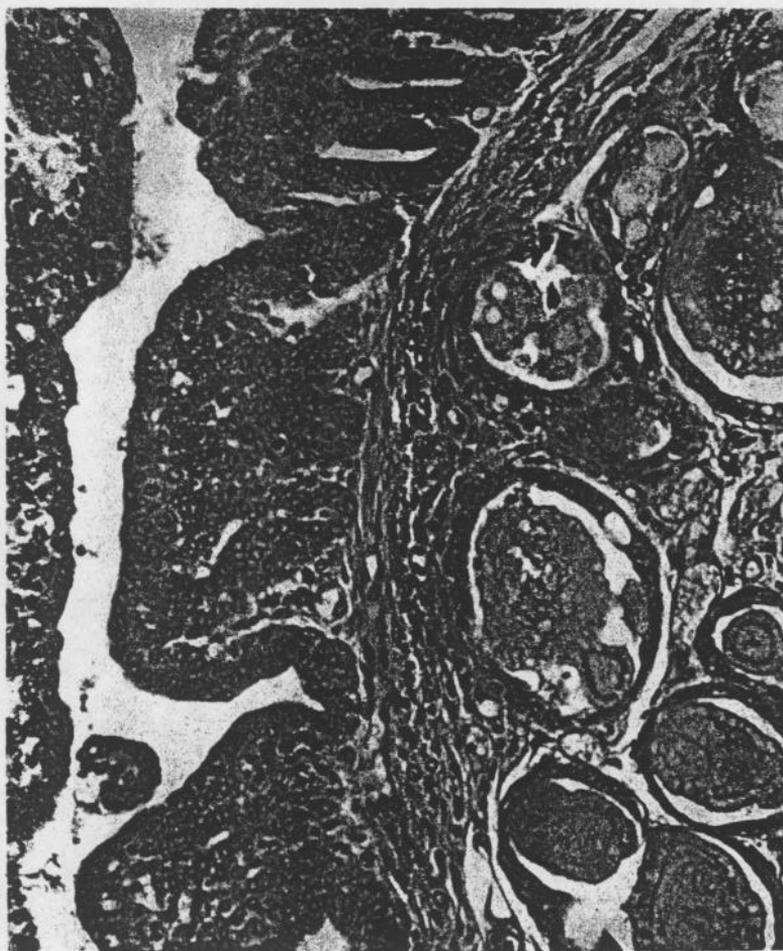


FIG. 27. *Rabbit*. Adenocarcinoma, showing on the right glandular spaces filled with secretion, and on the left papillary ingrowth. $\times 265$.

studied sufficiently (Figs. 26, 27, 28, 29). The fact that the morphology of an abnormal growth, a neoplasm, should in some way be determined by the genetic constitution of the host in which it arises, demands attention. The type of mammary tumor found may be as characteristic of a species as any normal structure.

A brief summary will be given of the prominent morphologic features of mammary tumors in other species, and important references, especially those that review preceding descriptions.

Rat. The most frequent type is a "fibroadenoma," a mixed tumor having a connective tissue and an epithelial component. The balance or imbalance in these components has inspired much experimental work, and attempts have been made to modify the relative amounts and degree of differentiation of the two elements by varying the conditions during transplantation. This work has been well summarized



FIG. 28. *Dog.* "Mixed" tumor. Central portion shows poorly preserved epithelial glandular tissues, and upper left and lower right corners show a mucoid stroma, in which cartilage formation is beginning. $\times 275$.

by Emge.⁶⁶ The morphology of the tumors appears to be influenced by hormonal variations in the host. Shay and co-workers¹⁰⁵ have reviewed previous work of this type and reported observations of their own. In rats which had received gastric instillation of methylcholanthrene they found a more glandular type of tumor in females and castrated males treated with female hormones. Breast tumors have been

found occasionally in old male rats, which received no treatment, but had other lesions indicating hormonal imbalance.¹⁰⁷

Rabbit. A series of papers has been published by H. S. N. Greene on familial mammary tumors in the rabbit.⁷⁴ He divided them into two distinct types. One was distinguished by periods of engorgement passing on to cystic mastitis and finally to

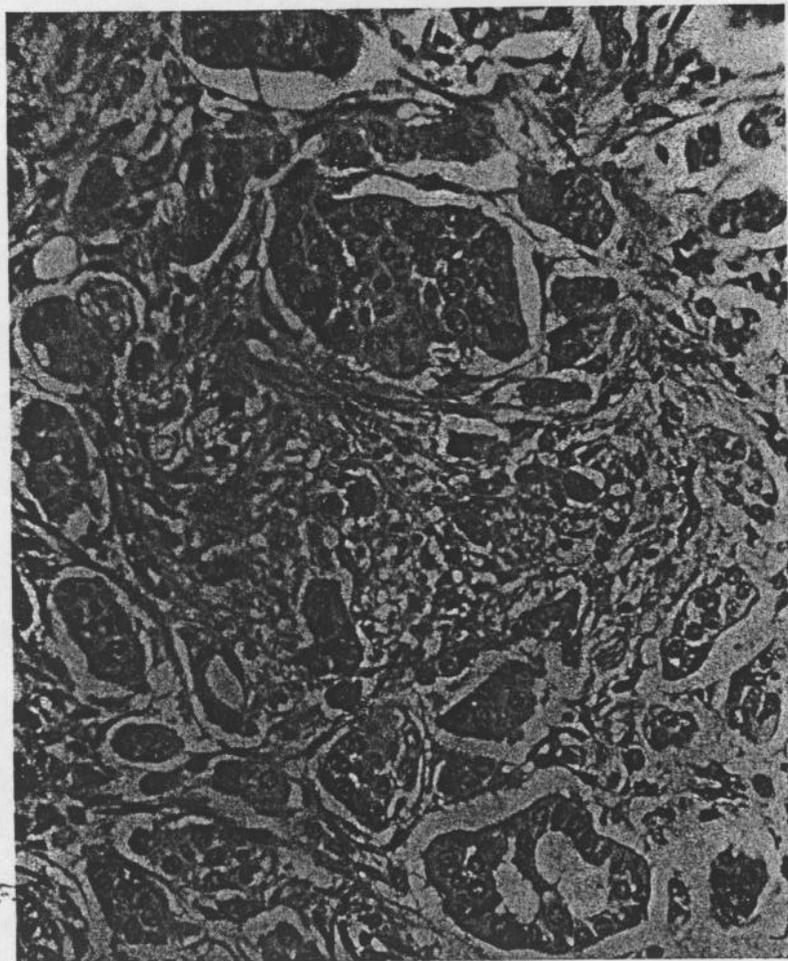


FIG. 29. *Human female.* Masses of adenocarcinoma cells are infiltrating connective tissue. A lumenated glandular structure appears at lower right. $\times 260$. All stained with hematoxylin and eosin.

neoplasms with a papillary structure. The other originated in clinically normal mammary tissue and was characterized by an adenomatous structure.

Guinea pig. Very few reports have been published, possibly because guinea pigs are rarely kept to old age. A few tumors were described in control and chronically irradiated guinea pigs surviving over 2.5 years at the National Cancer Institute.⁶⁷

Both males and females were affected. The tumors were classified as adenocarcinomas, and metastases were frequent.

Dog. Mammary tumors are the most frequent neoplasm in female dogs. Mulligan⁹¹ divided them into carcinomas, mixed tumors, duct papillomas, and myoepitheliomas. The mixed tumors were the most frequent. They were described as heterogeneous, containing epithelial, myoepithelial, and connective tissue elements. Hyalin cartilage and bone were formed in the connective tissue. Cotchin⁵⁹ is now studying benign tumors of the mammary gland. Complex tumors, in which a purely epithelial adenomatous component is accompanied by a component which seems to be myoepithelial, are the most frequent. Mucin, cartilage, or bone is often present.

Moulton⁸⁹ has also reviewed a group of 107 canine mammary tumors. He divided them into four main types, (1) mixed tumor, 72, (2) adenocarcinoma, 27, (3) fibrosarcoma, 5, and (4) osteochondrosarcoma, 3. Duct papilloma also appeared as a component of mixed tumor or adenocarcinoma. Transitions between different forms were frequent and the individual types were complex. In Moulton's series, myoepithelial cell proliferation could not be distinguished from fibroblastic or pleomorphic epithelial cell proliferation.

The histogenesis of the complex mixed tumors in dogs warrants further study.

The rarity of mammary tumors in domesticated animals, except for the dog, has often been noted. Jackson⁷⁷ reported one in a mule. Among captive wild animals, one was reported in a bear, and one in an opossum.¹⁰¹

PRESENT LINES OF RESEARCH

Although fashions in cancer research may come and go, the mammary tumor of the mouse continues in favor, because it can be adapted to almost any type of investigation. It is invaluable also because of the accumulated knowledge we already have regarding this one tumor, and on this foundation future work can be based. At the present time two popular lines of endeavor are the relation of viruses to cancer, in which the mammary tumor and the milk agent figure, and the effects of hormones in initiating and perpetuating new growths. The effect of radiation is also receiving intensive study. Perhaps of most significance is the effort being made to find out the underlying principles of malignant growth. Much of this work is too recent for critical evaluation and much of it is still in progress. Future results can be awaited with interest.

More evidence is being accumulated on the milk agent. The testing of a mammary tumor for the agent is an exacting and tedious procedure, requiring a year or more for a positive, and even longer for a negative, answer. In spite of the delay the evidence is now conclusive that many mammary tumors of the mouse have no demonstrable tumor agent.⁶² This is evidence that even in the mouse, elimination of the tumor agent will not eliminate all mammary tumors.

Further attempts have been made to visualize the mammary tumor agent by means of the electron microscope. Particulate bodies have been seen in epithelial cells from mammary tumors of a high tumor strain grown in tissue culture.⁴⁰ Such bodies have also been found in tissues and fluids known to carry the agent, and similar bodies were said to be absent in fluids extracted from normal tissues.³⁸

Cytoplasmic inclusion bodies in mammary tumors in irradiated mice have been described by Guérin.⁷⁹ These bodies had the characteristics usually attributed to viral inclusions, and Guérin thought that they might be an evolutionary cycle in the Bittner virus, which was made evident by the irradiation. Gutrin and Vigier⁷¹ obtained a filtrate from a transplanted mammary tumor. It was suspected that this filtrate contained an active agent, since it induced mammary tumors in adult mice of a different strain, and apparently multiplied when cultivated on eggs. The morphology of the resulting tumors was described as undifferentiated, though some showed keratinization. Inclusion bodies were found in these tumors, but a relation to the agent was not proved. The virus had characteristics like those of the Bittner virus.

Bernhard,⁴⁴ in collaboration with Guérin and others, made electron microscope studies of mammary tumors. Two types of bodies were seen, an intracytoplasmic paranuclear type and an extracellular type. Both types were thought probably related to the milk agent. The authors concluded that while their experiment did not furnish proof that these bodies were the virus, they were pathological structures, and probably the Bittner virus, since they could be transmitted by a cell-free filtrate. The bodies were thought to be identical with those shown by Dmochowski and coworkers.⁶³ More work is yet needed before it is proved that the milk agent can be recognized as an intracellular particle in mammary tumor cells and identified as the cause of the neoplastic change.

Andervont⁴⁹ has demonstrated that the wild house mouse carries a mammary tumor agent, but this is low in concentration or activity. The wild mice nursed by their own mothers developed only a few tumors. When they were nursed by agent bearing strain C3H mothers, they developed an increased number of tumors and at an earlier age. These tumors were all of Type A or Type B. Agent-free inbred mice foster-nursed by wild mice had an increased incidence of mammary tumors, indicating that they obtained the agent from the wild foster mothers.

The part played by hormones and genetic constitution on the development of mammary tumors can now be measured more accurately since it is possible to remove the mammary tumor agent. The powerful effect of the agent has probably overwhelmed and obscured the weaker influence of some other modifying conditions.

The estrogenic hormones have received especial attention. It is proved that estrogenic stimulation of the mammary gland is required for the initiation of mammary tumors and that many of the tumors appear to be secreting as if capable of a functional response. However, once initiated, the tumors usually become independent of any external hormonal stimulus. To explain this independent growth capacity, Pullinger⁴² suggested that the tumors may create their own intracellular hormone, and that it is this hormonal stimulus that perpetuates them. Much more concrete evidence is required to establish this hypothesis but the mammary tumor seems to be the best available means of testing it.

Pullinger⁴⁹ has tried to determine the relative importance of age and parity in the development of "spontaneous, benign and malignant mammary tumors" in RIIB mice (without the agent). Increase in pregnancies, rather than age, in-

creased the incidence and total numbers of "benign" growths of all kinds. Only twelve spontaneous mammary carcinomas occurred in 482 breeders that lived to 13 months or more, and each of which had borne 3 or more litters. Six of the carcinomas were of acinar structure, 3 were adenoacanthomas, 2 were squamous carcinomas, and 1 was a carcinosarcoma. Recognition of the importance of pregnancy, once the milk agent is removed, is in agreement with results from other laboratories.^{81, 87}

Pullinger,¹⁰⁹ again using RIIIb breeders, has investigated the effect of aging alone and of ovariectomy at 9 to 11 months without further treatment, or followed by estrogen. Ovariectomy greatly decreased the incidence of benign mammary growths, which are similar to hypertrophic nodules. When estrogen was given to the ovariectomized mice, the incidence was comparable to that in intact breeders in old age. Malignant growths were too rare to warrant a comparison.

Mühlbock⁸⁸ has given particular attention to hormones and mammary cancer. In mice without the agent, pregnancy could be recognized as an important factor, but there were marked differences in various strains of mice. Excessive hormonal stimulation produced by weekly implantation of hypophyses removed from other mice produced mammary cancer in virgin females of strains C3Hf, DBAf and O20, all of which lacked the agent. Estrogens and progesterone did not produce tumors in DBAf and O20, although estrogenic hormone alone was effective in C3Hf. Morphologically, these tumors were in no way different from mammary tumors occurring spontaneously after pregnancy. The work of Mühlbock's laboratory and the significance that it may have in relation to breast cancer in women are well summarized in a recent paper.⁸⁷ The basic principles in man and small animals are assumed to be identical.

Jull⁸⁴ found that the combination of progesterone with estrogen treatment significantly increased the incidence of mammary cancer in normal males and in ovariectomized females, when they received a limited dose of 20-methylcholanthrene applied to the skin. It was concluded that many of the tumors arose from the duct epithelium, and changes considered to be precancerous were more common in ducts than in acini.

The effects of estrogen, and hormones derived from the placenta have been investigated in the Division of Cancer Research at the University of Perugia in Italy. The precancerous changes following estrogenic treatment were similar to precancerous changes in untreated mice. The early structure of the tumors was usually cystadenocarcinoma.⁹⁵ With hormones derived from the placenta in high-mammary tumor strain C+ and RIII, the first change was in the ducts; they showed lateral budding, dilatation, and secretory activity. Lymphocytic infiltration appeared in the connective tissue. Later, acini and hyperplastic acinar nodules developed. The nodules appeared at an earlier period than in untreated mice.⁵⁶

Cohen and Cohen⁵⁸ have published a series of papers on the radiobiology of the mouse mammary carcinoma. The mammary tumor was used as a test object by means of which various factors relating to radiosensitivity and the host-tumor relationship could be determined. Of special interest in relation to morphology and biological behavior was the report of a spontaneous mutation in a homoplast,

which resulted in an increased radiosensitivity, yet no morphologic change accompanied this biologic alteration.

Goldfeder⁷⁴ also has tested the radiosensitivity of mammary tumors. A smaller dose was required for a tumor grown in strain DBA/H than for one grown in C3Hb. Following irradiation, the DBA/H tumor, which was of acinar structure, showed diffuse fibrosis, while the C3Hb tumor showed no fibrosis after the same dose.

The mammary tumor has been used in two recent investigations of some of the underlying principles of neoplasia. Dumbell and Rous⁶⁴ exposed "two polymorphous carcinomas of the milk factor type, with the characteristic tendency to form acini and tubules" to methylcholanthrene. This was done by implanting the tumor cells together with a 1 per cent solution of the carcinogen in olive oil. The cells of the tumor underwent no significant change. A similar procedure was carried out with lung tumors and urethane. Further neoplastic changes occurred in some of the tumors, but these did not seem to be caused by the carcinogen. It was stated that the superimposed neoplastic changes might well represent further steps in a sequential process begun primarily by carcinogenic stimulation but taking place independently of this thereafter.

Reference has already been made to recent studies by Foulds.⁷⁰ Palpable subcutaneous masses in a group of hybrid mice showed progressive growth during periods of pregnancy, and regression or quiescence when the pregnancy terminated. The growths might again become palpable with another pregnancy. Eventually many of these tumors seemed to be freed of their dependence on the hormonal state of the host and grew progressively after the pregnancy had ended; Foulds has taken these tumors as examples, "or special cases, which illustrate certain general principles of neoplasia." This study of the mammary tumor, "root and all," where the process of neoplasia can be observed in a comparatively simple form, may help in understanding the complex development of other neoplasms. Progression, the process by which tumors advance from an early responsive stage to unresponsive and independent growth, is well illustrated by the tumors arising during pregnancy. The complexities of histological structure and biological behavior of mammary tumors can be explained as expressions of the numerous and diverse unit characters that make up neoplasms. Though unrestricted capacity for growth is the character—usually has the most serious consequence to the host, it is only one of the many alterations in a cancer cell. The unit characters in a tumor can combine together in a great variety of ways, yet within wide limits, they are independently variable and liable to independent progression. This hypothesis as to the cause of the morphologic variation of tumors, particularly mammary tumors of the mouse, is the most satisfactory so far proposed. Analysis of the morphology of mammary tumors disposes forever of the idea that cancer is usually a unit alteration in a single cell, which reproduces itself unchangeably.

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