Mammary Tumors of the Rat: A Review

R. L. Noble and J. H. Cutts

(Dept. of Medical Research, Collip Laboratory, University of Western Ontario, London, Canada)

Mammary tumors in the rat have been studied in detail by only a few groups of workers. There is, however, a considerable amount of information on the subject contained in isolated papers and scattered throughout the scientific literature. Apparently, no previous attempt has been made to review this field of interest. In the past, the attention of geneticists, virologists, endocrinologists, and biologists has been focused on the mammary tumors of the mouse, and a vast literature has accumulated in which these tumors are characterized very exactly. The rat has been somewhat neglected in this concentration of effort on the smaller species, although it would appear worthy of greater and more serious attention. The need for a tabulation of rat data may be appreciated from the appearance in 1954 of a paper in Science, containing no references, entitled “Successful Transplantation of an Apparently Benign Neoplasm.” A rat fibroadenoma apparently was transplanted successfully. This achievement should not have been unexpected in view of Loeb’s report in 1902 of autotransplantation and in 1916 of homotransplantation of similar tumors in rats.

Rat mammary tumors have many points of interest and differ from those of the mouse in many ways. Histologically, both benign and malignant tumors occur spontaneously in the rat, or may be induced by a number of means. No viral factors have been demonstrated, and most rat tumors are curiously sensitive to hormone manipulation. Their possible value in chemotherapeutic screening of hormone derivatives has only recently received attention. All these considerations will be pointed out in the following review.

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countered, and mixed tumors combining adenocarcinoma. Fibrosarcomas are more frequently encountered, and mixed tumors combining adenocarcinoma and squamous-cell carcinoma, and, rarely, squamous-cell carcinoma, and two adeno-acanthoma), 12 per cent as mixed tumors (three sarcoma with adenocarcinoma and two with added squamous-cell carcinoma), and 59 per cent as fibrosarcoma (28). Bullock and Curtis (9), in examining 94 spontaneous breast tumors occurring in 489 rats of mixed origin, noted two cystadenocarcinoma with squamous epithelium, two carcinosarcomas, and three adenomas exhibiting sarcomatous change. Dunning and Curtis (28), in a study of 15,625 rats derived from twenty generations of pure strain Copenhagen 2331 and Fischer 344 rats and their reciprocal F1 and backcross hybrids, also observed 32 mammary tumors. Of these, only two were malignant: one was an adenocarcinoma and one a fibrosarcoma.

In 149 mammary tumors arising in random-bred Albany strain of hooded rats, only one adenocarcinoma was found (8, 144). In 468 rats of Wistar origin exhibiting 273 mammary tumors, eleven mammary adenocarcinomas and two sarcomas were noted (119). Similarly, of 94 spontaneous tumors of the breast occurring in 489 rats only two were classed as cystadenocarcinoma and two as carcinosarcoma (9). Probably the highest incidence of spontaneous adenocarcinoma of the breast has been reported in 150 female rats of the Sprague-Dawley random-bred strain. Over an average life span of 760 ± 21 days, mammary tumors were found in 72 rats (54 per cent) and, of these, seven (10 per cent) were classed as adenocarcinoma. The over-all incidence of carcinoma of the breast in these female rats therefore was 4.6 per cent. On the other hand, a smaller group of 40 male animals of the same strain developed only two mammary tumors (16). Metastases from spontaneous malignant tumors have occasionally been noted (8, 28).

3. Benign tumors of epithelial or mesodermal origin.—Benign spontaneous mammary tumors constitute a much larger group than do the preceding. Adenoma and adenolipoma have been described occasionally, but fibroadenoma and fibroma are apparently the most common tumor. Of the 32 mammary tumors found in 15,625 rats previously referred to (28), two were adenomas, two adenolipomas, five fibromas, and 21 fibroadenomas—a total of 93.7 per cent benign mammary tumors. Similarly, Bullock and Curtis (9) examined 94 tumors of the breast and noted eight adenomas, one adenolipoma, and 78 fibroadenomas or fibromas (93.4 per cent benign tumors). In 150 Sprague-Dawley female rats, 90 per cent of 72 breast tumors were classed as benign—one adenoma, 46 adenofibromas, and eighteen fibromas (16). Curtis, Bullock, and Dunning (13), after examining over 30,000 rats from seven distinct strains, found a total tumor incidence of 9.6 per cent, and, of these, mammary fibroadenoma contributed 12.8 per cent. Of the 6410 female rats reaching an age of 14 months (considered to be the minimum time of appearance), 0.9 per cent showed benign fibroadenomas. Of 919 female Wistar rats bearing mammary tumors and averaging 650 days of age, 206 were classed as benign tumors. The growth rate of the spontaneous fibroadenomas varied greatly. Of twenty tumors, eleven increased 2–10 times their original volume in 90 days (119). Metastases have not been observed.

4. Factors influencing incidence of spontaneous tumors.—

Age: The age of the rat, as might be suspected, influences the incidence of spontaneous tumors found at any time. Studies in which the animals have been allowed to live their normal life span have been the most informative. It was noted that 80 per cent of fibroadenomas developed in female Wistar rats between 64 and 128 weeks of age, whereas carcinomas occurred somewhat earlier in life (119). In the Albany strain of rats, benign tumors arose chiefly between 52 and 88 weeks of age, although three tumors were noted in rats only 20 weeks of age (144). In a comparison of seven different strains of rats, fibroadenomas were encountered in 0.9 per cent of 6410 females reaching 14 months of age (13).

Sex: As may be noted from the preceding discussion, the sex of the rat may markedly influence the occurrence of mammary tumors. Female animals were much more predisposed to this type of tumor, although all types of tumors have been encountered in males. The relative frequency of mammary tumors has been low in males, possibly from 1 to 6 per cent of all mammary tumors.

While exact experiments are not available, the implication is strong that the cause of the higher incidence in females is related to the influence of the sex hormones. As will be seen later, estrogens and progesterone may stimulate growth or be essential for successful takes of transplanted spontaneous mammary tumors, particularly the fibroadenomas. On the other hand, it may be noted that mammary tumors induced by sex hormones are usually multiple, and practically all are classed as
carcinoma. Some of the earlier workers were impressed with the possible role of a disturbed hormone balance as a cause of spontaneous tumors in the rat (70, 96) and believed that forced breeding led to a higher incidence of tumors (2, 83). On the contrary, a high incidence of benign mammary tumors in the Albany hooded strain of random-bred rats was believed to be related to a decreased fertility of unexplained cause (8). Further studies attempted to develop an inbred line of Albany rats with a higher incidence of tumors and to determine the endocrine imbalance responsible (144).

Diet: A few papers of considerable interest have presented evidence that the diet, particularly the lipide content, may definitely affect the incidence of mammary tumor development. Benson, Lev, and Grand (4) noted that the addition of 20 per cent olive oil to a chow diet increased the incidence of fibroadenoma in Sprague-Dawley female rats from 7 to 28 per cent by the time they were 28 months of age. Groups of 43 controls and 129 oil-fed animals were compared. No tumors appeared before 18 months of age. Similarly, an incidence of 57 per cent in 150 control Sprague-Dawley females was increased to 80 per cent in 100 animals fed a diet containing 10 per cent corn oil (16). Diets containing purified casein (118) or a low protein content (75) also have been found to lead to a higher incidence of breast fibroadenoma. In the latter paper, however, it may be noted that the low protein diet contained about 15 per cent fat from the addition of lard and vegetable oil, whereas the control chow diet would contain less than 5 per cent fat. In view of the preceding reports it seems likely, therefore, that the higher fat content of the experimental diet may have been the major factor contributing to the higher tumor incidence.

B. Transplanted Mammary Tumors of Spontaneous Origin

1. Malignant tumors.—Epithelial origin. The rarity of the occurrence of spontaneous malignant tumors has allowed only a few papers describing their characteristics. These, however, have been complete studies from which generalizations can be made for the behavior of this type of tumor when propagated in pure strains of rats. Transplantable mammary tumors of various origin in the rat have been included in the survey by Dunham and Stewart (19). Undoubtedly, the most widely known transplanted mammary tumor of the rat has been the Walker carcinosarcoma 256. Because of the wide use of this tumor for purposes beyond the scope of this review, no attempt has been made to summarize the literature. It is possibly pertinent, however, to note that the tumor has been described as being discovered in 1928 on the lower abdomen of a pregnant albino rat. It apparently regressed in size during lactation but grew rapidly again after the young were weaned. Histologically, it was an adenocarcinoma with a large proportion of connective tissue elements. The tumor, during successive transplantation, has lost the original organized epithelial element and now appears as an highly anaplastic growth (35). The behavior of the transplanted tumor has been studied by many workers. "Takes" have been obtained readily in up to 96 per cent of rats of either sex. The growth rate was rapid and reproducible, particularly on intramuscular implantation. It could be transplanted to rats of most strains, and it would grow, at a somewhat slower rate, even in the wild rat (127, 136). The tumor has been widely used for the screening of tumor inhibitors (140), and it may show limited hormone responsiveness (107).

Eisen (36, 38) has described the transplantation of mammary carcinoma in the August strain of inbred rats, and his observations may be considered as typical of other tumors of this type. Three carcinomas arising in females of 233–253 days of age and one tumor in a male 748 days old were described. Transplantation was effected in 58–78 per cent of rats of the August strain. Age, sex, pregnancy, or lactation did not influence tumor growth. The growth rate was rather slow. The tumors became palpable in 2–3 weeks, measured 2–5 cm. in diameter by 8 weeks, and killed the host in 3–4 months. Metastases were not observed. Intraperitoneal injection was followed by papillary nodular development on the omentum, whereas intravenous inoculation led to lung deposits. No change in the morphology or growth pattern was found over a 3-year period, in a comparison of the 3d and 27th transplant generation of one tumor, R2428. Similarly, the moderate number of takes (38 per cent) in related strains was unchanged, as was the occasional take (2 per cent in seven alien inbred strains). Growth of the transplant in rats of alien strains, when it did occur, was at a reduced rate and associated with increased necrotic changes (38). Dunning, Curtis, and Maun (28) described a mixed tumor, R2572, with adenocarcinoma and squamous-cell carcinoma components, which arose in an 849-day-old A × C female rat. This remained as a mixed tumor through four transplant generations, but subsequently the two components were successfully dissociated and maintained separately as a pure adenocarcinoma and an osteochondrosarcoma.

2. Benign tumors.—In contrast to the few reports of spontaneous malignant tumors main-
tained by transplantation, a large number of studies have been made on benign tumors. Also in contrast is the tendency for fibroadenoma to alter, morphologically, on repeated transplantation and frequently to show a marked response to the administration of, or lack of, various hormones. Benign tumors may be transplanted readily as originally described by Loeb (82-84). The best results have been obtained by transplanting relatively large, multiple pieces or slices of tumors, rather than cell suspensions, in rats of the strain of origin of the tumor. From 64 to 95 per cent successful takes in large series of female animals have been described (61, 67, 80, 90). Usually, prolonged latent periods and slow growth rates have been noted. The characteristics of transplanted tumors originating from different spontaneous tumors, however, have shown great variation. These in turn are probably related to the tissue element predominating in the original tumor, the frequent diminution in epithelial elements during successive transplantation, and the related variations in response to hormones. Some tumors, however, have been described which, over many generations, have retained a constant pattern of growth and morphology. Examples may be found in the adenoma and adenofibroma, unchanged after transplantation for many years in inbred Wistar rats (15), a transplantable adenoma which showed lactation (63), in an adenolipoma (114), or the fibroadenoma described by Huggins and collaborators in Sprague-Dawley rats (80) and used by others for quantitative studies (61). Others, working with benign tumors of different origin, have been impressed with alterations in growth, hormone responsiveness, and morphology, over succeeding generations (68, 70, 90). The transition of the benign tumor to a carcinoma (120) or a sarcoma (41, 44, 92, 113, 129) following repeated transplantation has been frequently noted.

Transplanted fibroadenomas may or may not (61, 80) show wide variation in their response to hormones, depending upon the individual characteristics of the tumor studied. In addition, alteration in response over successive generations may occur (90, 107). Most transplanted tumors show a hormone dependence on female sex hormones and initially will transplant with limited success or not at all into male rats or ovariectomized females (54, 64, 67, 80, 90, 120). The growth rate of the tumor may be reduced markedly by ovariectomy and still further following adrenalectomy or hypophysectomy (61, 80). Pregnancy has been found to stimulate the growth rate of transplanted tumors in many cases, but great variation has been reported (40, 43, 45, 63, 72, 82, 84, 90, 120). Most fibroadenomas are affected by estrogens. These hormones usually have been found to shorten the latent period for growth in male and female rats and to increase the number of takes following gonadectomy. Tumor growth stimulation has been noted by many workers, particularly in the early generations of a tumor line (61, 70, 80, 97, 98, 144), although negative findings have been reported when older generations have been used (42, 43, 72, 104).

Evidence of morphological changes during treatment has also been noted (68, 70). The interpretation of the effects of estrogens on tumor growth has been complicated by the demonstration by Millar and Noble (89, 91) that, although small doses of estrogens increased the growth rate, larger doses caused a marked depression of tumor growth. Stilbesterol or estradiol in doses from 1 to 10 ug. were stimulatory to early transplant generations, but levels of 50-100 ug. arrested growth. A similar biphasic effect on the growth response of tumors has been presented by Huggins et al. (80). It should be noted that the inhibition of tumor growth by nonphysiological doses of estrogens is associated with a marked reduction in body growth. Before ascribing a specific action to the estrogen (or other inhibitory steroid) comparison must be made with control rats reduced to the same growth rate by dietary restriction (99). Progesterone has been reported to have no effect on tumor growth (91), to inhibit the epithelial part of the tumor (71), or to stimulate growth, particularly when administered with estrogens (78, 80). Most reports agree that androgens suppress tumor development and growth (61, 62, 69-71, 78) and may increase the tendency toward fibroma and sarcoma formation (69-71, 97, 99). Cortisone and cortisol have little effect on tumor growth but may inhibit the number of takes (61, 91). Reference will be made later to the use of a transplanted fibroadenoma for screening steroids for chemotherapeutic activity.

Some findings strongly suggest that pituitary hormones may affect directly the growth of transplantable fibroadenoma (78, 80, 91). From the work of Huggins et al. (79, 80) it may be seen that, following hypophysectomy, tumor growth, although inhibited initially, eventually proceeded at a slow but progressive rate. Estrogen and progesterone combination therapy caused only a moderate acceleration in growth rate. With the additional treatment of growth hormone, the tumor growth rate was restored to normal. Luteotropic hormone (prolactin) could not be substituted for growth hormone, and none of these hormones alone had any effect on tumor growth (79).
Transformation of fibroadenoma to sarcoma may take place during hormone treatment, and it has been noted that any injections which markedly slowed the growth rate favored sarcomatous transformation (69, 70, 92). With such transformation, the hormonal susceptibilities of the original fibroadenomas were largely or completely lost (44, 92, 114). Growth was rapid, and transplantation could readily be effected into rats of either sex (112) and even into rats of different strains (67). Treatment of rats bearing transplanted fibroadenoma with 1,2,5,6-dibenzoanthracene has been reported (15). In male animals no change was observed, but in females the growth rate of the tumor was slowed. No consistent morphological changes in the tumors were found. Sarcoma developed at the injection site. Millar and Noble 1 injected 9,10-dimethylbenzanthracene into slowly growing, transplanted fibroadenoma. In two cases adenocarcinomas were induced in the benign tumor. This type of malignant change had never been observed in control tumors.

INDUCED MAMMARY TUMORS

Mammary tumors may be induced readily in a high percentage of animals by a number of means. The mammary tissue of the rat appears to be highly susceptible to malignant change following treatment with aminofluorene and related compounds, carcinogenic hydrocarbons, and various hormones. Curiously enough, induced tumors, in contrast to the more common spontaneous tumors, are generally carcinomas. Varying degrees of hormone responsiveness have been observed.

1. Aminofluorene compounds.—The orally active carcinogen, 2-acetoaminofluorene, when fed at a level of 0.03 per cent in the diet for periods longer than 95 days, was observed to induce cancer of various tissues by Wilson, DeEds, and Cox (142). They reported three mammary adenocarcinomas, of which one metastasized, in 39 female inbred albino rats of the Slonaker strain. This strain of rats only rarely showed spontaneous tumors of the breast, and these were fibroadenoma. Many workers have confirmed the induction of mammary cancer by feeding 2-aminofluorene (2-AAF), 2-acetylamino-fluorene (2-AAF), or related compounds. The incidence of mammary tumors thus induced has varied from 0 to 100 per cent owing to a number of controlling factors. Female animals were more susceptible than males, and certain strains of rats showed a varied susceptibility. Symeonidis (135) studied five different strains of rats fed 0.03 per cent of 2-AAF for an average of 350 days. In female animals surviving 120 days, none of the nineteen A X C line rats developed mammary tumors. However, 9 per cent of 21 Marshal (M-520) strain, 28 per cent of fourteen Buffalo strain, 64 per cent of 22 Sprague-Dawley, and 62 per cent of 21 Osborne-Mendel strain rats developed mammary tumors. In smaller groups of male animals, the incidence was 0, 0, 0, 35 and 26 per cent, respectively. Dunning, Curtis, and Madsen (26) also investigated the response of five inbred strains including the Copenhagen, August, and Fischer strains. The Fischer rats were the most susceptible, the Marshal and Copenhagen rats were resistant, while the August and A X C strains showed intermediate sensitivity. Bielschowsky (5, 6) noted a striking difference in an incidence of 60 per cent in Wistar strain females compared with only 4 per cent in a piebald strain after the feeding of 2-AAF. Sherman and Wistar rats have been found to exhibit approximately the same susceptibility (11). The induction period for mammary tumors with the more active carcinogens at dietary levels of 0.03 per cent was from 28 to 31 weeks, although this may be shortened by dietary measures (51).

As indicated in the preceding results, female rats have been found to be consistently more susceptible than male animals (7, 11, 65, 134). Some observers have been impressed with the inverse ratio of the induction of liver and breast tumors in female rats by these compounds (135). Animals free from liver tumors may survive sufficiently long to allow breast changes, but on the other hand a possible failure to inactivate estrogens by an altered liver function might be considered as an etiological factor. When 2-AAF was administered in doses of 100 mg. for 3 days it did not show estrogenic activity (5). The age at which feeding of the carcinogen was commenced had an important bearing on subsequent mammary tumor formation (7, 48). In a comparison of the first generation of three strains of rats (which began eating 2-AAF at 117 days of age), with the second generations, which started at 31 days, the incidence increased from 28 to 87 per cent, 64 to 91 per cent, and from 62 to 90 per cent, respectively (135). As might be expected, the dose of the carcinogen consumed in the diet was important. Levels of 2-AAF below 0.001 per cent were not effective in producing tumors, whereas 0.004 per cent induced tumors in 200–300 days. Feeding for periods of only 25 or 50 days lengthened the latent period for tumor development. Some mammary tumors were found in these studies (143). The oral feeding of carcinogens of this type was not essential for the production of mammary tumors, as these have been described following treatment by injections (141).

1 Unpublished observations.
The relative resistance of the male animal to the induction of these tumors has implicated the sex hormones in their production. Breeding females of low tumor strains have been found to be more susceptible than virgin animals (135). Pregnancy was associated with an increased growth rate of the tumor. During lactation, however, growth was arrested, and some regression took place, although normal tumor growth was resumed when the young were weaned. Lactogenic hormone administration was without effect (7). Gonad removal in either sex markedly reduced the incidence of induced mammary tumors—one in eleven ovariec-tomized compared with 23 in 36 normal female Wistar rats; none in five castrated compared with three in 41 normal males (5). The effect of hormones was extensively studied by Kirby (81) and Cantero, Sta-sney, and Paschkis (11, 134). When Sherman or Wistar rats fed 2-AAF were used, a mammary tumor incidence of 30 per cent in females and 0 per cent in males was observed (11). Treatment with 0.125 mg. of estradiol dipropionate 3 times weekly had little effect on the incidence in females, but allowed 6 per cent of males to develop tumors. The administration of 0.5 mg. of testosterone propionate, 3 times a week, prevented any tumor induction in female rats. On the other hand, administration of progesterone (0.5 mg. on the same dose schedule) increased the tumor incidence in female rats to 85 per cent, and the tumors showed a more rapid growth rate. Progesterone did not enhance tumor formation in males or in ovariec-tomized females. Chorionic or pregnant mare serum gonadotrophin did not affect mammary tumor incidence (11, 134). Kirby did not detect any effect of androgens or estrogens on tumor induction (81).

In many cases the tumors induced by aminofluorenes were found to be multiple and in different breasts of the rat. In most reports the mammary tumors were classed as adenocarcinoma, with an occasional squamous, ductal, or papillary-cell carcinoma, carcinosarcoma, or fibroadenoma (6, 7, 11, 74, 102, 103, 143). In one report black-haired random-bred rats showed a predominance of fibroadenomas (121). Transplantation of primary induced tumors has been reported by several workers. In two cases, more slowly growing adenocarcinemomas required estrogen treatment of the host for successful transplantation, although a more rapidly growing tumor arising in a male was apparently hormone-independent (5). The use of litter-mates was required for successful transplantation in one case (143). With inbred Buffalo strain rats, a transplanted tumor (No. 1648) was maintained for over twelve generations (102). Whereas in the first eight generations the tumors tended to remain localized, thereafter they became more invasive and faster growing. Subcutaneous, intra-muscular, or intravenous transplants were all successful. Morphologically, the tumor changed from a carcinoma, with well defined acini and small amounts of stroma, to a tumor showing areas of anaplasia, with sarcoma formation. Alterations in growth rate after successive transplantation have also been described in an induced tumor in AES rats (12). The feeding of 2-AAF to rats bearing transplanted mammary fibroadenoma did not affect their growth rate or induce malignant changes (73). Metastasis from induced tumors occurred not infrequently, particularly if the animals were allowed to survive with large tumors (7, 26, 142).

The diet used for the rats during the experimental period may affect the incidence of mammary tumors induced by 2-AAF (47). It would appear that a lowered incidence may be associated with a restricted caloric intake and that ordinary chow diets may not be entirely adequate for maximum tumor production, or may exert a protective action. Engel and Copeland (51) noted, in two strains of rats, that the use of purified diets allowed a shorter induction time and higher tumor incidence when compared with rats eating stock diets. They believed the stock diets exerted a protective action against tumor formation. Others (137) have supported this view. Dietary riboflavin had little effect (49), but a low fat or high protein level reduced the tumor incidence (141). A simple restriction of caloric intake, irrespective of fat content, was later shown to be associated with reduced induction rate of mammary tumors (50).

Extensive chemical studies have been reported on series of compounds related to 2-AF and 2-AAF. Although the primary motive of the experiments was not concerned with mammary tumors, it may be noted that many compounds showed the capacity to induce typical mammary adenocarcinoma (66, 74, 94, 103, 123, 124). The feeding of hexanitrophenylamine to Wistar rats was believed to cause an increased incidence of multiple breast fibroadenoma (138).

2. *Carcinogenic hydrocarbons.*—

a) Subcutaneous Injection: The injection of carcinogenic hydrocarbons in the rat typically leads to malignancy close to the site of injection. The injection, into areas of mammary tissue, of 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, or 3-methylcholanthrene, in solutions of warm paraffin or wax, has given rise only very occasionally to carcinoma of the breast (24, 25). Geschickter (37) inserted pellets made of 1,2,5,6-dibenzanthracene and 3,4-benzpyrene into the mammary tissue of
male and female rats. Tumors were found in nearly all rats in from 260 to 418 days, but these were all sarcomas, and the adjacent mammary tissue was of normal microscopic appearance. There is little evidence of a general systemic action on the breast from these forms of administration, although the appearance of sarcoma locally may reduce the life span of the animal below the latent period for mammary tumor development.

b) Intramuscular injection: During attempts to modify the local action of the carcinogen, 7,12-dimethylbenz[a]anthracene (DMBA) (formerly referred to as 9,10-dimethylbenzanthracene) was mixed with cholesterol before intramuscular injection. Under some conditions the induction of local sarcoma was inhibited or markedly delayed, and in these rats a high incidence of mammary tumors developed. Rats of a black-hooded random-bred strain received a single injection, intramuscularly into the thigh, of a solution of 5 mg. of DMBA and 10 mg. of cholesterol in 0.5 cc. of sesame oil. After 7 months, twenty female rats had developed mammary tumors, frequently multiple. Histologically, all were typical benign adenofibromas. Transplantation was readily effected into rats of either sex of the same strain (106). It is curious that tumors induced by this means should be adenofibroma, since, as will be seen subsequently, mammary tumors induced by carcinogenic hydrocarbons have, with few exceptions, been adenocarcinomas. The strain of rats used was the same as that found by others to develop fibroadenoma after 2-acetylaminofluorene feeding, to contrast again to the usual adenocarcinoma (121). On the other hand, the long induction period, up to 7 months, is in contrast to that following the intravenous injection or gastric installation of carcinogenic hydrocarbons and may favor the development of histologically benign tumors. Intramuscular injections of DMBA in oil alone in the same black-hooded strain was followed by the development, near the site of injection, of adenocarcinoma of the breast in four of 21 female rats in 4-5 months. In nineteen hypophysectomized animals similarly treated, no mammary tumors were observed (111).

c) Intravenous injection: Repeated intravenous injections of carcinogenic hydrocarbons were first shown by Geyer and collaborators (59) to be followed by the development of mammary tumors in rats. Using Wistar or Sprague-Dawley rats of 150 gm. in weight, they administered twelve weekly injections. In female rats, 24 adenocarcinomas or adenomas and one fibroadenoma were noted, an incidence of 32 per cent. The tumors were often multiple and invaded the surrounding tissues. DMBA was the only effective carcinogen tested. Negative results were obtained under comparable conditions with 1,2-benzanthracene, 3-methylcholanthrene, 1,2,5,6-dibenzanthracene, and p-dimethylaminobenzene. In a later paper (60) emulsions containing 0.22 mg. of DMBA were given by three intravenous injections over 1 week and repeated 3 weeks later. In female Sprague-Dawley rats an incidence of 80 per cent mammary tumors was noted in 48 weeks. The addition of a total of 0.6 mg. of a-estradiol to the injection mixture led to a higher incidence and earlier appearance of tumors. Similarly, diethylstilbestrol, added in a total dose of 5.28 mg., caused an increase in the total number of tumors so that 90-94 per cent of the rats showed tumors in 19 weeks. The estrogens administered alone in 5-6 times the dose did not induce tumors after 25 weeks. The tumors which developed were mostly adenocarcinoma or adenoma (92 in 127 rats), although sixteen fibroadenomas also were found. Scholler (125, 126), using a similar intravenous technic for Wistar rats, induced 89 per cent tumors in 14 weeks, the median time of appearance being 59 days. Pregnancy and lactation did not alter the incidence or growth rate. Physiological and pharmacological doses of estradiol, stilbestrol, progesterone, and estradiol plus progesterone were similarly without demonstrable effect. Transplantation of induced tumors was rarely successful and was not influenced by the treatment of the host. Recipients included rats of both sexes and of various age groups, castrate males, pregnant rats, and rats conditioned by hormone or DMBA treatment (126).

d) Oral administration: Shay and collaborators (131-33) first observed the development of mammary tumors in rats receiving repeated gastric instillations of methylcholanthrene in oil, starting when the rats were 60-80 gm. in weight. In female, random-bred Wistar rats receiving 2 mg. of carcinogen daily, 6 days every week, 82 per cent of the treated rats developed tumors. Male rats and ovariectomized females were much less susceptible. In sixteen of eighteen female rats, tumors developed in an average of 195 days. Tumors in six of fourteen males did not appear until 386 days, and in females spayed when immature only three of eight showed tumors at 466 days. The earliest tumors were observed after 150 days, and multiple growths occurred frequently. Occasionally, metastases to the lung occurred. When steroid pellets implanted in the subcutaneous tissue were used as a form of therapy, it was believed that progesterone and testosterone decreased the occurrence of tumors in females, whereas injection of follicle-stimulating hormone increased the incidence in
males. Adenocarcinomas were most frequently encountered in female rats, but fibroadenomas were more common in males treated with estradiol. It was believed that the hormone balance maintained by treatment influenced the type of tumor which developed. Huggins and collaborators (76, 77) have achieved more rapid induction of tumors in 100 per cent of Sprague-Dawley rats, by the daily oral administration of 10 mg. of 3-methylcholanthrene in sesame oil. In a large series of rats, treatment was started at 50 days of age, and all animals had developed tumors in 60 days. If 10 mg. was given daily for only 10 days, 100 per cent of animals developed tumors by 5 months. Three doses/week were apparently as effective as daily administration. DMBA given orally at a dose level of 1.0 mg. daily induced tumors in all of nine rats by 79 days. Of 680 induced mammary tumors, 678 were classed as carcinoma and two as fibrosarcoma. The normal mammary epithelium showed increased epithelial proliferation. Metastasis was not seen, but infiltration of the tissue was frequent. Most of the tumors studied showed hormonal dependency, since they decreased in size after ovariectomy, and especially following hypophysectomy. A few tumors showed greater autonomy and continued to grow after ovariectomy. Histologically these tumors showed areas of atrophy with other areas of progressive growth. Treatment with 0.1 \( \mu g \) daily of estradiol-17/\( \beta \) allowed a normal incidence of tumors in ovariectomized females. A dose of 1 \( \mu g \) delayed the appearance of tumors; 20 \( \mu g \) caused a further delay, and only one-third of the animals developed tumors. Progesterone, at a dose of 4 \( \mu g \) daily, apparently accelerated the time of appearance, whereas dihydrotestosterone, 1 \( \mu g \) daily, delayed the onset and inhibited tumor development. The stimulatory effects of progesterone could be blocked by estradiol. The growth rate of established tumors 25 days after the cessation of administration of the carcinogen was also studied. Tumors in control rats continued uninterrupted growth. Ovariectomy reduced the growth rate, but this could be accelerated by 5 \( \mu g \) daily of stilbestrol. Hypophysectomy caused a marked slowing in growth, and dihydrotestosterone, 1 or 2 \( \mu g \) daily, also reduced the rate of tumor growth. Mammary cancer development was apparently prevented in hypophysectomized rats over an 8-month period (76). This finding and the similar observation that an injected carcinogen was not effective in inducing mammary cancer in hypophysectomized in contrast to intact rats (111) suggests that pituitary hormones may play an essential role in tumorigenesis of the mammary gland of the rat. Pituitary removal may be looked on as a form of prophylaxis against this type of induced cancer in the rat. Dao and colleagues (14) in studies using orally administered methylcholanthrene have noted an increase in size and number of the induced mammary tumors during pregnancy in Sprague-Dawley rats—an increase from 50 per cent in controls after 65 days to a 90 per cent incidence. Following parturition all tumors were seen to regress immediately and some disappeared completely in 1–3 weeks.

e) Skin painting: Carcinogenic hydrocarbons have been applied to the skin of rats by Orr and colleagues (115–17), and it was noted that this was followed by mammary tumor induction. Rats of three different strains were painted with a solution of either 0.5 or 1.6 per cent DMBA. Mammary tumors developed in 73 per cent of animals in both groups. With the higher concentration of carcinogen, there were more multiple tumors, and the average time of appearance was 12.6 weeks, whereas with the lower concentration 27 weeks was required for tumor induction. A single application of a 1.6 per cent solution of DMBA in olive oil produced six tumors in nine female rats after about 13 months. Solutions of methylcholanthrene when applied by the same technic failed to induce tumor formation in 15 months. Histologically, all the tumors were adenocarcinomas except for two collagenous and one fibrosarcoma. One tumor was transplanted through three generations but exhibited a very slow growth rate. A study of the estrous cycle of the treated rats failed to show any indication of estrogenic action of DMBA. In view of the other work which has been reviewed it would seem likely that skin painting with DMBA was followed by general absorption of the carcinogen so that the breast was influenced by a systemic, rather than a local action.

3. Hormones.—
a) Growth hormone: Evans and Simpson (52) studied the action of prolonged administration of the growth hormone which was available in 1931 and first called attention to the induction of mammary fibroadenoma in rats of the Long-Evans strain. Tumors appeared in ten of sixteen animals treated over a 16-month period. No tumors were observed in similarly treated male rats. In a more recent report (100), highly purified pituitary growth hormone was administered for periods up to 485 days to female rats of the same strain. After 380 days of treatment, commencing with 0.4 mg. daily and gradually increasing to 5.0 mg. of growth hormone, eight of fifteen animals had developed mammary fibroadenoma or fibroma, in addition to tumors of other organs. In a control group, three of fifteen rats showed similar mammary tumors.
Of these, two had an additional pituitary adenoma. It was noted that many of the treated animals showed a local or generalized development of the mammary tissue. The tumors were frequently multiple, and two rats each had eleven tumors. In a similar experiment using hypophysectomized rats, no tumors were observed (101). It would appear that in this strain of rats growth hormone increased the frequency of fibroadenoma above that occurring normally.

b) Estrogens: The continuous administration of estrogen to rats has been followed by a high incidence of mammary tumors, usually carcinoma. It may be noted that secondary changes in the secretion of hormones by the pituitary gland and pituitary adenoma formation also occur following estrogen treatment. Pituitary hormones, therefore, may be implicated in mammary tumor development, and mammotrophin hormone-secreting pituitary tumors have been studied extensively by Furth and collaborators. Although such transplanted tumors may cause a marked stimulation of mammary tissue in Fischer rats, only occasional evidence of tumor formation has been observed under the conditions of the experiments, which were not primarily designed to induce tumors (55). The occasional development of mammary tumors was noted by some workers following the administration of estrogens (39, 86, 88).

Geschickter (56, 57), Noble, McEuen, and Collip (110), on the other hand, observed that a high incidence of tumors could be induced if estrogens were injected or, preferably, implanted subcutaneously as pellets. Additional studies on the induction of mammary tumors by estrogens have been reported by many workers (21, 22, 27, 29, 30, 32–34, 57, 58, 85, 86). Geschickter (56, 57), using an inbred strain of albino rats, produced mammary tumors in all animals by the injection or pellet implantation of estrogens. The time of appearance of the tumors varied with the dose. Injection of 30 μg. daily, of estrone required 600–700 days for the appearance of tumors, whereas 200 μg. daily required only 150–200 days. Pellets of 3–10 mg. stimulated tumor development in 50–200 days, one tumor being noted after only 21 days. Daily injections of 100 μg. of estradiol required 185 days for tumors to appear; the same dose of stilbestrol required 200 days, and, likewise, estrone required 375 days. The tumors were mainly of com edo type, duct and scirrhous carcinoma, frequently multiple, and many metastasized. In a later paper (38) it was shown that the time of appearance of the tumors was inversely proportional to the age of the rat at the start of the experiment. Tumors required 293 days to develop in 1-month-old rats, but only 90 days in those treated when 20 months of age. In total, 202 tumors were induced in 555 rats of either sex, ovariectomized or castrated, and these were mainly carcinomas, although some fibroadenomas were noted. Neither progesterone nor testosterone treatment induced tumors. Dunning and colleagues (32–34) found that different strains of inbred rats showed different susceptibilities to tumor development following estrogen pellet implantation. The August line 990 was the most susceptible but tolerated the treatment with estrone pellets poorly. In the A × C line 9935, 25 per cent of males and 18 per cent of females developed tumors. In Fischer line 344, 7 per cent of males and 16 per cent of females were susceptible, whereas the Copenhagen line 2331 was completely resistant. Pellets made from stilbestrol mixed with cholesterol were more effective than those made from estrone alone. The A × C rats and August rats showed 80–85 per cent tumors, the Fischer 17–22 per cent, while the Copenhagen were again totally resistant. Multiple tumors were found more frequently after stilbestrol than after estrogen treatment. Of 133 tumors examined histologically, 111 were adenocarcinomas, eleven adenocarcinomas and squamous-cell carcinomas, six solid carcinomas, one interductal carcinoma, and four were unclassified. The Copenhagen rats showed a stimulation of mammary tissue by estrogens, but tumor formation did not take place. Nelson (105) observed 68 tumors induced by estrogen in 103 rats of the Long-Evans strain after 300 days treatment. Metastasis occurred in 33 animals. These tumors were classified as duct carcinoma in 42 rats, adenocarcinoma in eight, combined duct and adenocarcinoma in thirteen, and carcinoma simplex in five. Others have found a different susceptibility in inbred and random-bred rats (85), but the Copenhagen line appears to be the only resistant one described. This form of induced carcinoma apparently may metastasize readily (105), but in some reports only occasional secondary deposits have been noted (37, 57, 58, 108). Transplantation has not been studied extensively, but difficulty has been experienced with random-bred rats (108, 109).

The influence of diet on tumor induction by estrogens has been extensively studied by Dunning, Curtis, and collaborators (22, 29, 30). Tumors were induced in the A × C rat by the subcutaneous implantation of cholesterol-diethylstilbestrol pellets. Caloric reduction by 26–38 per cent of the control animals did not reduce the tumor incidence in 67 rats surviving 180 days. Of these, 87 per cent showed multiple mammary cancers, as 236 gross tumors and 337 microscopic tumors. The
latent period for the appearance of tumors was, however, extended to 400 days in the low-calorie group, from a control time of 300 days. Increasing the fat content of the diet tended to reduce the latent period and to accelerate the growth rate of the tumors (29). The tryptophan content of the diet was also considered to be important (22, 30). The addition of 1.4 per cent ni-tryptophan increased the incidence of tumors from 77 to 100 per cent, the control rats having a total of 51 tumors compared with 79 in the experimental group. The treatment of 1.4 per cent DL-tryptophan increased the incidence of tumors from 77 to 100 per cent, the control rats having a total of 51 tumors compared with 79 in the experimental group. The treated group survived only 316 days compared with 363 days for the controls. Of the 161 tumors induced, 107 were papillary cystadenocarcinomas, 44 adenocarcinomas and solid carcinomas, nine showed varying amounts of squamous-cell cancer, and one was unclassified (30). A further study, using a purified diet deficient in tryptophan, indicated that deficient rats did not show as high an incidence of estrogen-induced mammary tumors (56 per cent) as did controls (85 per cent), but survival times were considerably reduced on the deficient diet (22).

The hormonal dependence of tumors in experimental animals was first described in 1941 with estrogen-induced tumors in rats (108). The subcutaneous implantation of estrone pellets into random-bred black, hooded rats was followed by development of adenocarcinoma in 28 of 49 rats, the first tumor appearing after 226 days. In four rats, the surgical removal of the pellet containing the hormonal stimulus was followed by a rapid regression of all mammary tumors. Later reimplantation of pellets into two of these animals led to the reappearance of mammary tumors. Progesterone treatment caused a slowing in growth of the induced mammary tumors in four rats. These experiments were repeated and confirmed (109). Nelson (105) noted tumors induced by estrone injections, in seven of twelve animals, which apparently continued to grow after the cessation of injections, but residual amounts of estrogen might be expected to remain in the animal for a considerable period of time.

**IS A VIRUS FACTOR IMPLICATED IN RAT MAMMARY TUMORS?**

Despite the extensive observations on the role of a viral agent in spontaneous mammary tumors in certain strains of mice, only a few similar studies have been made on rats. These show little evidence that a virus plays any role in the occurrence of mammary tumors. One reference to a translation from a Russian paper states, "a group of Russian workers found a virus-like agent in rat mammary tumors induced by administration of sex hormone" (145). On the other hand, electron microscopical examination of a breast carcinoma (T422), induced in a rat following the intraperitoneal injection of radioactive gold, failed to show any evidence of virus-like particles (128). The most convincing and direct experiments were reported by Dunning, Curtis, and Madsen (21, 27). These observers used inbred Copenhagen rats as a strain totally resistant to estrogen-induced mammary cancer, and the August and A × C strains as sensitive ones (as previously described). They then studied the induced tumor incidence in reciprocal F1 hybrids between resistant and sensitive strains. In a large series of animals the hybrids from both series showed an equal incidence and similar latent periods of tumor development. They concluded, "If the susceptibility of the hybrid to the induced neoplasms was controlled by a factor transmitted in the milk of the susceptible parent (such as obtained in some inbred strains of mice) the percentage of individuals with induced mammary cancer among the progeny of the mothers of the susceptible strain should have equalled that obtained in rats of the susceptible parental strain and should have been zero or relatively low among the progeny of mothers from the resistant strain." Since no differences were found in the two groups of hybrids, no evidence was found to indicate a maternally transmitted etiological factor. It was also pointed out that, unlike mammary cancers in agent-free mice, the rat tumors showed very little variation in morphology. Of a total of 351 tumors examined, 343 were papillary cystadenocarcinomas. In a different type of experiment Ambrus and Harrison (1) inoculated Sherman rats with an extract of mouse mammary glands containing the milk agent. There was no effect on the rat offspring. Normal rats were allowed to foster-nurse young virus-free mice. These remained free of mammary tumors so that an effective agent for the mouse was not present in rat milk.

**MAMMARY TUMOR-HOST RELATIONSHIP**

A few disconnected observations on the response of the host to mammary tumors have been reported. Dickinson, Begg, and Millar (3, 18) noted that liver catalase values were essentially normal for Sprague-Dawley rats bearing transplanted fibroadenomas. However, the sarcomatous transformation of similar transplanted tumors was followed by a 50 per cent loss of liver catalase activity. This change was not explained by an alteration in liver size or growth rate of the tumor. They
also noted that the fibroadenomas caused a significant degree of anemia in the host. Field (53) observed a reduction in the prothrombin time of Sprague-Dawley rats bearing spontaneous fibroadenomas. Increased size of the liver and pituitary gland was also associated. Following tumor removal, all values returned to normal. The cathartic activity of the liver and kidneys has also been found to be increased in the Buffalo strain of rats bearing a transplanted aminophsene-induced tumor (87). The spleens of such animals showed increased extra-medullary hematopoiesis. Rats of the August strain bearing transplanted adenocarcinoma R 2426 were offered a free choice of various diets (10). No effects of diet were noted on tumor growth, nor did the tumor-bearing rats select any diet preferentially. After 6 weeks’ growth the transplants averaged 11.05 gm. in weight per rat. The radiosensitivity of the preceding tumor has been studied by Eisen (38). The tumor proved to be considerably more resistant than the Walker tumor in Wistar rats (17). Irradiation of 5,500 r in vitro was required to abolish proliferative capacity of R 2426. With less irradiation, some tumors grew slowly after a prolonged latent period. Subsequent transplantation showed a growth rate like the original, so that no induced changes in the cells, altering their growth rate, had occurred. Treatment of growing tumors with 12,000 r did not always cause complete regression. Heiman (67) compared the sensitivity to x-radiation of fibroadenoma and fibrosarcoma arising from them. He noted that 5500 r and 3500 r, respectively, were required to effectively destroy the cells.

Two reports concern a study of metastasis. In one (31) the transplantable adenocarcinoma R 2426 was successfully implanted into the prostate gland. Metastasis was not noted after estrogen stimulation, although induced prostatic carcinoma in the strain of rats used regularly metastasized to bone. Wallace (139) studied the metastasis from a group of sarcomas, all of which originated from a transplantable mammary fibroadenoma. The occurrence of spontaneous metastasis varied with the different tumors observed and could be increased by mechanical means. Intravenous inoculation was followed by metastasis but only in the sublines which were capable of spontaneous metastasis. Either the tumors possessed different intrinsic properties to metastasize, or the resistance of the host was differently affected.

A high content of estrogenic substance occurring in transplanted mammary fibroadenoma in rats has been reported (72). Others, however, failed to confirm this observation (95, 144).

CHEMOTHERAPEUTIC STUDIES

Recent interest has centered on the possible use of a transplantable mammary tumor of the rat which might serve for chemotherapeutic studies. Some type of hormonally sensitive tumor would be required to screen compounds of steroid structure. As indicated in this review, certain tumors, especially the fibroadenoma and the carcinoma induced by orally administered carcinogens, have been used by Huggins and collaborators for assay purposes. The tumors which appear suitable for further consideration will be briefly summarized. Eisen (37) originally noted that estradiol benzoate, 0.166 μg. twice weekly, caused an inhibition of growth rate of adenocarcinoma R 2426 carried in the August strain of rats. Caloric restriction of controls, however, resulted in similar alteration in the tumor growth rate. Testosterone had no effect. Dunning (20) more recently has used the same tumor and found that estrone, 20 μg. daily, and progesterone, 1 mg. daily, had no effect on tumor growth; deoxycorticosterone acetate, 6 mg. daily, had a slight stimulatory effect. Cortisol, 2–4 mg. daily, and testosterone, 2 mg. daily, caused a significant reduction in the growth rate. The estrogen-induced carcinoma, which showed such a striking dependence on estrogens for continued growth, has not yet been used for steroid assay purposes (108). Unless such tumors would retain their hormonal dependency after transplantation, possibly in pure strain rats, their usefulness would be limited.

The benign fibroadenoma studied by Huggins et al. (78, 80) showed a consistent growth rate over successive transplant generations and has been found to be particularly hormone-sensitive. Growth stimulation followed treatment with small doses of estrogen and progesterone. Its particular value, however, was for the assay of the growth-inhibiting properties of many compounds of the androstanne series. Huggins and Mainzer (78) found 2-a-methyl-dihydrotestosterone to be the most active inhibitor tested and presented evidence indicating that the property of inhibiting tumor growth did not necessarily parallel androgenic activity. This tumor has recently been studied by others (61) and has also been used to screen steroids of the androstane series (62). The quantitative method described appeared to yield consistent results and was readily performed. With some compounds the antitumor action was again not related to androgenic activity. The potential danger in using fibroadenomas for quantitative assay procedures has been previously pointed out. Any tendency for alterations in growth or hormone re-
sponsiveness in progressive transplantations must be noted.

Adenocarcinomas induced by 2-acetylamino-fluorene have not been used for steroid assay purposes, although, as has been pointed out, some tumors showed a marked stimulation by progesterone (11).

Tumors induced by the oral administration of carcinogenic hydrocarbons appear to fill most of the requirements for quantitative assay studies (77). It has already been noted that these induced adenocarcinomas respond to the female sex hormones, and inhibiting effects of an androgen have been demonstrated (77). The primary induced tumors could be used for assay purposes, or possibly further studies will show that transplants may retain hormonal responsiveness.

REFERENCES


38. ———. The Occurrence of Benign and Malignant Mammary Lesions in Rats Treated with Crystalline Estrone. Ibid., pp. 632–44.
50. FIELD, J. B. Prothrombin Activity in Rats with Mammary Lesions in Rats Treated with Crystalline Estrogen. Ibid., 1:457–64, 1941.
68. ———. Growth of Transplanted Mammary Fibroadenoma in Castrated Rats Injected with Hormones. Ibid., 39:172–77, 1940.


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R. L. Noble and J. H. Cutts