A Genetic Analysis of the Induction of Tumors by Methylcholanthrene

III. Local and Remote Induction of Carcinoma of the Mammary Gland*

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In 1939, Strong and Smith (21) reported on the local induction of carcinoma of the mammary gland in mice by methylcholanthrene. Ten female mice that were being used as breeders developed such tumors at or near the site where the carcinogen had been injected subcutaneously (1 mgm. dissolved in 0.1 cc. sesame oil and injected at 60 days of life). Eight of these mammary tumors were in NH strain mice; 2 were in mice of the JK strain. It was noteworthy that such mammary carcinomas had been induced in only 2 out of 7 distinct inbred strains of mice used in the experiment. It was further significant that these 2 strains (NH and JK) were genetically related and were both characterized by a very low incidence of spontaneous tumors of the mammary gland.

Burdette (5) obtained 1 adenocarcinoma of the mammary gland in a CBAN breeder female mouse by the biweekly painting of a 1 per cent solution of methylcholanthrene dissolved in benzene to the skin of the back for 139 days. Kirschbaum (10) obtained 5 similar tumors in F strain female mice. These tumors occurred in 3 of 5 mice which had been injected intravenously with a suspension of methylcholanthrene in horse serum and 2 occurred in mice injected intraperitoneally with the same carcinogen and vehicle. Mice of the F strain are similarly very resistant to the occurrence of spontaneous tumors of the mammary gland. Mice of the CBAN strain show an intermediate degree of susceptibility. It has recently been reported by Bonser (2) that the injection of relatively large amounts of methylcholanthrene (2 mgm. dissolved in lard given subcutaneously in the right flank in 2 doses at an interval of 5 weeks) will produce tumors including adenocarcinomas of the mammary gland in mice. In a later report in which the histology of these tumors was described, Bonser and Orr (3, 4) stated that 23 of the 160 tumors induced in the above series were adenocarcinomas of the mammary gland. Nine of these were unassociated with other types of neoplasias and all occurred in female mice at the site of injection of the carcinogen. It is not stated whether or not the female mice were being used for breeding.

The occurrence of mammary tumors in rats subsequent to the implantation of paraffin pellets containing variable amounts of methylcholanthrene has been reported by Dunning, Curtis, and Eisen (8). The changes in the mammary tissue adjacent to the wax cysts were described and the major effect appeared to be upon the duct epithelium. Examination of a representative number of the nodular areas which occurred immediately proximal to the implanted material revealed that from 15 to 20 per cent of the masses contained mammary tissue. Among these, from 12 to 45 per cent contained areas of squamous epithelium. In from 5 to 30 per cent there was evidence in the mammary ducts of the transition of columnar into squamous epithelium. Areas of metaplastic squamous epithelium were more frequent in nodules from female hosts but were not confined to them. The earliest changes were observed in females, 9 to 30 days after injection. These consisted of the partial transformation of the epithelium of the mammary ducts and the formation of squamous epithelium in hyperplastic mammary ducts. In the total number of tumors examined, 159 or 17 per cent contained areas of breast tissue, of which approximately one-half showed hyperplastic changes, and about 30 per cent of which contained squamous epithelium. In only 5 per cent, however, was the relationship between the mammary ducts and the presence of squamous epithelium uninvolved with tumor growth. These investigators stated further “the mechanism of the action of methylcholanthrene on breast epithelium is unknown but a direct irritative effect appears probable. The reaction is not comparable with the effect of the estrogens.”

Perry and Ginzton (14), using unpedigreed albino stock mice (origin unknown), reported carcinomas of the breast in spayed female mice painted with a 0.3 per cent benzene solution of 1,2,5,6-dibenzanthracene and theelin. They did not obtain mammary carcinomas by the carcinogen alone in normal female mice, but did report six obtained in normal mice with the carcinogen and theelin. They conclude that the “incidence of carcinoma of the skin is chronologically related to the development of carcinomas of the breast.”

Materials and Methods

One of us (16, 18) has been making a survey of the specific types of tumors and an analysis of the frequency distribution induced in mice of the NH descent by 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil and injected subcutaneously at 60 days of life. Ultimately it is intended to analyze this material genetically, in order to ascertain whether or not genetic factors are involved in the origin of these specific types of induced tumors.

This communication presents observations on 800 mice of the NH strain, of which 384 were females and 416 were males. The mice were weaned at 30 days of age and placed in reserve until 30 days later,
at which time, 60th day of life, 1 mgm. of methylcholanthrene dissolved in 0.10 cc. of sesame oil was injected subcutaneously on the right side. The sexes were not separated and the ensuing young were discarded within 24 hours post-partum (forced breeding).

Female mice of the NH descent have, up to the present time, never developed spontaneous tumors of the mammary gland (either in the virgin state or under forced or normal breeding). The present series of 800 methylcholanthrene-injected mice includes all mice in lineal descent. Consequently variability as brought about by selection following hybridization cannot be a factor in influencing frequency distribution and specific types of tumors induced in this present experiment.

Results

The data obtained with reference to mammary gland changes (hyperplasia, metaplasia, and neoplasia) are given in Table I. Forty-five mice showed definite evidence of carcinoma of the mammary gland, 40 of which were the usual adenomatous type found in mice (Figs. 1 and 2) and 5 were of the scirrhous type, less frequently found in cases of spontaneous mammary carcinoma. Eleven of the carcinomas of the adenomatous type showed squamous metaplasia (Figs. 3, 4, and 5). Of the 45 cases of carcinoma of the mammary gland, 21 occurred unaccompanied by any other type of tumor, whereas 24 were associated in varying degrees of anatomical intimacy with other types of tumors as follows: 12 with spindle cell sarcoma, 8 with carcinoma of the skin, 1 with primary carcinoma of the lung and rhabdomyosarcoma, and 3 with spindle cell sarcoma and carcinoma of skin.

In addition to these 45 definite carcinomas of the mammary gland, two other changes in mammary tissue were also found. These were (a) 9 cases of nonmalignant hyperplasia and (b) 11 cases of squamous metaplasia of mammary tumors (all in tumors of the adenomatous type). These alterations occurred in mice showing tumors other than those of mammary origin as follows: 7 cases of mammary hyperplasia associated with spindle cell sarcoma (Fig. 6); 2 cases of hyperplasia with spindle cell sarcoma and carcinoma of the skin; 3 cases of squamous metaplasia of mammary tumors occurring along with carcinoma of the skin; and 8 cases of squamous metaplasia of neoplastic mammary tissue unassociated with any other type of tumor (Figs. 3 and 4).

In all instances squamous metaplasia was associated with cyst-like formations, marked keratinization, and extensive inflammation. In certain areas the mastitis was in a definitely suppurative state; in others, small accumulations of polymorphonuclear leukocytes were the only evidence of inflammatory processes. The general picture, however, indicated that in all of the metaplastic tumors an extensive mastitis had been present for a considerable time.

All mammary changes occurred only in female mice. The frequency distribution of mammary tumors is compared to those for other types of tumors in these 800 NH mice in Fig. 7.

Table I: Data on Tumors and Lesions of Mammary Gland Observed in 800 Female NH Mice

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>No. of mice</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Mammary gland carcinoma</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>a. Usual adenomatous carcinoma of mice</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>b. Scirrhous type</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>c. Squamous metaplasia (These mice included under a.)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>II. Mammary gland carcinoma, alone or associated with other tumors</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>a. Alone, with no other tumor</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>b. With spindle cell sarcoma only</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>c. With carcinoma of the skin only</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>d. With lung tumor and rhabdomyosarcoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>e. With spindle sarcoma and carcinoma of the skin</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>III. Nonmalignant hyperplasia of mammary gland</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>a. With spindle cell sarcoma</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>b. With carcinoma of the skin</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>c. With spindle cell sarcoma and carcinoma of the skin</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>IV. Squamous metaplasia in mammary gland carcinoma</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>a. With carcinoma of the skin</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>b. With spindle cell sarcoma</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>c. In mammary gland carcinoma alone, no other tumor in animal</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

The average age of those mice which developed mammary carcinoma alone was 159.8 days following the injection of the methylcholanthrene (146.6 days for those which showed squamous metaplasia; 167.9 days for the adenomatous type). The average age of mice showing both mammary tumors and an associated other type of tumor was 146.1 days. Forty-two of these mammary tumors arose at sites adjacent to injection points (locally induced); 3 on the opposite side of the body (remote induction).

General Discussion

The occurrence of mammary gland changes as a result of the injection of estrogens in both male and female mice parallels genetic susceptibility, as stated in reviews by Gardner (9) and Loeb (12); that is,
Figs. 1 and 2.—The more typical mammary tumors which occurred in the mice of the NH strain. Mag. X 100 and X 150, respectively.

Figs. 3 and 4.—A mammary tumor which has undergone considerable squamous metaplasia. Note polymorphonuclear leukocytes and cornification. There were no skin lesions in this animal. Mag. X 120 and X 100, respectively.

Fig. 5.—Subcutaneous area of mammary hyperplasia, adjacent to a metaplastic (squamous) mammary carcinoma. No skin lesions in animal. Mag. X 100.

Fig. 6.—Focal area of mammary hyperplasia adjacent to a spindle cell sarcoma. Mag. X 150.
the full development of neoplasias of mammary origin in a male mouse has not, with few exceptions, been brought about by estrogens except in mice of strains highly susceptible to carcinoma. There are no reports of the production of mammary tumors by estrogen treatment alone in mice belonging to a strain genetically resistant to cancer. Thus it is indicated that the response to the estrogen may serve as a measure of genetic susceptibility. Twombley (22), however, with a combination of foster nursing and estrogen treatment, has been able to obtain carcinoma of the mammary gland in male mice of the C57 black (cancer-resistant) strain. Other criteria, however, such as (a) tolerance to salicylaldehyde (17, 19), (b) changes in hemoglobin levels at different ages (15), and (c) abundance of porphyrins in the Harderian glands (20) also parallel and may be used as an index of genetic susceptibility to carcinoma.

That a possible estrogenic effect of the carcinogen used may be a factor in the origin of carcinoma of the mammary gland of this series must be taken into consideration. Cook and Dodds (7) reported that 100 mgm. of three phenanthrene derivatives (9,10-dihydroxy-9,10-di-n-butyl-9,10-dihydro-1,2,5,6-dibenzoanthracene, 5,6-cyclopenteno-1,2-benzanthracene and 1,2-benzpyrene) dissolved in sesame oil brought about estrous in mice. They conclude that since massive doses were used the reaction must be considered qualitative and not quantitative as compared with estrone. Carminati (6) was unable to find estrogenic activity with 1,2-benzpyrene in rats. Perry (13), however, using 31 mgm. of 3,4-benzpyrene (1,2-benzpyrene) in 0.75 cc. of lard was able to report cornified vaginal smears within 48 hours of the subcutaneous injection of this carcinogen. In view of (a) the massive doses used and (b) the discrepancies of results obtained by several investigators in measuring estrogenic activity of carcinogens, it is perhaps unwarranted to maintain that the induction of mammary carcinomas in this present series was brought about by an estrogenic effect of the carcinogen upon mammary tissue (only 1 mgm. of methylcholanthrene was injected once and the tumors arose later after several weeks). Lewis and Turner (11) have, perhaps, indicated a possible explanation in that they find that 1.5 mgm. of 1,2,5,6-dibenzanthracene, even though non-estrogenic, will bring about mammary growth. They conclude that, "in this particular it compares with the mammogenic duct growth factor of the anterior pituitary."

The present experiment may indicate another mechanism in the origin of mammary tumors. So far these mammary tumors have occurred only in female mice of cancer-resistant strains, which have been used for breeders. Whether they would arise in virgins of the same strain is not known as yet. There is sufficient evidence available to demonstrate that mammary tumors never arise in male mice of the NH strain after the injection of methylcholanthrene. On the other hand, the induction of mammary tumors in breeder female mice (45 out of 384 or 11.7 per cent) is highly suggestive. That these tumors have been obtained in three strains (JK, NH, and F), all of which are highly refractory to the spontaneous occurrence of mammary tumors, indicates that another mechanism than the one accepted for the induction of mammary tumors by estrogens may be operating. It is not likely (although not impossible) that the presence of methylcholanthrene should take the place of genetic susceptibility. Neither does it seem likely that methylcholanthrene could destroy or inactivate genetic resistance to mammary tumors.

The present experiment also suggests that in the origin of the spontaneous tumors of mammary tissue there may be a carcinogenic agent which carries the process of oncogenesis further than does the intrinsically produced estrogen. In other words, the estrogen stimulates mammary tissue and maintains the pubertal and gestational growth phases but some other agent initiates the true neoplastic changes in the physiologically altered (estrogen-stimulated) mammary tissue. Such a concept would bring together the observations on (a) spontaneous mammary tumors arising in different genetic strains, (b) occurrence of tumors of similar origin in both males and females by estrogens (only in genetically susceptible strains), (c) the induc-
tion of similar tumors by methylcholanthrene in genetically resistant strains, and (d) the "physiological use" factor of Bagg (1).

**SUMMARY**

In a series of 800 mice of the NH descent injected with 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil at 60 days of age, 45 showed definite evidence of carcinoma of the mammary glands. These carcinomas all occurred only in female mice. In addition to these neoplasms, hyperplasia of mammary tissue and squamous metaplasia of mammary tumors were also found in the treated animals. These types of mammary tissue response occurred separately and in combination with other types of tumors, such as (a) spindle cell sarcoma, (b) carcinoma of the skin, and (c) rhabdomyosarcoma. Mice of the NH descent are characterized by showing a high resistance to spontaneous tumors of mammary origin.

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