Acceleration of the Development of Mammary Carcinomas in Mice by Methylcholanthrene*

J. Engelbreth-Holm

(From the Cancer Research Laboratory of the Danish Anti-Cancer League, Copenhagen, Denmark)

(Received for publication November 16, 1940)

An account was given in the previous article (7) of a series of experiments in which 9,10-dimethyl-1,2-benzanthracene, painted on the skin or subcutaneously injected, assisted or accelerated the development of leukemia not only in an inbred strain of mice, Aka, in which leukemia develops spontaneously in a large number of the animals that are over 8 months old, but also in Little's dilute brown strain, in which spontaneous leukemia occurs rarely, with an incidence of about 2 per cent. In the latter strain accelerated development was also observed in the case of mammary carcinoma, which occurs spontaneously in about 50 per cent of the females above the age of 9 months. It was doubtful, however, in this case whether the treatment had actually increased the incidence of mammary carcinoma, though there was no doubt that the animals treated developed breast cancer considerably earlier than those not treated.

Meanwhile, in another series of experiments, an undoubted acceleration of mammary carcinoma was attained, as was evident both from the increased number of tumors and from their earlier appearance.

Material and Technic

Mice of Little's dilute brown strain were used, and in this strain, as already stated, mammary carcinoma develops spontaneously in about 50 per cent of breeding females over 9 months old. Most of the tumors did not develop before the age of 12 months. In a few cases, about 2 per cent, leukemia developed spontaneously in both male and female animals that were over 6 months old.

Animals from 4 to 6 weeks old were painted once a week with a 0.5 per cent solution of methylcholanthrene in benzene in a manner similar to that employed by Murphy and Sturm (15) and by Morton and Mider (13), the substance being applied at different places so as to avoid, to some extent, the development of painting tumors in the skin. The painting was continued for 23 weeks, after which the animals were given no further treatment. At this point some of the animals had already developed mammary carcinoma, although others did not develop it until later, a period of freedom thus existing between the cessation of the treatment and the development of the tumor. In the cases of 2 animals this interval lasted for as long as 2 months.

Results

Twenty-seven females and 26 males were treated as described above. During the experimental period 8 females and 2 males died from intercurrent diseases, mostly enteritis or pneumonia.

All the remaining 19 females died from mammary carcinoma, 17 of them with multiple carcinomas. Those that survived longest were 10½ months old. One of the animals, No. 458, also had incipient lymphogenous leukemia when it died. This, in view of its low age, only 6 months, may also, presumably, be considered as due to the treatment. The changes in the individual animals are tabulated in Table I.

Table II shows the ages at death of these 19 animals compared with 19 animals, from the same generations of the strain as those from which the test animals originated, dying with mammary carcinoma.

It appears from Table II that the treatment with methylcholanthrene caused a very considerable acceleration of the tumorous development, which became manifest in the animals treated far earlier than is usual in this strain. Additional evidence of this acceleration was the fact that all the 19 females that survived the first 5 months of the experiment developed mammary carcinoma, while only 50 per cent of the untreated animals of this strain did so. Finally, the accelerated development was shown by the occurrence of more than 1 mammary carcinoma in each of 17 of the 19 test animals. Double breast cancers, and on one rare occasion a triple cancer, were occasionally observed in the untreated females, in about 10 per cent of the cases, whereas among the 19 test animals 5 were observed with 2, 8 with 3, 1 with 5, 2 with 6, and 1 with 7 breast cancers.

While an undoubtedly great acceleration of the development of mammary carcinoma was observed

* This investigation was aided by a grant from The Lady Tata Memorial Trust and an anonymous grant for the study of breast cancer.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/2368014/crs0010020109.pdf by guest on 13 August 2022

TABLE 1: CHANGES IN 19 FEMALE MICE, DLB, PAINTED WITH METHYLCHOLANTHRENE, 0.5 PER CENT IN BENZENE, AT VARIOUS PARTS OF THE SKIN.

Mouse no.	Age at death, in months	Findings
440	8	6 mammary carcinomas, in right axilla, right side of the neck, both inguinal regions, all about 15x15x18 mm., also 2 small ones in perineum. No metastases.
442	10	3 mammary carcinomas, towards the back of the neck, 26x24x20 mm., in both axillae 10x10x10 mm. No metastases. Adenocarcinomas.
443	8	5 mammary carcinomas, in left axilla 27x 24x18 mm., ulcerated, right axilla, 18x 15x10 mm., perineum 10x8 and 10x6x 3 mm. Spleen slightly enlarged normal. Heart large, pale, myocarditis with small abscesses. Metastasis in the lung. Adenocarcinomas.
445	8	3 mammary carcinomas, in left axilla 5x 5x5 mm., left inguinal region 14x3x 10 mm., and right inguinal region, 30x20x18 mm., ulcerated. Adenocarcinoma with metaplasia to squamous cell epithelium. No metastases.
446	5½	Mammary carcinoma, in perineum 20x11x 11 mm. No metastases. Adenocarcinoma.
450	10½	3 mammary carcinomas, in right inguinal region, right axilla, 7x5x4 mm., left axilla, 6x5x5 mm. No metastases. An adenocarcinoma of the usual type, 2 mammary carcinomas with great metaplasia to squamous cell epithelium, partly with cornification.
451	7	2 mammary carcinomas, in right and left axillae, 15x15x10 and 18x11x10 mm. No metastases. Adenocarcinoma.
454	10	3 mammary carcinomas, in left axilla, 22x 14x11 mm., left inguinal region, 16x 14x12 mm., right axilla 10x10x10 mm. Adenocarcinomas. One of these with metaplasia to squamous cell epithelium. The mouse previously had small nodes, presumably papillomas, on the snout and cheek. These disappeared spontaneously. No metastases.
455	10½	2 mammary carcinomas, in the left side of the neck, 17x17x10 mm., right inguinal region, 23x22x17 mm. No metastases. Adenocarcinomas.
457	8	2 mammary carcinomas, in right side of the neck, 12x12x9 mm., left inguinal region 19x14x10 mm. No metastases. An adenocarcinoma with patchy metaplasia and a solid squamous epithelial carcinoma, apparently originating from mammary glands.
458	6	Mammary carcinoma. Leukemia. An adenocarcinoma, 21x18x18 mm. in right inguinal region. No metastases. Spleen large, 24x7 mm., dark. Liver slightly enlarged. Lymphnodes normal. Lymphogenous leukemic infiltration in liver and spleen.
459	10	2 mammary carcinomas, on the right side of the neck, 19x13x12 mm. and in perineum, 12x10x8, and right axilla, 27x21x14 mm. No metastases.
469	7	7 mammary carcinomas from 8 to 17 mm. in diameter along the lymphatics on the left side of the neck, in left axilla, left inguinal region, 2 in perineum, right side of the thorax, right inguinal region.
472	8	3 mammary carcinomas, in perineum, 28x 16x14 mm., right inguinal region, 15x 13x6 mm., right axilla, 6x6x6 mm. No metastases. Adenocarcinomas.
473	10	2 mammary carcinomas, in right axilla, 29x21x15 mm., and left axilla 5x5x 4 mm., both ulcerated. No metastases. The large tumor an adenocarcinoma of the usual type, the small one a solid mammary carcinoma without metaplasia to squamous cell epithelium.
474	7	2 mammary carcinomas, in right axilla, 26x22x15 mm., and right inguinal region, 8x7x7 mm. No metastases. Adenocarcinomas with diffuse transition to squamous epithelial carcinoma.
478	9	2 mammary carcinomas, in right axilla, 27x18x8 mm., right inguinal region, 12x10x10 mm., and in left axilla, 14x 14x10 mm. Spleen and liver slightly enlarged, thymus normal, lymphnodes about 6 mm., large. No metastases. Adenocarcinomas, one with a sarcoma-like stroma very rich in cells. Leukemoid reaction in liver, spleen, lymphnodes.
481	8½	6 mammary carcinomas, in left side of neck 20x15x15 mm., ulcerated, in both axillae, both inguinal regions, and in perineum, tumors 10 to 18 mm. in size. No metastases. Adenocarcinomas with varying, very pronounced metaplasia to squamous cell epithelium.
493	8	3 mammary carcinomas, in both inguinal regions, 45x22x22 mm. and 14x12x 8 mm. Some metastases in the lungs. Adenocarcinomas with metaplasia to squamous cell epithelium. Myocarditis with diffuse abscesses.

in the females, as was evident not only in the increased number of tumors in the individual animals, but also in the generally increased incidence and earlier appearance of the tumors, the same treatment applied to 26 males of the same strain did not produce any case of breast cancer.

Two of the 26 males died from intercurrent diseases; the remaining 24 animals had all survived 9 months of experiment when they were 10 months old, whereas nearly all the females had by then died from mammary carcinoma. Later, at the age of 11 or 12 months, 6 of these male animals had died with small or large skin papillomas situated on the snout, cheek, neck, breast,

Downloaded from http://aacrjournals.org/cancerres/article-pdf/2368014/crs0010020109.pdf by guest on 13 August 2022

or abdomen. Large ulcerated skin carcinomas with much cornification were found in a couple of them; these must, presumably, be considered as local painting tumors. In no case was breast cancer or leukemia observed.

TABLE II: DISTRIBUTION OF AGES AT DEATH OF 19 DLB MICE TREATED WITH METHYLCHOLANTHRENE AS COMPARED WITH 19 UNTREATED MICE DYING OF SPONTANEOUS MAMMARY CARCINOMA

Age at death in months	6	7	8	9	10	11	12	13	14	15	16	17	Total number of mice
Mice treated with methylcholanthrene	2	3	6	2	4	2	..	..	..	..	..	..	19
Untreated mice with spontaneous mammary carcinoma	..	..	..	1	1	2	3	3	4	4	..	1	19

DISCUSSION

The development of mammary carcinoma in inbred strains of mice is dependent on at least 3 factors: hereditary disposition; hormone milieu, especially estrogenic hormones; and a factor, as yet unexplained, that is transmitted through the mother's milk during the suckling period. Observations on these subjects and reviews of the literature have been presented in the papers of Bittner (1), Lacassagne (10), and Gardner (8).

In addition to these factors, it is necessary to take into consideration exogenous influences in many cases. Carcinogenic hydrocarbons are the best known examples of these exogenous influences but there undoubtedly are others. While many investigations have been made into the importance of heredity and the influence of mother's milk, and the significance of estrogenic hormones in the development of breast cancer has been much discussed, exogenous influences have rarely been studied with reference to the development of breast cancer.

It is certainly doubtful whether any of these factors that have been shown to be of significance in the development of mammary carcinoma can, *per se,* produce cancer. The malignant tumor does not develop until 2, or all 3, of the factors act simultaneously. It is possible, furthermore, that these 3 influences, even in cooperation, cannot produce a tumor without some exogenous influence, which, however, need only be very slight. From the experiments reported here, there can be no doubt that exogenous carcinogenic influences may be of importance, even of great importance, in the development of mammary carcinoma in mice.

Previous research has rarely shown such acceleration of the development of tumors. Kreyberg (9) and Bonser and Connal (3) painted mice of Kreyberg's white label strain with tar, but found no influence on the mammary carcinomas occurring spontaneously in this strain. Cramer (5) found in similar experiments a rather lower incidence of mammary carcinoma in tarred mice than in untreated animals. Dobrovolskaïa-Zavadskaïa and Adamowa (6) also found that the incidence of breast cancer was unchanged, or even lower, when a strain with about 50 per cent mammary carcinoma was treated with tar, 1,2,5,6-dibenzanthracene, or radon.

On the other hand, Maisin and Coolen (12) reported that painting with methylcholanthrene and with benzpyrene, in addition to producing tumors at the site of painting, accelerated the development of mammary carcinoma to some extent; but it is difficult to judge the results of their experiments, as there is no mention of controls.

Experiments conducted in 1939 at the Marie Curie Hospital (11) showed that estrone alone was not able to produce mammary carcinoma, but the simultaneous action of estrone and 1,2,5,6-dibenzanthracene produced a number of breast cancers, the carcinogenic hydrocarbons reinforcing, or supplementing, the action of estrone.

Such considerable acceleration of the development of mammary carcinoma by the influence of carcinogenic hydrocarbons as in the experiments here described has, however, not been observed before. It is difficult to explain why the previous experiments, mentioned above, did not show a similar acceleration of the development of tumorous processes. It may be that the various carcinogenic substances do not act uniformly in this respect, or that the mode of application and the dosage of the substance are important. The facts are still somewhat obscure. In previous experiments (7) 7 cases of acceleration of mammary carcinoma, 3 cases of leukemia, and several cases of papilloma or carcinoma at the site of painting were produced in 16 female mice of the same strain as those in the experiments reported here, by painting the skin of the back 2 or 3 times a week with 9,10-dimethyl-1,2-benzanthracene. In the present experiments mammary carcinoma appeared early, and was often multiple, in all the females painted on various sites with methylcholanthrene. The difference may perhaps be explained by the method of painting, for it is possible that the avoidance of local tumors at the site of application may explain the increased number of mammary carcinomas. These experiments, however, shed no light upon the undetermined question of the antagonism between tumors produced by painting and mammary carcinoma.

The difference between the results obtained by Mider and Morton (13) from painting dilute brown mice with methylcholanthrene and the results reported here is still more extraordinary. Exactly the same technic and the same carcinogenic hydrocarbons were employed in both experiments. While Mider and

Downloaded from http://aacrjournals.org/cancerres/article-pdf/2368014/crs0010020109.pdf by guest on 13 August 2022

Morton obtained a large number of accelerated leukemias in their experiments, accelerated mammary carcinomas appeared in the present experiments in all the females, whereas only 1 case of leukemia developed. Since the same technic was used for both experiments, the difference must be sought in the strains of mice employed; both of these were certainly dilute brown strains, but they were nevertheless different, since Mider and Morton stated that in their strain "breast cancer developed spontaneously in from 80 per cent to 90 per cent of the breeding females at a mean age of 10.6 months and that a 'lymphoblastoma' occurs commonly in both sexes between 650 and 800 days." In the strain used for the present experiments only about 50 per cent mammary carcinoma is observed in the breeding females and from 1 per cent to 2 per cent leukemia in the untreated animals of both sexes.

Both these strains originate from Little's dilute brown strain, and yet they are clearly distinct, both with regard to the incidence of spontaneous mammary carcinomas and leukemias and with regard to their reaction to painting with methylcholanthrene. That such a "cleavage" can occur in an inbred strain has been shown for dilute brown mice by Burrows (4), who found 4 lines with 93 per cent mammary carcinoma and 1 with only 33 per cent in 5 lines originating from 5 pairs belonging to this strain.

These facts show that the acceleration of the spontaneous tumors of a given strain that can be obtained by treatment with carcinogenic hydrocarbons is dependent on the genetic constitution of the animals. That, with regard to the mammary carcinomas, it is also dependent on the presence of estrogenic hormones, is suggested by the fact that in these experiments mammary carcinomas appeared in all the females but in not a single male. Whether it is also dependent on a factor transmitted with mother's milk is not yet known.

The experiments reported here show that the development of mammary carcinoma in an inbred strain of mice can be accelerated to a pronounced degree and can also be accentuated by the influence of methylcholanthrene. This acceleration of the development of tumors has been observed only in females, not in males. The results, together with those of other workers, indicate that this action of carcinogenic hydrocarbons is dependent on the genetic constitution of the animals.

Summary and Conclusions

Twenty-seven females and 26 males of Little's dilute brown mice were painted once a week with 0.5 per cent methylcholanthrene in benzene on various parts of the skin. All the 19 surviving females developed mammary carcinoma. Seventeen of the animals had more than 1 breast cancer. The tumors developed far earlier than is usual in this strain, the animals dying when from 8 to 10 months old as contrasted with the untreated mice which survived 13 to 15 months. No mammary carcinoma occurred in the males, but after the age of 10 or 12 months, there were some cases of papillomas and carcinomas of the skin.

Methylcholanthrene thus accelerated the development of mammary carcinoma in the females.

References

1. Bittner, J. Relation of Nursing to the Extra-Chromosomal Theory of Breast Cancer in Mice. Am. J. Cancer **35**: 90-97. 1939.
2. Bittner, J. "Influences" of Breast-Cancer Development in Mice. Pub. Health Rep. **54**:1590-1597. 1939.
3. Bonser, G. M., and K. I. Connal. Effect of the Presence of a Malignant Tumour Upon the Development of a Second Malignant Tumour. J. Path. & Bact. **48**:263-274. 1939.
4. Burrows, H. Spontaneous Cancer of the Breast in Mice. British Empire Cancer Campaign, 16th Ann. Rep. 46. 1939.
5. Cramer, W. On an Antagonism in the Development of Malignancy in Two Different Organs. J. Path. & Bact. **43**:77-89. 1936.
6. Dobrovolskaïa-Zavadskaïa, N., and N. Adamova. Réaction, à différents agents cancérigènes, de souris appartenant à la même lignée cancéreuse. Bull. Assoc. franç. p. l'étude du cancer **28**:76-107. 1939.
7. Engelbreth-Holm, J., and H. Lefèvre. Acceleration of the Development of Leukemias and Mammary Carcinomas in mice by 9,10-Dimethyl-1,2-benzanthracene. Cancer Research **1**:102-108. 1941.
8. Gardner, W. U. Estrogens in Carcinogenesis. Arch. Path. **27**:138-170. 1939.
9. Kreyberg, L. On the Susceptibility to Cancer Development in the Skin and in the Mammary Gland in Two lines of Inbred Mice. Am. J. Cancer **24**:554-565. 1935.
10. Lacassagne, A. A Relationship of Hormones and Mammary Adenocarcinoma in the Mouse. Am. J. Cancer **37**: 414-424. 1939.
11. Marie Curie Hospital, Cancer Research Committee. British Empire Cancer Campaign, 16th Ann. Rep. 121. 1939.
12. Maisin, J., and M. L. Coolen. Au sujet du pouvoir cancérigène du méthyl-cholanthrène. Compt. rend. Soc. de biol. **123**:159-160. 1936.
13. Mider, G. B., and J. J. Morton. The Effect of Methylcholanthrene on the Latent Period of Lymphomatosis in Dilute Brown Mice. Am. J. Cancer **37**:355-363. 1939.
14. Morton, J. J., and G. B. Mider. The Production of Lymphomatosis in Mice of Known Genetic Constitution. Science **87**:327-328. 1938.
15. Murphy, J. B., and E. Sturm. Primary Lung Tumors in Mice Following the Cutaneous Application of Coal Tar. J. Exper. Med. **42**: 693-700. 1925.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/2368014/crs0010020109.pdf by guest on 13 August 2022