

Influence of Carcinogens on the Age Incidence of Leukemia in the High Leukemia F Strain of Mice*

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Percutaneous application of methylcholanthrene, 3,4-benzpyrene, and 9,10-dimethyl-1,2-benzanthracene dissolved in benzene caused an increase in the total incidence, and the precocious appearance, of leukemia in mice of the dba strain (7, 10, 11). Approximately 30 per cent of untreated mice of this strain develop the disease (7). Similar treatment with methylcholanthrene (6) and benzpyrene and methylcholanthrene (3) of two high leukemia strains hastened the appearance of the disease but decreased the total incidence, since many mice died from induced neoplasms other than leukemia. Mice of the low leukemia Rf stock responded to skin painting with and subcutaneous injection of methylcholanthrene and benzpyrene by exhibiting both a higher incidence and an earlier appearance of the disease than controls. Hybrids between a high (Ak) and a low leukemia (Rf) stock responded in a similar manner (3). Several strains (NH, C57, CHI) with a low incidence of spontaneous leukemia failed to respond to the leukemia-inducing activity of carcinogens (6, 7, 11). On the other hand, some strains (Swiss, Rf, C3H, Buffalo) with a very low incidence of spontaneous leukemia developed a high incidence following treatment with carcinogenic chemicals (3, 11). Three of 4 strains (dba, Ak, F, C58) which have a high incidence of the spontaneous disease developed leukemia earlier in life when treated with methylcholanthrene, benzpyrene, or 9,10-dimethyl-1,2-benzanthracene (2, 3, 6, 7, 10, 11, 12).

The present report concerns further work on the reaction of the high leukemia F strain to carcinogens, a preliminary report (6) having been given on the reaction of this strain and 3 low leukemia strains to the percutaneous application of methylcholanthrene.

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MATERIALS AND METHODS

A 0.5 per cent solution of 3,4-benzpyrene in benzene was painted twice weekly on the skin of 32 strain F mice according to the technic of Morton and Mider (10). In 7 mice the treatment was begun at birth, in the other 25 at an average age of 35 days. Thirty-nine male mice of the low leukemia C3H strain were treated similarly. One hundred and seven strain F mice were painted in like manner with methylcholanthrene. A preliminary report has appeared on 83 of these mice (6). Thirty strain F mice were painted twice weekly with methylcholanthrene beginning at birth. Thirteen strain F mice were painted twice weekly, beginning at approximately 35 days of age, with a 0.3 per cent solution of 1,2,5,6-dibenzanthracene dissolved in benzene. Twenty strain F mice were painted similarly with benzene, the vehicle for the carcinogens; 20 mice received weekly injections of 0.001 cc. of benzene in sesame oil, a treatment which is said to have induced leukemia in market mice (8, 9). Thirteen strain F mice, 6 females and 7 males, received at 6 weeks of age 2 intravenous injections of methylcholanthrene dispersed in horse serum (a total of 0.2 cc. of a dispersion containing 1 mgm., more or less, per cc.). The dispersion of methylcholanthrene in horse serum was made by the method of Andervont and Lorenz (1). A total of 212 strain F mice served as controls. All mice were maintained on the same diet, Purina fox chow.

Mice were examined daily. Diagnosis was made from gross findings and tissue imprints stained with a May-Grünwald-Giemsa combination (5). A cytological study was made in every instance.

RESULTS

Twenty-four of 107 strain F mice (22.4 per cent) developed leukemia before 200 days of age when painted with methylcholanthrene dissolved in benzene beginning at 35 days of age, and 32 of the 107 (30 per cent) before 300 days of age. Five of 32 (16 per cent) developed leukemia before 200 days of age when

painted with 3,4-benzpyrene, and 15 of 32 (47 per cent) before 300 days of age. Only 5 of 212 (2.3 per cent) untreated strain F controls developed the disease before they had reached the age of 200 days, 29 of 212 (13.7 per cent) before 300 days. No mice of a group of 12 painted with dibenzanthracene developed leukemia before 300 days. In the instances given above the carcinogens were dissolved in benzene. Controls similarly treated with benzene alone developed leukemia no earlier than untreated controls: 2 of 29, or 7 per cent, before 300 days of age. Animals injected with benzene dissolved in sesame oil developed leukemia at perhaps a somewhat earlier age than untreated controls: 0 per cent before 200 days of age, but 30 per cent before 300 days of age. Table I summarizes these results.

TABLE I: OCCURRENCE OF LEUKEMIA IN CONTROL STRAIN F MICE AND STRAIN F MICE RECEIVING TREATMENT WITH CARCINOGENS AND BENZENE

Treatment	Number of mice	Number developing leukemia before 300 days of age	Number of leukemias	Number dead without leukemia	Number still alive
None	212	29	108	70	34
Methylcholanthrene in benzene *	107	32	3	75 †	0
Methylcholanthrene in benzene ‡	30	10	10	20 †	0
3,4-Benzpyrene in benzene	32	15	17	15 †	0
1,2,5,6-Dibenzanthracene in benzene ...	12	0	5	7	0
Benzene	29	-	2	Mice living beyond 300 days of age killed at 440 days of age	
Benzene in sesame oil §	20	6	7	Mice living beyond 300 days of age killed at 375 days of age	
Methylcholanthrene in horse serum ...	13	1	6	7	0

* Treatment started at 35 days of age.

† High death rate due to skin tumors.

‡ Treatment started at birth.

§ Subcutaneous injection.

|| Intravenous injection.

The period of skin painting with methylcholanthrene before leukemia appeared was approximately 130 days, whether treatment was begun at birth or at 35 days of age. The average age at which leukemia appeared was 134 days if painting was begun at birth, 162 days if it was begun at 35 days of age. The average age at which the disease appeared in the benzpyrene-painted animals was 235 days, after slightly more than 200 days of treatment. If only the first 33 per cent of the animals to develop leukemia are considered (to make the figures comparable with the methylcholanthrene group) the number of days of painting required was 175, or 40 more than in the methylcholanthrene group. The latent period in the dibenzanthracene group was about 375 days.

Animals treated with 3,4-benzpyrene developed more mediastinal lymphosarcomas than did those treated with methylcholanthrene. Methylcholanthrene favored the appearance of systemic leukemia in young ani-

mals; in untreated strain F mice the usual disease in very young animals is mediastinal lymphosarcoma. Lymphosarcoma and leukemia have been tabulated together as "leukemia" (Tables I, II, and III).

The efficiency of the carcinogen, when dissolved in benzene and applied to the skin, in hastening the appearance of leukemia could be correlated with the potency of the carcinogen in inducing the development of skin tumors in these mice. No mice survived skin painting with methylcholanthrene for more than 200 days without developing either skin cancer or leukemia, whereas 12 of 32 mice treated similarly with 3,4-benzpyrene did not develop either skin cancer or leukemia until after 200 days of treatment. In those treated with dibenzanthracene no skin tumors appeared before 200 days of skin painting had elapsed.

Among all mice treated by percutaneous application, 53 of the 64 that developed leukemia (Table III) were free of skin tumors, whereas only 8 of the 117 treated

TABLE II: AVERAGE DAYS OF PAINTING PRECEDING LEUKEMIA WITH 3 DIFFERENT CARCINOGENS

Carcinogen	Average days of painting preceding leukemia
Methylcholanthrene	134 days (30-33 per cent developed leukemia)
3,4-Benzpyrene	175 days for first 33 per cent; 200 days for 53 per cent
1,2,5,6-Dibenzanthracene ...	375 days

mice that did not develop leukemia were free of either skin carcinomas or papillomas. In all cases suppuration was associated with the skin cancer. No lung tumors were found on gross examination. Most of the 30 C₃H male mice which were treated twice weekly with

3,4-benzpyrene developed leukemia.

Intravenous treatment of methylcholanthrene did not decrease the incidence of leukemia. Leukemia developed in 10 of 10 mice before 300 days of age (one case in females). Leukemia had been all mice before 300 days of age.

tumor was carcinoma of the skin.

py (1 of 10) of the 10 mice.

3,4-benzpyrene developed skin tumors, but none developed leukemia.

Intravenous injection at 6 weeks of age of 0.2 mgm. of methylcholanthrene dispersed in horse serum did not decrease the latent period for the development of leukemia. Of 11 strain F mice so treated only one developed leukemia before 200 days of age, and this was the only mouse of the group to develop leukemia before 300 days of age. The incidence of spontaneous mammary cancer in the F strain is less than 1 per cent (one case in 111 control female breeders), but 3 of 6 females receiving methylcholanthrene intravenously developed mammary cancer. All these animals had been allowed to breed and rear young. Two of these mammary cancers appeared at 180 days of age, and the animals were sacrificed at 208 days of age, when the

carcinogen relatively more mice died of skin tumors before 200 days of age. Dibenzanthracene painting did not alter the latent period (as found in untreated animals) for leukemia. The effectiveness of these carcinogens in decreasing this latent period was proportional to their effectiveness in producing skin cancer in this strain.

In studying the influence on leukemia in the F strain of an artificial nongenetic factor, 3,4-benzpyrene thus far appears to be the carcinogen of choice since it is better tolerated than methylcholanthrene (fewer skin tumors at an early age) and more effective than dibenzanthracene. It has not yet been determined whether the total incidence of leukemia in the F strain can be increased by lowering the concentration of the solution of benzpyrene in benzene, thus perhaps

TABLE III: SKIN TUMORS IN RELATION TO THE APPEARANCE OF LEUKEMIA IN STRAIN F MICE RECEIVING PERCUTANEOUS APPLICATIONS OF CARCINOGENS DISSOLVED IN BENZENE

Carcinogen	Number of mice	Leukemic mice—64		Nonleukemic mice—117	
		No skin tumors	Skin tumors	No skin tumors	Skin tumors
Methylcholanthrene†	107	29	3	5	70
Methylcholanthrene‡	30	9	1	1	19
Benzyrene	32	10	7	1	14
Dibenzanthracene	12	4	1	1	6
Totals	181	52	12	8	109

* "Skin tumors" includes carcinomas and papillomas of appreciable size.

† Treatment begun at 35 days of age.

‡ Treatment begun at birth.

tumors had reached a considerable size. One tumor was an adenocarcinoma, the other a squamous cell carcinoma. The third mammary cancer, a squamous cell carcinoma, appeared at 478 days of age. In both squamous cell carcinomas the origin of the cancers could be traced from ductal epithelium. The carcinogen had been injected intravenously into the tail vein, a site distant from the axillary region, in which all the mammary tumors arose.

DISCUSSION

Following the percutaneous application of 3,4-benzpyrene to mice of the dba strain, Morton and Mider (11) found the latent period preceding the appearance of leukemia to be greater than with methylcholanthrene. The incidence of the disease also was lower. In the present experiments with the F strain it was found too that the latent period was longer with 3,4-benzpyrene than with methylcholanthrene, but the total incidence of leukemia was greater. The latter findings can perhaps be attributed to the longer latent period preceding the development of skin tumors in the F strain when benzpyrene rather than methylcholanthrene was used. In the case of the latter car-

decreasing the incidence of skin tumors without too appreciably affecting the power of the carcinogen to induce leukemia.

Apparently those treated F mice that did not develop skin tumors were more susceptible to the induction of leukemia than were those that did (Table III). It is possible that the development of skin cancer exerted some inhibitory action on the development of leukemia, or that the suppuration associated with the skin tumors represents an inhibitory mechanism. In contrast to these findings, however, Engelbreth-Holm and Lefèvre (2) have observed that in mice of the Ak and Dlb strains painted with 9,10-dimethyl-1,2-benzanthracene, "no antagonism was observed between the occurrence of the painting tumor and the development of mammary carcinoma or leukemia."

While the percutaneous application of benzene did not significantly alter the age incidence of leukemia in a group of 29 strain F mice, there is evidence that perhaps this vehicle for the carcinogens can appreciably shorten the preleukemic latent period and increase the incidence of leukemia in the dba strain, although not to the same extent as either methylcholanthrene or 3,4-benzpyrene (11). Morton and Mider (11) also

found that the carcinogens were more effective when dissolved in benzene than in acetone. It is likely that in the series of F mice injected with benzene in sesame oil (Table I) the appearance of leukemia was accelerated, but not nearly so decidedly as in animals receiving methylcholanthrene or 3,4-benzpyrene dissolved in benzene.

In our experience the response of mice to carcinogens with respect to the development of leukemia has been in direct proportion to the susceptibility of each strain of mice to spontaneous leukemia (6). In the C3H strain painting with methylcholanthrene raised the incidence of leukemia from 1 per cent (3 of 280 animals) to 33 per cent (3 of 94 animals). The disease in untreated animals appeared when they were between 400 and 600 days of age, whereas induced leukemia appeared in mice between 100 and 300 days of age (6). Among 39 C3H mice treated similarly with 3,4-benzpyrene no leukemias appeared within the first 300 days of life. It is of interest that treatment with appropriate doses of estrogenic hormones raised the incidence of leukemia appreciably in the C3H strain (4). In the C3H stock used by us the estrogens represent a more potent leukemia-inducing agent than either methylcholanthrene or 3,4-benzpyrene. Neither the NH nor C57 strain, both low leukemia stocks, developed leukemia when treated with methylcholanthrene (percutaneous application). Other investigators have observed, however, that the incidence of leukemia can be definitely increased in certain low leukemia stocks by treatment with carcinogens (2, 7, 11).

The latent period between the institution of treatment and the appearance of leukemia was not significantly different, whether percutaneous application was begun at birth or at 35 days of age. This observation is in keeping with the findings of other investigators, who have noted, in addition, that older animals are perhaps not so responsive to the leukemia-inducing action of carcinogenic agents as those 2 months of age or younger (7, 11).

The intravenous injection of methylcholanthrene did not affect the age incidence of leukemia in the small group of mice used. It is possible that either the route of administration or the vehicle used was responsible for the ineffectiveness of the carcinogen. Also it is probable that the total quantity of carcinogen made available to the animal was not sufficient to effect the early appearance of leukemia. The development of mammary cancer in 3 out of 6 females is a finding in keeping with the observations of Strong and Williams (13); namely, that treatment with methylcholanthrene increases the incidence of mammary cancer in females of a low mammary cancer strain.

SUMMARY

The efficiency of methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene, when dissolved in benzene and applied to the skin, in hastening the appearance of leukemia in the high leukemia F strain of mice bore a direct relation to the potency of the carcinogens in inducing other tumors in these mice. Both methylcholanthrene and benzpyrene were effective in shortening the preleukemic latent period, methylcholanthrene being the more active in this regard. Dibenzanthracene did not decrease the preleukemic latent period, although this carcinogen does induce skin tumors. The latent period (between institution of treatment and the appearance of leukemia) was the same whether treatment was begun at birth or at 35 days of age. The effect on the appearance of leukemia could be attributed to the action of the carcinogens and not to the vehicle, benzene. The treated mice which did not develop skin tumors readily were more susceptible to the induction of leukemia than those which developed skin papillomas and carcinomas relatively early. Intravenous injection of 0.2 mgm. methylcholanthrene at 6 weeks of age did not influence the age of appearance of leukemia in F strain mice, but 3 of 6 female breeders developed mammary cancer. The incidence of mammary cancer in untreated female breeders of the F strain is less than 1 per cent.

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