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Some Effects of Carcinogenic Agents on Mice Subject to Spontaneous Leukoses*†

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During the past 2 years we have been investigating the effect of painting with methylcholanthrene on the development of leukosis in dilute brown (strain Dba) mice (4, 5). The strain has been extensively studied by Little and Murray (3, 6, 7). Although it is generally considered to have a high incidence of spontaneous mammary carcinoma, several sublines have been developed in which the tumor incidence varies. Line 212 has a relatively high incidence of spontaneous "lymphoblastoma" or lymphomatosis while in line 122 breast tumors predominate. Pulmonary tumors are rarely found. The exact incidence of spontaneous nonepithelial tumors is not known. They occur less frequently than in the C57 black mice. In the latter strain, investigated at the Roscoe B. Jackson Memorial Laboratory, 13.79 per cent of 877 mice developed nonepithelial tumors when 700 to 750 days old (3).

Spontaneous lymphomatosis appears in dilute brown mice at an age of 650 to 800 days at the Jackson Laboratory. It is found in the dilute brown stock at the New York State Institute for the Study of Malignant Disease in mice more than 500 days old. The leukosis is characterized grossly by splenomegaly and enlargement of 1 or more groups of lymph nodes. In the spontaneous disease the mesenteric, retroperitoneal, and mediastinal nodes are involved more frequently than the cervical, axillary, or inguinal groups. The microscopic picture is that of leukemic infiltration of organs and tissues with cells resembling lymphoblasts.

The original experiments were performed on a group of 50 dilute brown mice obtained from the Jackson Laboratory. They represented a mixture of various lines of the strain. They were painted twice weekly until death with a 0.5 per cent solution of methylcholanthrene in benzene at 9 successive sites. Three different types of leukosis were produced in 77 per cent of the group (Table I).

The most frequent lesion (75.5 per cent) was a general lymphomatosis in which most of the lymph nodes of the body were enlarged. Splenomegaly and hepatomegaly were present. Anasarca occurred as a terminal event. The cell population in the affected tissue was similar to that seen in the spontaneous disease. The abnormal cells replaced the lymph nodes and bone marrow completely and infiltrated liver, spleen, intestine, kidneys, bladder, genitalia, pancreas, skin, muscle, and subcutaneous tissues. Hyperleucocytosis from 50,000 to 300,000 was present. Transplantation of affected tissues to mice of the same strain reproduced the widespread adenopathy and infiltrations of the original disease.

TABLE I: LEUKOSIS IN DILUTE BROWN MICE

Agent	Number of mice	General lymphomatosis	Mediastinal lymphoma	Reticulo-endotheliosis	Total leukoses
Methylcholanthrene 0.5% in benzene	48	28	8	1	37
3,4-Benzpyrene 0.5% in benzene	48	20	10	0	30
Benzene	50	10	1	0	11

Solitary mediastinal lymphomas were found in 21.5 per cent of the mice that developed leukosis. Other than a large mediastinal mass, no gross tumor was demonstrable. Abnormal cells that appeared to be identical with those previously described were found only in the mediastinal tumor. The bone marrow, lymph nodes, and blood stream were not involved.

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Transplantation of 3 of these tumors to 30 other dilute brown mice failed.

The 3rd type of lesion was a pathological curiosity which has been termed reticulo-endotheliosis. We have observed only 1 case. The gross features resembled those of general lymphomatosis. The type of cell that characterized this condition was large, acidophilic, and frequently multinucleated. It appeared to be actively phagocytic. The distribution was that seen in general lymphomatosis. The hematopoietic elements of the bone marrow were depleted.

A similar group of dilute brown mice was painted in the same manner with a 0.5 per cent solution of 3,4-benzpyrene in benzene. Only 62.5 per cent of them developed leukosis. General lymphomatosis comprised 66.6 per cent. Half of these showed no gross lymphadenopathy although the characteristic type of cell was present in the usual distribution. The variation following benzpyrene resembled that found in the spontaneous disease. This might have been due to the difference in chemical structure, the diminution in the total stimulus, or to substrain differences in the mice that were painted.

Fifty more dilute brown mice were painted with benzene alone. This experiment has been in progress for more than 540 days. Six mice are still alive and appear healthy. Ten of the 44 that have died had a modified form of general lymphomatosis. Generalized lymphadenopathy was found in 2 of these. In the other 8 only abdominal lymph nodes were involved. Infiltration of liver, spleen, lung, and kidneys was noted, yet the bone marrow showed little or no change. The mean age at which the lymphomatosis was found, 496.3 days, leads one to question whether benzene alone had any pronounced influence on the latent period [1] of the spontaneous disease.

These preliminary observations established that some carcinogenic agents could be made to influence the latent period of leukosis in dilute brown mice. A breeding colony of line 212 stock was obtained from the Jackson Laboratory. This facilitated study of the disease since the lineage of each animal obtained by brother-sister mating was known. It is unfortunate that adequate controls are lacking. We have not had a single case of spontaneous leukosis in any of our breeding colony to date. Thirty of them have lived more than 500 days. Mammary carcinomas, primary lung tumors, and a sarcoma of the uterus have been found.

One hundred fifty-six identified line 212 dilute brown mice survived painting with a 0.5 per cent solution of methylcholanthrene in benzene for more than 60 days. The incidence of leukosis in these animals was 98.7 per cent. Two died without tumor, 150 had general lymphomatosis, and 4, mediastinal lymphoma. The sex did not influence the reaction. The incidence and latent period of leukosis were almost identical for males and females (Table II).

TABLE II: EFFECT OF SEX ON INDUCED LEUKOSIS IN LINE 212 DILUTE BROWN MICE

	Males	Females	Total
Incidence, per cent	98.7	98.6	98.7
Mean latent period, days	86.1 ± 1.4	90.0 ± 1.4	87.7 ± 0.9
Standard deviation	13.4	11.1	12.4
Variation	15.5%	12.3%	13.7%

The effect of the age of the mouse at the time that painting was commenced has been investigated (Table III). Little variation in the latent period was found before the mice were 80 days old. In the following age groups, 81-100 and 100+ days, the latent period was prolonged. Further experiments with older mice have been commenced. One might expect that painting older mice would hasten the appearance of the disease. The increase in time observed suggests that the animals developed some degree of resistance either to the action of methylcholanthrene or to the tumor cells. Although this question cannot be decided at present, the following evidence may be cited.

TABLE III: EFFECT OF AGE ON LATENT PERIOD OF INDUCED LEUKOSIS IN LINE 212 DILUTE BROWN MICE

Age in days when painting started	Number of mice	Latent period, in days
1-20	8	89.6
21-40	41	86.7
41-60	31	86.2
61-80	45	91.9
81-100	11	95.8
101+	14	99.5
Total	150	87.8

The age of the mouse within 60 to 160 days did not influence the latent period or growth rate of transplanted lymphomatosis. If the prolonged latent interval in older mice was due to relative increase in resistance to the neoplasm, then the period from onset to death should be longer. This factor is subject to considerable variation but no significant change in relation to age could be demonstrated.

In the subsequent experiments the line 212 mice were divided into identified litter mate groups. One of these groups was painted with a 0.5 per cent solution of methylcholanthrene in benzene, the other with the chemical or solvent under investigation (Table IV). The incidence of leukosis was not

[1] Latent period refers to the time elapsed between the first painting with methylcholanthrene solution and the appearance of detectable lymphadenopathy.

altered, but the latent period was lengthened when a 0.25 per cent solution of methylcholanthrene in benzene was used. An equal concentration of methylcholanthrene in acetone resulted in approximately the same latent period as did the benzene solution, but the incidence of leukosis was reduced. More mediastinal lymphomas occurred than in any previous group. Five of the 22 females that were painted developed breast tumors without evidence of lymphomatosis. Breast carcinomas were present in groups painted with carcinogenic agents in benzene solution also. These, however, were associated with general lymphomatosis. If the mice with mammary tumors were added to those with leukosis, the tumor incidence, 92.8 per cent, approached that found for the other groups painted with methylcholanthrene. Acetone solutions of methylcholanthrene might have a different action than benzene solutions. Benzene affects the hematopoietic system. When already damaged it might be more susceptible to the action of methylcholanthrene than one that remains relatively normal when painted with acetone. This problem is receiving further consideration at present.

TABLE IV: LEUKOSIS IN LINE 212 DILUTE BROWN MICE

Agent	Number of mice	General lymphomatosis: Number	General lymphomatosis: Latent period, in days	Mediastinal lymphoma: Number	Mediastinal lymphoma: Latent period, in days	Per cent leukosis
Methylcholanthrene 0.5% in benzene	156	150	87.8	4	91.7	98.7
Methylcholanthrene 0.25% in benzene	44	41	100.1	2	89.0	97.7
Methylcholanthrene 0.25% in acetone	53	38	103.1	5	114.6	81.6
3,4-Benzpyrene 0.5% in benzene	49	38	126.4	1	113.0	79.5

Control.....No leukosis in 30 mice living more than 450 days.

The experience with a 0.5 per cent solution of 3,4-benzpyrene in benzene bore out the differences between the action of methylcholanthrene and benzpyrene described in the literature. The latent period was prolonged and the incidence of leukosis was decreased. The 10 mice that did not develop leukosis lived at least 122 days and the mean duration of painting them was 156 days. The general lymphomatosis that followed painting with benzpyrene was essentially the same as that induced by methylcholanthrene. The variation noted in the unidentified group of dilute brown mice must have been due to a mixture of different substrains, probably a difference in the genetic pattern of the ancestors.

Identified dilute brown mice of line 122 (high mammary carcinoma) were painted in the same manner with a 0.5 per cent solution of methylcholanthrene in benzene. Characteristic general lymphomatosis developed in each of the 12 mice that were painted. The latent period was 122 days. No breast tumors were obtained.

Little is known concerning the reaction of the blood leucocytes to painting with methylcholanthrene in benzene. The mice with fully developed lymphomatosis had a leucocytosis exceeding 100,000 cells per cu. mm. More than 90 per cent of them were immature cells resembling lymphoblasts. Serial leucocyte counts indicated that the characteristic abnormal cells appeared in the circulating blood 2 to 4 weeks before lymphadenopathy could be recognized. Their numbers were small at first but increased rapidly. The neutrophilic elements became depleted before the lymphocytes did. Some of the mice showed evidence of an anemia. Megaloblasts and normoblasts appeared in the peripheral blood. In a mouse that developed a breast tumor without leukosis in the course of painting the leucocytes rose rapidly. This was due to an increase in adult segmented neutrophiles. Myeloblasts and myelocytes were rarely seen. The organs of this mouse did not show evidence of leukemic infiltration. The bone marrow was hyperplastic but showed no evidence of a maturation defect.

Five other strains of mice were painted with a 0.5 per cent solution of methylcholanthrene in benzene in the same manner as were the dilute browns. C albino, C3H and C57 black strains were obtained from the Roscoe B. Jackson Memorial Laboratory, and the old and new Buffalo strains were from the Biological Station of the New York State Institute for the Study of Malignant Diseases. All of the mice were between 4 and 6 weeks old when painting was started. The results indicated that the occurrence of spontaneous leukosis in a mouse strain does not mean that painting with methylcholanthrene will increase the incidence or decrease the latent interval of production (Table V). The incidence of spontaneous leukemia is relatively high in C albino and C57 black stock, yet no cases were obtained after the administration of methylcholanthrene. The incidence of induced leukosis was low in the other 3 strains. Again, adequate controls are lacking so that no comparison with the spontaneous rate may be made. Leukoses followed painting with methylcholanthrene only in those strains of mice that had a high incidence of spontaneous mammary carcinoma.

The leukosis produced in old Buffalo, new Buffalo, and C3H mice by methylcholanthrene was morphologically identical with that seen in the dilute brown strain. The spontaneous disease affecting the C57 black

family appears to be the same also. While we have termed them lymphomatoses this designation is open to criticism. The identification of immature mouse leucocytes by staining reactions is fallible. Morphologic studies are inconclusive and facilities for studying the characteristic cells *in vitro* have been lacking. Such an error in interpretation of the fundamentally affected cell would account for the bizarre results.

None of the mice that developed a primary lung tumor had concurrent lymphomatosis. Death from pulmonary neoplasm cannot be the sole reason why these animals did not develop leukosis. The latent period for each of the tumors was approximately the same. Skin tumors, epidermoid carcinomas, and subcutaneous sarcomas, usually occurred somewhat earlier than did leukosis. It is possible that they killed the mice before a leukosis had an opportunity to appear. The presence of concurrent skin and lung tumors or skin tumors and leukosis was infrequent.

TABLE V: EFFECT OF METHYLCHOLANTHRENE 0.5 PER CENT IN BENZENE ON 5 STRAINS OF MICE

Strain	Number painted	Days painted	Leukosis	Tumors			No tumor
				Skin	Lung	Breast	
Old Buffalo	37	125	2	21	18	0	9
New Buffalo	29	125	5	14	13	0	8
C3H	46	174	5	38	11	1	5
C albino	42	174	0	21	16	0	14
C57 black	34	250	0	8	0	0	26

In the C albino and C57 black strains nonmalignant extramedullary myelopoiesis was a common autopsy finding. This lesion, described by Lewis (2), and by Barnes and Sisman (1), was characterized by enlargement of the liver and spleen. The lymph nodes were not grossly altered. The histologic appearance of the organs was similar to that seen in myeloid leukosis. Evidence of erythropoiesis and a preponderance of adult neutrophiles readily distinguished the lesion from a true leukosis. The bone marrow was hyperplastic but evidence of maturation was seen. The heterotopic cells which characterized the methylcholanthrene leukosis were not encountered. The condition could not be transmitted to mice of the same strain by subcutaneous transplantation of affected organs or tissues. Extramedullary myelopoiesis was also found in dilute brown mice that were painted with benzene alone.

SUMMARY AND CONCLUSIONS

The appearance of leukosis in dilute brown mice may be hastened by painting them with: (a) a 0.5 per cent solution of methylcholanthrene in benzene, (b) a 0.25 per cent solution of methylcholanthrene in benzene, (c) a 0.25 per cent solution of methylcholanthrene in acetone, or, (d) a 0.5 per cent solution of 3,4-benzpyrene in benzene. Painting with benzene alone does not have an effect of the same magnitude. The incidence of leukosis in line 212 of the dilute brown strain is greater after painting with methylcholanthrene than after painting with benzpyrene. Preliminary reports of the alterations in the blood leucocytes that precede the disease are presented.

Leukosis was found in old Buffalo, new Buffalo, and C3H mice after painting with a 0.5 per cent solution of methylcholanthrene in benzene but not in the C57 black or C albino strains. All of these mice are known to develop spontaneous leukosis although the incidence is not established.

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