THE EFFECT OF METHYLCHOLANThRENE ON THE LATENT PERIOD OF LYMPHOMATOSIS IN DILUTE BROWN MICE

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Some carcinogenic agents may alter the type, incidence, or time of appearance of spontaneous mouse tumors. Lung tumors have been found to appear at an earlier age and in higher incidence among susceptible mice after treatment with tar (16), 1:2:5:6-dibenzanthracene (1, 2, 9, 19), or methylcholanthrene (2). Maisin and Coolen (11) observed an increase in the occurrence of breast tumors among female mice painted with methylcholanthrene. Definite data regarding the influence of carcinogens on leukoses in mice are, however, lacking.

Mercier (12, 13) noted the appearance of lymphosarcoma after the intraperitoneal injection of tar in mice of a strain in which the disease also developed spontaneously. Andervont (3) observed lymphocytomas after subcutaneous injection of 1:2:5:6-dibenzanthracene in C albino mice, which are known to have a high incidence of spontaneous leukosis. Perry and Ginzton (17) found leukemia among the stock mice that they treated with 1:2:5:6-dibenzanthracene and estrogens, and Burrows and Cook (5) have described leukemia and sarcoma following subcutaneous injection of 1:2:5:6-dibenzanthracene-9:10-endo-αβ succinate. The intrasplenic injection of crystalline 3:4-benzpyrene in mice of the S strain was followed by a leukemia with atypical cells (4). The influence of the carcinogenic agent in the genesis of these leukoses has not been studied. This paper will present a study of the effect of methylcholanthrene on the production of tumors in a strain of genetically uniform mice susceptible to spontaneous lymphomatosis.

Dilute brown (dba) mice were obtained from the Roscoe B. Jackson Memorial Laboratory, where the strain has been maintained by brother-sister mating for more than 25 generations. Between 80 and 90 per cent of the breeding females of this strain develop breast cancer at a mean age of 10.6 months (standard deviation 2.7 months) (14). Among virgin females the incidence of mammary cancer is 51 per cent (15). Lung tumors occur in less than 5 per cent of the animals. Data regarding the occurrence of other spontaneous neoplasms in the strain are incomplete. "Lymphoblastoma" (8) occurs commonly in both sexes between 650 and 800 days. The abdominal lymph nodes are most frequently involved. Mediastinal lymphadenopathy occurs more often than cervical, axillary, or inguinal, usually as a part of a generalized process. The liver is sometimes involved by metastasis or by a primary tumor classified as an endothelioma.

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Figs. 1A and B. General lymphomatosis in dilute brown mouse, characteristic gross appearance; liver, spleen (S), and lymph nodes (L) enlarged.

Figs. 2A and B. Transplanted lymphomatosis: graft (T) twenty-one days old; lymph nodes (L) enlarged; liver and spleen normal.

Fig. 3. Great enlargement of liver and spleen (S) in general lymphomatosis; lymph nodes also enlarged.

Fig. 4. Mediastinal lymphoma (T): liver and spleen (S) of normal appearance.
The mice were between four and five weeks old at the beginning of the experiment. They were not allowed to breed. Methylcholanthrene, 0.5 per cent in commercial benzene, was painted on the skin twice weekly until death by the method of Murphy and Sturm (16), the site being changed with each application so that the same area was not painted twice in a month. Contiguous regions were not painted in succession. The order was: head, left hind leg, right hind leg, left foreleg, right foreleg, sacral region, abdomen, interscapular region, anterior thorax. The first tumor appeared on the 69th day of the experiment. The last two mice died on the 226th day. All animals were autopsied. The principal tissues were fixed in Zenker's fluid. Sections were stained with hematoxylin and eosin.

Of the 60 animals with which the experiment was commenced, 48 developed one or more malignant neoplasms. Ten died without tumors between the 26th and the 169th day. Two were lost.

Forty mice had leukoses. This group of diseases comprised four distinct types, of which general lymphomatosis was the most frequent. It occurred in 28 animals (16 male, 12 female) between the 69th and the 204th days. In these animals a generalized lymphadenopathy was present at autopsy (Figs. 1 and 3). The nodes were gray-white, soft and cellular, frequently measuring more than 1 cm. in diameter. The cervical, axillary, and inguinal lymph nodes were involved most frequently. The mesenteric, retroperitoneal, and mediastinal groups rarely attained as great a size. The livers were moderately large, pale and soft. The spleens were greatly enlarged, red-gray, soft and cellular. The other organs showed no gross anatomical changes. Most of the animals in this group developed anasarca. The fluid was clear and watery. A greatly enlarged liver was always associated with anasarca.

**Fig. 5. General Lymphomatosis: Characteristic Appearance of Atypical Cells from Lymph Node.** × 390

**Fig. 6. Mediastinal Lymphoma: Cells of Same Morphologic Appearance as in General Lymphomatosis.** × 390
Histologic changes were constant among the group (Figs. 5, 7, 9, 11, 13). They appeared to be identical with those found in dilute brown mice dying with the spontaneous disease. The architecture of the lymph nodes was obliterated by masses of large atypical cells which showed no attempt at struc-
The bone trabeculae have almost disappeared. The marrow is packed with atypical cells which infiltrate the contiguous tissues. \( \times 20 \)

The bone trabeculae are well defined. The cells are confined to the marrow spaces, without infiltration of adjacent tissues. \( \times 20 \)

tural arrangement. The cytoplasm was homogeneous, scanty, and weakly basophilic. The nuclei were large, round, and vesicular, with the chromatin condensed at the periphery. Nucleoli were prominent. The cells spread through the node capsule and infiltrated the surrounding tissues.

Cells of the same type obliterated the structure of the spleen. Megakaryocytes were more numerous than usual. Hemosiderin was increased. The characteristic lymphoid cells packed the sinusoids of the liver and infiltrated the periportal areas. When the sinusoidal collections were numerous the hepatic parenchyma was atrophic. Infiltration was also found in the skin, bladder, coagulating gland, uterus, ovary, salivary glands, muscle, and rarely in the lungs and kidneys.

The bone marrow presented a striking picture. The marrow spaces were filled with atypical cells, which spread through the cortical bone into the vertebral canal and surrounding soft parts. Granulocytes had almost disappeared. The bone trabeculae were thin. The femora contained collections of atypical cells that lifted the periostium from the bone.

The blood vessels showed a pronounced increase in leukocytes in most of the cases. Their lumina contained the characteristic cells found in the tissues. Ante-mortem leukocyte counts were performed on 8 mice. In 3 the total was within normal limits. In 5 it ranged from 50,000 to 300,000, with 85 to 90 per cent of the lymphoid series. Many immature forms were present.

Only one attempt was made to transmit this leukosis to other mice. Pieces of a lymph node from an animal dying on the 107th day of generalized lymphomatosis were inoculated subcutaneously into 10 dilute brown mice. Six of
the animals developed large local tumors at the site of implantation within ten to fourteen days. They died ten to fourteen days later with general lymphadenopathy but no anasarca or enlargement of the liver or spleen. The histologic characteristics of the transplanted growth were identical with those of the original tumor, including infiltration of spleen, liver, bone marrow,
muscle, and kidney. The mouse from which the original material was obtained had a leukocyte count of 15,500, but 2 mice in which the transplants grew had 190,000 leukocytes, among which abnormal forms were seen. In the second transplant generation 3 of 10 grafts grew. The gross and histologic pictures were unaltered but no hyperleukocytosis was found. In the third generation 4 of 10 animals developed the disease. The highest leukocyte count was 67,500. In all of the transplanted material the latent period of tumor production was ten to fourteen days and the mice died twenty to twenty-eight days after injection or ten to fourteen days after growth was first noted. No growth was obtained in the fourth generation. An attempt to transplant the tumor to C57 black and Swiss albino mice was unsuccessful.

The second type of reaction was observed in 8 mice (6 males, 2 females), which had localized mediastinal lymphoma. The mass was gray-white, soft and cellular, compressing the adjacent lung tissue (Fig. 4). No other lymphadenopathy was found in these animals. The livers and spleens were not enlarged. Anasarca did not occur. The histologic appearance of the tumor was identical with that of the lymph nodes among the mice having general lymphomatosis. The cell type was the same. Hemosiderin was present in the spleen but the architecture was unaltered. The liver appeared normal. Anaplastic cells could be traced directly from the mediastinal mass to the heart and lungs. The bone marrow contained both erythropoietic and leukopoietic cells. Leukocyte counts were within normal limits. (Figs. 6, 8, 10, 12, 14.)

Non-malignant extramedullary myelopoiesis, the third type of reaction, occurred in 3 mice (2 males, 1 female). No lymphadenopathy was present. Splenomegaly was most pronounced in this group. The livers were enlarged.
Infiltration was present in the spleen, liver, and kidneys. The bone marrow was extremely hyperplastic, containing granulocytes in all stages of development. The spicules of bone were greatly thinned. In one case the cells had penetrated the periosteum and were invading the surrounding tissues. No blood counts were performed, but leukocytes, particularly polymorphonuclear cells, were abnormally numerous in the peripheral blood vessels.

One mouse, a female, died on the 130th day of the experiment (Figs. 15, 16, 17, 18). The gross picture resembled general lymphomatosis with lymphadenopathy, splenomegaly, hepatomegaly and anasarca but the histologic appearance was quite different. The lymph nodes and spleen were filled with large polygonal cells with well defined cell membranes, homogeneous acidophilic cytoplasm, and one or more round vesicular nuclei. The cells had no structural arrangement. Many contained erythrocytes, leukocytes, cellular débris, or needle-like highly refractile bodies. The same type of cell was found about the portal areas of the liver and in the sinusoids. A few were present in the bone marrow, but hematopoietic elements were almost entirely absent. The cells bore no resemblance to megakaryocytes. They appeared to be of reticulo-endothelial origin. The lesion is similar to that described by Simonds as lymphogranulomatosis (20), but the changes are not those commonly associated with the disease in man.

Two lung tumors were found, both consisting of convoluted strands of polygonal cells forming imperfect acini as described by Murphy and Sturm (16). Mammary adenocarcinoma occurred in 2 of the animals. The 21 skin tumors were typical squamous-cell carcinomata. The 2 sarcomata were of the fibrous type.

Fifty dilute brown mice were painted in the same manner with commercial benzene to control the results. Forty-five of them are still alive after the 225th day of painting. One died on the 113th day with extramedullary myelopoiesis. No lymphomatosis has been found and the survivors appear healthy.

Two families of dilute brown mice, substrain 212, were bred brother to sister and the progeny were treated with methylcholanthrene as in the original experiment. The substrain has a high incidence of spontaneous "lymphoblastoma," but in other respects retains the characteristics of the dilute brown strain. Of the 14 mice that were painted when they were ten days old, 7 survived the first month of painting. All of these mice, 5 males and 2 females, developed typical general lymphomatosis between the 92d and 114th days of life. The gross and histologic pictures were the same as those previously described. Neither litter mates nor the parents of these animals have shown any evidence of tumor formation to date.

**DISCUSSION**

The leukoses observed in the dilute brown mice were similar to those recorded by previous investigators for other strains (6, 7, 10, 18, 20, 21). The peculiar case of reticulo-endotheliosis is unique. Its significance is not obvious. No histologic difference could be detected between the spontaneous lymphomatoses and those that followed painting with methylcholanthrene. The chief anatomical difference was the preponderance of external lym-
phadenopathy in the induced lesions as contrasted with internal lymphadenopathy in the spontaneous disease. Apparently the solitary lymphoma is also more prevalent in mice that have been treated with methylcholanthrene.

The appearance of leukoses in strains of mice susceptible to spontaneous mammary cancer has been noted before. Breast cancer occurs at 10.5 months in dilute brown mice while lymphomata do not appear in any considerable number before the twentieth month. After painting the skin with methylcholanthrene the natural sequence of events is reversed. The animals die of leukoses before the age period in which breast tumors develop. That methylcholanthrene is responsible for the shortened latent period is most probable, since a comparable effect was not obtained with benzene alone. The reduction in the period is significant. The mice developed the disease in less than one-half the time required for the spontaneous disease to appear.

The reaction of the ten-day-old mice to methylcholanthrene was surprisingly uniform. The 7 animals surviving the first month died within 18 days of one another. All presented the same pathologic features. Whether the incidence of lymphomatosis is increased by methylcholanthrene is not known since quantitative data regarding the spontaneous disease are still incomplete.

SUMMARY
Forty-eight of 60 dilute brown mice that were painted with methylcholanthrene in benzene developed leukoses. Four types of reaction are described: general lymphomatosis, localized mediastinal lymphoma, extramedullary myelopoiesis, reticulo-endotheliosis. Spontaneous lymphomatosis occurs in members of the strain that live 650 to 800 days. Painting with methylcholanthrene reduces the latent period of lymphomatosis by more than one-half. A comparable effect is not obtained by painting with benzene alone.

BIBLIOGRAPHY
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