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Influence of Methylcholanthrene on Age Incidence of Leukemia in Several Strains of Mice.*

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It has been reported that a large number of mice of the dba strain developed leukemia when painted on the skin with methylcholanthrene or 9:10 dimethyl-1:2 benzanthracene dissolved in benzene.[1] There are other reports concerning the effect of carcinogens on the induction of leukemia in mice.[2]

The question arises as to whether carcinogens actually induce leukemia in mice of non-leukemic strains or whether the administration of these agents accelerates the appearance and perhaps increases the incidence of this disease only in mice showing a tendency towards its spontaneous development. Mice of the inbred F strain which shows a high incidence of spontaneous leukemia[3] were painted with methylcholanthrene twice weekly according to the technic of Morton and Mider.[1] Mice of the C3H strain received similar treatment. Other non-leukemic strains (NH, CHI, CBAN and C57) were painted once a week. Painting with methylcholanthrene was begun at an average age of 30 days. The essential findings are summarized in Table I.

Most of the painted F mice which did not develop leukemia were sacrificed or died because of the development of large ulcerating skin tumors which appeared between the ages of 100 and 200 days. It was not possible with this technic, therefore, to determine whether the incidence of leukemia in the F strain can be increased by administration of methylcholanthrene. In the C3H strain 3 lymphoid neoplasms appeared before 300 days of age among 94 mice painted. Of 280

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TABLE I.
Age Incidence of Leukemia in Untreated Mice and Mice Painted with Methylcholanthrene (F strain—leukemic; C3H strain—non-leukemic).

Age in days	No. of Leukemias	Type of disease			No. of Leukemias	Type of disease		
		ML*	LL†	MedLym‡		ML*	LL†	MedLym‡
	Untreated F mice—125 animals				F mice painted with MC—83 animals			
0-100	0	0	0	0	1	1	0	0
100-200	4	0	1	3	24	11	10	3
200-300	9	0	2	7	4	0	2	2
300-400	18	4	8	6				
400-500	16	3	12	1				
500-600	17	7	10	0				
600-700	5	3	1	1				
Totals	69	17	34	18	29	12	12	5
	Untreated C3H mice—280 animals				C3H mice painted with MC—94 animals			
0-100	0	0	0	0	0	0	0	0
100-200	0	0	0	0	2	0	0	2
200-300	0	0	0	0	1	0	1	0
300-400	0	0	0	0				
400-500	1	0	1	0				
500-600	2	0	2	0				
600-700	0	0	0	0				
Totals	3	0	3	0	3	0	1	2

*Myelogenous leukemia.
†Lymphatic leukemia.
‡Mediastinal lymphosarcoma—very large lymphoid tumor in the mediastinum, usually relatively little systemic disease.

controls no leukemias or lymphosarcomata were observed in this age group (Table I). No leukemias appeared in 90 mice of the CHI, NH, C57 and CBAN strains which were painted once a week for from 3-14 months.

The age at which leukemia appeared and the type of leukemia developed in F mice were influenced by painting with methylcholanthrene. In untreated mice of the F strain only 4 of 69 cases of spontaneous leukemia appeared before 200 days of age. Of the 29 leukemias observed following painting with methylcholanthrene, however, 25 appeared before 200 days of age. Myelogenous leukemia appears in untreated F mice only after 300 days of age (17 cases—Table 1).‡ In mice painted with methylcholanthrene, on the other hand, a case of myelogenous leukemia appeared at 97 days of age after 73 days treatment, a total of 12 cases appearing before 200 days of age. (Two of these were transplanted, myelogenous leukemia developing in the inoculated mice.) Most untreated F mice which develop lymphoid neoplastic disease before 300 days of age exhibit massive mediastinal tumors. The proportion of such tumors was not

‡ Myelogenous leukemia in the F strain has been described.[3]

increased in mice of this age group when treated with methylcholanthrene. These experiments demonstrated that mice of the F strain, when painted with methylcholanthrene will develop between 97 and 200 days of age a type of leukemia characteristic for mice of an older age group (300-700 days).

Summary. Leukemia appeared at an earlier age than in controls when mice of the F strain were painted twice weekly with methylcholanthrene. Myelogenous leukemia, which does not occur in untreated F mice before 300 days of age, appeared as early as 97 days after birth in treated mice. Only 3 cases of leukemia occurred in 184 mice of non-leukemic strains treated in a similar manner. The effectiveness of methylcholanthrene in influencing the appearance of leukemia in young mice depended on the genetic susceptibility of mice to the disease.

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Microestimation of Leucine, Isoleucine, and Valine.

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Arginine, histidine, lysine, cystine, methionine, tyrosine, tryptophane, phenylalanine, glycine, alanine, and threonine can all be determined by available micro methods[1, 2] in only 2 to 4 g of protein, but until recently methods were not available for the estimation of 3 of the essential amino acids: leucine, isoleucine, and valine in small quantities of protein. Last year Fromageot and Heitz[3] described a procedure for the determination of leucine and valine, involving deamination to the corresponding hydroxy acids with nitrous acid, oxidation to acetone with chromate, and measurement of the acetone colorimetrically after reaction with salicylaldehyde. Leucine and valine were estimated in the presence of each other by carrying out the oxidation with chromate on 2 aliquots under conditions such that the proportionate yield of acetone from the 2 amino acids differed

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