INTRODUCTION

The purpose of this paper is to present certain heterogeneous observations recorded during an investigation of leukemogenesis in irradiated mice. Inasmuch as some of this data is a prerequisite to the proper planning of other experiments on induced leukemia, it is felt that this communication may help other investigators in this field to avoid experimental pitfalls.

Radiation has been established as one of the most important exogenous leukemogenic agents in mice (8, 12, 16), and there is evidence suggesting that it exerts a similar influence in man (13, 22, 30). The experimental literature on spontaneous and induced leukemia and lymphoid tumors has been summarized in recent years by Furth (5), Kirschbaum (15), and Engelbreth-Holm (4).

The sequence of events which occurs during the latent period between exposure to the agent and the appearance of the tumor or leukemia is still a subject of speculation. However, a number of factors which control or modify the incidence of the disease have been elucidated, among which may be listed the dosage of the agent (9, 14), the genetic constitution of the mice (16, 23), the nutritional state (18, 28), and the maternal age at parturition (19). Sex differentials in the incidence of lymphoid tumors have been reported in some strains (21, 24) but not in others (17). That the sex hormones play an important role is attested by the fact that estrogens are capable of inducing these tumors in certain strains of mice (9). Moreover, ovariectomy has been shown to decrease, and orchidectomy to increase, their incidence in other strains (21, 24).

However, little is known about the relationship between the age at the time of exposure to the agent and the subsequent incidence of lymphomas. Cowdry and Sun:zeff (2) found that the percentage of methylcholanthrene-induced epidermoid tumors varied with age in one strain of mice but not in another. An experiment designed to demonstrate a similar age-dependence in the case of induced lymphoid neoplasms might establish age as a new variable, and might give some clue as to the mechanism of action of the leukemogenic agent. The results of one such experiment are here reported. In view of the strain variation noted by Cowdry and Sun:zeff, and in order to further investigate this age-dependence factor, work is now being repeated with larger numbers of mice per age group, a larger series of groups, and a different strain of mice.

The results of two initial experiments intended to determine the influence of thymectomy and splenectomy, respectively, on the incidence of radiation-induced lymphomas are also reported here because the data pertaining to age-dependence render them inconclusive, thus illustrating the importance of controlling and suitably selecting age in designing such experiments. Furth and his co-workers (6, 21) found that thymectomy greatly reduced the incidence of spontaneous lymphoid tumors in strain Ak mice, while splenectomy had no influence. The data to be reported here are equivocal but would suggest that removal of the thymus slightly decreased the incidence and markedly delayed the appearance of radiation-induced lymphomas, while splenectomy reduced the incidence but did not affect the latent period.\footnote{The term "latent period" is used synonymously with "induction time" here, and both terms refer to the lapse of time between the first x-ray exposure and the observation of a grossly detectable lymphoid tumor, either at autopsy or at the onset of pronounced dyspnea in a subsequently autopsied mouse.}

Incidental observations pertaining to the sites of origin of these neoplasms, their probable unicentric initial development, and their dissemination through the body are also presented.

MATERIALS AND METHODS

The age-dependence study will be referred to as Experiment I, that on thymectomy as Experiment II, and that on splenectomy as Experiment III.

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and that on splenectomy as Experiment III. Strain A mice, initially obtained from Dr. L. C. Strong and subsequently bred in the laboratory, were used for all experiments. In previous work with this strain, the incidence of spontaneous lymphoid tumors in 80 untreated animals was 3.8 per cent; no lymphoid tumors were observed in 55 animals treated with methylcholanthrene (16). Spontaneous lymphomas are quite rare in strain A mice less than 1½ to 2 years old.

In Experiment I, a total of 198 mice, equally divided by sexes, were irradiated by groups at 2 weeks, 1 month, and 2, 3, 4, and 6 months of age, respectively. Roentgen radiation was given in fractionated, daily doses of 50 r on 12 consecutive days for a total of 600 r of whole body radiation; the physical factors were 125 K.V.P., 6 Ma., 3 mm. Al filter, 30 cm. distance, 43 r per minute. The mice were placed in perforated cardboard boxes measuring 5 × 6 × 1½ inches which were then carefully positioned for treatment, each box containing 5 to 7 mice. Deaths attributable to irradiation occurred in a small number of animals in anesthesia, by the method of Segaloff (29). Of these, 35 were operated upon 1 week before irradiation, and 38 were subjected to thymectomy about 1 month after the end of x-ray exposure. Similarly, in Experiment III, 36 mice were splenectomized one week before irradiation, and 48 others 1 month after, for a total of 84 animals. The controls for these experiments served also as the 2 and 3 month age groups of Experiment I. Both sexes were equally represented among the animals surviving through the latent period. The diet and general environment paralleled the conditions of Experiment I.

RESULTS

The results of Experiment I are summarized in Table I, together with incidental observations on the occurrence of ovarian and mammary tumors, and the net incidence of lymphoid tumors by age groups is graphically presented in Fig. I. It can be seen that the greatest incidence of lymphoid tumors occurred in the group irradiated at one month of age, and that there is a sharp drop between 1 and 2 months. The number of tumors developing in the 1 month group was greater than the combined total among all of the older age groups. There were 10 lymphomas among 38 surviving animals treated at 1 month of age or younger, an incidence of 26.4 per cent, and only 7 tumors (6.0 per cent) among 117 mice 2 months of age or over at the time of irradiation. The average induction time for the entire series of 17 tumors was 215.0 days. The 10 tumors in the combined younger group had an average induction time of 207.9 days, against an average of 225.4 days in the older age groups. The minimum induction time was 157 days, in a mouse of the 1 month old group, and the maximum, 301 days, in a mouse of the 3 month old group. Ten of the 17 tumor-bearing mice were males, and 7 were females. This sex difference is not considered significant.

<table>
<thead>
<tr>
<th>Table I: Age and Sex in Relation to Lymphoma Incidence</th>
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<td>Age at onset of irradiation</td>
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<td>Total initial No. of mice</td>
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<td>Deaths within 4 months after irradiation</td>
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<td>Net No. of mice</td>
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<td>Lymphoid tumors No.</td>
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<td>Net incidence, %</td>
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<td>Ovarian tumors No.</td>
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<td>Mammary tumors No.</td>
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Each group except for the 2 week old mice, which succumbed in such large numbers that an inadequate residue remained for observation. Following irradiation, the mice were maintained with no further experimental treatment and observed for the development of lymphoid tumors. All mice surviving approximately 1 year after irradiation were killed and the experiments terminated. The maximal age of the animals was therefore 12 to 18 months. During this period the mice were caged in groups of 4 to 6 and received Purina fox chow checkers and water ad libitum.

Experiments II and III were set up concurrently with Experiment I, and the same roentgen dosage was employed. The mice in these experiments, however, were all 2 to 3 months of age at the time of irradiation. A total of 73 mice were thymectomized, under ether anesthesia, by the method of Segaloff (29). Of these, 35 were operated upon 1 week before irradiation, and 38 were subjected to thymectomy about 1 month after the end of x-ray exposure. Similarly, in Experiment III, 36 mice were splenectomized one week before irradiation, and 48 others 1 month after, for a total of 84 animals. The controls for these experiments served also as the 2 and 3 month age groups of Experiment I. Both sexes were equally represented among the animals surviving through the latent period. The diet and general environment paralleled the conditions of Experiment I.
Of 73 thymectomized and irradiated mice, a total of 45 (22 males, 23 females) survived for at least 5 months after radiation. Only 2 surviving mice of this group (4.2 per cent) developed lymphoid tumors, with induction times of 379 and 437 days, respectively. One of these had been thymectomized prior to x-ray treatment; the other, 1 month after radiation. No residual or recurrent thymic tissue was found at autopsy in control or experimental animals. The results in the thymectomized animals must be compared with those in intact irradiated controls of the same age at the time of treatment. The combined incidence for the 2- and 3-month old control groups was 7.4 per cent, and the latent periods ranged from 162 to 301 days, with an average of 229 days. The difference in incidence is of doubtful significance, but the difference in induction time appears quite pronounced. Ten thymectomized and irradiated mice are still alive more than a year after irradiation, and it is possible that additional tumors may still appear in this small group. Thymectomized but nonirradiated controls were omitted because of the very low incidence of spontaneous lymphomas in nonirradiated mice of this strain. Likewise, in Experiment III, no splenectomized, nonirradiated mice were maintained.

In Experiment III, 57 mice (29 males, 28 females) of an initial total of 84 survived at least 5 months, and only one of these (1.7 per cent) developed a lymphoma 231 days after treatment. Using the control data cited in the paragraph above for comparison, there appears to have been a slight decrease in incidence, of questionable significance, without a corresponding increase in induction time following splenectomy. It is apparent, however, that results of greater significance might have been obtained had the age been so selected as to yield a maximal number of tumors in the analogous controls. Unfortunately, the age-dependence data were not available when these experiments were initiated, and the marked discrepancy between the incidence of the 1- and 2-month-old groups was not suspected. These experiments are now being repeated using younger mice of strains A and C57 black.

The tumors were all lymphomas of the stem-cell, lymphoblastic, or lymphocytic varieties, apparently identical with those induced by methylcholanthrene (20) or by estrogens (9). No myeloid or monocytic forms were seen. The thymus is by far the organ most commonly involved. The spleen is distinctly less often infiltrated, and certain non-lymphoid organs (the lung, liver, kidney, heart, and adrenal, in order of frequency) are invaded more often that the superficial lymph nodes. These nodes, which are usually involved early in the course of the spontaneous disease, are often completely devoid of lymphoma, both macroscopically and microscopically, in the radiation-treated animals. The mediastinal lymph nodes are not uncommonly invaded by direct extension from the thymus, and the mesenteric nodes are distinctly more often affected than the superficial nodes. There is invasion of the peripheral blood to give a true leukemia in a considerable number of instances, and white blood cell counts as high as 800,000 have been observed. The exact percentage of tumor-bearing mice in which leukemia becomes manifest is unknown because the disease was undetected until autopsy in a number of instances. The brain and testis have rarely been infiltrated in the animals observed to date; in 2 animals, focal collections of tumor cells were noted in the meninges. Several of the tumors have been successfully transplanted, by subcutaneous, intravenous, intraperitoneal, or intraocular inoculation, into other strain A mice, F1 hybrids of strain A parentage, and rarely into Bagg albino mice. They have not grown on inoculation into the anterior chamber of the guinea pig eye, following the technic of Greene (11).

The route of dissemination of the disease is not clear. It might be surmised, from the fact that frank leukemia occurs not uncommonly, that the tumor enters the blood stream and reaches distant organs by the hematogenous route. However, histological study reveals that the tumor collections in the lungs occur in and ultimately occlude the peribronchial and perivascular lymphatic channels (Fig. 4), and secondarily spread to the subpleural lymphatics; parenchymal pulmonary...
The predominant and early involvement of the thymus in certain spontaneous instances and in most of the estrogen- or x-ray-induced tumors would in itself suggest that the thymus may be the site of a monocentric origin of the disease. This thesis received additional support when McEndy, Boon, and Furth (21) demonstrated that thymectomy greatly reduced the incidence of spontaneous leukemia in mice of the Ak stock, but that splenectomy had no such effect. In the course of the present investigation, several animals have been killed near the end of the latent period because of dyspnea, and a gross examination revealed no abnormalities except for minimal enlargement of the thymus and perhaps a slight change in its consistency. On histological examination, however, one lobe of the thymus was seen to be completely replaced by a lymphoma while the adjacent lobe exhibited intact architecture and no definite evidence of infiltration (Figs. 2 and 3). All of the other organs, including the lymph nodes and spleen, were apparently free of disease. These chance observations clearly indicate that a lymphoid tumor may arise and proliferate in one lobe of the thymus at a time when the other lobe, and all of the other tissues, are histologically uninvolved. In another instance, one lobe of the thymus was of normal size, the opposite lobe was enlarged and lymphomatous, and two adjacent lymph nodes were intensely hyperplastic but apparently contained no tumor. That the thymus is merely the most susceptible and not the sole site of origin is also indicated by the infrequent development of primarily splenic lymphomas in both intact and thymectomized mice. To date, no instance of a radiation-induced lymphoma apparently originating in a peripheral lymph node has been observed. Bioassay studies during the latent period, using the thymus glands of irradiated mice, should yield additional information concerning the problem of the unicentric origin of lymphomas.

INCIDENTAL OBSERVATIONS

The development of ovarian tumors after irradiation of mice has previously been reported by Furth and his co-workers (7, 8). Ten mice in Experiment I developed tumors of the ovary which were bilateral in four instances. Additional ovarian neoplasms might...
FIGS. 2 TO 5

Kaplan—Radiation-Induced Lymphoid Tumors of Mice
have been expected if the experiment had been maintained longer. Six of these 10 mice had been irradiated at 1 month of age, suggesting that age may also modify the incidence of radiation-induced ovarian tumors. These neoplasms, occurring in anovular ovaries, were predominantly composed of sheets of cells having a small round nucleus and rather large amounts of clear cytoplasm; this type has been previously designated as a luteoma (Fig. 5). Less often, atypical granulosa cell tumors have been observed. In a number of instances, these two cellular types have occurred side by side in the same ovary. No metastases have been observed to date. The latent period has been approximately 6 to 9 months for most of these neoplasms.

Breeding female mice of Strain A, having the milk influence, are highly susceptible to the development of spontaneous mammary tumors. The incidence of these tumors is sharply reduced by irradiation. In Experiment I, only females that were old enough to be breeding at the time of irradiation (the 3 to 6 month age groups) bore any of these tumors, and the total incidence was only 4 in 77 surviving females, or approximately 3 per cent. Only 1 animal had both an ovarian and a mammary tumor. Whether the inhibition caused by roentgen radiation was due not only to sterilization but in part also to diminution of circulating levels of estrogen is not known. It is well known that after doses of x-rays adequate to destroy all ovarian follicles, estrogen production persists in quantities adequate to permit the continuation of estrus cycles (10, 25). It is probable, however, that some decrease in estrogen secretion occurs after radiation.

DISCUSSION

The results presented are preliminary and do not warrant extensive discussion at this time. The age dependence of radiation-induced lymphoid tumors, if verified on reinvestigation, would suggest some interesting implications. The apparent critical period of susceptibility is somewhere between 1 and 2 months of age, which, in a mouse, roughly corresponds with the time of puberty. Inasmuch as puberty is a period of intense endocrine activity, it is possible that radiation at this time can more easily disturb the inter-relationships of the endocrine glands and throw their activities out of balance. It is known that endocrine imbalance contributes to the development of other neoplasms, and that the lymphoid tissues are under hormonal influences, particularly those of the adrenal and pituitary (3), gonads, and thyroid (27). Andreasen (1) studied the development and involution of the lymphoid organs of the white rat in relation to age, and found that the thymus reached its maximum size at 2 to 3 months of age, or approximately at the time of puberty. The alternate possibility cannot yet be excluded that the high incidence at or about the time of puberty is due merely to the presence of a greater mass of lymphoid tissue in the thymus at this age.

SUMMARY

1. Strain A mice in groups of various ages yielded a maximum incidence of lymphoid tumors in animals irradiated at 1 month of age with a sharp decrease at 2 months and later.

2. Thymectomy of strain A mice irradiated at 2 to 3 months of age resulted in a considerable increase in the latent period and a very slight decrease in the incidence of lymphomas as compared with the low incidence in intact irradiated control mice of the same age. In a parallel experiment, splenectomy was followed by a slight decrease in incidence without change in latent period.

3. The tumors are lymphocytic lymphomas which, in most instances, appear to arise in the thymus, to disseminate to the spleen, lungs, liver, kidneys, and lymph nodes, and to cause leukemia in a considerable percentage of animals.

4. Incidental observations on radiation-induced ovarian tumors and on inhibition of spontaneous mammary tumors following x-ray treatment are presented.

REFERENCES


Observations on Radiation-Induced Lymphoid Tumors of Mice

Henry S. Kaplan