In several strains of mice, spontaneous and induced lymphoid tumors exhibit a tendency to arise in the thymus gland. Furth and his associates (1, 8) found that the incidence of spontaneous lymphomas in a susceptible strain was sharply reduced after thymectomy. Subsequently, Kaplan (4) and Law and Miller (6, 7) reported a similar influence of thymectomy on lymphoma development under other experimental conditions. Furth (1) and Kaplan (4) attributed this phenomenon simply to removal of the tissue of maximal susceptibility. However, Law and Miller (6, 7) reported that the incidence of lymphoid tumors could be restored to essentially normal levels in thymectomized mice by autologous or homologous implants on Lymphoid Tumor Incidence in C57BL Mice*

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In all these investigations, thymectomy was performed before exposure to the tumor-inciting agent, or, in the spontaneous lymphoma studies, at a time when the endogenous agents were presumably still active. Moreover, Law and Miller implanted their thymic grafts prior to the start of carcinogen application; thymic tissue was therefore present in the animal during exposure.

It seemed of interest to study the effect on lymphoid tumor incidence of thymectomy at varying intervals after irradiation and to determine whether homologous thymic tissue implanted after irradiation can restore the lymphoma susceptibility of thymectomized mice. This paper is concerned with two such experiments.

MATERIALS AND METHODS

Strain C57BL mice of both sexes, aged 33 ± 3 days at the start of irradiation, were used in both experiments. Littermates were distributed at random among the various experimental groups. The animals were identically caged and had free access to Purina Laboratory Chow and water. Animals that died following operation or irradiation, or were too autolysed to permit autopsy diagnosis, have been considered indeterminate. Except for animals dying with grossly typical lymphomas, tissues taken at autopsy were routinely subjected to histological examination.

In Experiment I, eight groups of sixteen mice each were treated with four total-body x-ray exposures of 168 r at intervals of 8 days. One remained intact and served as an irradiated control group. The others were designated for thymectomy at 4, 6, 8, 10, 12, and 16 weeks, respectively, after the last irradiation; an additional group of irradiated "spares" was assigned to the 6-week group when operative deaths in this group became excessive. The technic of thymectomy was identical with that previously described (4). The freshly removed thymus glands were minced and suspended in 0.6 cc. of ice-cold Locke's solution; 0.2 cc. of this thick suspension was then injected intraperitoneally into each of three normal C57BL recipient mice about 1 month old as a bioassay for the presence of lymphoid tumor cells (9). The surgical survivors and bioassay recipients were then maintained without further treatment and were observed for the development of lymphoid tumors. When it became apparent that many thymus glands removed 18 weeks after irradiation contained obvious tumors, the group designated for thymectomy at 16 weeks was sacrificed control group. The others were designated for thymectomy at 16 weeks was sacrificed 12 weeks after irradiation contained obvious tumors, the group designated for thymectomy at 16 weeks was sacrificed and autopsied instead; representative tissues were studied histologically to determine the number of lymphoid tumors present at that time (157 days after the start of irradiation).

In Experiment II, seven groups, each containing 50-60 mice, were employed. All but one of these received total-body irradiation in four doses of 168 r at 4-day intervals. Four groups were thymectomized by the usual technic (4); the other three were subjected to a sham operation in which the thorax was opened but the thymus was left intact. All operations were performed 4 days before the start of irradiation. Whole thymus glands were quickly excised from donor C57BL mice 33 ± 3 days of age; some were then rapidly given a single x-ray dose of 900 r in vitro, after which one entire irradiated or nonirradiated thymus was implanted through a small cutaneous incision into the axillary subcutaneous tissue of each mouse of the recipient groups within 1-2 hours after the last total-body x-ray exposure. The animals were then observed for the appearance of lymphoid tumors.

RESULTS

The results of Experiment I, as tabulated about 12 months after the start of irradiation, are indicated in Table 1. Tumor incidence in the irradiated control group reached 100 per cent at 10 months. No tumors occurred in animals thymec-

1 Physical factors were: 120 kvP; 9 ma.; 0.25 mm. Cu + 1.0 mm. Al added filter; 30 cm. mouse-target distance; 32 r/min.; HVL = 0.39 mm. Cu.
tomized 4 weeks after irradiation. Thymic lymphomas were encountered sporadically as early as 6–8 weeks after irradiation. They were present in the excised thymuses of one-third of animals operated upon at 10 weeks and in over one-half of those thymectomized at 12 weeks.

Thymectomy proved to be a strikingly effective prophylactic measure. Excluding animals that already had lymphomas at the time of surgery, all other survivors, even those thymectomized as late as 12 weeks after irradiation, have remained completely free of lymphoid tumors. Moreover, thymectomy appeared to be of therapeutic value in a small number of mice, presumably as a result of fortuitous removal of an early lymphoma still confined to the gland. In two instances, the excised thymus yielded a positive bioassay, establishing the presence of tumor within it, but no evidence of recurrent or disseminated lymphomas has appeared in the donor mice during a postoperative observation period of over 9 months. In addition to these two apparent “cures,” prolonged postoperative survivals of from 6 to 12 weeks or more have occurred in at least three other mice found to have a gross thymic tumor at operation. In these three instances, residual tumor ultimately disseminated to cause death, however. Since the bioassay method proved to be quite insensitive, and in several instances failed to yield growth of well established tumors, it seems probable that there were other, undetected “cures” following thymectomy.

One group of 24 mice was sacrificed, in lieu of thymectomy, at experimental day 137, 16 weeks after their last irradiation, to obtain data on the incidence of lymphoid tumors at this time. Only ten appeared on gross inspection to have a definite or probable thymic lymphoma, but 23 (96 per cent) were positive on histologic examination. At least three of these lymphomas were so early that they were still confined entirely to one thymic lobe, confirming similar instances reported earlier (3). More recently, in another group of similarly irradiated mice killed at day 120 for biochemical studies, over 80 per cent were found to have lymphoid tumors in the thymus, several of which were still limited to one lobe. In some instances, the tumor cells had not yet completely replaced the normal architecture, and it appeared that involvement of the medulla was incomplete, suggesting that the disease first appears in the cortex of the gland.

The results of Experiment II, which was terminated after about 20 months, are summarized in Table 2. Tumor incidence in all the sham-operated groups exceeded 90 per cent, and was not affected by the presence or absence of an exogenous thymic implant. In striking contrast, there was only one lymphoma among 31 determinate mice (3.2 per cent) in Group III, which received no implant after thymectomy and irradiation, and a similarly low incidence (within the range of spontaneous lymphoma incidence for the strain) in nonirradiated thymectomized Group IV animals which received an irradiated thymic implant. Of particular interest, however, is the occurrence of twelve lymphomas among 31 determinate mice of Group I (38.7 per cent) and of eight lymphomas among 39 determinate animals of Group II (23.1 per cent), which received nonirradiated or irradiated thymic implants, respectively, subsequent to thymectomy and systemic irradiation. Statistical comparison with the results in Group III is complicated by the different rates of operative, post-irradiation, and miscellaneous deaths.

If we calculate $\chi^2$ (with 6 degrees of freedom) for Groups I, II, and III in Table 2, using the total number of determinate mice without reference to the presence of tumors, we are testing the hypothesis that the three treatments (I, II, III) are equivalent with respect to incidence of various causes of
indeterminacy. The value of $x^2$ is 8.062, which is not significant, in that the probability of a larger value is about 0.25, and we find no reason for regarding the treatments as different in this respect.

If then we calculate $x^2$ for the table with determinate mice subdivided into tumor and nontumor, we obtain a value of $x^2$ (with 8 degrees of freedom) equal to 18.685. This is significant at the level of 0.02, and we conclude that the treatments do differ as to effect on tumor incidence. It is clear that the difference arises in the contrast between Group III and the other two groups on the tumor versus nontumor breakdown.

The histologic appearance of the uninvolved and tumor-infiltrated thymic implants was essentially similar to that described by Law and Miller (7). The implants were involved by tumor to about the same degree and with roughly the same frequency as the superficial lymph nodes, and distinctly less often than the abdominal or thoracic viscera. Lymphomas had in most instances replaced the mediastinal structures, even in the thymectomized animals of Groups I and II. Incomplete removal and subsequent regeneration of the thymus may have been responsible for the appearance of some of the tumors in these groups. However, there was no recognisable factor of selection in the experimental design which would have contributed to a greater frequency of incomplete operations in these groups as compared to Group III. The fact that some thymic implants were plainly unaffected by tumor in animals with disseminated lymphomas confirms the observation of Law and Miller that the tumors do not originate in the implants (Fig. 1).

It is of interest that the latent period for lymphoid tumor development exhibited a much greater range in Groups I and II than in the highly susceptible sham-operated groups or the refractory Group III animals. Although it is reasonable to suspect that the earliest tumors in Groups I and II occurred in incompletely thymectomized mice, the considerable number of late tumors cannot be explained on this basis. All the late tumors were lymphocytic lymphomas; none of the reticulum-cell and Hodgkin's-like tumors to which aged mice of this strain are prone were included as lymphomas in any group.

**DISCUSSION**

The design of Experiment II was distinctly more stringent than the experiments reported by Law and Miller (6, 7) in that thymic implants were not introduced until exposure to the inciting agent (x-rays) had been completed. Perhaps because the experimental conditions were more rigorous, thymic implants failed to restore lymphoma incidence to the level of sham-operated irradiated controls, but they did nonetheless significantly augment susceptibility. The mechanism whereby thymic implants exert this influence can only be conjectured. The thymus has for so many years frustrated the efforts of investigators seeking to ascribe endocrine activity to it that such an interpretation, though perennially attractive, seems premature at this time. It would seem, however, that the thymus plays a dual role in lymphoma pathogenesis; it is the foremost site of origin for such tumors in certain strains of mice, and its presence appears to condition susceptibility, independently of the site of tumor origin, in all strains thus far tested.

Moreover, as indicated by Experiment I, removal of the thymus as late as 12 weeks after irradiation effectively prevents subsequent lymphoma development in C57BL mice. It is likely that the main effect of thymectomy in this strain is the removal of the tissue of maximal susceptibility, but the indirect thymic influence probably plays some part as well. That this indirect mechanism may be radiosensitive is suggested by the lesser
effectiveness of thymic implants pre-irradiated in vitro (Group II, Experiment II).

The data of Experiment I help to establish parameters with respect to the time of lymphoid tumor onset in irradiated C57BL mice. As previously noted (3), tumors are present sporadically in the thymus as early as 6 weeks after the end of irradiation (experimental day 66), attaining an incidence of 96 per cent at day 137. Under conditions of maximal susceptibility, it would appear that the factors responsible for lymphoid tumor development are operative for a relatively brief period of the order of a few weeks or less. Moreover, collateral evidence (5) suggests that the tumorigenic process may be reversible during much of this time. More intensive and detailed investigation of the altered morphologic and functional interrelationships of the lymphoid tissues and endocrine organs during this critical time period therefore seems indicated.

**SUMMARY**

Two experiments concerning the role of the thymus in lymphoid tumor genesis in irradiated C57BL mice are reported. In Experiment I, thymectomy as late as 12 weeks after irradiation completely suppressed subsequent lymphoid tumor development and effected apparent cures of two mice whose excised thymuses were shown by bioassay to contain tumor. Tumors were encountered sporadically in thymuses removed at 6 weeks, in about one-third of those removed at 10 weeks, and in the majority of those removed 12 weeks after irradiation.

In Experiment II, thymectomized mice implanted after irradiation with homologous thymic grafts, either intact or irradiated in vitro, had a significantly higher incidence of lymphoid tumors than litter-mates similarly treated but receiving no thymic implants. Incidence remained far below the levels of sham-operated, irradiated groups, and was lower for the irradiated than for the intact implants.

**REFERENCES**


**Fig. 1.—Subcutaneous thymic implant, still exhibiting cortical and medullary zones and no evidence of lymphoid tumor infiltration. This implant was removed from a sham-operated, irradiated animal which died with a mediastinal thymic lymphoma 165 days after the start of the experiment.**
Influence of Postirradiation Thymectomy and of Thymic Implants on Lymphoid Tumor Incidence in C57BL Mice

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