Effect of Hypothyroidism and Thyroid Grafts on Lymphoid Tumor Development in Irradiated C57BL Mice*

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Endocrine factors condition susceptibility of some strains of mice to the induction of lymphoid tumors by various carcinogenic agents (reviews by Kaplan et al. [14], Gardner et al. [6]). In previous work adrenalectomy or orchietomy augmented, whereas cortisone, hydrocortisone, or testosterone inhibited, the leukemogenic process (9, 10, 13). Hypophysectomy failed to influence the incidence of radiation-induced lymphoid tumors in C57BL mice (16).

Alterations in thyroid activity have been reported to influence lymphoid tissue growth and involution (2). Grad (8) has reported that the influence of hyper- or hypothyroidism on the incidence of spontaneous lymphatic leukemia in AKR mice is indirect and dependent on the effect of treatment on body weight: decrease in body weight resulted in a decreased incidence of the disease.

It has been demonstrated by Kaplan and co-workers (11) that lymphomas develop in thymectomized, irradiated C57BL mice bearing implants of isologous thymus glands from nonirradiated mice. The incidence, latent period, morphology, and distribution of these tumors have been found to resemble those of lymphomas arising in the thymuses of intact, irradiated mice of this strain (12).

The present experiments were undertaken to study the influence of radiothyroidectomy (I131) on the incidence of thymic implant lymphomas in thymectomized-irradiated C57BL mice.

MATERIALS AND METHODS

Experiment 1.—A preliminary experiment was set up to test the effects of thymectomy, thyroidectomy, and irradiation on young C57BL mice. The surviving animals were then observed for lymphoid tumor incidence.

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Strain C57BL mice of both sexes were weaned and randomized into four groups at 21 ± 3 days of age (Table 1). Groups I and III were placed on Remington’s low-iodine diet (19) and distilled water for 16 days and then on Purina chow and tap water. Groups II and IV were on Purina chow and tap water throughout the experiment. At approximately 30 days of age mice of all four groups were thymectomized. Four days later Groups I and III received 30 μC. I131, intraperitoneally, while Groups II and IV did not. Starting 16–17 days later, Groups I and II were given a total-body x-ray dose of 480 r, and Groups III and IV were given a total dose of 672 r, in four equally fractionated exposures at 7-day intervals. Immediately after the last x-ray exposure thymuses from 1–3-day-old C57BL mice were implanted subcutaneously in the right axillary region. Lymphoid tumor development was observed by checking the mice twice weekly and noting growth of the thymic implant. When progressive growth was noted, the animals were sacrificed and tissues were fixed in Bouin’s solution for histological examination. The observation period was approximately 1 year. Latent periods for lymphoid tumors were calculated from the day of thymic implantation and, as an incidental observation, for pituitary tumors from the day of I131 administration.

Experiment 2.—Conditions of this experiment were modified slightly from Experiment 1, although the same general plan was carried out. Strain C57BL mice of both sexes at 33 ± 3 days of age were distributed at random among three experimental groups, and all were thymectomized (Table 2). Groups I and II were radiothyroidectomized, following the procedure given for Experiment 1, while Group III was not. Seven to 8 days later, mice of Group I received thyroid grafts from normal C57BL donors of the same age and sex, while those in Groups II and III did not. Thyroid grafts were made subcutaneously in the left axillary

1 Physical factors were: 120 kvP, 7.5 ma., 0.25 mm. Cu + 1.0 mm. Al added filter, 30 cm. target-distance, output 34 r/min.
or inguinal region. At approximately 2 months of age, all groups were given total-body x-radiation (672 r in four equally fractionated exposures at 7-day intervals). Immediately after the last x-ray exposure thymuses from 1–2-day-old C57BL donors were implanted subcutaneously in the right axillary region. Animals that died during treatment were considered indeterminate. Tissues from all mice in this experiment were prepared for histologic examination, including the thyroid-trachea region and thyroid grafts. The observation period was approximately 1 year, and latent periods for lymphoid and pituitary tumor development were calculated as in Experiment 1.

To determine the extent of thyroid damage and the activity of the thyroid grafts, $^{131}$I uptake measurements were made on a representative number of mice that developed lymphoid tumors. A tracer amount (1.0 μc.) of $^{131}$I was administered intraperitoneally 24 hours before sacrifice. The thyroid-trachea region, thyroid graft, and a small piece of gastrocnemius muscle of each mouse were dissected and placed in 2-dram plastic vials for uptake measurements in a scintillation well counter. The $^{131}$I uptake of the thyroid has been expressed as per cent of the injected dose.

Serial body weight measurements were taken every 4 weeks and plotted as the average change from the mean initial weight in grams against experimental day.

### RESULTS

**Experiment 1.**—At either x-ray dose (480 r or 672 r), mice which had been radiothyroidectomized (Groups I and III) developed appreciably fewer lymphomas than did the animals which were not thyroidectomized (Groups II and IV, Table 1). The very high treatment mortality in the males of Group I reduced the net number of mice to 9. Comparison of the pooled data from the radiothyroidectomized Groups I and III (five of 60) and the control groups II and IV (24 of 81) indicates a highly significant difference in lymphoid tumor incidence ($P < 0.005$). However, the difference between these groups was statistically significant only in the females ($P < 0.025$); in the males a trend in the same direction was seen, but the results were not significant ($P < 0.10$).²

Of the 55 mice which did not develop lymphoid tumors in Groups I and III (thyroidectomized), 51 developed pituitary tumors by the end of the 12–13-month observation period (Table 1). No pituitary tumors were observed in Groups II and IV (thyroid intact). The earliest grossly visible pituitary tumor was seen at 195 days after $^{131}$I administration, and the mean latent period was 368 days.

**Experiment 2.**—The inhibiting effect of thyroidectomy on lymphoid tumor development was also significant ($P < 0.005$). No lymphoid tumors developed in the radiothyroidectomized groups II and IV, whereas all of the control groups II and IV developed lymphomas.

### Table 1

**Lymphoid Tumor Incidence in Nonirradiated Thymic Implants and Pituitary Tumor Incidence in Radiothyroidectomized, Thymanectomized, Irradiated C57BL Mice**

<table>
<thead>
<tr>
<th>Treatment*†</th>
<th>Sex</th>
<th>Initial NO.</th>
<th>NET NO.</th>
<th>NO.</th>
<th>PER cent</th>
<th>LympHOMAS</th>
<th>Pituitary</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MOUSE (days)</td>
<td></td>
<td></td>
<td></td>
<td>Mean latent period (days)</td>
<td>No. mice</td>
<td>Mean latent period (days)</td>
</tr>
<tr>
<td>Group I:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Radiothyroidectomy and x-ray (480 r)</td>
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<td>20</td>
<td>17</td>
<td>1</td>
<td>0</td>
<td>137</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>20</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>8</td>
<td>347</td>
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<tr>
<td>Total</td>
<td>40</td>
<td>26</td>
<td>1</td>
<td>4</td>
<td>137</td>
<td>23</td>
<td>21</td>
<td>587</td>
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<tr>
<td>Group II:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>X-ray</td>
<td>♀</td>
<td>24</td>
<td>24</td>
<td>8</td>
<td>35</td>
<td>177</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>20</td>
<td>16</td>
<td>2</td>
<td>13</td>
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<td>14</td>
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<tr>
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<td>40</td>
<td>10</td>
<td>25</td>
<td>175</td>
<td>30</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiothyroidectomy and x-ray (672 r)</td>
<td>♀</td>
<td>23</td>
<td>17</td>
<td>3</td>
<td>18</td>
<td>188</td>
<td>14</td>
<td>14</td>
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<tr>
<td></td>
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<td>23</td>
<td>17</td>
<td>1</td>
<td>0</td>
<td>190</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>34</td>
<td>4</td>
<td>22</td>
<td>192</td>
<td>30</td>
<td>30</td>
<td>587</td>
</tr>
<tr>
<td>Group IV:</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>♀</td>
<td>20</td>
<td>19</td>
<td>8</td>
<td>42</td>
<td>248</td>
<td>11</td>
<td>0</td>
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<tr>
<td></td>
<td>♂</td>
<td>20</td>
<td>22</td>
<td>6</td>
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<tr>
<td>Total</td>
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<td>14</td>
<td>34</td>
<td>219</td>
<td>27</td>
<td>0</td>
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</tr>
</tbody>
</table>

* All mice were thymectomized and received isologous thymic grafts.
† Total whole-body x-ray: four equally fractionated doses of 168 r or 120 r every 7 days.
‡ Mice which survived the period of treatment.

1 Fisher's exact $\chi^2$ test used for males; $\chi^2$ with continuity correction used for females and total. Significant values shown are for a two-sided test. The authors are grateful to Dr. Lincoln E. Moses, Associate Professor of Statistics, Stanford University, for the statistical analysis.
roidectomy on thymic implant tumor incidence in females was confirmed in this experiment (Table 2). Only three of fifteen female mice of Group II (radiothyroidectomized) developed thymic implant tumors, while fifteen of nineteen female mice of the controls (Group III, thyroid intact) developed lymphomas. There was no difference in tumor incidence between the hypothyroid males of Group II and the control males of Group III (six of nineteen and seven of nineteen, respectively). When thyroid activity was restored by grafting a normal thyroid after radiothyroidectomy (Group I), the lymphoid tumor incidence was comparable to that of the control mice of Group III (~3 of 33 and ~ of 38, respectively). These results indicate that the observed inhibition in females was due to reduced thyroid function rather than to other radiation effects which might be attributable to the administration of radioactive iodine. The mean latent period for thymic tumor development was 146 days in Groups I (thyroid graft) and III (thyroid intact), while the mean latent period for Group II (thyroidectomized) was 120 days. Pituitary tumors developed in ~ of ~5 hypothyroid, lymphoma-free mice of Group II, with a mean latent period of 349 days; none occurred in the other groups.

I\textsuperscript{131} uptake of thyroid grafts in thyroidectomized animals (Group I) was the same as that of thyroid from intact animals of Group III (approximately 6 per cent at 24 hours after administration), indicating that the thyroid grafts were functioning as actively as intact thyroids in control animals.

Body weight determinations at monthly intervals are shown in Chart 1. Little difference was noted between body weights of thyroidectomized mice bearing active thyroid grafts and those of control animals; however, a significant decrease in body weight was exhibited by the hypothyroid animals of Group II.

Treated, grafted, and control thyroids were examined histologically. Extensive fibrosis and destruction of thyroid follicles were seen in thyroids from mice which had received "thyroidectomy" doses of I\textsuperscript{131}. In contrast, the thyroid grafts had active thyroid follicles similar to those seen in the controls.

**DISCUSSION**

Thymic implant lymphoma development in female thymectomized-irradiated C57BL mice was significantly inhibited by radiothyroidectomy. Although the lymphoma incidence in males receiving identical treatment was reduced, the observed results were not statistically significant at the 0.05 level. The interpretation of these observations is ambiguous, since some of the known factors which influence leukemogenesis, such as nutrition and other endocrine disturbances secondary to the inhibition of thyroid activity, were not controlled. Inanition or caloric restriction may inhibit leukemogenesis (20, 21). Recently, Grad (8) reported that the influence of hyper- and hypothyroidism on the incidence of spontaneous lymphatic leukemia in AKR mice may be indirect and dependent on the effect of treatment on body weight—a decrease in body weight resulting in decreased incidence of leukemia. Although in the present experiments no attempt was made to control food intake or body weight, it seems probable that the observed inhibitory effect was indeed attributable to hypothyroidism, for the following reasons: (a) lymphoma incidence returned to control levels in radiothyroidectomized mice whose thyroid was restored by grafting a normal thyroid after radiothyroidectomy (Group I), the lymphoid tumor incidence was comparable to that of the control mice of Group III (~3 of 33 and ~ of 38, respectively). These results indicate that the observed inhibition in females was due to reduced thyroid function rather than to other radiation effects which might be attributable to the administration of radioactive iodine. The mean latent period for thymic tumor development was 146 days in Groups I (thyroid graft) and III (thyroid intact), while the mean latent period for Group II (thyroidectomized) was 120 days. Pituitary tumors developed in ~ of ~5 hypothyroid, lymphoma-free mice of Group II, with a mean latent period of 349 days; none occurred in the other groups.

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Body weight determinations at monthly intervals are shown in Chart 1. Little difference was noted between body weights of thyroidectomized mice bearing active thyroid grafts and those of control animals; however, a significant decrease in body weight was exhibited by the hypothyroid animals of Group II.

> **TABLE 2**
>
> | TREATMENT*† | SEX | NET NO. | MEAN PERCENT LATENT PERIOD | MEAN PERCENT LATENT PERIOD |
> |-------------|-----|---------|---------------------------|---------------------------|
> | Group I:    |     |         |                           |                           |
> | Radiothyroidectomy | ♀ | 17      | 15                        | 88                        | 156                       | 2                         | 0                         |
> | +Thyroid graft | ♀ | 16      | 8                         | 50                        | 156                       | 8                         | 0                         |
> | +X-ray                                      | Total | 33      | 25                        | 70                        | 146                       | 10                        | 0                         |
> | Group II:   |     |         |                           |                           |
> | Radiothyroidectomy | ♀ | 15      | 3                         | 20                        | 125                       | 12                        | 11                       | 346                      |
> | +X-ray                                      | ♀ | 19      | 6                         | 31                        | 117                       | 13                        | 11                       | 351                      |
> | Total                                              | 34      | 9                         | 26                        | 120                       | 25                        | 22                       | 349                      |
> | Group III: |     |         |                           |                           |
> | X-ray (thyroid intact)               | ♀ | 19      | 15                        | 79                        | 134                       | 4                         | 0                         |
> |                                             | ♀ | 19      | 7                         | 37                        | 161                       | 12                        | 0                         |
> | Total                                              | 38      | 22                        | 58                        | 147                       | 16                        | 0                         |

* All mice were thymectomized and received isologous thymuses after the last x-ray exposure.
† Whole-body x-ray: 168 r × 4, every 7 days.
function was restored by thyroid grafts; (b) if the inhibitory effect of hypothyroidism were due to a decrease in body weight per se, the thyroidectomized males, which had as great a decrease in body weight as the females of the same group, might have been expected to show an equally profound inhibition of lymphoma development; (c) earlier studies have demonstrated that the incidence of radiation-induced lymphomas is not similarly reduced in hypophysectomized animals, despite the fact that they weigh 30–50 per cent less than their intact controls (16).

The possibility of secondary alterations in adrenal, gonadal, or pituitary activity following thyroidectomy must not be overlooked, since the thyroid indirectly controls their function through the pituitary (15). The following endocrine factors are known to influence lymphoma development in irradiated C57BL mice: (a) castration augments, whereas ovariection has no effect (14); (b) testosterone inhibits, whereas estrogen augments the lymphomagenic action of x-radiation in both males and females (10, 22); (c) adrenalectomy enhances and adrenal cortical steroids (cortisone and hydrocortisone) suppress lymphoma development (9, 13); (d) hypophysectomy has no effect (16). Thus, the fact that lymphoma incidence was significantly inhibited in hypothyroid females, but not in males, may indeed be attributable to secondary alterations of gonadal and/or adrenal activity. In the males, thyroid ablation is likely to have produced a significant decrease in secretion of androgenic hormone, which exerts a detectable inhibitory influence in the intact animal. The loss of this inhibitory factor may have been sufficient to balance the inhibitory effect of thyroidectomy per se on lymphoma development. Conversely, in the females the associated inhibition of ovarian estrogen secretion after thyroid ablation would have been expected to be additive with the inhibitory effect of hypothyroidism. It seems reasonable to conclude that hypothyroidism inhibits lymphoma development in both sexes, but the effect may be masked by secondary endocrine changes.

Radiothyroidectomy of immature and mature C57BL mice did not significantly alter adrenal, gonadal, uterine, or seminal vesicle weights. Studies on hypothyroid-gonadectomized or adrenalectomized animals maintained on physiological doses of gonadal or adrenal steroids should provide more information on this subject.

The high incidence of pituitary tumors in our

3 C. S. Nagareda and H. S. Kaplan, unpublished observations.
radiothyroidectomized-irradiated animals confirms the work of other investigators (1, 3–5, 7). It has been reported by Rawson and co-workers (18) that the thymus and lymph nodes in vivo are capable of inactivating thyrotropic hormone and that the extent of inactivation is surpassed only by that of the thyroid itself. The possibility that high levels of thyrotropic hormone may have altered the metabolic activity of the thymus by some as yet unknown mechanism and rendered the cells less susceptible to the induction of lymphoid tumors should be explored. Observations in our laboratory on irradiated-adrenalectomized-gonadectomized mice treated with thyrotropin have given equivocal results. In one study a trend toward enhancement of lymphoma incidence was observed (17), while in another, slight inhibition was noted. Further studies are needed to clearly establish the influence of thyrotropic hormone.

SUMMARY

Studies on the effect of radiothyroidectomy and thyroid grafts on the incidence of thymic implant lymphomas in thymectomized-irradiated C57BL mice are reported. Hypothyroidism significantly inhibited thymic implant tumor development in females. A similar reduction of lymphoma incidence in hypothyroid males was not statistically significant. When thyroid activity was restored by grafting normal thyroids to radiothyroidectomized animals, lymphoma incidence returned to the level seen in euthyroid animals.

I\(^{131}\) uptake measurements were made on a representative number of thyroids and thyroid grafts. There was no significant uptake by the I\(^{131}\)-treated thyroids. Thyroid grafts were just as active as thyroids from control animals.

Body weight decreased significantly in hypothyroid animals and was restored to control euthyroid levels in radiothyroidectomized animals by thyroid grafts. The possible influence of secondary nutritional and endocrine disturbances on leukemogenesis are discussed; it seems likely that the observed inhibition is attributable to hypothyroidism per se, rather than to secondary influences on nutrition or other endocrine imbalances.

Incidental observations on pituitary tumor development in lymphoma-free radiothyroidectomized animals are also reported. Pituitary tumor development was completely prevented by thyroid grafts after radiothyroidectomy.

REFERENCES


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